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The effect of temperature on mitochondrial respiration in permeabilized cardiac fibres from the freshwater turtle, *Trachemys scripta*

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ABSTRACT

Ectothermic animals regularly experience fluctuations in body temperature, which have profound effects on biochemical and metabolic processes. To cope with cold environments, the freshwater turtle, *Trachemys scripta*, exhibits inverse thermal compensation, where physiological processes exhibit exaggerated Q_{10} effects and are actively downregulated to limit energy requirements. The present study aimed to identify potential sites of temperature sensitivity in mitochondria from the turtle heart. The effect of acute temperature change on ADP sensitivity and respiratory flux through different components of the electron transport chain (Complexes I, II and IV) was analysed in permeabilized cardiac fibres from the turtle ventricle. An acute decrease in temperature significantly reduced the acceptor control ratio and maximum respiration rate ($\dot{M}_{O_2}$) through all complexes of the electron transport chain. Calculated Q_{10} values for $\dot{M}_{O_2}$ across the three experimental temperatures tested (5, 13 and 21 °C) were in the range of 1.19–3.65, and although there was a tendency for exaggerated Q_{10} values in the lower temperature range (5–13 °C), there were no significant differences in Q_{10} between any temperatures or complexes examined. These results suggest the large-scale reductions in turtle cardiac function and high Q_{10} values at acutely low temperatures are likely due to a reduction in energy demand (contractile function), rather than supply (mitochondrial respiration).

1. Introduction

Fluctuations in environmental temperature have profound effects on biochemical and metabolic processes. For ectothermic animals that experience daily and/or seasonal thermal challenges, adaptations to temperature are crucial to maintaining physiological status and thermal range. A well studied example is the freshwater turtle, *Trachemys scripta*. Natural populations of *T. scripta* range southward to some Caribbean islands and to northern South America, reaching Missouri, Illinois and Indiana (Ultsch, 2006). While summer temperatures in these areas can reach up to 38 °C, some populations of *Trachemys* must endure several months under ice-covered lakes and ponds where temperatures range from 0 to 4 °C (Ultsch, 2006). In some cases, the cold winter conditions in these aquatic habitats can lead to severely limited dissolved oxygen, causing hypoxic and even anoxic conditions (Ultsch, 2006).

Non-hibernating mammals and birds respond to acutely cold temperatures by increasing physiological and metabolic processes to compensate for the reduction in activity of temperature sensitive processes (Johnston and Bennett, 1996). In contrast, ectothermic vertebrates conform to environmental temperature, and some species, such as the turtle, exhibit inverse thermal compensation, where physiological processes not only decrease with cold temperature, but in addition, are actively suppressed (Beall and Privitera, 1973; Rotermund and Privitera, 1972). Indeed, as temperature is reduced to 3 °C, metabolism falls in the turtle at a Q_{10} of 2–3, but at temperatures below 10 °C an exaggerated Q_{10} of 8–10 is observed so that aerobic metabolism is depressed 16 fold to 5% of the original value (Herbert and Jackson, 1985; Jackson, 2002). This extreme temperature sensitivity, in conjunction with active downregulation, permits large energy savings and reduces thermogenic demands when food availability is at its lowest. The exact cellular mechanisms behind the hypersensitivity to temperature remain largely unknown. As the site of aerobic respiration, mitochondria are an obvious candidate for regulating metabolic rate suppression. Indeed, similar to the whole animal, acute temperature reductions in turtle heart mitochondria cause reduced respiration rates with exaggerated Q_{10} effects (up to 5) in the lower temperature range (Almeida-Val et al., 1994). In line with this observation, reducing temperature or cold acclimation leads to a colossal decline in heart rate, cardiac output and cardiac power output by 5–15 fold (Q_{10} up to 8.8) (Stecyk et al., 2008). Clearly, inverse compensation and high temperature sensitivity actively depress cardiac activity and decrease ATP demand for mechanical work by almost 10-fold (Stecyk et al., 2008).

