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ORIGINAL ARTICLE

A near-infrared spectroscopy (NIRS) device unveils oxygenation levels in skeletal muscle and brain of a conscious Harris's Hawk (*Parabuteo unicinctus*)

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Abstract
We measured the regional saturation of oxygen of hemoglobin (rSO_2) and the total hemoglobin index (HbI) in the brain and skeletal muscles of a conscious Harris's Hawk by a near-infrared spectroscopy device (NIRS). The oxygenation levels of the breast were significantly lower than the cerebral parts. A flight exercise significantly increased the rSO_2 and HbI of the breast. The breast surface temperature significantly increased in response to flight training by a thermography. NIRS enabled us to measure changes in the oxygenation levels of brain and skeletal muscles in a conscious Harris's Hawk before and after a moving task.

Keywords Biophotonics · Skeletal muscle · Brain

Zusammenfassung
Nahinfrarot-Spektroskopie (NIRS) macht den Oxygenierungsgrad in Skelettmuskulatur und Gehirn eines bei Bewusstsein befindlichen Wüstenbussards (*Parabuteo unicinctus*) sichtbar
Wir maßen die lokale Sauerstoffsättigung des Hämoglobins (rSO 2) sowie den Gesamthämoglobinindex (HbI) im Gehirn und in der Skelettmuskulatur eines bei Bewusstsein befindlichen Wüstenbussards mithilfe eines Gerätes zur Nahinfrarot-Spektroskopie (NIRS). Der Grad der Sauerstoffsättigung lag im Brustgewebe signifikant niedriger als im zerebralen Bereich. Eine Flugübung führte zu einer signifikanten Erhöhung der rSO 2- und HbI-Werte im Brustgewebe. Die Thermografie zeigte eine signifikante Erhöhung der Brustoberflächentemperatur in Reaktion auf die Flugübung. NIRS ermöglichte es uns, Änderungen im Oxygenierungsgrad des Gehirns und der Skelettmuskulatur an einem bei Bewusstsein befindlichen Wüstenbussard vor beziehungsweise nach einer Bewegungsübung zu messen.

Introduction

Biomedical optics is a growing technology used to diagnose and treat diseases. In veterinary medicine, biophotonics approaches have been used to examine skin and subcutaneous tumors and to predict the prognosis of lymphoma (Cugmas and Spigulis 2019). We recently demonstrated that an examiner's finger-mounted NIRS device is feasible to analyze the oxygenation revels of brain and skeletal muscles in conscious Chihuahua dogs in a study of microcirculatory physiology among companion animals (Hiwatashi et al. 2017). In that study, we proposed that Chihuahuas are suitable models for measuring the oxygenation levels of the brain due to the existence of the skull's molera in this breed. Functional NIRS is also available for examining dogs to investigate changes in their behavior (Gygax et al. 2015).

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In addition to NIRS, the use of a thermography device is a helpful noninvasive method for diagnosing dysfunctions of peripheral circulation, the growth of mammary cancer, and pain in patients (Ring and Ammer 2012). In veterinary medicine, the detection of changes in the surface temperature by thermography has enabled the identification of orthopedic and infectious diseases, showing that a free-moving thermography device is advantageous and can be used without sedation or anesthetics (Tessier et al. 2003; Sathiyabarathi et al. 2016).

Harris's hawk is one of the popular birds for falconry. The owners of falconry birds pay close attention to the health of the birds. Thus radiographs, fine-needle aspirates, and biopsies are all used in the maintenance of avian health to prevent and diagnose wild avian diseases (e.g., infections, tumors, and unexpected bone fractures). However, it would be suggested that these methods have some disadvantages including radiation damage, unexpected bleedings, and pains. It is known that dysfunction of blood flow due to a cardiovascular disturbance can cause disease in both humans and animals; however, there has been no noninvasive method in veterinary medicine for detecting and quantifying changes in the peripheral blood flow of birds. We hypothesized that the flight task in falconry training would increase not only the blood flow and oxygenation levels but also the surface temperature in breast muscles of a Harris's hawk, and that cerebral autoregulation may govern the brain's circulation to be constant in response to exercise. We conducted the present study to investigate changes in the surface temperature of a Harris's hawk by thermography and to examine oxygenation levels using an NIRS device in the hawk's brain, breast, and legs before and after falconry training.

