The Journal of Experimental Biology 208, 317-325
Published by The Company of Biologists 2005
doi:10.1242/jeb.01397

Energetics of a long-distance migrant shorebird (*Philomachus pugnax*) during cold exposure and running

Eric Vaillancourt[1], Sophie Prud'Homme[1], François Haman[1], Christopher G. Guglielmo[2] and Jean-Michel Weber[1,*]

[1]*Biology Department, University of Ottawa, Ottawa, Ontario, Canada* and [2]*Biological Sciences, University of Montana, Missoula, Montana, USA*

*Author for correspondence (e-mail: jmweber@science.uottawa.ca)

Accepted 15 November 2004

Summary

The metabolic consequences of cold exposure and exercise are not well characterized in birds. Ruff sandpipers *Philomachus pugnax* are migrant shorebirds traveling between Africa and Siberia for up to 30 000 km annually. Our goal was to quantify the fuel selection pattern of these remarkable athletes during shivering and terrestrial locomotion. We used indirect calorimetry and nitrogen excretion analysis to measure their rates of lipid, carbohydrate and protein oxidation at different temperatures (22, 15, 10 or 5°C) and different treadmill speeds (15, 20, 25, 30, 35 or 40 m min^{-1}). Results show that lipid oxidation supplies nearly all the energy necessary to support shivering and running, and that the pattern of oxidative fuel selection is independent of shivering or running intensity. During shivering, total ATP production is unequally shared between lipids (82%), carbohydrates (12%) and proteins (6%). During running, lipids remain the dominant substrate (66%), with carbohydrates (29%) and proteins (5%) playing more minor roles. The prevailing use of lipids during intense shivering and high-speed running is not consistent with the fuel selection pattern observed in exercising and cold-exposed mammals. The exact mechanisms allowing birds to use lipids at extremely high rates are still largely unexplored, and quantifying the relative importance of different fuels during long-distance flight remains a major challenge for future research.

Key words: oxidative fuel utilization, fuel selection, lipid, carbohydrate, animal energetics, indirect calorimetry, energy expenditure, shivering, exercise, ruff sandpiper, *Philomachus pugnax*.

Introduction

The metabolic consequences of cold exposure and exercise have been well characterized in mammals (Himms-Hagen, 1996; Rowell and Shepherd, 1996), but little information is available for birds (Bicudo et al., 2001; Butler, 1991; Hohtola et al., 1998; St-Laurent and Larochelle, 1994), and the physiological changes caused by shivering and exercise are even less well understood for migrants (Jenni-Eiermann et al., 2002; Klaassen, 1996; Kvist et al., 2001; Piersma et al., 2003; Ramenofsky, 1990; Ward et al., 2002). Ruff sandpipers *Philomachus pugnax* are long-distance migrant shorebirds that fly from wintering areas in Africa to nesting grounds in North-eastern Siberia; this annual round trip can reach 30 000 km (Cramp and Simmons, 1983). These remarkable athletes have been able to extend the physiological limits of endurance capacity, but nothing is known of their pattern of metabolic fuel selection. Quantifying the relative contributions of the different fuels to total metabolism has proved very difficult, mainly because methods routinely used in mammalian research are not easily adaptable to birds.

Migration flights are energetically very demanding, but other activities than flying also offer significant physiological challenges. For instance, metabolic rate must be increased when low temperatures are encountered (during the night or at high altitude), or when the birds are rapidly building fat reserves and spend a lot of time running while feeding. In some species, leg muscles are even known to hypertrophy during stopovers (Piersma et al., 1999). Therefore, shivering thermogenesis and terrestrial locomotion are two ecologically relevant activities regularly performed by ruff sandpipers.

Acclimation to cold environments has been the subject of many bird studies (Ballantyne and George, 1978; Block, 1994). For acute cold exposure, however, most of the work has been carried out on juvenile birds, and it has been shown that they cannot thermoregulate (Østnes et al., 2001). As they age, shivering thermogenesis develops in leg and pectoral muscle, and the latter becomes a major site of heat production (Marjoniemi and Hohtola, 1999), although few studies have determined which oxidative fuels are being used. One study provides indirect information on metabolic fuels during cold exposure, and reports respiratory exchange ratios (RER) of

0.70 and 0.77 (RER=$\dot{V}_{CO_2}/\dot{V}_{O_2}$) in fasted and fed Arctic terns *Sterna paradisaea*, suggesting that lipid oxidation is dominant (Klaassen et al., 1989). However, the RER values given were not corrected for protein oxidation, and results from a single species exposed to two temperatures cannot be generalized. It is possible that the relative use of carbohydrates (CHO) for thermogenesis is higher in other species and at lower temperatures. For example, the use of glycogen as a thermogenic fuel during extreme cold exposure was investigated in the pectoral muscle of pigeons (Parker and George, 1975). The authors concluded that carbohydrates could become a significant fuel for heat production during high-intensity shivering.

Most of the information available on avian fuel metabolism during exercise is based on measurements of metabolite concentrations and total body composition in captive birds, or in wild animals caught at various stages of migration (Guglielmo et al., 2002; Jenni and Jenni-Eiermann, 1998; Jenni-Eiermann et al., 2002). All these studies have shown that birds can use lipids at very high rates, but they have not provided much information on carbohydrate metabolism, on individual birds (i.e. only groups of animals are compared), or on the time course of changes in fuel utilization (only start and end points have been measured). In a few cases, it has been possible to exercise birds in flying wheels (red junglefowl, Chappell et al., 1996; Hammond et al., 2000; house sparrow, Chappell et al., 1999) or in wind-tunnels (pigeon, Rothe et al., 1987; Rothe and Nachtigall, 1987; thrush nightingale, Klaassen et al., 2000; Lindström et al., 1999; European starling, Ward et al., 2001; barnacle and bar-headed geese, Ward et al., 2002; red knot, Jenni-Eiermann et al., 2002; Kvist et al., 2001). Changes in metabolite concentration reported in many of these studies are very useful, but they only provide an estimate of fuel utilization, and conclusions based on such measurements can be misleading (e.g. see Haman et al., 1997). In contrast, dynamic changes in substrate oxidation over time can be followed using indirect calorimetry, and this is the main reason it was selected for our experiments. Unfortunately, this method has rarely been applied to bird exercise studies in the past and the RER values reported have never been used to calculate fuel oxidation (Brackenbury and Vincent, 1988; Rothe et al., 1987; Ward et al., 2002, 2001). In this study, our goal was to quantify the rates of lipid, carbohydrate and protein oxidation in ruff sandpipers during cold exposure and terrestrial locomotion, using indirect calorimetry and nitrogen excretion measurements. Even though it is well established that long-distance flight is predominantly supported by lipid oxidation, the fuel selection patterns of running and shivering birds are not known. Information presently available shows that the neural and hormonal pathways regulating body temperature and thermogenesis are very different between birds and mammals (Hissa, 1988). Therefore, we anticipated that fuel selection of birds and mammals would also be different. The experimental approach selected in our study has enabled characterization of how the duration and the intensity of running and cold exposure affect fuel metabolism.