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The purpose of this study was to investigate more closely the temperature sensitivity of turtle heart mitochondria by analysing the fluxes through different components of the electron transport chain (ETC) to identify potential sites of temperature sensitivity. Rather than using isolated mitochondria, we used permeabilized cardiac fibres so that the mitochondria and their contacts with the cytoskeleton are left intact, providing a physiologically relevant preparation. To broaden the study further, we have also measured the effect of temperature on ADP sensitivity.

2. Materials and methods

2.1. Animals

Adult red eared slider turtles, *T. scripta* ($n=6$, body mass$=492.1 \pm 41.1$), were obtained from Niles Biological Inc. (Sacramento, CA, USA). Turtles were transported by air freight to the University of British Columbia and held in aquaria at room temperature (21 °C), with free access to basking platforms, heat lamps and diving water. Animals were fed several times a week with commercial turtle food pellets. All turtles were held under a 12/12 h photoperiod. Treatment of all experimental animals was in accordance with the guidelines of the Canadian Council on Animal Care and the University of British Columbia Animal Care Committee.

2.2. Solutions

The solutions described below were made according to the Oroboros "selected media and chemicals", on line source.

Skinning solution (solution A), in mM: Ca-K_2-EGTA 2.8, K_2-EGTA 7.2 (free [calcium]$=0.1$ μM), Na^+ATP 5.8, $MgCl_2$ 1.4, imidazole 20, taurine 20, potassium 2-(N-morpholino)ethanesulfonic acid (K-MES) 50, Na^+phosphocreatine 15 mM, dithiothreitol (DTT) 0.5, pH 7.1, adjusted with 5 N KOH.

Respiration medium (solution B), in mM: EGTA 0.5, $MgCl_2$ 1.4, taurine 20, KH_2P0_4 10, HEPES 20, BSA 1%, K-MES 60 mM, sucrose 110 mM, pH 7.1, adjusted with 5 N KOH.

2.3. Isolation of permeabilized cardiac fibres

Protocols for permeabilizing cardiac muscle have been exhaustively reviewed (Kuznetsov et al., 2008). In brief, animals were decapitated and the heart was excised. A sample of ventricular tissue from each experimental group was frozen in liquid N_2 for metabolite analysis (see below) and stored at −80 °C. The remaining ventricular tissue was placed into ice-cold skinning solution (solution A), and muscle pieces (2 mm in length) were cut from the spongy myocardium and gently separated into small cell bundles. Bundles were incubated for 30 min with gentle shaking in S-solution containing 50 mg ml^{-1} saponin. The saponin concentration and the incubation time were chosen based on previous experiments on turtles (Birkedal and Gesser, 2003; Birkedal and Gesser, 2004). After permeabilization, the fibre bundles were washed in respiration medium (solution B) for 10 min to remove all adenine nucleotides and phosphocreatine (PCr). This latter procedure was repeated twice and the bundles were then left in fresh solution B until use. All these preparative procedures were carried out at 4 °C.

2.4. Respiration of mitochondria and cardiac fibres

Permeabilized muscle fibre respiration was measured with an Oroboros Oxygraph 2-k high resolution respirometry system (Oroboros Instruments, Innsbruck, Austria). Oxygen electrodes were calibrated daily with air-saturated solution B at each experimental temperature. Zero calibrations were achieved by injecting yeast into the experimental chambers and allowing them to completely deplete the oxygen in the chamber. Oxygen solubility in the assay medium at each temperature was calculated as described previously (Gnaiger, 1983). Two identical respiration chambers (chamber A and chamber B) held at the same temperature were run in parallel for each experimental run. Fibre bundles (wet weight 6–8 mg) were added to each chamber containing 2 ml of solution B.

2.5. Protocol for permeabilized fibre respiration

The two respiration chambers containing the permeabilized fibres were randomly assigned to one of the following protocols:

Protocol 1: assessment of respiratory flux through complexes I, II and IV of the ETC. Malate (0.25 mmol l^{-1}) and pyruvate (5 mmol l^{-1}) were used as substrates to spark the citric acid cycle and provide a carbon substrate, respectively. State III respiration was achieved through addition of saturating ADP to a final concentration of 1 mmol l^{-1}. Once maximal ADP stimulated respiration was achieved and in a steady state, rotenone (0.5 μmol l^{-1}) was added to inhibit complex I (rotenone inhibited state 4 respiration), and succinate (10 mmol l^{-1}) was added to assess respiratory flux through complex II. Antimycin A (5 μmol l^{-1}) was then added to block complex II, and flux through complex IV was assessed by adding the electron donor tetramethyl-phenylenediamine (TMPD, Sigma, 0.5 mmol l^{-1}). To avoid oxidation of TMPD, ascorbate (0.5 mmol l^{-1}) was added to TMPD prior to injection. Finally, to assess the functional integrity of the outer mitochondrial membrane, cytochrome C (10 μmol l^{-1}) was added to the chamber. A representative trace depicting protocol 1 can be seen in Fig. 1A.

Protocol 2: determination of the apparent K_m of ADP stimulated respiration. Pyruvate (5 mmol l^{-1}), malate (0.25 mmol l^{-1}) and succinate (10 mmol l^{-1}) were used to provide maximum substrate stimulation. ADP was then added in steps from 0.025 mM to 1 mM and respiration rate was monitored at each [ADP]. A representative trace depicting protocol 2 can be seen in Fig. 2A.

Cardiac fibre preparations were assayed at 3 different temperatures; 5, 13 and 21 °C. As the experimental protocol contains irreversible pharmacological inhibitors, only one temperature could be assayed in one set of fibre bundles. Therefore, three sets of fibre bundles were initially isolated from the heart and were kept on ice (unpermeabilized) until needed. During preliminary experiments, measurements of maximum ADP stimulated respiration and acceptor control ratios (ACR, see below for definition and calculation) demonstrated that permeabilized fibres kept on ice were viable for up to 10 h after fibre isolation. Thus, all assays were performed within this time frame. The order of experimental test temperature was randomized for each experiment. At the end of experimentation, dry weights of fibres were determined by drying at 65 °C for 90 min and respiration rates are expressed as nmol O_2 min^{-1} mg dry $weight^{-1}$.

2.6. Metabolite analysis

To confirm the health and metabolic status of the turtles, analyses of lactate, glycogen, ATP, PCr and intracellular pH (pHi) were performed on frozen heart. Tissue was broken into small pieces under liquid N_2 with an insulated mortar and pestle, aliquots were transferred into 1.5 mL tubes and stored at −80 °C. An aliquot of tissue was further ground under liquid N_2 into a fine power and used for the determination of pHi with the methods of Portner et al. (1990) and a thermostatted (21 °C) Radiometer BMS3 Mk2 capillary microelectrode with a PHM84

