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Keywords: Oxidative phosphorylation, Liver, Adenine nucleotide translocase (ANT), Proton leak, Archosaurs

Summary

The primary objective of this study was to assess whether adenine nucleotide translocase (ANT) content could be associated with phylogenetic disparities in mitochondrial coupling efficiency, within liver mitochondria obtained from rats, crocodiles, and ducklings. Our measurements included mitochondrial membrane conductance, ANT content, and oxidative phosphorylation fluxes at various steady-state rates. We observed significant variations in ANT content in liver mitochondria across the three species, and these variations correlated with interspecific differences in mitochondrial oxidative phosphorylation activity and coupling efficiency. These findings expand upon previous research by highlighting the pivotal role of ANT in modulating mitochondrial efficiency on an interspecific scale.

1. Introduction

Thyroid hormone levels, temperature, and body size represent major determinants of the mitochondrial oxidative phosphorylation activity and coupling efficiency, deeply ingrained in phylogenetics. For instance, mitochondria from endothermic vertebrates demonstrate higher mitochondrial proton conductance compared to ectotherms (Brand et al., 1991; Brookes et al., 1998; Hulbert et al., 2002; Polymeropoulos et al., 2017). This proton conductance activity, which does not generate ATP, can lower mitochondrial coupling efficiency, i.e., the ATP/O ratio, and consequently increase the energy expenditure for mitochondrial ATP synthesis. In conjunction with greater mitochondrial oxidative and enzyme activities (Bennett, 1972; Akhmerov, 1986; Paital and Samanta, 2013; Wiens et al., 2017; see however Berner, 1999), these findings suggest that mitochondria from endotherms convert energy from reduced coenzymes into ATP at a higher energy cost than ectotherms. However, it was reported that the contribution of proton leak to the resting metabolic rate of hepatocytes is as high in endotherms as it is in ectotherms (Brand et al., 1991; Stuart et al., 2001; Hulbert et al., 2002), suggesting

similar mitochondrial coupling efficiency, and ultimately, no substantial difference in the cost of mitochondrial ATP synthesis in cells. Furthermore, the proportion of cellular oxygen consumption allocated to mitochondrial proton leak (Porter and Brand, 1995; Else et al., 2004a), as well as the maximal effective coupling efficiency of mitochondria (Boël et al., 2019; Boël et al., 2023; Barbe et al., 2023), remains similar in birds and mammals regardless of their size, despite the negative correlation between mitochondrial proton conductance negatively and body mass in these endothermic species (Porter and Brand, 1993; Brand et al., 2003). All these data indicate that phylogenetic or allometric differences in the magnitude of mitochondrial proton conductance may not necessarily lead to alterations in mitochondrial coupling efficiency, i.e., in the energy cost of ATP production.

Mitochondrial coupling efficiency can also be influenced by other characteristics of the mitochondrial function capable of altering the proton motive force, thus the proton leak activity across the inner mitochondrial membrane. These characteristics may include, among others, oxidative capacity (Nogueira et al., 2001; Roussel et al., 2018) or the abundance of mitochondrial anion carrier proteins such as adenine nucleotide translocase (ANT) (Schönfeld, 1990; Cadenas et al., 2000; Brand et al., 2005; Bertholet et al., 2019). For instance, previous research has indicated that mitochondrial contents of cytochrome-c oxidase (e.g., cytochrome a) and ANT contribute to interspecific variations in mitochondrial function (Guderley et al., 2005; Hulbert et al., 2006). Expanding upon these observations, we examined whether ANT content could be linked to phylogenetical differences in mitochondrial coupling efficiency, i.e., the ATP/O ratio, in liver mitochondria isolated from rats (*Rattus norvegicus*), crocodiles (*Crococylus niloticus*), and ducklings (*Cairina moschata*). We assessed the capacity for succinate oxidation in these mitochondria, measuring their membrane proton conductance and ANT content. Additionally, we quantified oxygen consumption, ATP synthesis, and the resulting coupling efficiency (ATP/O ratios) at both maximal and submaximal oxidative

phosphorylation rates. Our findings reveal that liver mitochondria from crocodiles display high coupling efficiency and low inner membrane proton conductance compared to rat mitochondria. Conversely, rats demonstrate high coupling efficiency and similar inner membrane proton conductance when compared to duckling mitochondria. These interspecific variations in mitochondrial coupling efficiency can be attributed to differences in mitochondrial ANT content.

