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Recovery from an activity-induced metabolic acidosis in the American alligator, *Alligator mississippiensis*

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Abstract

The metabolic acidosis resulting from an intense exercise bout is large in crocodilians. Here we studied recovery from this pH perturbation in the American alligator. Metabolic rate, minute ventilation, arterial pH and gases, and strong ion concentration were measured for 10 h after exhaustion to elucidate the mechanisms and time course of recovery. Exhaustion resulted in a significant increase in lactate, metabolic rate, and ventilation, and a decrease in arterial P_{CO_2}, pH and bicarbonate. By 15 min after exhaustion, oxygen consumption returned to rest though carbon dioxide excretion remained elevated for 30 min. Arterial P_{O_2}, $[Na^+]$, and $[K^+]$, increased following exhaustion and recovered by 30 min post-exercise. Minute ventilation, tidal volume, $[Cl^-]$, and respiratory exchange ratio returned to resting values by 1 h. The air convection requirement for oxygen was elevated between 15 and 60 min of recovery. Breathing frequency and pH returned to resting values by 2 h of recovery. Lactate levels remained elevated until 6 h post-exercise. Arterial P_{CO_2} and $[HCO_3^-]$ were depressed until 8 h post-exercise. Compensation during recovery of acid-base balance was achieved by altering ventilation: following the initial metabolic acidosis and titration of bicarbonate, a relative hyperventilation prevented a further decrease in pH.

Keywords: Acid-base; Acidosis; *Alligator mississippiensis*; Arterial blood gases; Exercise; Metabolic rate; pH; Ventilation

1. Introduction

The activity capacity of many reptile species is largely supported by anaerobic metabolism (Bennett, 1978) and as a consequence, a metabolic acidosis results. A significant period of time is required to recover from this acid-base perturbation. Several factors influence recovery including duration of activity (Gleeson, 1991; Gleeson and Hancock, 2002) and body temperature (Tattersall and Boutilier, 1999; Wagner et al., 1999). Ventilatory adjustments and lactate levels during and following activity are fairly well described in reptiles (Gleeson and Bennett, 1982; Gleeson, 1991; Farmer and Carrier, 2000b,a; Nedrow et al., 2001), but changes in blood chemistry coupled with ventilatory adjustments throughout recovery have not been reported. In this experiment we address the following questions. What is the magnitude of the acidosis caused by an exhaustive activity bout? How long does it take for the acidosis to be recovered? What are the relative contributions of respiratory and metabolic (here, strong ion exchange) processes to recovering acid-base balance? We chose the American alligator (*Alligator mississippiensis*) as our model for this study because its acid-base regulation is well understood for a variety of physiological perturbations, namely digestion (Busk et al., 2000), varied gas tensions (Powell and Gray, 1989; Hicks and White, 1992; Wang and Warburton, 1995), graded exercise (Farmer and Carrier, 2000b), and temperature changes (Douse and Mitchell, 1991).

2. Materials and methods

2.1. Animals

American alligator eggs were collected from the Rockefeller Wildlife Refuge in Grand Chenier, LA, USA and were incubated at 30 °C. After hatching, alligators were housed in 1×4×1 meter fiberglass tanks with free access to

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water and basking sites; temperatures ranged from 28 to 32 °C. Animals were fed fish (goldfish or smelt), mice, and/or chicken pieces to satiation once weekly. Six alligators (mass = 479 ± 50 g) were used in this study. Approval for animal use in this study was given by the University of California, Irvine's Institutional Animal Care and Use Committee (protocol #2123).

2.2. Surgery

For serial blood sampling, a chronic arterial cannula was inserted into the femoral artery of the left hind limb. Alligators were lightly anesthetized by placing them in a container with gauze soaked in Isoflurane (Isoflo, Abbott laboratories, North Chicago, IL, USA), then intubated with a tracheal tube and artificially ventilated with 2% Isoflurane and room air (SAR-830, CWE Inc., Ardmore, PA, USA; Dräger, Lubeck, Germany). The incision site was scrubbed with Prepodyne (Iodine scrub, WestAgro, Kansas City, MO, USA), and a 3 cm incision was made through the skin. Superficial muscle groups were separated by blunt dissection, exposing the femoral artery. Flexible tubing (polyethylene tubing, I.D. 0.023, O.D. 0.038 cm Harvard Apparatus, Inc., Holliston, MA, USA) was inserted into the artery, and secured with 3-0 silk suture (Ethicon, Somerville, NJ, USA). The incision was stitched and sealed with a cyanoacrylate adhesive (Vetbond, 3M, St Paul, MN, USA). The cannula was looped and sutured at intervals to the skin to prevent tangling during subsequent activity. Analgesics (Flunixamine, Fort Dodge, Madison, NJ, USA) were administered for pain relief and antibiotics (Baytril, Bayer Corporation, Shawnee Mission, KS, USA) to prevent infection. Following surgery, the animal was ventilated with room air until voluntary breathing resumed. Animals were then allowed to recover from surgery for at least 48 h at 30 °C prior to experimentation.