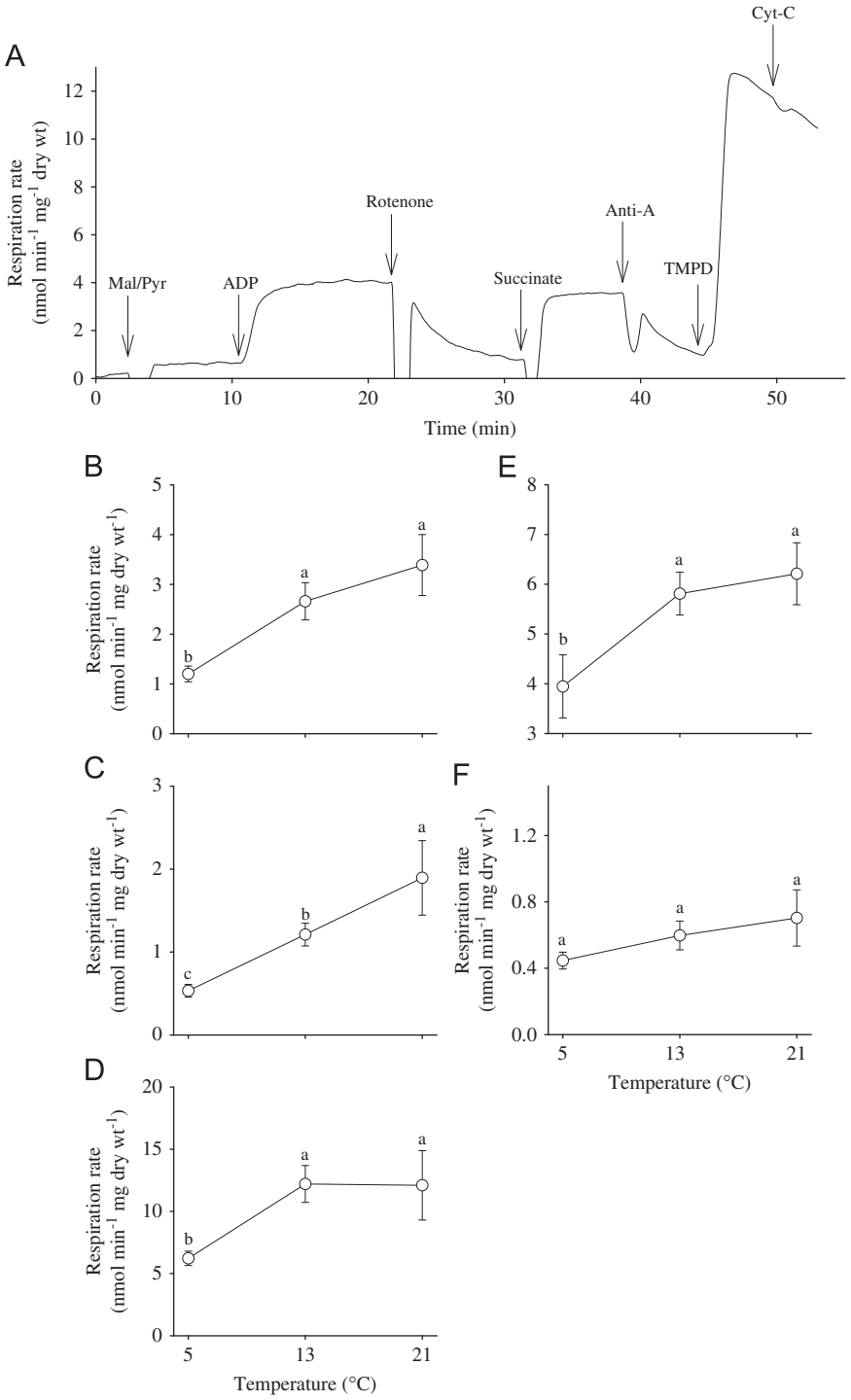

Fig. 1. Respiratory parameters in cardiac fibres from red eared slider (*Trachemys scripta*) turtles. Fibres were acutely tested at 5, 13 and 21 °C. (A) Representative trace of respiration rates in cardiac fibres from a red eared slider turtle tested at 21 °C. Mean data ± SEM for: (B) maximum respiration rate through complex I–IV with pyruvate and malate as substrates; (C) respiration rate through complex II–IV with succinate as a substrate; (D) respiration rate through complex IV via the electron donor TMPD; (E) acceptor control ratio (ACR); and (F) state II respiration rate with pyruvate and malate as substrates. Dissimilar letters indicate a significant effect of acutely changing temperature. $P < 0.05$, $n = 6$.

pH meter (Radiometer, Copenhagen, Denmark). For the extraction of metabolites, 0.5–1 ml of ice-cold 1 M $HClO_4$ was added to a microcentrifuge tube containing 50–100 mg of frozen tissue, and the mixture was immediately homogenised on ice with a Syngene homogenizer for 20 s followed by sonication for a further 20 s with a Kontes sonicator set to its highest setting. A 200 μL aliquot of the homogenate was then taken and frozen at −80 °C for glycogen content. The remaining homogenate was centrifuged at 10,000g for 10 min at 4 °C, and the supernatant was neutralised with 3 M K_2CO_3. The neutralised extracts were assayed spectrophotometrically for ATP, PCr and lactate according to methods described by Bergmeyer (1983). Total creatine (Cr) was measured on tissue extracts (Bergmeyer, 1983) after treatment with 1 N KOH and total Cr was 7 μmol/g wet weight. Samples for glycogen