2. Materials and methods

2.1. Animals

In the present work, animals of similar body weight were used to limit confounding allometric effects on liver mitochondrial bioenergetics as described in endotherms and ectotherms (Porter and Brand, 1993; Brand et al., 2003; Roussel et al., 2015; Boël et al., 2023). Nile crocodiles of approximately 12-week old (*Crocodylus niloticus*) were captive-bred juveniles kindly donated by a crocodile farm (Pierrelatte, France). Crocodiles were chosen because their mitochondria can be assayed at high temperature (Guderley and Seebacher, 2011). They were maintained at 28-30°C for at least 2 weeks prior to the experiments, and were fed on meat. Muscovy ducklings of ~ 2-week old (*Cairina moschata*) were obtained at the age of 1 day from a commercial stockbreeder (Eclosion Grimaud Frères, Roussay, France), maintained at thermoneutrality for at least 10 days, and fed *ad libitum*. Sprague-Dawley rats (~ 6-week old) were housed in cages at ~25°C and fed *ad libitum*. All animals had access to water *ad libitum*.

2.2. Mitochondrial isolation

Liver mitochondria were isolated at 4°C by differential centrifugation as previously described (Salin et al., 2010; Roussel et al., 2015). We focus our study on liver because that organ performs identical physiological function and contributes to a significant part of the resting

metabolic rate in the three species. In addition, a large amount of mitochondria can be isolated from this organ, which was necessary to perform the different biochemical protocols described below. Liver was rapidly dissected, weighed, and homogenized with a Potter-Elvehjem homogenizer (3 passages) in ice-cold isolation buffer (250 mM sucrose, 1 mM EGTA, 20 mM Tris-base, and pH 7.3 at 4°C). For crocodile, liver samples from two individuals were pooled for each mitochondrial preparation. The liver homogenate was centrifuged at 800×g for 10 min. The supernatant was centrifuged at 1000×g for 10 min, filtered and re-centrifuged at 8,700×g for 10 min to pellet mitochondria. The mitochondrial pellet was re-suspended in ice-cold isolation buffer and re-centrifuged twice at 8,700×g for 10 min. The final pellet was re-suspended in 100 µL isolated buffer and protein content of mitochondrial suspension was assayed at 540 nm using the biuret method with bovine serum albumin used as standard. Of note, the mitochondrial preparation from crocodile liver contained a dark pigment which absorbs at 540 nm. Therefore, for each sample, the absorbance of the same volume of mitochondria assayed in Biuret solution without copper sulfate (i.e. 0.6% K-Na-tartrate and 3% NaOH) was used as a blank.

2.3. Mitochondrial oxidative phosphorylation activity and efficiency

Mitochondrial oxidative phosphorylation efficiency was assessed at 37°C by measuring the rates of oxygen consumption and ATP synthesis in a respiratory buffer (120 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 2 mM $MgCl_2$, 0.3 % fatty acid-free bovine serum albumin, 3 mM Hepes, pH 7,4) supplemented with 1.5 U/ml hexokinase, 20 mM glucose and 5 µM rotenone. Respiration was initiated by adding succinate (5 mM), then mitochondrial ATP synthesis was initiated by the addition of 500µM, 100 µM, 25 µM, 10µM or 5 µM ADP. Respiration rates were measured in a thermostatically regulated glass chamber containing a Clarke-type oxygen electrode (Rank Brothers Ltd, Cambridge, UK). After recording the phosphorylating respiration

