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The Effects of Skeletal Muscle Contractions and Paralysis on Physiological Responses to Head Immersion in Pekin Duck

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ABSTRACT. This study investigated whether increases and decreases in pectoral muscle metabolism influence underwater endurance time and cardiovascular responses to head submersion in Pekin ducks. Muscle metabolic rate (anaerobic ATP synthesis) was estimated using ^{31}P magnetic resonance spectroscopy, and was manipulated by direct electrical stimulation and by pharmacological paralysis. These manipulations had no significant effect on underwater endurance times or cardiovascular responses to head immersion (heart rate, mean arterial blood pressure). Anaerobic ATP synthesis was not detectable in paralyzed muscle during head immersion, implying that muscle metabolic rate was reduced to less than 10% of normal resting values. Despite this, underwater endurance times were not significantly extended. We conclude that muscle metabolic rate *per se* does not influence the maximum breath-hold time in this species. comp biochem physiol 118B;4:765–770, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. ATP turnover, skeletal muscle, duck, diving responses, breath-hold time, metabolic depression, ^{31}P-NMR, neuromuscular blockade

INTRODUCTION

Depression of whole-body aerobic and anaerobic metabolic rate has long been considered an important mechanism contributing to the pronounced breath-hold capacity of the domestic duck (*Anas platyrhynchos* var.) (2,15,16). In one study (15), the estimated heat production was so low during head immersion (up to 90% depression from resting values), that it could only be explained by a metabolic depression in all nonperfused tissues, including skeletal muscles. Stephenson and colleagues, however, did not observe any decrease in ATP turnover in Pekin duck pectoral muscle during head immersion (19) and during cardiac arrest induced ischemia (21), casting doubt upon the existence of metabolic depression during prolonged submersion (6).

The latter conclusion was difficult to reconcile, however, with the frequent finding that body temperature does not increase during head immersion (2,15,16,17,23) despite reduced thermal conductivity (23). Heat loss would be expected to decrease during head immersion, owing to the cessation of breathing (which eliminates convective and evaporative respiratory heat loss) and the reduction or cessation of blood flow to the body surface and extremities (7). This should lead to a rise in body temperature unless metabolic heat production is reduced (17,18). However duck core body temperature remains unchanged or decreases slightly during prolonged head immersion (2,15,16,17,23).

The longest underwater endurance times often tend to be associated with "quiet" dives (2,16,17) in which the animals refrain from struggling. This suggests that metabolic depression and extended breath-hold times may only occur when skeletal muscle contractile activity is minimal. If this is true, then it is possible that our previous experiments yielded spurious results due to muscular contractions associated with struggling (19) or cardiac-arrest–induced contractures (21). We therefore decided to investigate further the effect of muscle metabolic rate on underwater endurance capacity and correlated cardiovascular responses. Pectoralis muscle metabolism was evaluated using phosphorus magnetic resonance spectroscopy during head immersions under two different conditions: electrically stimulated contractile activity and drug-induced paralysis. We hypothesized that large differences in muscle metabolism would yield large differences in breath-hold capacity.

MATERIALS AND METHODS

Experimental protocols conformed to the recommendations of the Canadian Council on Animal Care. Pekin ducks of

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either sex were used and were provided with food and water *ad libitum*. Six ducks used in the muscle stimulation experiments were obtained from King Cole Ducks Ltd, Ontario. They were approximately 3 months old and weighed 2.65 ± 0.11 kg. Six ducks used in the muscle paralysis experiments were bred at the University of British Columbia. They were over 6 months old and weighed 2.48 ± 0.08 kg.

One or more days before experiments both brachial arteries and one brachial vein were cannulated using PE 160 tubing pretreated with TDMAC anticoagulant (Polysciences Inc., Warrington, PA, U.S.A.) to inhibit clot formation. The feathers overlying the left pectoral muscle were trimmed. In the group of ducks used for paralysis experiments, the interclavicular air sac was intubated (3 ml plastic syringe barrel). All of these procedures were performed under general anaesthesia (halothane, 1–3% in a 50:50 mixture of air and oxygen) or local anaesthesia (lidocaine, 1%) using sterile materials, and the ducks were given an injection of broad spectrum antibiotic into the leg musculature (50 mg · kg^{-1} ampicillin sodium; Ayerst Laboratories, Montreal, Quebec, Canada).