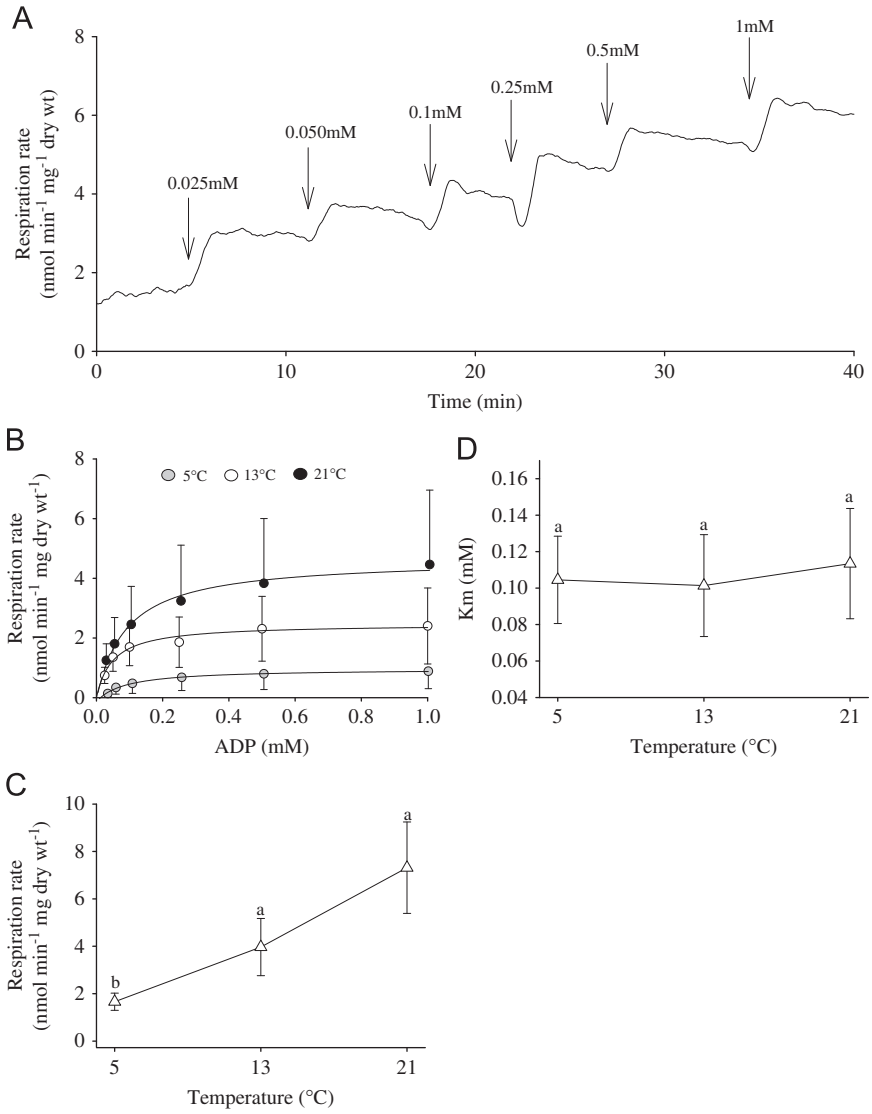

Fig. 2. ADP affinity in cardiac fibres from the red eared slider turtle (*Trachemys scripta*). Fibres were acutely tested at 5, 13 and 21 °C. (A) Original representative trace from cardiac fibres tested at 21 °C showing protocol (see the materials and methods section for details). Mean data ± SEM for (B) effect of ADP concentration on maximum respiration rate at 5 °C (grey circles), 13 °C (white circles) and 21 °C (black circles). (C) Maximum ADP stimulated respiratory flux rate (V_{max}) and (D) ADP concentration giving half maximal respiration rate ($K_{m\ ADP}$). Dissimilar letters indicate a significant effect of acutely changing temperature (1-way ANOVA, +post-hoc), $P < 0.05$, ($n = 6$).

analysis were thawed on ice, partially neutralised with 3 M K_2CO_3, digested with amyloglucosidase and measured for glucose as previously described (Hassid and Abraham, 1957).

2.7. Calculations and statistics

All reported respiration rates are normalised to dry weight. ACR were calculated as (v0+Vm)/v0, where Vm is the maximal respiration rate at saturating ADP concentrations (state III), and v0 is the basal respiration rate of mitochondria before addition of ADP (state II).