rate for at least 2 min, four 300 µL samples of mitochondrial suspension were withdrawn from the suspension every 2 min and were quenched in 200 µL perchloric acid solution consisting of 10% $HClO_4$ and 25 mM EDTA. After centrifugation (15,000 *g*, 5 min) and neutralization of the resulting supernatant with a KOH solution (2 M KOH, 0.3 M MOPS), ATP production was determined from the glucose-6-phosphate content of samples as described previously (Salin et al., 2010; Roussel et al., 2015). To ensure that mitochondrial rates were specific to mitochondrial ATP synthase activity, we determined oxygen consumption and ATP synthesis rates in the presence of oligomycin (2 µg/mL). These values were taken into account to calculate the rate of mitochondrial ATP synthesis. The maximal activity of the electron transport system was measured as the carbonyl-cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP)-induced maximal respiration rate in mitochondria respiring on succinate (5 mM) in the presence of 5 µM rotenone and 2 µg/mL oligomycin.

2.4. ANT content of mitochondria

The ANT content was determined by titrating the rate of phosphorylating respiration with increasing concentrations of carboxyatractyloside, an irreversible inhibitor of ANT activity. Mitochondria (1.35 ± 0.17 mg/mL for crocodile, 0.76 ± 0.11 mg/mL for rat, and 0.50 ± 0.01 mg/mL for ducklings) were incubated in respiratory buffer supplemented with 1.5 U/mL hexokinase, 20 mM glucose, and energized with 5 mM succinate in the presence of 5 µM rotenone. After 3-5 minutes, 100 µM ADP was added to initiate phosphorylating respiration. The mitochondrial content of ANT protein was determined by the amount of carboxyatractyloside required to completely inhibit the rate of phosphorylating respiration (Schönfeld, 1990; Roussel et al., 2000).

2.5. Mitochondrial membrane potential

Mitochondrial respiration and membrane potential were measured at 37°C in respiratory buffer supplemented with 5 µM rotenone, 2.5 µg/mL oligomycin, 60 ng/mL nigericin, 2 µM triphenyl-methyl-phosphonium ($TPMP^+$). The $TPMP^+$ electrode was calibrated by four sequential 0.5 µM additions of $TPMP^+$, then 5 mM succinate was added to start the reaction. The kinetic response of proton leak to proton-motive force was determine by titration of oxygen consumption with malonate, up to 10 mM (Salin et al., 2010; Roussel et al., 2018). Membrane potentials were calculated as previously described by Brand (1995), assuming a $TPMP^+$-binding correction of 0.42, 0.54 and 0.66 $(\mu L/mg \text{ of protein})^{-1}$ for liver mitochondria of rats, ducklings and crocodiles, respectively (Brookes et al., 1998; Hulbert et al., 2002; Brand et al., 2003).

2.6. Statistical analysis

Data were tested with analysis of variance (ANOVA) for independent values, followed by protected least significance tests (Staview v4.5 statistical software). The relation between some of the mitochondrial parameters and the mitochondrial content of ANT was compared using linear regression. Data are reported as means ± S.E.M. with significance considered at $p < 0.05$.

3. Results and Discussion

Table 1 provides information on the body weight and liver mass of the animals. As expected (Else and Hulbert, 1985), the liver mass relative to body weight was lower in crocodiles than in rats or ducklings. Fig. 1A shows that the steady state rates of ADP-induced phosphorylating oxygen consumption were significantly different between species, being the lowest in crocodiles and the highest in ducklings. These disparities in the phosphorylating respiration among species were associated with variations in the maximal respiratory capacity of mitochondria, as determined by FCCP-induced maximal uncoupled respiration (Fig. 1B). These findings align with comparative studies that have reported lower mitochondrial activity

in ectotherms compared to endotherms (Akhmerov, 1986; Hulbert et al., 2002; Guderley et al., 2005; Paital and Samanta, 2013; Wiens et al., 2017; see however Berner, 1999). Thyroid hormone level and membrane lipid composition are among mechanisms well known to cause variation in metabolic rate and mitochondrial activity (Brookes et al., 1998; Nogueira et al., 2002; Brand et al., 2003). Hence, a lower level of thyroid hormones and/or a lower proportion of docosahexanoeic acid (C22:6n-3) within membrane are mechanisms able to cause lower mitochondrial activity in crocodiles compared to endotherms. Interestingly, these mechanisms are, more generally, involved in the evolution of endothermy (Else et al., 2004b; Little and Seebacher, 2014).