On the day of the experiment, an NMR probe was positioned on the bald skin overlying the left pectoral muscle. At the same time, arterial and venous cannula extensions (approximately 1.5 m long), a ventilated face mask with attached tubes for filling and draining with water, a unidirectional lung ventilation system (paralysis group only), a pair of subcutaneous copper wire electrodes (35 SWG, 6 cm long) (stimulation group only), and a cloacal thermocouple were fitted to the animal. Unidirectional ventilation was achieved using an animal ventilator (Harvard Apparatus). Humidified air at 25°C flowed into the trachea at approximately 1 l · min^{-1} and exited via the intubated interclavicular air sac. Arterial blood gas tensions were regulated by adjustment of respiratory air flow and, when necessary, by bleeding oxygen into the inhaled air.

The duck was then placed in dorsal recumbency on a foam pad and positioned inside the bore of the magnet so that the NMR probe and pectoral muscle were near the isocentre of the magnetic field. The ambient temperature within the bore of the magnet was maintained at approximately 25°–30°C.

Arterial blood pressure was monitored using a P10EZ or EM751A pressure transducer, the output of which was recorded on a Harvard Universal Oscillograph with use of an appropriate transducer interface. Blood samples were analyzed for blood gas tensions and pH using a Radiometer BMS3 or Instrumentation Laboratories Micro 13 pH/blood gas analyzer. Deep (cloacal) body temperature was measured before and after NMR data acquisitions using a Model BAT-10 battery operated digital thermometer (Physitemp Instruments Inc., Clifton, NJ, USA).

Muscle stimulation was effected using a portable Respond II Neuromuscular Stimulating System Model 3108 (Medtronic Neuro Inc.). The muscle was stimulated at 6 Hz in 20 sec trains with 2-sec intervals between trains. Each stimulus was delivered at 90 mA for 300 μsec. Muscle contractions were monitored qualitatively by observing motion-induced pressure fluctuations in a saline-filled balloon taped against the muscle.

In the muscle stimulation experiments, ^{31}P magnetic resonance spectra were obtained on a CSI 2T Omega imager/spectrometer unit (Bruker Instruments, Inc.) with a 18 cm bore. The NMR probe consisted of a 2.5 cm i.d. insulated pair of phosphorus and proton surface coils tuned to 34.63 MHz and 85.56 MHz, respectively. Shimming of the magnetic field was performed with the proton surface coil to a water peak linewidth of 40 to 50 Hz. The phosphorus data were collected with an interpulse interval (t_r) of 2 sec, a pulse length of 160 μsec, an acquisition time of 512 msec, and a spectral width of 4 kHz. 15, 64, or 128 scans were taken per spectrum.

Complete paralysis was induced by slow bolus intravenous injection of the nondepolarizing neuromuscular blocking drug, pancuronium bromide. A dose of 0.2 mg · kg^{-1} was used, which in preliminary trials was found to effect paralysis for 20 to 30 min.

In the muscle paralysis experiments, ^{31}P magnetic resonance spectra were obtained on a Nicolet 1180 spectrometer with a 23 cm bore 1.5 T superconducting magnet (Oxford Instruments Inc.). The NMR probe consisted of a 3 cm i.d. surface coil tuned to 32.51 MHz for phosphorus. Shimming of the magnetic field was performed using a phosphoric acid standard solution. The phosphorus data were collected with an interpulse interval (t_r) of 1 sec, a pulse length of 35 μsec, an acquisition time of 50 msec, and a spectral width of 2 kHz, 40 or 192 scans were taken per spectrum.

In both experiments, partial saturation at each resonance was corrected using the t_r ratio method adopted previously (19). The correction factor was obtained from two control ^{31}P spectra with interpulse intervals of 15 sec and 1 or 2 sec, respectively. These untreated control (UC) spectra were accumulated under steady-state conditions before initiating stimulation or paralysis (untreated controls, UC)

Following acquisition of the UC spectra, stimulation or paralysis was initiated. After 5 min, a "treated control" spectrum was acquired (TC) then head immersion was initiated. The face mask was rapidly filled with cold tap water and, in the paralyzed ducks, the animal ventilator was simultaneously switched off. Cardiovascular and NMR data were collected continuously throughout the period of head submersion. Breath-hold was terminated when the heart rate increased above pre-immersion levels. This abrupt failure of the cardiovascular response has been shown to correlate with an iso-electric EEG and is followed within 10 to 15 sec by death unless the animal is allowed to resume lung ventilation (5). At this point, a blood sample was taken and then the mask was drained and, in the paralyzed birds, the animal ventilator was turned on at an elevated rate to effect rapid recovery of blood gas tensions.