Materials and methods

Animals

Adult European ruff sandpipers *Philomachus pugnax* L. were obtained from a captive colony (Dr David Lank, Simon Fraser University, Burnaby, British Columbia, Canada). The birds were kept indoors in a room allowing flight (2.1 m×3.9 m×2.4 m) with *ad libitum* access to food (Zeigler, Finfish Silver, Gardners, PA, USA; 42% protein; 10% lipid; 4% fiber) and water (67 cm×42 cm×11 cm deep water basin with ramp). The room had no windows and was only supplied with artificial light. Photoperiod (12 h:12 h L:D) and temperature (22°C) were kept constant. The animals were acclimated to these conditions for at least 2 months before starting experiments. Seven females and 3 males (mean mass ± S.E.M., 110±7 g) were used for the experiments. The physiological parameters reported in this study showed no gender differences, and therefore results for males and females were pooled. Average body masses for males and females were 166±6 g and 98±2 g, respectively, and were very similar to the values reported by Cramp and Simmons (1983) for wintering birds (December–February; 172 g for males and 99 g for females). Body mass is known to increase significantly before and during spring migration (March–April; 210 g for males and 132 g for females), and therefore our experiments were performed in lean ruffs that were not physiologically prepared for enhanced lipid oxidation associated with migration. All experiments were started 30 min after the animals had stopped having access to food.

Indirect calorimetry

For cold exposure, rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) were measured using an Oxymax system (Columbus Instruments, Columbus, OH, USA) supplied with room air at 2–3 l min^{-1} as detailed previously (Weber and O'Connor, 2000). For exercise, gas exchange was measured with Applied Electrochemistry analyzers (models S-3A/II and CD-3A; Pittsburgh, PA, USA), using an air flow rate of 2 l min^{-1}. Small fans ensured that the air was continuously mixed in the measuring chambers. Air flow rate was controlled by a mass flow regulator accurate to within 1% of full scale and calibrated using a reference volume meter (Porter Instruments, Edgemont, PA, USA). Oxygen and CO_2 concentrations were measured in the inflow and outflow air after removing water vapor through calcium sulphate columns (Drierite; W. A. Hammond, Xenia, OH, USA). New calcium sulfate was always exposed to air for 5 min before use to avoid CO_2 absorption during measurements. All analyzers were calibrated with known gas mixtures before and after each experiment. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were corrected for dry gas under standard temperature and pressure conditions (STPD). Both experimental systems were accurate to within ±3% after bleeding known amounts of CO_2 or N_2, or within ±2% by burning 99% ethanol in the respirometers. The method of indirect calorimetry was selected for these experiments because it is non-lethal, non-invasive, and allows us to measure changes in the rates of metabolic fuel utilization in individual

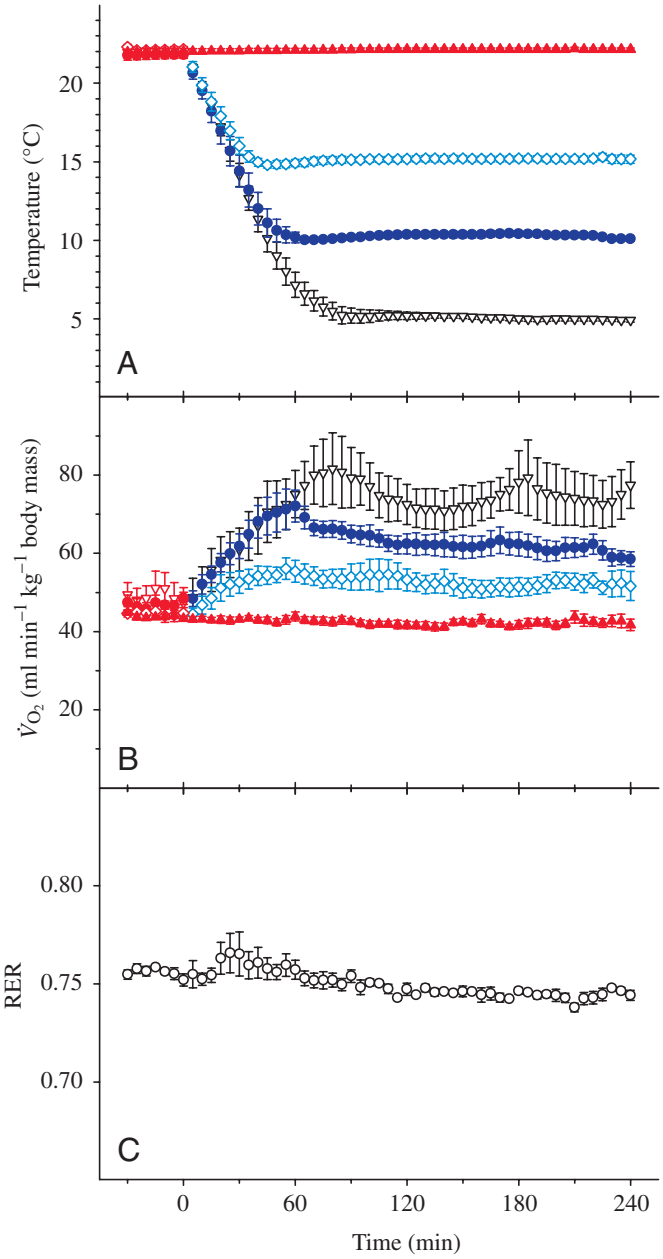

Fig. 1. Changes in (A) ambient temperature, (B) rate of oxygen consumption ($\dot{V}_{O_2}$) and (C) respiratory exchange ratio (RER) of adult ruff sandpipers. Treatment temperatures are indicated as follows: 22°C control (red triangles), 15°C (open blue diamonds), 10°C (blue circles) and 5°C (open inverted triangles). RER values were pooled (open circles) because they were not significantly different between treatments. Values are means ± S.E.M. (N=6).

animals over time (i.e. rates of carbohydrate and lipid oxidation after correcting for protein oxidation, itself estimated by measuring the rate of nitrogen excretion). These advantages are not provided by alternative methods commonly used in this field (i.e. changes in blood or tissue metabolite concentrations, or whole body composition analyses).