2.3. Protocol

To collect expired gases, a mask was made from the end of a falcon tube (Corning Inc. Life Sciences, Acton, MA, USA) cut to 4 cm. Two holes were drilled in the mask, and flexible tubing (Tygon® tubing, Saint-Gobain Performance Plastics, Akron, OH, USA) was glued into these holes. The mask was then affixed over the alligator's nostrils and was secured to the head with polyether impression material (Impregum F, 3M EPSE, St. Paul, MN, USA). The sides of the mouth were sealed with Impregum to prevent the loss of expired gases. Room air was pulled through the mask by aquarium pumps, passing through a Drierite (anhydrous calcium sulfate, Xenia, OH, USA) column to remove water vapor. Flow rate (1.2–2.6 L min^{-1}, depending on the size of the animal) was controlled with rotameters (Brooks Instruments, Hatfield, PA, USA). Alligators were then placed in a darkened plastic container with holes drilled in the lid through which the tubing from the mask and the cannulae were exteriorized. A minimum of 12 h of quietly resting in the box was allowed to alleviate the effects of handling stress. Metabolic rate, breathing frequency and tidal volume data were collected by Acknowledge data acquisition software (Biopac, Goleta, CA, USA). Ventilation was measured by pneumotachography (8311, Hans Rudolph, Inc., Kansas City, MO, USA); oxygen consumption and carbon dioxide excretion were measured with oxygen and carbon dioxide gas analyzers (S-3A, Applied electrochemistry Inc., Sunnyvale, CA, USA; CD-3A, Applied Electrochemistry Inc., Sunnyvale, CA, USA, respectively). Calibration curves for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were made by injecting known volumes of varied gas mixtures through the mask. Minute ventilation and tidal volume are reported at BTPS, $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ at STPD. Air convection requirement for oxygen and carbon dioxide were calculated from measured minute ventilation and metabolic rate values ($\dot{V}_E\ \dot{V}_{O_2}^{-1}$, $\dot{V}_E\ \dot{V}_{CO_2}^{-1}$). Blood gases, pH, and strong ion concentration were measured by a NOVA blood gas analysis system (Waltham, MA, USA) corrected for temperature with a Radiometer blood gas analysis system (Copenhagen, Denmark). Two 0.25 mL blood samples separated by at least 1 h were

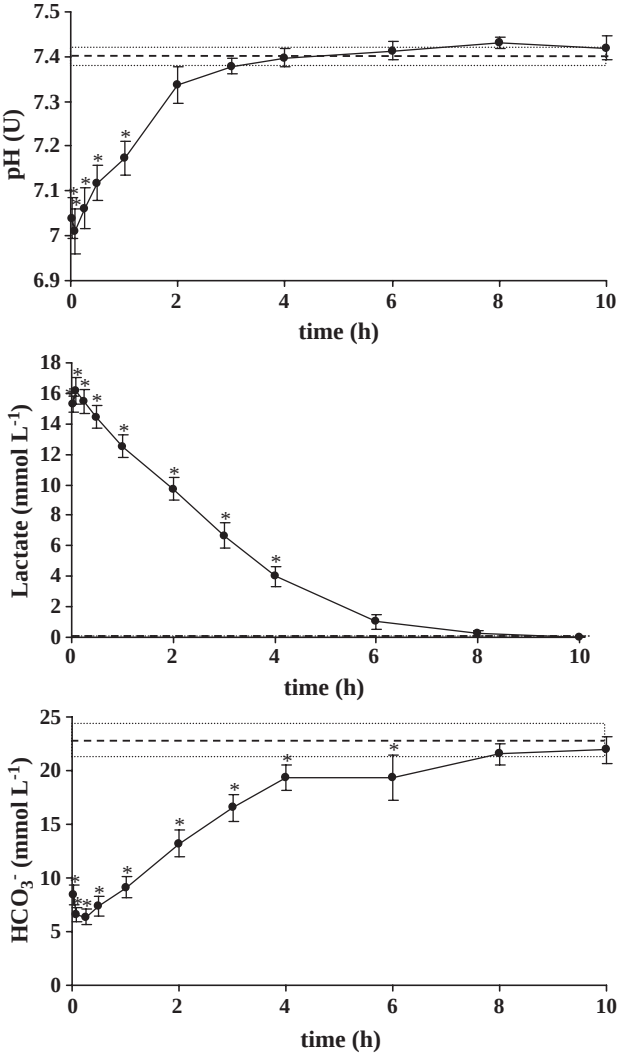

Fig. 1. Arterial pH, lactate, and bicarbonate levels throughout 10 h of recovery from exhaustive activity. Dashed line and dotted lines represent mean and standard error of the mean for resting levels, respectively. * indicates $P < 0.05$.