Free cytosolic [ADP] was calculated from measured values of [ATP], [PCr] and pHi assuming equilibrium of the creatine kinase reactions and according to published protocols (Golding et al., 1995; Teague et al., 1996). Free [Cr] was estimated by subtracting [PCr] from total [Cr]. The equilibrium constants for creatine kinase (K_{CK}) were corrected for experimental temperature, ionic strength, measured pH and free Mg^{2+} (assumed to be 1 mM; Van Waarde et al., 1990). Tissue [ATP], [PCr] and [Cr] were converted from μmol/g wet weight to molar concentrations using a cardiac intracellular fluid volume of 0.6 ml g^{-1} wet weight (Farrell and Milligan, 1986) and final values for [ADP_{free}] are reported in μM. A one-site saturation Michael–Menten non-linear regression model was used to describe the effects of changes in [ADP] on fibre respiration rate and to determine the $K_{m\ ADP}$ and V_{max} constants.

Statistical significance between different temperatures was assessed with a one or two-factor repeated measures ANOVA, as appropriate, and Student–Newman–Keuls post-hoc tests. Statistical tests were performed using SigmaStat software (version 4; Systat Software, San Jose, CA).

3. Results

3.1. Metabolite analysis

Turtle hearts were in good condition, with low [lactate] and high [glycogen] (Table 1). Values for heart [ATP], [PCr], pHi and [ADP_{free}] were similar to those published previously (Table 1; Stecyk et al., 2009).

Table 1
Metabolites from red eared slider turtle ventricular muscle.

	Lactate (μmol/g w.w.)	Glycogen (μmol glycosyl units/g w.w.)	ATP (μmol/g w.w.)	PCr (μmol/g w.w.)	Free Cr (μmol/g w.w.)	pH	ADP_{free} (nmol/g w.w.)	ADP_{free} (μM)
$n=8$	0.10 ± 0.30	165.7 ± 31.1	2.6 ± 0.4	2.1 ± 0.4	4.9 ± 0.4	7.15 ± 0.01	46.4 ± 14.0	77.3 ± 23.3

PCr, phosphocreatine; ADP_{free} is expressed in units of nmol/g w.w. and μM assuming 0.6 mL water/g wet weight of tissue. Free Cr is calculated from measurements of total creatine minus PCr.

Table 2
Q10 Values for the effect of acute temperature change on respiratory parameters of red eared slider turtle cardiac fibres.

	State II	$\dot{M}_{O_2}$ CI–IV	$\dot{M}_{O_2}$ CII–IV	$\dot{M}_{O_2}$ CIV	ACR	$K_{m\ ADP}$	V_{maxADP}
21–13 °C	1.29 ± 0.29	1.58 ± 0.60	1.90 ± 1.64	1.19 ± 0.43	1.10 ± 0.13	1.32 ± 0.40	2.53 ± 1.10
13–5 °C	1.48 ± 0.20	3.65 ± 1.49	3.54 ± 1.06	2.55 ± 0.50	1.86 ± 0.37	1.13 ± 0.36	2.24 ± 0.75
21–5 °C	1.40 ± 0.25	2.11 ± 0.40	2.42 ± 0.52	1.55 ± 0.28	1.36 ± 0.06	1.15 ± 0.19	3.20 ± 0.99

Q_{10} for maximal respiration rates ($\dot{M}_{O_2}$) through complex I–IV (CI–IV), complex II–IV (CII–IV) and state II respiration. ACR, acceptor control ratio. $K_{m\ ADP}$, ADP concentration that elicits half maximal respiration; V_{maxADP}, estimated maximal ADP stimulated respiration. Values are mean ± SEM, $n=6$