For data analysis each NMR spectrum was filtered in the

time domain with a 10 Hz exponential multiplication followed by Fourier transformation, phasing and baseline correction. Each spectral line was fitted with a lorentzian lineshape from which the chemical shift and area of each peak were calculated. The fitting procedure was based upon Marquardt's (13) nonlinear least squares fitting algorithm.

Muscle metabolite contents were estimated from the ^{31}P spectra by assuming that total NMR-visible ^{31}P was 59.4 μmol · g^{-1} wet tissue. This was determined previously by in vitro NMR analysis of Pekin duck pectoral muscle samples (19).

Intracellular pH (pH_i) was calculated from the ^{31}P spectra as follows:

$$pH_i = pK' + \log[(\Delta Pi - \partial_A)/(\partial_B - \Delta Pi)] \quad (1)$$

where, ΔPi is the difference between the chemical shifts (ppm) of the inorganic phosphate and phosphocreatine resonances, pK′ is the negative logarithm of the apparent acid dissociation constant for phosphates, and ∂_A and ∂_B are, respectively, the acidic and basic phosphate titration endpoints. pK′, ∂_A, and ∂_B were calculated at body temperature according to Kost (8).

The quantity of lactic acid that accumulated during ischaemia (ΔLa^-, μmol · g^{-1}) was calculated as follows (9):

$$\Delta La^- = \alpha(\Delta PCr) + \beta(\Delta pH_i) \quad (2)$$

where, α, the coefficient of H^+ consumption by net PCr hydrolysis, was calculated, following Alberty (1) and Lawson and Veech (12), to be from 0.4 to 0.7 depending upon the prevailing pH_i, and β, the nonbicarbonate buffering capacity, was taken to be 57.6 μeq · g^{-1} · ΔpH^{-1} (19).

The experiment by Stephenson and Jones (19) involved experimental conditions similar to those described above, with the exception that the pectoral muscle metabolic rate was not manipulated. Thus, the published data (19) are treated as control values for the purposes of statistical comparisons in the present study. Univariate analysis of variance was used, followed when appropriate, by the Scheffé test for post-hoc multiple pairwise comparisons. In some cases, a two-sample or paired *t*-test was employed when appropriate. Linear correlation between two variables was quantified using the Pearson correlation coefficient, r. Data were analyzed using Data Desk software (Data Description Inc., Ithaca, NY, USA). Results are presented as means ± SEM. Differences are considered statistically significant when $P < 0.05$.

RESULTS

Underwater endurance times were 7.6 ± 0.4 min in paralyzed ducks and 6.0 ± 0.5 min in stimulated ducks. The difference between groups was not statistically significant. Furthermore, the breath-hold capacities of both groups were statistically similar to that of the nonstimulated, nonparalyzed group studied previously [6.5 ± 0.3 min (19)].

There were no statistically significant differences in cardiovascular responses to immersion (Fig. 1). Before immersion, and before the initiation of stimulation and paralysis (UC values) heart rate of the stimulation group (231 ± 13 beats · min^{-1}) was significantly higher than that of the paralysis and control groups (144 ± 16 and 138 ± 7 beats · min^{-1}, respectively). Muscle stimulation increased heart rate significantly (to 287 ± 22 beats · min^{-1}), whereas paralysis had no effect. Before head immersion, there were no significant differences between groups (both for UC and TC) in mean arterial blood pressure, P_{aCO_2}, pH_a and pH_i.