Cold exposure experiments

Shivering experiments were carried out in a closed

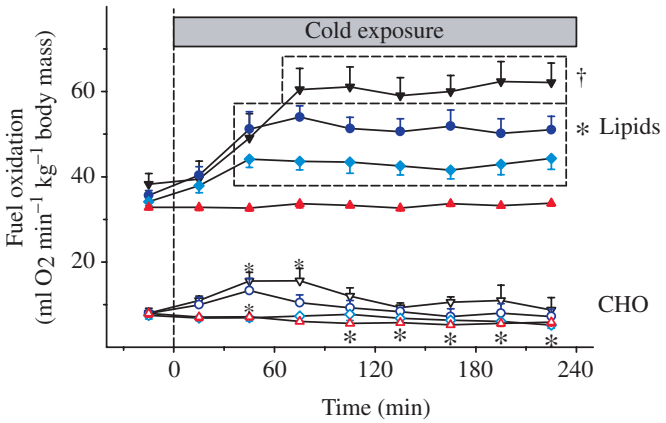

Fig. 2. Changes in rates of carbohydrate (CHO; open symbols) and lipid oxidation (filled symbols) over time for ruff sandpipers exposed to 22°C (control) (red triangles), 15°C (blue diamonds), 10°C (blue circles) and 5°C (black inverted triangles). Values are means + S.E.M. (N=6). Significant differences from baseline for individual values (CHO) or boxed groups (lipids) are shown; *P<0.05, †P<0.001.

respirometer (38 cm×26 cm×21 cm) connected to a cooling bath (PolyScience, Niles, IL, USA) containing Canadian windshield washer fluid. The refrigerated fluid was recirculated within the respirometer walls. All the cold exposure measurements were started between 9:00 and 11:00 h, and no food or water was available in the respirometer. While shivering, the birds stood quietly in place. No walking or jumping was observed. A piece of perforated Plexiglas covered the respirometer floor to protect the animal's feet. Before collecting data, each bird was placed in the respirometer for 5 h at 22°C on two separate occasions to familiarize it with the experimental set-up. After familiarization, each animal was measured at four temperatures in random order (22, 15, 10 and 5°C) with a minimum of 3 days between measurements. Each experiment included a 60 min baseline period at 22°C, a transition period of 30–60 min to reach the test temperature, and a 3 h period at the test temperature. Ambient respirometer temperature was recorded every 5 min.

Exercise experiments

The metabolism of running birds was quantified on a motorized treadmill enclosed in an acrylic respirometer (50 cm×28 cm×14 cm) at an incline of 8% (modified Simplex II, rat treadmill respirometer from Columbus Instruments). This incline was selected to reach as high a metabolic rate as possible without wing flapping. The animals were familiarized with the experimental set-up by running at different speeds for at least three practice sessions of 15–30 min. Baseline gas exchange values (speed 0) were obtained by leaving each animal quietly in the respirometer for 60 min in the dark. The last 10 min of this resting period were used to quantify pre-exercise $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. In each exercise session, two different running speeds were monitored until steady gas exchange values were reached (i.e. when the coefficient of variation remained <10% for at least 10 min).

Each bird was measured at speeds of 15, 20, 25, 30, 35 and 40 m min^{-1}. The order of speeds tested was randomized and successive sessions for the same animal were separated by at least 24 h.

Nitrogen excretion

The rate of nitrogen excretion was measured by collecting excreta for 6 h in eight fasting individuals kept at 22°C, and by quantifying the concentrations of uric acid (Marquardt, 1983) and urea (Kit 640-A; Sigma, St Louis, MO, USA). A mean value of 0.534±0.056 mg N min^{-1} kg^{-1} body mass (N=8) was measured and used for all birds in our calculations. Therefore, we have assumed that the rate of protein oxidation was not affected significantly by cold exposure or running.

Calculations and statistical analyses

Rates of carbohydrate and lipid oxidation (CHO_{ox} and FAT_{ox}, respectively) were calculated from $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and the rate of nitrogen excretion using the equations of Frayn (1983) modified for uricotelic animals (Walsberg and Wolf, 1995), and for the units used in our study:

$$CHO_{ox} = 3.39\dot{V}_{CO_2} - 2.39\dot{V}_{O_2} - 0.65\dot{N},$$

$$FAT_{ox} = 3.39\dot{V}_{O_2} - 3.39\dot{V}_{CO_2} - 5.28\dot{N},$$

where rates of carbohydrate and lipid oxidation are in ml O_2 min^{-1} kg^{-1} body mass, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are in ml min^{-1} kg^{-1} body mass, and $\dot{N}$ is the rate of nitrogen excretion in mg N min^{-1} kg^{-1} body mass.

Percentages were transformed to the arcsine of their square root before analysis. For cold exposure, mean $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, RER and percentages were compared using one- or two-way, repeated-measures analyses of variance (ANOVA). For running data, one-way ANOVA was used because sample size differed between speeds. For each bird, the cost of transport was measured and compared to values calculated from allometric equations for shorebirds only (Bruinzeel et al., 1999) or for birds in general (Taylor et al., 1982). Mean observed and predicted values were compared using a one-way ANOVA. Comparisons between test and control means were performed using Bonferroni's adjustment. Decisional threshold was set at P<0.05 and all the values presented are means ± S.E.M.

Results

Cold exposure experiments

Changes in environmental temperature, metabolic rate ($\dot{V}_{O_2}$) and RER during cold exposure are presented in Fig. 1. After a progressive decrease from baseline temperature of 22°C, the animals were kept under constant cold conditions for 3 h at 15, 10 or 5°C (Fig. 1A). While standing quietly at 22°C, metabolic rate was 46.1±1.0 ml O_2 min^{-1} kg^{-1} body mass. When temperature was decreased, metabolic rate increased proportionately with the intensity of cold exposure, and reached a maximum of 81.6±9.1 ml O_2 min^{-1} kg^{-1} body mass after 80 min at the lowest test temperature of 5°C (Fig. 1B). RER was not significantly different between temperatures (P>0.05), and therefore values for all treatments were pooled for this parameter (Fig. 1C). RER did not change from the mean baseline value of 0.756±0.003 over time (P>0.05). However, when the effect of time was tested separately for each temperature, RER was temporarily elevated from baseline levels between 25 and 30 min in the 5°C group (P<0.05).