3.2. Respiration of cardiac fibres

Cardiac fibres were of good quality with high respiration rates, high ACR values (Fig. 1E and F) and a negligible effect of cytochrome C addition on fibre $\dot{M}_{O_2}$ (< 1% increase in $\dot{M}_{O_2}$ following cytochrome C injection; data not shown). At all temperatures tested, fibre $\dot{M}_{O_2}$ was higher in the presence of pyruvate (assessing $\dot{M}_{O_2}$ based on electron flux from complex I to IV; Fig. 1B) than in the presence of succinate and rotenone (assessing $\dot{M}_{O_2}$ based on electron flux from complex II to IV; Fig. 1C). Cardiac fibre $\dot{M}_{O_2}$ was 3–6 times greater in the presence of TMPD and ascorbate (assessing $\dot{M}_{O_2}$ from electron donation directly to complex IV) compared with pyruvate and/or succinate stimulated $\dot{M}_{O_2}$ (Fig. 1B–D). Acute decreases in temperature from 21 to 5 °C significantly decreased all respiratory parameters with the exception of state II respiration, which was temperature insensitive (Fig. 1). Fibre $\dot{M}_{O_2}$ based on electron flux through complex I–IV and IV as well as fibre ACR were temperature sensitive only between 5°C and 13 °C (Fig. 1), whereas $\dot{M}_{O_2}$ from electron flux through complexes II–IV was temperature sensitive only between 21 and 13 °C (Fig. 1C). Calculated Q_{10} values across the three temperatures tested for $\dot{M}_{O_2}$ through complexes I, II and IV were in the range 1.2–3.7, and although there was a tendency for exaggerated Q_{10} values in the lower temperature range (5–13 °C), there were no significant differences in Q_{10} between any temperatures examined (Table 2).

3.3. Apparent ADP kinetics of cardiac fibre $\dot{M}_{O_2}$

At all temperatures examined, the addition of increasing [ADP] resulted in increasing fibre $\dot{M}_{O_2}$ (Fig. 2A and B). Acute changes in temperature had no effect on the estimated K_m for ADP stimulated respiration, but there was a large effect of temperature on calculated V_{max} values, with V_{max} at 5 °C lower than V_{max} at 13 and 21 °C (Fig. 2C and D). Despite the fact that temperature sensitivity of fibre $\dot{M}_{O_2}$ appeared to be greater at lower temperatures, there were no differences in Q_{10} values calculated between the three temperatures examined (Table 2).

4. Discussion

4.1. Temperature sensitivity of respiration through ETC complexes

Respiration rates of turtle cardiac fibres were found to be similar to those published previously for this species (Birkedal and Gesser, 2003, 2004). A reduction in temperature from 21 to 5 °C led to a slower rate of respiration, and the magnitude of the decline was similar through all of the individual ETC complexes. However, $\dot{M}_{O_2}$ was considerably less temperature sensitive (Q_{10}, 1.2–3.7) compared to values published on isolated turtle heart mitochondria (Q_{10}, 1.5–5.1; Almeida-Val et al., 1994) and in vivo cardiac power measurements (Q_{10} up to 8.5; Herbert and Jackson, 1985; Stecyk et al., 2008). Moreover, in contrast to these latter studies, turtle cardiac fibres did not experience an exaggerated Q_{10} in the lower temperature range, and ACR values declined rather than increased as temperature was reduced. Several possibilities may explain these discrepancies between the present study and previously published values. Firstly, the different results may simply be explained by different study species (*T. scripta* vs. *Chrysemys picta*; Almeida-Val et al., 1994). Secondly, the mitochondrial study found an exaggerated Q_{10} effect only within the range of 10–15 °C, a range that was not specifically measured in the present study (Almeida-Val et al., 1994). However, variable thermal sensitivities between permeabilized preparations and isolated mitochondria from the same species have been described before. Permeabilized rainbow trout cardiac fibres and isolated cardiomyocytes were virtually insensitive to temperature, while isolated mitochondria exhibited significant reductions in respiration rates with decreasing temperature (Sokolova et al., 2009). The authors of the study could not give an explanation for the variable effects of temperature in the different preparations. It seems likely that the interaction of mitochondria with cytoplasmic proteins or elements of the cytoskeleton in permeabilized fibres may be important for in vivo regulation of respiration, which may affect or control thermal sensitivity. Indeed, assessing mitochondrial properties in permeabilized preparations is considered far more representative of respiration in vivo (Kuznetsov et al., 2008), which substantiates our results.