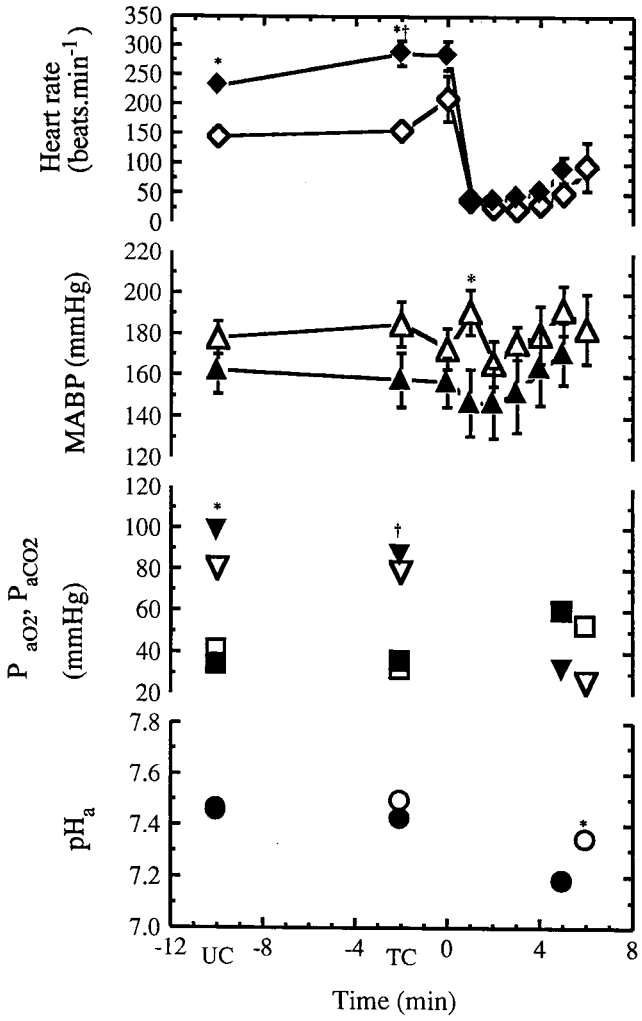

FIG. 1. Cardiovascular responses to involuntary head submersion in paralyzed (unfilled symbols) and stimulated (filled symbols) Pekin ducks. Mean values ± SEM ($N = 6$) are shown. UC: untreated control data measured before onset of stimulation, paralysis or head immersion; TC: treated control data measured 5 min after injection of neuromuscular blocking drug or initiation of electrical stimulation of the left pectoralis muscle; head immersion initiated at time = 0 min. MABP: mean arterial blood pressure; P_{aO_2} (triangles), P_{aCO_2} (squares), partial pressures of oxygen and carbon dioxide, respectively, in arterial blood; pH_a: arterial blood pH. *Statistically significant difference between groups; †statistically significant difference between TC and UC values.

During the untreated control period, P_{aO_2} was slightly, but statistically significantly, higher in the stimulation group than in the paralysis group. Muscle stimulation induced a small but statistically significant fall in P_{aO_2} before head immersion so that P_{aO_2} was the same in both groups at the start of head submersion (Fig. 1).

Head immersion had no statistically significant effect on mean arterial blood pressure in any group. Average heart rate during the period of head immersion was not significantly different between groups and was not significantly correlated with underwater endurance time within or between groups. At the end of the submersion, P_{aO_2} and P_{aCO_2} were not significantly different between groups. However, pH_a was statistically significantly higher in the paralyzed ducks than in the stimulated or control ducks at the end of submersion.

Muscle phosphagen concentrations and intracellular pH (pH_i) in the stimulation and paralysis groups are illustrated in (Fig. 2). ATP concentration did not differ between the three groups during the untreated control period before immersion. However, muscle paralysis induced a statistically significant increase in [ATP], while stimulation had no effect (TC). Intracellular phosphocreatine concentration ([PCr]) was similar in both groups during the untreated control period. Paralysis and muscle stimulation both caused a reduction in [PCr], but this was statistically significant only in the stimulated ducks.

[ATP] did not change significantly during head submersion in both groups of ducks. In the paralyzed group, phosphocreatine concentration and pH_i remained constant during head submersion, and net anaerobic ATP synthesis was therefore not detectable. In the stimulated group, phosphocreatine concentration decreased to approximately 50% of the pre-immersion value, and pH_i decreased significantly by approximately 0.4 pH units during head submersion. Lactate was calculated to accumulate in the stimulated muscles at an average rate of $6.8 \pm 0.7\ \mu mol \cdot min^{-1} \cdot g^{-1}$, which was significantly higher than that of the control ducks studied previously [$3.2 \pm 0.7\ \mu mol \cdot min^{-1} \cdot g^{-1}$ (19)]. Anaerobic ATP synthesis peaked at approximately $25.1 \pm 3.6\ \mu mol \cdot min^{-1} \cdot g^{-1}$ during the second minute of head submersion in stimulated ducks, and it declined progressively thereafter as muscle fatigue developed. Calculated average rate of anaerobic ATP synthesis was significantly greater in stimulated duck pectoral muscle ($10.2 \pm 1.0\ \mu mol \cdot min^{-1} \cdot g^{-1}$) than in that of paralyzed (undetectable) and control ducks [$5.9 \pm 1.1\ \mu mol \cdot min^{-1} \cdot g^{-1}$ (19)] during head submersion. Underwater endurance time was not significantly correlated with muscle anaerobic ATP synthesis rate (Fig. 3).