Changes in the rates of carbohydrate and lipid oxidation (CHO_{ox} and FAT_{ox}) during cold exposure are presented in Fig. 2. At all times, FAT_{ox} was more than 3.2-fold higher than CHO_{ox} (the average ratio of FAT_{ox}/CHO_{ox} was 5.6). Overall, CHO_{ox} was higher at 5°C than at all other temperatures (P<0.05). Cold exposure had no effect on CHO_{ox} over time (P>0.05), except between 45 and 75 min at 5°C, when it was higher than baseline (P<0.05). FAT_{ox} was increased in proportion with the intensity of cold exposure. All treatment temperatures were significantly different from each other (P<0.001), and each treatment showed a significant increase in FAT_{ox} over time, except for the control group kept at 22°C (P<0.001 for 5, 10 and 15°C; P>0.05 for control). Regression analysis shows that the slope of the relationship between FAT_{ox} and $\dot{V}_{O_2}$ is very different from 0 (slope=0.841; r^2=0.920; P<0.001). The relationship between CHO_{ox} and $\dot{V}_{O_2}$ is also significant, but the slope is much shallower than for FAT_{ox} (slope=0.174; r^2=0.338; P<0.01).

All the parameters measured in the cold exposure experiments reached steady-state values (see Fig. 1B), and are summarized in Table 1. These values were calculated by averaging measurements over the last 25 min at each

Table 1. *Oxygen consumption, carbon dioxide production, respiratory exchange ratio, carbohydrate and lipid oxidation in birds exposed to 22, 15, 10 or 5°C*

	Temperature (°C)			
	22 (Control)	15	10	5
$\dot{V}_{O_2}$ (ml min^{-1} kg^{-1} body mass)	42.9±1.3[a]	52.7±2.1[a,b]	61.4±2.4[b,c]	74.0±7.0[c]
$\dot{V}_{CO_2}$ (ml min^{-1} kg^{-1} body mass)	32.1±1.2[a]	38.8±1.4[a,b]	45.6±1.7[b,c]	54.9±5.7[c]
RER	0.747±0.005[a]	0.737±0.005[a]	0.742±0.008[a]	0.739±0.008[a]
CHO_{ox} (ml O_2 min^{-1} kg^{-1} body mass)	5.9±0.8[a]	5.2±0.8[a]	7.2±1.7[a]	8.7±3.0[a]
FAT_{ox} (ml O_2 min^{-1} kg^{-1} body mass)	33.8±0.7[a]	44.3±2.6[a,b]	51.1±3.1[b,c]	62.1±4.6[c]

$\dot{V}_{O_2}$, rate of oxygen consumption; $\dot{V}_{CO_2}$, rate of carbon dioxide production; RER, respiratory exchange ratio; CHO_{ox}, carbohydrate oxidation; FAT_{ox}, lipid oxidation.

Values are means ± S.E.M. (N=6). Values not sharing a common superscript letter are significantly different (P<0.05).

temperature, and they are presented for gas exchange ($\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and RER) and fuel utilization (CHO_{ox} and FAT_{ox}). Steady-state values were progressively higher for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and FAT_{ox} as cold exposure intensified. However, RER and CHO_{ox} were not affected by temperature (Table 1).

Exercise experiments

Oxygen consumption and RER of birds running at various speeds are shown in Fig. 3. Baseline values for animals resting on the treadmill at 22°C were 51.7±4.0 ml min^{-1} kg^{-1} body mass for $\dot{V}_{O_2}$ and 0.761±0.008 for RER (N=11). These resting rates were not different from baseline values measured in the cold exposure experiments (P>0.05; Fig. 1). $\dot{V}_{O_2}$ increased progressively with running speed (P<0.05; Fig. 3A), and the highest exercise $\dot{V}_{O_2}$ of 78.9±3.5 ml O_2 min^{-1} kg^{-1} body mass was measured at a speed of 35 m min^{-1}. RER was elevated from baseline at low and intermediate speeds (P<0.05 at 15, 30 m min^{-1}; P<0.001 at 25 m min^{-1}), but it was not different from baseline at the two highest speeds (P>0.05; Fig. 3B). The slope of the regression line for RER *vs* speed was not different from zero (P>0.05).

Fig. 4 shows CHO_{ox} and FAT_{ox} as a function of speed. At all times, FAT_{ox} was more than 1.6-fold higher than CHO_{ox} (the average ratio of FAT_{ox}/CHO_{ox} was 2.5). Baseline CHO_{ox} in animals resting on the treadmill was 9.07±1.28 ml O_2 min^{-1} kg^{-1} body mass. The slope of the regression line for the CHO_{ox} *vs* running speed was not different from zero (P>0.05). In contrast, FAT_{ox} increased progressively as speed increased (slope of regression line higher than zero; P<0.01).

Fig. 5 shows the relationship between the energy cost of locomotion per unit time (E_{metab}) and running speed. Mean resting rate of energy expenditure was 2.21±0.23 J s^{-1} (speed=0), and the slope of the linear regression between E_{metab} and speed was different from 0 (P<0.01). The slope of this relationship, or cost of transport (energy cost per unit distance) for running ruff sandpipers was 1.29 J m^{-1}. This value is significantly lower than predicted from the allometric equation for birds in general (2.49 J m^{-1}; P<0.01; Taylor et al., 1982). However, it is not different from the value predicted from the allometric equation specifically derived for shorebirds (1.68 J m^{-1}; P>0.05; Bruinzeel et al., 1999).

Fuel selection during cold exposure and exercise

The relative contributions of carbohydrate, lipid and protein oxidation to $\dot{V}_{O_2}$ during cold exposure and running are summarized in Fig. 6. In the cold exposure experiments (Fig. 6A), lipid oxidation accounted for more than 80% of $\dot{V}_{O_2}$, the same value observed in the control animals held at 22°C. Protein oxidation (4–7%) and carbohydrate oxidation (10–14%) had much lower relative contributions. The importance of carbohydrates and lipids did not vary between temperatures (P>0.05), but the percentage contribution of proteins decreased with temperature (P<0.001).