Materials and methods

An 8-month-old male Harris's Hawk was tested. The hawk was being reared for falconry and free flight. The hawk underwent a daily examination of its health condition and physical state by a veterinarian. A tissue oxygenation monitor (model KN-15, Toccare, Astem Co., Kawasaki, Japan) and its NIRS probe were used (Kanayama and Niwayama 2014). The Toccare device's measurement method depends on spatially resolved NIRS with two LED light sources (770 and 830 nm). Using the Toccare enabled us to measure the rSO_2 (0–99%) and the HbI (0–1.0) of each organ at 5 mm depth with a sampling interval of 0.5 s. To determine the surface temperature at several body parts of the hawk, we used a thermography device (FLIR ONE, FLIR, Wilsonville, OR, USA). This device enabled us to analyze noninvasive measurements of the surface temperatures during 3 s to obtain a single measure.

This study was performed after obtaining approval regarding animal care in accord with Japan's Act regarding the welfare and management of animals in research. Before onset of the present study, the hawk had experience for falconry and free flight since he was young. We also spent 3 weeks for habituation of the hawk to measure NIRS and thermography. For NIRS measurement during habituation and the present study, the hawk was retained by a falconry casting jacket which was custom-made by Japanese avian shop and set at a supine position. We loosened the jacket holding and exposed breast and leg parts during NIRS measurements. Additionally, we recognized that it was helpful for obtaining NIRS values to insert a slide glass (1.3 mm in thickness) between NIRS light sources and bird skin due to thin skeletal muscle layer of breast and leg. We determined the measuring point of NIRS after the value curve reached to plateau. Collectively, these processes and contrivances enabled us to obtain stable values of NIRS.

We measured the oxygenation revels by NIRS and the surface temperatures by thermography at three parts of the hawk: brain, breast, and leg. Before the NIRS values were measured, we captured the surface temperature at the hawk's brain, breast, and leg. The measured parts of temperatures were the same site of NIRS examinations. To tightly adhere the NIRS probe against the skin and to prevent feather effects, we gently pushed feathers aside using ethanol-wetted cotton without plucking feathers. After the NIRS probe was securely attached, we could obtain a stable NIRS value within 10 s in the conscious hawk. To confirm changes in the values of NIRS and surface temperature in the hawk, we designed a flight exercise. As the flight exercise (conducted as one 30-min session per day in the open air), we enforced ten flights by the hawk between the trainer's arm and the top of a pole. We measured the values of NIRS and surface temperature before the exercise (pre-Ex), after the 30 min exercise (post-Ex), and after approx. 90 min exercise (rest). Sets of measurements were taken on the same day for 7 consecutive days of training. The NIRS and surface temperature values for the 7 days of training ($n = 7$) are presented as the mean ± SE. To compare the values among pre-EX, post-EX, and rest in same measured parts, significant differences ($p < 0.05$) were determined by a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. To compare the values among brain, breast, and leg in same measured time points, significant differences ($p < 0.05$) were determined by an ANOVA followed by Tukey's post hoc test.

Results

Before the training exercise, i.e., at pre-Ex, the values of rSO_2 at the hawk's brain region ($46 \pm 1\%$, $p < 0.05$, $n = 7$) were significantly higher than those of the skeletal muscles (breast: $29 \pm 1\%$, $n = 7$, and leg: $24 \pm 2\%$, $n = 7$). There were no significant differences in the rSO_2 between the breast and leg (Fig. 1a). The rSO_2 values of the skeletal muscles were significantly increased after exercise (post-Ex) and returned to the pre-Ex condition when the hawk was at rest (Fig. 1a). Even we enforced the flight exercise, the hawk's brain rSO_2 exhibited no changes (Fig. 1a).