Fig. 2A reports the linear relationship between the rates of ATP synthesis and oxygen consumption in liver mitochondria from crocodiles, rats, and ducklings. The maximal rates of ATP synthesis and the corresponding oxygen consumption (i.e., the highest points on the right side of the linear relationships in Fig. 2A) were lowest in crocodiles and highest in ducklings, with the order being crocodile < rat < duckling. Crocodile mitochondria exhibited approximately 2.5 and 3.5 times lower rates of ATP synthesis and oxygen consumption, respectively, compared to rat and duckling mitochondria. The slopes of the linear relationships did not show significant differences between species ($P/O_{crocodile}$ = 1.54 ± 0.12; P/O_{rat} = 1.34 ± 0.06; $P/O_{duckling}$ = 1.36 ± 0.04; p=0.18), indicating that these relationships were parallel. However, the rates of non-phosphorylating respiration (the points were the curves intersects the x-axis) significantly varies among the three species (crocodile < rat < duckling; see Table 1 and Fig. 2A). Consequently, the linear curves for crocodile mitochondria and duckling mitochondria were notably shifted to the left and right, respectively, compared to the curve for rat mitochondria (Fig. 2A). This suggests that at any given steady-state rate of ATP production, crocodile liver mitochondria consumed less oxygen, whereas duckling mitochondria required more oxygen than rat mitochondria. This difference is better illustrated in Fig. 1B, which shows

that for any given rate of oxygen uptake, the ATP/O of liver mitochondria was higher in crocodiles and lower in ducklings compared to rats. Overall, crocodile liver mitochondria exhibited greater efficiency, whereas duckling mitochondria displayed lower efficiency than rat mitochondria (Fig. 2). In the present work, we used juveniles that were of the same body mass but not of the same age due to growth rate differences between species. In juvenile brown trout, it has been shown that individuals with a higher growth performance also exhibited a higher coupling efficiency in their liver mitochondria (Salin et al., 2019). In common frog, an intraspecies comparison of populations having large differences in body mass at the same age found that frogs with high growth rates and large body sizes exhibited higher mitochondrial coupling efficiency compared to the smaller frogs (Salin et al., 2012). In contrast, differences in growth performance in broilers were not associated with mitochondrial efficiency variations in skeletal muscle and liver (Bottje et al., 2002). In rat, values of thermodynamic efficiency of liver mitochondrial oxidative phosphorylation did not seem to vary in 40- to 180-day-old rats (Lionetti et al., 2004; Iossa et al., 1998). In endotherms. Altogether these studies suggest that in the present study, the difference in stage of development between juveniles would not fully explain differences in mitochondrial coupling efficiency between the three species.

As shown in Fig. 2B, the effective ATP/O ratio displays a non-linear relationship with mitochondrial oxidative activity. It increases sharply as the phosphorylation rate rises before leveling off and reaching a maximum. This non-linear relationship results from the fact that proton leak pathways and ATP synthesis compete for the same driving force, known as the proton motive force (Brand, 2005). Consequently, during the dynamic transition from the basal non-phosphorylating state to the maximal phosphorylating state (Fig. 2), proton leak significantly decreases as oxidative phosphorylation increases, leading to better mitochondrial efficiency (ATP/O ratio). Therefore, there is no fixed value for the ATP/O ratio that can describe effective coupling efficiency, as it closely depends on the degree of oxidative