In the exercise experiments (Fig. 6B), lipid oxidation contributed 75% of $\dot{V}_{O_2}$ at speed 0. This contribution was lower at 15, 25 and 30 m min^{-1} (58–62%; P<0.05) but did not differ from the resting value at the other speeds measured (65–72%; P>0.05). Exercise caused an increase in relative carbohydrate oxidation above resting levels (P<0.001), but only at the lower running speeds (15–30 m min^{-1}). The relative contribution of

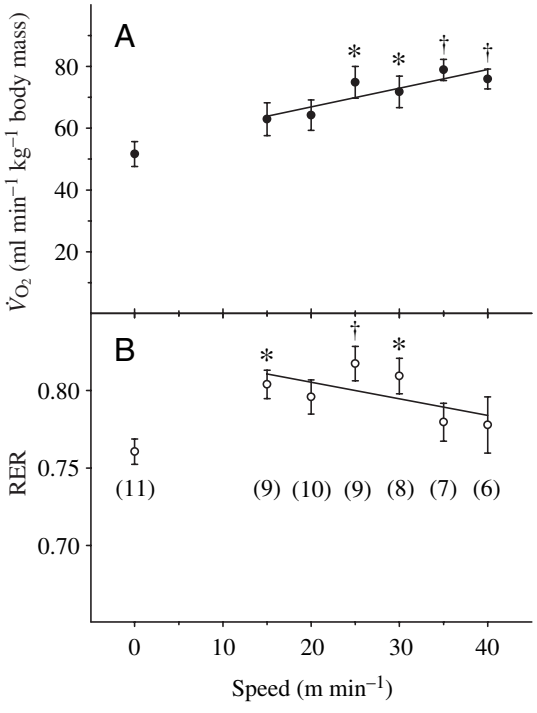

Fig. 3. (A) Rate of oxygen consumption ($\dot{V}_{O_2}$) and (B) respiratory exchange ratio (RER) of ruff sandpipers at rest and during running at various speeds. The lines indicated were fitted by linear regression on the exercise values only (A: y=0.64x+54.07; B: y=0.01x+0.824). Values are means ± S.E.M.; sample sizes are indicated in parentheses. Significant differences from values at rest are shown; *P<0.05, †P<0.001.

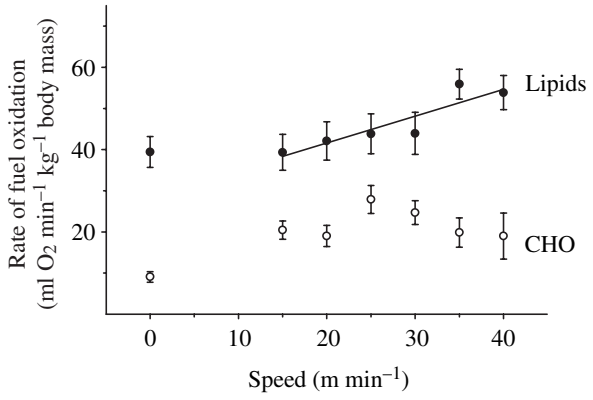

Fig. 4. Rates of carbohydrate (open circles) and lipid oxidation (filled circles) of ruff sandpipers at rest and during running at various speeds. The lines indicated were fitted by linear regression on the exercise values only (y=0.64x+29.53). Values are means ± S.E.M. (sample sizes as in Fig. 3).

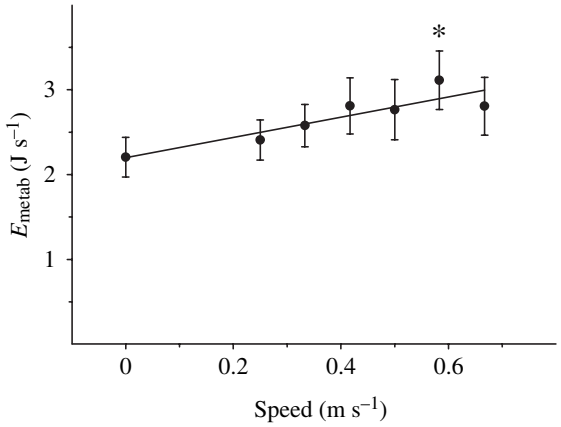

Fig. 5. Rate of energy expenditure of ruff sandpipers at rest (speed 0) and during running at various speeds. Line indicated was fitted by linear regression for exercise values only (y=0.02x+2.19). The cost of transport of running ruffs (=slope of line) is 1.29 J m^{-1}. Values are means ± S.E.M. (sample sizes as in Fig. 3). *Significant difference from control value at speed 0 (P<0.005).

protein oxidation to $\dot{V}_{O_2}$ was decreased during exercise compared to rest (P<0.001).

Discussion

Our goal was to quantify the pattern of oxidative fuel utilization of a long-distance migrant shorebird during cold exposure and running. Results show that shivering thermogenesis and land locomotion of ruff sandpipers are predominantly supported by lipid oxidation. The large lipid reserves of this endurance athlete provide most of the energy for heat generation and running, even at the lowest temperature and at the highest exercise intensity tested in our experiments.

Cold exposure

The metabolic rate of ruff sandpipers is stimulated in proportion with the intensity of cold exposure (Fig. 1B) and lipids are responsible for fueling shivering thermogenesis (except for a minor, transient contribution from carbohydrates; see Figs 2, 6A, and Table 1). This conclusion is further supported by the observation that the slope of the regression line between FAT_{ox} and $\dot{V}_{O_2}$ is much higher than for CHO_{ox} *vs* $\dot{V}_{O_2}$ (0.841 and 0.174, respectively). This study is the first to quantify the use of oxidative fuels for heat production in an avian species exposed to cold. It shows that the relative use of lipids and carbohydrates is not affected by shivering, and is therefore independent of environmental temperature (Fig. 6A). More than 80% of $\dot{V}_{O_2}$ is accounted for by lipid oxidation at all temperatures, even during intense shivering at 5°C. At the highest FAT_{ox} measured here (~62 ml O_2 min^{-1} kg^{-1} body mass), and assuming that the same pattern of fuel utilization is maintained, we can calculate that a 110 g ruff sandpiper with lipid reserves of 20% body mass (or half the maximal value observed just before migration; Van Rhijn, 1991) could shiver continuously for 4.5 days.

