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The metabolic cost of fever in Pekin ducks

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ABSTRACT

Fever is an energetically expensive component of the mammalian immune system's acute phase response. Like mammals, birds also develop fever when exposed to pathogens, but, as yet, the energy requirements of febrile mediation in birds are not known. We injected ducks (*Anas platyrhynchos*; $n=8$) with 100 μ kg^{-1} LPS or sterile isotonic saline and recorded their core body temperatures while measuring their O_2 consumption and CO_2 production in an open-flow respirometric circuit. Lipopolysaccharide elicited robust increases in the core body temperatures of our birds. The metabolic rate of the ducks increased about 80 min after treatment with LPS, relative to the metabolic rate of saline injected birds, and peaked 100 min later when the highest body temperatures were recorded. Our ducks increased their energy expenditure by 33.1% for about 3 h to mount a febrile response that, on average, increased their body temperature 1.4 °C. Studies with humans and rats, kept at thermoneutral temperatures, found a 10–15% increase in metabolic rate for every 1 °C increase in body temperature. The increase in metabolic rate, reported here (23%/°C), is noticeably higher and we conclude that febrile mediation is metabolically more expensive in Pekin ducks than in mammals.

1. Introduction

Fever is a component of the acute phase response that is thought to increase survival to an immune challenge, but at the cost of a substantial amount of metabolic energy from the host (Ardawi and Newsholme, 1985; Bilbo and Nelson, 2002; Demas et al., 1997; Moret and Schmid-Hempel, 2000). Febrile mediation in mammals is reported to increase the metabolic rate by 10–15% for every 1 °C increase in body temperature (Banet, 1981; Barr et al., 1922; Kluger and Rothenburg, 1979; Roe and Kinney, 1965). Like mammals, birds also develop fever when exposed to bacterial endotoxin (Gray et al., 2005; Jones et al., 1983; Koutsos and Klasing, 2001; Maloney and Gray, 1998; Nomoto, 1996). However, no one has ever measured the metabolic cost of fever in birds.

Knowledge regarding the metabolic energy requirements of fever in birds would contribute to a better understanding of the evolutionary development of the febrile mechanism and will provide insight into the energetic trade-offs involved in immune defences in this homeothermic class. Also, knowing the metabolic cost of immune defences in this class is necessary to accurately calculate metabolic energy requirements in ecological prediction models.

Therefore, the aim of this study was to determine the metabolic cost of fever in an avian class. We hypothesized that the metabolic cost of febrile mediation in birds would be similar to the metabolic cost of febrile mediation in mammals because the underlying physiological mechanisms responsible for fever in birds and in mammals are comparable (Gray et al., 2005; Nomoto, 2003; Romanovsky et al., 2005).

For our study, we used Pekin ducks (*Anas platyrhynchos*) as experimental animals. Pekin ducks are phylogenetically old birds from the lineage galloanserine, which is thought to be the basal lineage from which all later orders of birds developed (vanTuinen et al., 2000). Pekin ducks develop robust fevers when injected with bacterial endotoxin (Gray et al., 2005; Maloney and Gray, 1998; Marais and Gray, 2009) and the febrile mechanism in ducks has been shown to extend to other non-passerines (Gray et al., 2005; Jones et al., 1983; Koutsos and Klasing, 2001; Maloney and Gray, 1998; Nomoto, 1996).

We challenged ducks with LPS, or a saline control, and then monitored their core body temperatures remotely, using biotelemetry and abdominally implanted temperature data loggers, at the same time as measuring O_2 consumption and CO_2 in an open-flow respirometric system.

2. Materials and methods

2.1. Ethical approval

The procedures of this study were approved by the Animal Ethics Committee of the University of the Witwatersrand (approval no: 2010/4/04).

2.2. Animals

Eight female Pekin ducks (*Anas platyrhynchos*) were used. The ducks, from different flocks, were obtained from a commercial

Abbreviations: EE, energy expenditure; LPS, lipopolysaccharide; RH, relative humidity; TRI, thermal response index

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poultry farm when they were 6 months old and experimentation commenced after a period of acclimatization, when the birds were ~9 months old. (Pekin ducks reach maturity by 14 weeks of age and have a life-span of 10–12 years (Cherry and Morris, 2008).) Our ducks had a mean body mass of 2.4 ± 0.4 kg. They were housed together in an indoor room and exposed to a 12 h:12 h light:dark cycle with lights on at 6 am and an ambient temperature of 22 ± 1 °C. The ducks had *ad libitum* access to water and dry chicken food enriched with minerals and vitamins.

2.3. Body temperature measurements

Core body temperature was measured with two methods: (1) abdominally implanted bio-telemetry units (model E-mitter, Minimitter, Sunriver, OR, USA.). Recordings were made at 1 min intervals with the use of a peripheral processor (VitalView System, Minimitter) connected to a personal computer and, (2) abdominally implanted miniature temperature data loggers (Tidbit, Onset Computer, Bourne, MA, USA). The data loggers have a limited data storage capacity and they could be programmed to continuously record body temperature every 10 min. For us, this was sufficient for body temperature recordings of our ducks to compare experimental days to days when they were not used in experimentation. However, on days of experimentation, we wanted to record core body temperature more frequently at 1 min intervals, and we therefore relied on body temperature data that was transmitted from the implanted bio-telemeters. This enabled us to accurately synchronize core body temperature data with metabolic rate measurements.