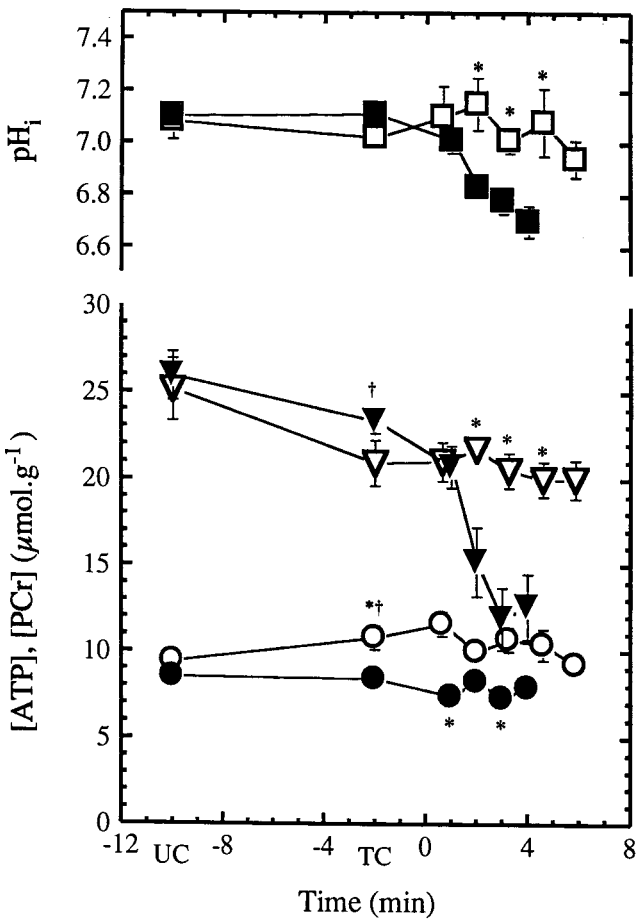

FIG. 2. Effect of head immersion on pectoral muscle intracellular pH (pH_i) and metabolite concentrations in paralyzed (unfilled symbols) and stimulated (filled symbols) Pekin ducks. Mean ± SEM ($N = 6$) are shown. UC: untreated control data measured before onset of stimulation, paralysis, or head immersion; TC: treated control data measured 5 min after injection of neuromuscular blocking drug or initiation of electrical stimulation of the left pectoralis muscle; head immersion initiated at time = 0 min. [PCr]: phosphocreatine concentration; [ATP]: adenosine 5′ triphosphate concentration. *Statistically significant difference between groups; †statistically significant difference between TC and UC values.

DISCUSSION

This study has found that underwater endurance times are not significantly influenced by skeletal muscle metabolic rate in the Pekin duck. The underwater endurance times of the paralyzed ducks, which ranged from 6.4 to 8.8 min, were similar to or shorter than those of nonparalyzed ducks in this and other studies [*e.g.*, (5,10,23)]. Hudson and Jones (5) found that unmanipulated Pekin ducks of the size used in this study will tolerate head submersion for approximately 8.4 min. The endurance times of the paralyzed ducks were not statistically different from this predicted value. This indicates that muscle metabolic depression does not cause prolongation of underwater endurance time. In the absence of detectable anaerobic ATP synthesis, the paralyzed pectoral muscle may have relied entirely upon the small amount of oxygen bound to myoglobin (19). This implies that ATP turnover was less than 10% of the resting nonparalyzed rate during head immersion in paralyzed ducks.