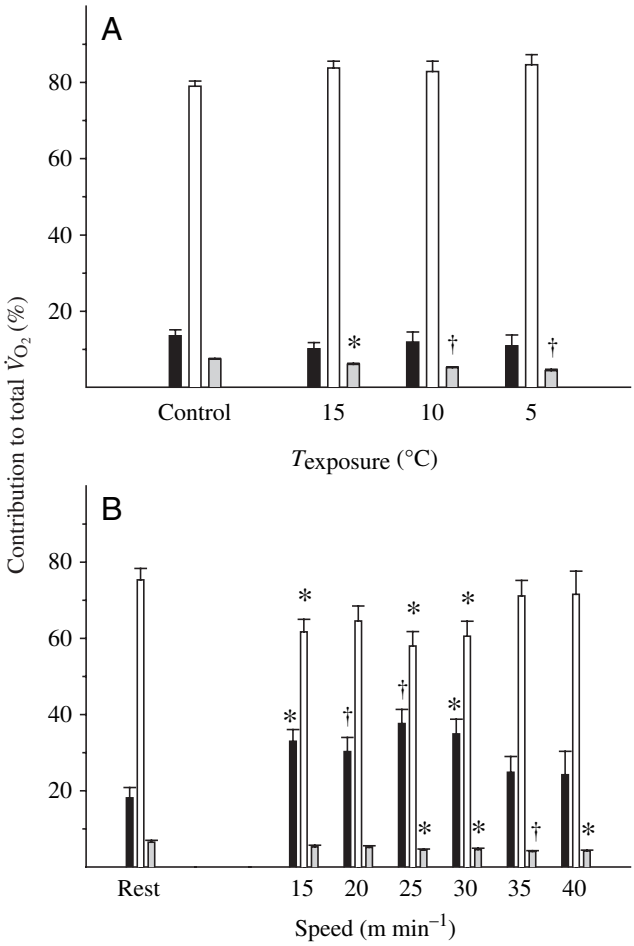

Fig. 6. Relative contributions of carbohydrates (CHO) (black bars), lipids (white bars) and proteins (grey bars) to total energy expenditure of ruff sandpipers during (A) cold exposure and (B) running. Values significantly different from controls (22°C for cold exposure and speed 0 for exercise) are indicated; *P<0.05, $^{\dagger}P$<0.001. Values are means + S.E.M. (N=6 for cold exposure and as in Fig. 3 for exercise).

How does this fuel selection pattern compare with what has been observed in other cold-exposed endotherms? In the absence of information on birds, we looked for shivering studies on mammals of similar size (and surface to volume ratio) as our sandpipers, but without much success. Using arterio–venous differences in substrate concentrations, the only study we were able to find reports that lipid oxidation accounts for more than 90% of $\dot{V}_{O_2}$ in cold-exposed rats (Adán et al., 1995). However, comparing these results with ours is not appropriate because the rats were acclimated to 22°C and kept in individual cages. Under such housing conditions, rats are known to produce significant amounts of brown adipose tissue, because they are well below their thermoneutral zone (29–31°C for Wistar rats; Romanovski et al., 2002) and cannot use social thermoregulation. Therefore, heat production from brown adipose tissue may explain the high FAT_{ox} reported for rats by Adán et al. (1995), whereas the presence of this specialized thermogenic tissue has never been demonstrated in birds (Saarela et al., 1991). In addition, we have recently

carried out experiments on group-housed rats acclimated to 28°C, to eliminate the thermogenic contribution of brown adipose tissue. Under these conditions, lipids were responsible for 52% $\dot{V}_{O_2}$ and carbohydrates for 37% $\dot{V}_{O_2}$ during prolonged exposure to 5°C (E. Vaillancourt, F. Haman and J.-M. Weber, unpublished).

Surprisingly, the only other mammal whose fuel metabolism has been well characterized during shivering is the adult human. In this experimental model, it was established that lipids play a much less important role than observed in ruff sandpipers. For cold exposure conditions eliciting a twofold increase in metabolic rate, lipid oxidation is responsible for 50% of $\dot{V}_{O_2}$ in humans (Haman et al., 2002). The exact reasons for these discrepancies are unknown, but may be due to differences in the fiber composition of shivering muscles. In humans, it has been shown recently that CHO_{ox} for thermogenesis depends on the specific recruitment of type II, fast glycolytic fibers, responsible for 'burst shivering' (Haman et al., 2004). In long-distance migrant birds, large pectoral muscles produce most of the heat, and the metabolic machinery of their fibers is specifically geared for lipid oxidation. Therefore, the high metabolic capacity of flight muscles for fat catabolism could explain why, on their own, lipids account for over 80% of $\dot{V}_{O_2}$ in cold-exposed ruffs. This high capacity for lipid oxidation and the large lipid reserves available in migrant birds mean that carbohydrates and proteins only play minimal roles during shivering, each accounting for less than 14% of $\dot{V}_{O_2}$. Taken together, these results show that lipids probably play a much more prominent role for shivering thermogenesis in migrant birds than in mammals.

At the lowest temperature tested in our study, metabolic rate appeared to fluctuate with a period of ~90 min (Fig. 1B) and this pattern may be related to a well-known heat-saving strategy previously observed in birds. Similar cyclic variation in $\dot{V}_{O_2}$ and leg temperature has been observed in cold-exposed pigeons; these changes are caused by vasoconstriction/vasodilation cycles of the leg geared to decrease heat loss from this uninsulated region of the body. Interestingly, pigeons exposed to a lower temperature than tested here (–10°C) showed a shorter period of only 20 min (Østnes and Bech, 1998). Further research is needed to establish whether this energy-saving strategy is found in all birds or only in species regularly exposed to cold conditions.

Exercise

As experimental models, long-distance migrant birds provide a unique vantage point to study the extreme performance of metabolic systems during exercise. Unfortunately, making physiological measurements during migration or simulating prolonged flight in the laboratory are extremely difficult and rarely attempted. As a compromise, and because little *in vivo* information is presently available on avian exercise, we reasoned that investigating land locomotion would be an important first step towards a better understanding of fuel metabolism in exercising migrants. However, it is clear that running does not simulate migration because leg and flight muscles are different, and maximal metabolic rates achievable during running are low compared to flight.

Indirect calorimetry measurements in running or flying birds have only been performed in a few studies that report $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (or RER), but these parameters have never been used to determine oxidation rates of metabolic fuels (Brackenbury and Vincent, 1988; Rothe et al., 1987; Suarez et al., 1990; Tucker, 1968; Vincent and Brackenbury, 1988; Ward et al., 2002, 2001). In the present study, we have quantified $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and nitrogen excretion to calculate absolute rates of fat, carbohydrate and protein oxidation as well as the relative contribution of each fuel to $\dot{V}_{O_2}$ during running (Figs 3, 4 and 6B). Results show that, as for cold exposure, lipids play a dominant role in energy metabolism during running (Fig. 6B). When exercise intensity is increased, FAT_{ox} is augmented whereas CHO_{ox} remains independent of running speed (Fig. 4). The overall fuel selection pattern of exercising ruff sandpipers stays relatively constant across speeds, with lipids providing more energy than all other fuels combined (58–72% $\dot{V}_{O_2}$) and carbohydrates being responsible for about half the contribution of lipids (24–38% $\dot{V}_{O_2}$; Fig. 6B). The increased reliance on carbohydrates associated with higher running speeds commonly observed in mammals does not occur in birds, showing that the fuel selection patterns of exercising birds and mammals are different. Even though lipids are well known to provide most of the energy for sustained flying in long-distance migrant birds (Guglielmo et al., 2002; Jenni and Jenni-Eiermann, 1998, 2002), the present study is the first to investigate running, and to provide detailed information about the use of all oxidative fuels under controlled exercise conditions of different intensities.