Whatever the reason for the discrepancy, it seems counter-intuitive for mitochondria of a highly active organ, such as the heart, to exhibit exaggerated Q_{10} of respiration. Although previous studies have shown cardiac function is profoundly reduced during cold exposure (Stecyk et al., 2008), especially over long periods, the heart has to remain continually active, and limited ATP supply under stressful conditions may lead to cardiac failure. Thus, results from our study suggest the large-scale reductions in cardiac function and high Q_{10} values at acutely low temperatures are likely due to a reduction in energy demand, rather than supply. In line with this contention, Birkedal and Gesser (2004) reported an increase in state III respiration rate in cold acclimated turtle cardiac fibres, suggesting ATP supply is upheld and possibly even upregulated during exposure to chronic cold.

4.2. Apparent K_m of ADP affinity

Permeabilized preparations of mammalian heart tissue and cardiomyocytes are known to exhibit diffusion restrictions with respect to ADP, such that $K_{m\ ADP}$ is substantially higher than when it is measured in isolated mitochondria (Andrienko et al., 2003; Scheibye-Knudsen and Quistorff, 2009). These restrictions have been attributed to low permeability of the outer mitochondrial membrane and cytosolic diffusion restrictions (Andrienko et al., 2003; Saks et al., 1995). Additionally, the different $K_{m\ ADP}$ of permeabilized preparations may simply be a result of insufficient cell separation or clustering of fibres in the respirometer (Sokolova et al., 2009). Ectothermic cardiomyocytes have a relatively simple morphology with low volumes and high surface-area to volume ratios (Galli et al., 2006), but intracellular diffusion restrictions have also been reported in the rainbow trout. For example, the ranges of $K_{m\ ADP}$ for rainbow trout permeabilized fibres (224–783 $\mu mol\ l^{-1}$) and cardiomyocytes (86–93 $\mu mol\ l^{-1}$) are orders of magnitude higher than that of the mitochondria (6–50 $\mu mol\ l^{-1}$) (Birkedal and Gesser, 2003; Guderley and St Pierre, 1999; Sokolova et al., 2009). In our study, we calculated turtle cardiac fibre $K_{m\ ADP}$ to be within the range of 100–110 $\mu mol\ l^{-1}$, which are values usually associated with cardiomyocyte preparations. This is at odds with Birkedal and Gesser (2003), who found the $K_{m\ ADP}$ of cardiac fibres from *T. scripta* to be higher than our values (300 $\mu mol\ l^{-1}$). The addition of 20 mM creatine substantially reduced the $K_{m\ ADP}$ from this very high value to values that were closer to those observed in the present study ($<90\ \mu M$; Birkedal and Gesser 2003), suggesting that ADP diffusion may have been limiting in the preparations without added creatine. Furthermore, the apparent $K_{m\ ADP}$ calculated in the present study is reasonably close to the calculated cardiac [ADP_{free}] determined in our study ($\sim 77\ \mu M$; Table 1). This close association between the apparent $K_{m\ ADP}$ and [ADP_{free}] suggests that mitochondrial respiration is strongly controlled by available ADP in vivo, which is consistent with published literature (Saks et al., 1995) and further supports the validity of the apparent $K_{m\ ADP}$ reported herein. It must be noted that our total creatine pool is only ~30% phosphorylated, which is low compared with other studies (Overgaard and Gesser, 2004). The low PCr/total Cr ratio in our study may be due to a degradation of PCr during heart dissection (cutting through the turtle shell took ~2 min), which could affect our calculations of [ADP_{free}]. If we assume 60% phosphorylation of our total Cr pool and recalculate [ADP_{free}], it is reduced by ~4 fold (from ~77 μM to ~20 μM), which is still within the range where mitochondrial respiration is highly influenced by [ADP_{free}].

4.3. Effect of temperature of $K_{m\ ADP}$

Turtle cardiac fibre $K_{m\ ADP}$ was insensitive to temperature. This result has been observed previously (Birkedal and Gesser, 2003), and sets the turtle apart from other ectothermic species, which show a significant decrease in the affinity for ADP at low temperatures (Birkedal and Gesser, 2003). This may represent an adaptation for the turtle, as it would suggest the range of temperatures that the animal encounters will not affect ADP kinetics.

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