phosphorylation activity. Nonetheless, this non-linear mitochondrial property can be quantified by fitting the relationships between ATP/O ratios and the rates of oxygen consumption with a mono-exponential function [$ATP/O = ATP/O_{max} \times (1 - e^{-mtEC \times (JO - JObasal)}$; Mogensen and Sahlin, 2005], where J_O represents the phosphorylating oxygen consumption rate, J_{Obasal} is the non-phosphorylating oxygen consumption rate (the intersection point with the *x*-axis), and "mtEC" is the gradient of the non-linear curve. This factor has been termed the mitochondrial efficiency channeling (mtEC) factor because it describes how rapidly mitochondrial coupling efficiency reaches its maximum. The mtEC factor value can then be used as a single mitochondrial parameter to describe the overall variation in effective coupling efficiency with changes in mitochondrial activity or power (Boël et al., 2019). In the present study, mtEC was highest in crocodiles and lowest in ducklings (Fig. 2B inset). These results show that the increase in effective ATP/O ratios was steeper, indicating better coupling in crocodiles than in ducklings, with rat mitochondria falling in between.

Differences in mitochondrial proton conductance may contribute to variations in mitochondrial coupling efficiency (Beavis and Lehninger, 1986; Brand et al., 1991; Nogueira et al., 2002). This is supported by the fact that the shifts in the relationships depicted in Fig. 2 seem to be driven by differences in the rates of non-phosphorylating respiration, a respiratory state characterized by the absence of ATP synthesis, a high proton motive force, and maximal proton leakage (Brand and Nicholls, 2011). In the present study, the inner membrane proton conductance of liver mitochondria was higher in rats than in crocodiles (Fig. 1S.A), but there was no significant difference between rats and ducklings (Fig. 1S.B). At comparable potential values, the permeability of the inner membrane to protons was approximately three times greater in rats than in crocodiles. These results align with previous studies that have shown that mitochondria from small reptile species (< 2-3 kg) exhibit lower proton conductance than mitochondria from mammals of similar body weight (Brand et al., 1991; Brookes et al., 1998).

It is worth noting that these differences between reptiles and mammals are less pronounced when comparing larger species (Hulbert et al., 2002; Polymeropoulos et al., 2017). Further insight, the higher proton conductance in rat mitochondria was associated with a lower value of effective ATP/O ratio at maximal phosphorylating activities (the highest points to the right of the non-linear curve in Fig. 2B) than in crocodile mitochondria (ATP/O_{Rat} = 1.14 ± 0.05 versus $ATP/O_{Crocodile}$ = 1.26 ± 0.11). Although the difference did not reach significance, this result aligns with previous studies showing that agents that increase mitochondrial proton conductance, such as thyroid hormones or mitochondrial uncouplers, also decrease the effective coupling efficiency at maximal phosphorylating activity (Beavis and Lehninger, 1986; Nogueira et al., 2001; Nogueira et al., 2002). Taken together, these observations suggest that differences in membrane conductance to protons may partly explain the differences in mitochondrial efficiency between crocodiles and rats. In contrast to ectothermic species, mitochondrial proton conductance is less distinct between bird and mammal species (Brookes et al., 1998; Brown et al., 2009; Polypopoulos et al., 2017). In the present study, the mitochondrial inner membrane proton conductance was not different between rats and ducklings (Fig. 1S.B). This result implies that difference in coupling efficiency between rats and ducklings was mainly linked to a difference in the mitochondrial oxidative capacity. This hypothesis is largely supported by previous studies showing that mitochondria with a lower oxidative phosphorylation capacity often exhibit a higher coupling efficiency, such as in the presence of low dose of cyanide or nitric oxide or following caloric restriction (Zangarelli et al., 2006; Clerc et al., 2007; Roussel et al., 2018).