For comparison, we have calculated FAT_{ox} and CHO_{ox} from published bird exercise studies reporting $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values, but without correcting for protein oxidation (because rates of nitrogen excretion are not available). The only two treadmill studies we could find show that CHO oxidation accounts for >80% of $\dot{V}_{O_2}$ in running chickens (Brackenbury and Vincent, 1988; Vincent and Brackenbury, 1988). The divergent fuel selection patterns observed in highly aerobic ruff sandpipers and sedentary, domesticated chickens can probably be explained by differences in the fiber composition of leg muscles (and associated enzymatic machinery), a parameter known to vary with species, age and gender (Guglielmo et al., 2002; Olson, 2001). From studies on flying birds, we calculated that lipid oxidation accounts for >65% of $\dot{V}_{O_2}$ (budgerigar, Tucker, 1968; pigeon, Rothe et al., 1987; European starling, Ward et al., 2001; barnacle and bar-headed goose, Ward et al., 2002), except for hovering hummingbirds that can temporarily rely entirely on carbohydrates while feeding on nectar (Suarez et al., 1990). Migrant birds must clearly rely predominantly on lipids during non-stop, long-distance flights because alternative fuels are only stored in very small quantities, and would therefore be rapidly depleted.

Land locomotion is particularly important for long-distance migrant shorebirds because it allows them to replenish energy reserves rapidly during short stopovers. Strong selection

pressure for decreasing the cost of walking and running may therefore be responsible for the very low cost of transport (energy cost per unit distance) observed here in ruff sandpipers. This cost is 48% lower than predicted from the allometric equation established for birds in general (Taylor et al., 1982), but it is not different from the value predicted from the allometric equation for shorebirds only (Bruinzeel et al., 1999). This observation suggests that economical running is not an exclusive attribute of migrants, but that it is a common feature of all shorebirds, possibly related to their particular leg morphology.

Conclusions

The energy necessary to support shivering and running in ruff sandpipers is provided almost exclusively by the oxidation of lipid reserves. Their pattern of oxidative fuel selection does not depend on shivering or running intensity. During shivering, total ATP production is unequally shared between lipids (82%), CHO (12%) and proteins (6%). During land locomotion, lipids remain the dominant substrate (66%), with CHO (29%) and proteins (5%) playing more minor roles. The prevailing use of lipids during intense shivering and high-speed running is not consistent with the fuel selection pattern observed in exercising and cold-exposed mammals. Long-distance flying is well known to be supported primarily through lipid oxidation and this study shows that the same source of fuel is also dominant during other activities like intense running and shivering. The exact mechanisms allowing birds to use lipids at extremely high rates are still largely unexplored, and quantifying the exact importance of proteins and carbohydrates during long-distance flight remains a major challenge for future research.

This work would not have been possible without help from David Lank (Simon Fraser University) who provided essential advice and the animals used in this study. The research was supported by an NSERC discovery grant to J.-M.W.

References

Adán, C., Ardévol, A., Remesar, X., Alemany, M. and Fernández-López, J. A. (1995). Hindleg muscle energy and substrate balances in cold-exposed rats. *J. Exp. Biol.* **198**, 1243-1251.

Ballantyne, J. S. and George, J. C. (1978). An ultrastructural and histological analysis of the effects of cold acclimation on vertebrate skeletal muscle. *J. Therm. Biol.* **3**, 109-116.

Bicudo, J. E. P. W., Vianna, C. R. and Chaui-Berlinck, J. G. (2001). Thermogenesis in birds. *Biosci. Rep*. **21**, 181-188.

Block, B. A. (1994). Thermogenesis in muscle. *Ann. Rev. Physiol.* **56**, 535-577.

Brackenbury, J. H. and Vincent, R. (1988). Utilisation of energy substrates in treadmill-exercised domestic fowl (*Gallus gallus domesticus*): blood glucose. *Br. Poult. Sci.* **29**, 457-468.

Bruinzeel, L. W., Piersma, T. and Kersten, M. (1999). Low costs of terrestrial locomotion in waders. *Ardea* **87**, 199-205.

Butler, P. J. (1991). Exercise in birds. *J. Exp. Biol.* **160**, 233-262.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269-2279.

Chappell, M. A., Zuk, M. and Johnsen, T. S. (1996). Repeatability of aerobic performance in red junglefowl: Effects of ontogeny and nematode infection. *Funct. Ecol.* **10**, 578-585.

Cramp, S. and Simmons, K. E. L. (1983). *Philomachus pugnax* Ruff. In *Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic*, vol. III. *Waders to gulls* (ed. S. Cramp and K. E. L. Simmons), pp. 385-402. New York: Oxford University Press.

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* **55**, 628-634.

Guglielmo, C. G., Haunerland, N. H., Hochachka, P. W. and Williams, T. D. (2002). Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a migratory shorebird. *Am. J. Physiol.* **282**, R1405-1413.

Haman, F., Legault, S. R. and Weber, J.-M. (2004). Fuel selection during intense shivering in humans: EMG pattern reflects carbohydrate oxidation. *J. Physiol.* **556**, 305-313.

Haman, F., Péronnet, F., Kenny, G. P., Massicotte, D., Lavoie, C., Scott, C. and Weber, J.-M. (2002). Effect of cold exposure on fuel utilization in humans: plasma glucose, muscle glycogen, and lipids. *J. Appl. Physiol.* **93**, 77-84.

Haman, F., Zwingelstein, G. and Weber, J.-M. (1997). Effects of hypoxia and low temperature on substrate fluxes in fish: plasma metabolite concentrations are misleading. *Am. J. Physiol.* **273**, R2046-R2054.

Hammond, K. A., Chappell, M. A., Cardullo, R. A., Lin, R. and Johnsen, T. S. (2000). The mechanistic basis of aerobic performance variation in red junglefowl. *J. Exp. Biol.* **203**, 2053-2064.

Himms-Hagen, J. (1996). Neural and hormonal responses to prolonged cold exposure. In *Handbook of Physiology*, vol. 1 (ed. M. J. Fregly and C. M. Blatteis), pp. 439-480. New York: Oxford University Press.

Hissa, R. (1988). Controlling mechanisms in avian temperature regulation: a review. *Acta Physiol. Scand.* **132**, 1-148.