The bio-telemeters and data loggers were calibrated by water immersion against a high-accuracy thermometer (Quat 100, Heraeus, Germany), to an accuracy of 0.1 °C before implantation. The bio-telemeters were coated with silicone by the supplier and we coated the data loggers with wax (Sasol Wax, Sasolburg, RSA) before implantation. The ducks were anaesthetized with a gaseous induction of 2% Isoflurane (Safe Line Pharmaceuticals, Wadeville, JHB, RSA) in oxygen for surgical implantation of telemeters and data loggers. For this a plain endotracheal tube (internal dia. 33 mm) was positioned in the trachea to secure the duck's airway. Once the duck was fully anaesthetized the abdominal feathers were plucked and a 5 cm longitudinal incision was made in the belly, extending from the apex of the duck's keel, inferiorly. Data loggers were gently pushed through the opening, created by the incision. Thereafter, the surgical wound was closed with absorbable sutures. At the end of the surgical procedure the endotracheal tube was removed and the duck allowed to regain full consciousness before it was released in its housing pen. We commenced with experimental procedures three weeks after surgery.

At the end of experimentation the ducks were euthanized and the implanted data loggers were retrieved and the data downloaded for analysis.

2.4. Experimental procedure

The study was completed over a period of two months during which each duck was given one intramuscular injection of 100 μ kg^{-1} Lipopolysaccharide (LPS) from *Escherichia coli* (Sigma-Aldrich, St. Louis, MO, USA) dissolved in sterile isotonic saline and, on another occasion, a control injection of saline. The dose of LPS was based on the results of previous fever studies in birds (Gray et al., 2005; Johnson et al., 1993a; Maloney and Gray, 1998; Nomoto, 1996). Ducks were treated on two occasions, once with a control injection of saline and once with an injection of LPS. The order of these treatments was randomized and injections were given at 2 pm on the days of experimentation. Ducks were placed in a metabolic chamber at 12 pm and allowed 2 h to acclimatize, before receiving a treatment. Metabolic rate measurements continued for 12 h after treatment. We allowed at least two weeks between treatments.

2.5. Measuring energy expenditure

We used open circuit respirometry to continuously measure the rate of O_2 consumption (ml O_2 STPD kg^{-1} min^{-1}) and CO_2 production (ml CO_2 STPD kg^{-1} min^{-1}) of the ducks after injections of LPS or saline.

On the days of experimentation the birds were weighed to the nearest 1 g (FU series weighing scale, Snowrex, Taipei, Taiwan), and placed in a blackened 96 L airtight metabolic chamber (inside dimensions: $60 \times 40 \times 40$ cm). An oil pan ($60 \times 40 \times 6$ cm) filled with mineral oil (Sasol Oil, Johannesburg, RSA), and positioned at the bottom of the chamber, served as a trap for excrement. The birds were positioned on a grid above the oil pan. The ambient temperature of the laboratory, where we conducted these experiments, was kept constant at 23 °C which is within the thermoneutral zone of Pekin ducks (Simon-Opperman et al., 1978). The air flowing into the chamber was dried with silica gel (Sigma-Aldrich, St. Louis, MO, USA) before it entered the chamber. The rate of airflow into the chamber was controlled at 3 L/min STPD with a mass flow controller (GFC series, Aalborg, NY, USA).

The temperature and Relative Humidity (RH) of the air flowing into, and out of, the chamber was recorded every 60 s using two data loggers (Hobo, Onset Computer Corporation, Boston, USA) situated in the air line at the air inlet and outlet, respectively.

A sample of in-flowing air, taken from the air supply line at a point before the air passed through the mass flow controller, was analyzed for $\%O_2$ and $\%CO_2$ content with a gas analyzer (PowerLab, ADInstruments, Sydney, Australia). We used a sub-sampler (SS4, Sable Systems, Las Vegas, USA) to continuously extract 200 mL from the air flowing out of the chamber. This air sample was dried with silica gel and Drierite (Sigma-Aldrich, Munich, Germany) and then analyzed for O_2 and CO_2 content (FC-10 oxygen analyzer, CA-10 carbon dioxide analyzer, Sable Systems, respectively). All the gas analyzers were interfaced to a PC via a data acquisition system (PowerLab 8/30, ADInstruments). Data acquisition software (LabChart, ADInstruments) was used to record $\%O_2$ and $\%CO_2$ in the in-flowing and in the out-flowing air. We obtained stable readings for $\%O_2$, $\%CO_2$ within 30 min of putting a duck into the metabolic chamber. We recorded the $\%O_2$ and the $\%CO_2$ in in-flowing as well as out-flowing air every 60 s.

O_2 consumption was calculated according to: $\dot{V}O_2 = \dot{V}_i \times (F_iO_2 - F_eO_2)/(1 - F_e \times (1 - RQ)$ (Koteja, 1996) and CO_2 production according to: $\dot{V}CO_2 = \dot{V}_i \times F_eO_2$ (Arch et al., 2006). Energy consumption (kilojoules per hour) was calculated by multiplying the $\dot{V}O_2$ by the energy equivalents of 21.1, 20.08 and 19.7 kJ/L O_2 for respiratory quotient values of 1, 0.8 and 0.7, respectively, depending on the RQ we measured during experimentation (Arch et al., 2006).

The gas analyzers were calibrated daily, before the start of experimentation, against certified gas mixtures (Afrox, South Africa).

2.6. Data analysis

All data are reported as mean ± SD or ± SEM, where appropriate. Values of $P < 0.05$ are considered to be statistically significant.

2.6.1. Core body temperature data

Body temperatures were analyzed for a period of 12 h after each injection. Body temperatures following treatment were also compared with body temperatures for the corresponding time on a "normal" day, when ducks were not subjected to any experimentation and remained in their home pens. For this, we used repeated