The pre-Ex brain HbI values (26 ± 3%, $p < 0.05$, $n = 7$) were notably higher than those of the pre-Ex skeletal muscles (breast: 3 ± 0%, $n = 7$, leg: 2 ± 0%, $n = 7$). There were no significant differences in the rSO_2 between the breast and leg (Fig. 1b). The brain rSO_2 values were 1.6 times higher (vs. breast) and 1.9 times higher (vs. leg), and the brain HbI values were 8.6–13 times higher than those of the other parts.

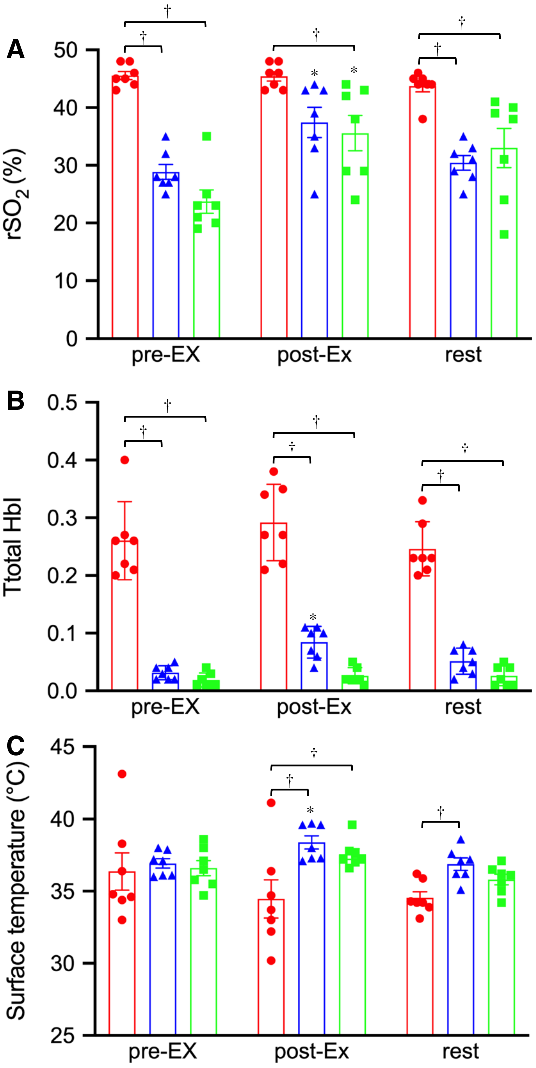

Fig. 1 Effects of flight exercise on the changes in rSO_2 (**a**), total HbI (**b**), and surface temperature (**c**) at brain (red columns and symbols, $n = 7$), breast (blue columns and symbols, $n = 7$), and leg (green columns and symbols, $n = 7$) parts of a Harris's Hawk. $^*p < 0.05$ from pre-EX in the same part and $^\dagger p < 0.05$ among parts in the same period (color figure online)

A significant increase in the HbI was observed only at the breast between before and after the flight exercise (Fig. 1b).

Unlike the rSO_2 and HbI values, the pre-Ex surface temperatures were not significantly different among the brain, breast, and leg, with values distributed around 36 °C. The post-Ex surface temperatures at the breast were significantly increased (from pre-Ex 36.9 ± 0.4 °C to post-Ex 38.4 ± 0.5 °C) (Fig. 1c). At the rest period, the surface temperature values of the breast and leg returned to the pre-Ex level (rest, Fig. 1c).

Discussion

Brain NIRS is a useful and advantageous tool for detecting cerebral oxygenation levels in local and microcirculatory spaces through the skin and bones, and it is currently used as a noninvasive clinical method for patients. In veterinary medical sciences, brain NIRS has been conducted in dogs (Cugmas and Spigulis 2019). Morphologically, avian bones have unique characteristics such as a pneumatized structure' of bones, because birds evolved to fly. NIRS could detect changes in the cerebral oxygenation levels of songbirds in response to physiological stimuli and hypercapnia (Mottin et al. 2011; Vignal et al. 2008). The results of our present study of a Harris's Hawk also demonstrated that the Toccare enabled the measurement of brain oxygenation levels. Before exercise, the brain rSO_2 of the Harris's Hawk examined herein was 46 ± 1%. We also found that variances of rSO_2 at the hawk's brain region were small compared to the breast and leg (Fig. 1a), suggesting that the Toccare may be a superior to measure brain NIRS device for avians. Our previous study demonstrated that the brain rSO_2 values of healthy Chihuahuas at rest were approx. 60% (Hiwatashi et al. 2017), suggesting that there is room for further study regarding potential heterogeneity of cerebral oxygen levels between these species.