Hohtola, E., Henderson, R. P. and Rashotte, M. E. (1998). Shivering thermogenesis in the pigeon: the effects of activity, diurnal factors, and feeding state. *Am. J. Physiol.* **275**, R1553-R1562.

Jenni, L. and Jenni-Eiermann, S. (1998). Fuel supply and metabolic constraints in migrating birds. *J. Avian Biol.* **29**, 521-528.

Jenni-Eiermann, S., Jenni, L., Kvist, A., Lindström, Å., Piersma, T. and Visser, G. H. (2002). Fuel use and metabolic response to endurance exercise: a wind tunnel study of a long-distance migrant shorebird. *J. Exp. Biol.* **205**, 2453-2460.

Klaassen, M. (1996). Metabolic constraints on long-distance migration in birds. *J. Exp. Biol.* **199**, 57-64.

Klaassen, M., Bech, C. and Slagsvold, G. (1989). Basal metabolic rate and thermal conductance in Arctic tern chicks and the effect of heat increment of feeding on thermoregulatory expenses. *Ardea* **77**, 193-200.

Klaassen, M., Kvist, A. and Lindström, Å. (2000). Flight costs and fuel composition of a bird migrating in a wind tunnel. *Condor* **102**, 444-451.

Kvist, A., Lindström, Å., Green, M., Piersma, T. and Visser, G. H. (2001). Carrying large fuel loads during sustained bird flight is cheaper than expected. *Nature* **413**, 730-732.

Lindström, Å., Klaassen, M. and Kvist, A. (1999). Variation in energy intake and basal metabolic rate of a bird migrating in a wind tunnel. *Funct. Ecol.* **13**, 352-359.

Marjoniemi, K. and Hohtola, E. (1999). Shivering thermogenesis in leg and breast muscles of galliform chicks and nestlings of the domestic pigeon. *Physiol. Biochem. Zool.* **72**, 484-492.

Marquardt, R. R. (1983). A simple spectrophotometric method for the direct determination of uric acid in avian excreta. *Poult. Sci.* **62**, 2106-2108.

Olson, J. M. (2001). Ontogeny of catabolic and morphological properties of skeletal muscle of the red-winged blackbird (*Agelaius phoeniceus*). *J. Comp. Physiol. B* **171**, 527-542.

Østnes, J. E. and Bech, C. (1998). Thermal control of metabolic cold defence in pigeons *Columba livia. J. Exp. Biol.* **201**, 793-803.

Østnes, J. E., Jenssen, B. M. and Bech, C. (2001). Growth and development of homeothermy in nestling European shags (*Phalacrocorax aristotelis*). *Auk* **118**, 983-995.

Parker, G. H. and George, J. C. (1975). Glycogen utilization by the white fibres in the pigeon pectoralis as main energy process during shivering thermogenesis. *Comp. Biochem. Physiol.* **50A**, 433-437.

Piersma, T., Gudmundsson, G. A. and Lilliendahl, K. (1999). Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiol. Biochem. Zool.* **72**, 405-415.

Piersma, T., Lindström, Å., Drent, R. H., Tulp, I., Jukema, J., Morrison, R. I. G., Reneerkens, J., Schekkerman, H. and Visser, G. H. (2003). High

daily energy expenditure in incubating shorebirds on High Arctic tundra: a circumpolar study. *Funct. Ecol.* **17**, 356-362.

Ramenofsky, M. (1990). Fat storage and fat metabolism in relation to migration. In *Bird Migration: Physiology and Ecophysiology* (ed. E. Gwinner), pp. 214-231. New York: Springer-Verlag.

Romanovski, A. A., Ivanov, A. I. and Shimansky, Y. P. (2002). Ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality. *J. Appl. Physiol.* **92**, 2667-2679.

Rothe, H.-J., Biesel, W. and Nachtigall, W. (1987). Pigeon flight in a wind tunnel. II. Gas exchange and power requirements. *J. Comp. Physiol. B* **157**, 99-109.

Rothe, H.-J. and Nachtigall, W. (1987). Pigeon flight in a wind tunnel. I. Aspects of wind tunnel design, training methods and flight behaviour of different pigeon races. *J. Comp. Physiol. B* **157**, 91-98.

Rowell, L. B. and Shepherd, J. T. (1996). III. Control of energy metabolism during exercise. In *Handbook of Physiology*. Section 12. *Exercise: Regulation and integration of multiple systems* (ed. R. Terjung), pp. 839-1183. New York: Oxford University Press.

Saarela, S., Keith, J. S., Hohtola, E. and Trayhurn, P. (1991). Is the 'mammalian' brown fat-specific mitochondrial uncoupling protein present in adipose tissues of birds? *Comp. Biochem. Physiol.* **100B**, 45-49.

St-Laurent, R. and Larochelle, J. (1994). The cooling power of the pigeon head. *J. Exp. Biol.* **194**, 329-339.

Suarez, R. K., Lighton, J. R. B., Moyes, C. D., Brown, G. S., Gass, C. L. and Hochachka, P. W. (1990). Fuel selection in rufous hummingbirds: Ecological implications of metabolic biochemistry. *Proc. Natl. Acad. Sci. USA* **87**, 9207-9210.

Taylor, C. R., Heglund, N. C. and Maloiy, G. M. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 1-21.

Tucker, V. A. (1968). Respiratory exchange and evaporative water loss in the flying budgerigar. *J. Exp. Biol.* **48**, 67-87.

Van Rhijn, J. G. (1991). *The Ruff*. San Diego: Academic Press.

Vincent, R. and Brackenbury, J. H. (1988). Utilisation of energy substrates in treadmill-exercised domestic fowl (*Gallus gallus domesticus*): blood plasma free fatty acids. *Br. Poult. Sci.* **29**, 469-479.

Walsberg, G. and Wolf, B. (1995). Variation in the respiratory quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. *J. Exp. Biol.* **198**, 213-219.

Ward, S., Bishop, C. M., Woakes, A. J. and Butler, P. J. (2002). Heart rate and the rate of oxygen consumption of flying and walking barnacle geese (*Branta leucopsis*) and bar-headed geese (*Anser indicus*). *J. Exp. Biol.* **205**, 3347-3356.

Ward, S., Moller, U., Rayner, J. M. V., Jackson, D. M., Bilo, D., Nachtigall, W. and Speakman, J. R. (2001). Metabolic power, mechanical power and efficiency during wind tunnel flight by the European starling *Sturnus vulgaris*. *J. Exp. Biol.* **204**, 3311-3322.

Weber, J.-M. and O'Connor, T. (2000). Energy metabolism of the Virginia opossum during fasting and exercise. *J. Exp. Biol.* **203**, 1365-1371.