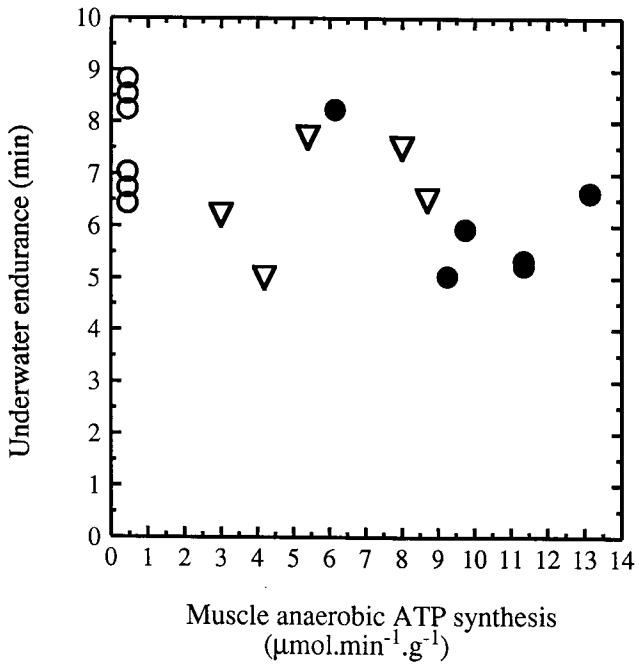

FIG. 3. Relationship between maximum underwater endurance and net anaerobic ATP synthesis in Pekin duck pectoral muscle. Paralyzed ducks (○); stimulated ducks (●); unmanipulated ducks (▽) from Stephenson and Jones (19). Net anaerobic ATP synthesis was undetectable in paralyzed ducks.

Muscle stimulation did not substantially reduce underwater endurance times, although the endurance times of both the stimulated and the "control" ducks (19) were significantly less than the values predicted by the allometric equations published by Hudson and Jones (5). The lack of a pronounced effect of muscle stimulation on the underwater endurance time also suggests that electrical stimulation of skeletal muscle does not cause vasodilation during head immersion, as was shown previously (22).

It seems likely that when a submerged duck is relaxed and the peripheral vasoconstriction is intense, whole body metabolic heat production may decrease (15,16,23). If the animal struggles, however, the average whole-body metabolic rate may rise above pre-immersion levels (19). The present study has shown, however, that this on it's own has little or no effect on underwater survival time. The apparent trend towards lower endurance times at higher muscle metabolic rates (Fig. 3) was not statistically significant. Thus, muscle contractions will only affect underwater endurance if they are also associated with vasodilatation. However, high muscle metabolic rate does not stimulate vasodilatation during head submersion (22) due to the powerful influence of sympathetic innervation and adrenal catecholamines (10,11). It is nevertheless tempting to speculate that *voluntary* muscle contractions (and perhaps "fictive" voluntary contractions in paralyzed birds), may induce peripheral vasodilatation, perhaps through a central neural command mechanism, that would accelerate oxygen depletion and therefore shorten underwater endurance time (22). The latter hypothesis remains to be tested.

We conclude that it is the efficacy of the oxygen conserving cardiovascular response, and not whole body metabolic rate, that is the main determinant of underwater endurance time since it is the former that determines the proportion of the oxygen store that is available for the hypoxia-sensitive CNS. The duck brain does not have an enhanced tolerance to hypoxia (3) and the entire brain is hyperperfused during apneic asphyxia (20). Two components of the cardiovascular response, the arterial blood pressure and the bradycardia, were similar in all three groups of ducks and this may explain why endurance times were similar in the present studies.

The metabolic functions of the viscera, including the liver, kidney, gut, and endocrine glands are dependent on blood flow since they are concerned with transport of materials into and out of the circulation. Therefore, when blood flow ceases during head submersion (7), so do the metabolic activities of these tissues (4,14,24). This is likely to be a major cause of the observed metabolic depression during "quiet" prolonged submersion. If, however, blood flow is sustained due to a weak vasoconstrictor response, these visceral tissues may retain some function, in which case they would act to deplete the oxygen stores at an accelerated rate. It is currently unknown whether the visceral vascular beds are subject to variable rates of perfusion during head submersion and, if so, how this is controlled and what effect it has on underwater endurance. In this context it is worth pointing out that a similar uncertainty applies to the dynamic control of skeletal muscle microcirculation during immersion. Any blood flow to the muscle would supply oxygen for use in oxidative phosphorylation and the ATP so produced would not have been detected in this study. However, this error is likely to be small because mean arterial blood pressure was maintained in the face of a more than 80% decrease in heart rate, indicating a profound increase in peripheral vascular resistance.

In summary, this study has shown that skeletal muscle paralysis does not extend breath-hold capacity, and skeletal muscle contractile activity does not substantially reduce underwater endurance in Pekin duck. We conclude that maximum breath-hold times are critically dependent upon the degree of peripheral vasoconstriction and that the metabolic rate of ischaemic tissues has little or no influence on the underwater survival time of the duck.

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