We also observed that at the pre-flight time point, the hawk's cerebral rSO_2, and HbI values were highest compared to the corresponding values of its skeletal muscles, suggesting that the blood flow supply to the brain and the oxygenation are maintained at high levels. The flight exercise significantly increased the rSO_2 and HbI in the hawk's breast but not in its brain. During the post-exercise rest period, the increased NIRS values returned to the pre-Ex levels. These findings revealed that there was an exercise-dependent redistribution of blood supply to the breast skeletal muscles (Katayama and Saito 2019) and an autoregulation of cerebral circulation (Willie et al. 2014; Jessen 2001) in the Harris's Hawk. Collectively, our present findings provide the first report that an examiner's finger-mounted NIRS device can detect changes in the oxygenation levels of the brain and skeletal muscles in a conscious Harris's Hawk. This device will be useful for understanding avian peripheral circulation in health and disease.

Unlike mammals, red blood cells of birds are nucleated, elliptical shape, and larger diameter. Since the birds did not require to eliminate nuclei from their red blood cells or change in shape/size due to their specific and efficient respiratory system (Gavrilov 2013). Additionally, there are a variety of hemoglobin types in birds. Although we could not identify the differences of Hb oxygenation levels between mammals and birds in the present study, a flight task against the hawk changed the values of rSO_2 and HbI measured by Toccare, suggesting that it will be helpful for resolving the differences to measure NIRS values of other bird strains. The values of HbI in skeletal muscles (breast and leg) were very low compared to the brain in the present study. Linear regression analyses of relationships between rSO_2 and HbI demonstrated brain, breast, and leg parts of a Harris's Hawk were $y=-0.13806+0.0089919x$ $R=0.33884$, $y=-0.069205+0.0038749x$ $R=0.76297$ and $y=0.005663+0.00057442x$ $R=0.35983$, respectively (data not shown). We speculate that blood supply to breast skeletal muscle of the hawk may be small but oxygenation level of Hb is relatively maintained. Further investigation will be needed to examine anatomical other parts, thicker muscles or larger body birds.

Unsurprisingly, the flight exercise significantly increased the surface temperature of the hawk's breast. Generally, physical activities by skeletal muscles' movement involve thermogenesis of the body due to enhancements of the blood flow and metabolic demand accompanied by an increase in oxygenation. The breast muscles of birds greatly contribute to the machinery of their wing muscles. In falconry, there is no quantitative evaluation based on avian physiology. Our present results also indicate that (1) the detection of thermogenesis in breast muscles could be helpful for the establishment of outcomes of daily falconry training, and (2) regular surveillance using thermography can be an effective noninvasive method for health and medical examinations of birds.

In mammals, selective brain cooling plays significant roles in the protection of the brain against thermal damage compared to other organs (Jessen 2001). The existence of a selective brain cooling system in birds has been a matter of controversy, because the environments of birds vary widely; for instance, diving-/non-diving birds and that live at high/low altitudes (Jessen 2001). Our present findings indicated that the hawk's brain oxygenation levels were maintained from before to after the flight exercise each day. We observed slightly reduced brain surface temperatures after the physical activity, whereas the breast exhibited opposite thermal changes. The advanced biophotonic techniques used in this study would provide new insight into avian selective brain cooling mechanisms. This study was conducted during the winter season in Japan. We plan to investigate the effects of ambient temperature on exercise-mediated changes in the blood flow and thermogenesis of birds. It is also necessary to investigate the effects of sensory stimuli on changes in the cerebral temperature, not only at the surface but also in deeper areas.

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