The mitochondrial coupling efficiency (ATP/O ratio) is not only negatively controlled by proton leak, but also by its sensitivity to changes in phosphorylation reactions, which exert roughly equal positive control. These reactions encompass several steps, including those involving the ATP synthase, the phosphate carrier, and the ANT (Brand et al., 1993; Roussel et

al., 2004). ANT is a major transport protein located in the inner mitochondrial membrane, supplying the cell with ATP during periods of high energy demand. Previous research has reported a correlation between ANT content correlates and interspecific variations in mitochondrial oxidative capacity in muscle mitochondria (Guderley et al., 2005). In the present study, the ANT content in liver mitochondria differed significantly among the three species, with the lowest content observed in crocodiles and the highest in ducklings (crocodile < rat < duckling; see Fig. 3).

While ANT's primary role is to export ATP from the mitochondrial matrix to the cytosol while importing cytosolic ADP when cellular energy needs are high, it can also catalyze proton leakage in the resting state when cellular energy needs are low (Bertholet et al., 2019). In addition, ANT can also facilitate fatty acid- or AMP-induced uncoupling in resting mitochondria (Schönfeld, 1990; Roussel et al., 2000; Cadenas et al., 2000; Bottje et al., 2009; Guderley and Seebacher, 2011). It has also been reported that ANT may contribute from half to two-thirds of the basal proton conductance in resting mitochondria (Brand et al., 2005). In the present study, the basal non-phosphorylating state of respiration, measured in the presence of oligomycin, was sensitive to CAT addition (Table 1). Based on these data, we can estimate that ANT may contribute to more than half of the basal non-phosphorylating respiration rate in rats, which was significantly higher than the values calculated in crocodiles and ducklings (Table 1). The basal non-phosphorylating respiration serve as a measure of the maximal proton leakage across the inner mitochondrial membrane. Hence, these data suggest that differences in mitochondrial ANT content may drive part of the differences in coupling efficiency between species. This hypothesis is reinforced by the fact that, when mitochondrial fluxes (ATP synthesis and corresponding oxygen uptake) are normalized by mitochondrial ANT content, the relationships between the rates of ATP synthesis and oxygen consumption from crocodiles, rats and ducklings overlap (Fig. 4). It must be kept in mind that the present results are based on

succinate oxidation. Previous studies have shown that ANT is a major controlling step of the oxygen-consumption flux in rat liver mitochondria respiring on succinate (Groen et al., 1982; Kunz et al., 1988; Rossignol et al., 2000). In contrast, it appears that ANT becomes a minor controlling step of the phosphorylating respiration in mitochondria respiring on pyruvate/malate (Rossignol et al., 2000). Whether differences in mitochondrial ANT contents between species would have mediated differences in coupling efficiency of mitochondria respiring on NADH-linked substrates remain an open question that deserves further experimental testing.

In conclusion, these results offer a comprehensive insight into the variations in oxidative phosphorylation activity, membrane proton conductance, and coupling efficiency among the major taxonomic groups. They also expand upon previous research by highlighting the significant role of ANT in regulating mitochondrial efficiency across different. ~~The question of the evolutionary pressures influencing ANT and the precise mechanisms through which it influences differences in mitochondrial energetics among major taxonomic groups remains unanswered but presents an intriguing avenue for further exploration in this field.~~

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Figure Legends

Figure 1: Mitochondrial oxidative capacity of liver mitochondria. (A) The rate of oxygen consumption as a function of ADP concentration from crocodiles (grey diamonds), rats (white circles), and ducklings (black triangles). (B) Maximal respiratory capacity measured in the presence of FCCP from crocodiles (grey bar), rats (white bar), and ducklings (black bar). Values represent means ± s.e.m. from (n) independent mitochondrial preparations; (n) is provided alongside the species name. Overall kinetics (A) or bars (B) with different superscript letters are significantly different at $P<0.05$.

Figure 2: Mitochondrial oxidative phosphorylation activity (A) and efficiency (B) in liver mitochondria from crocodiles (grey diamonds), rats (white circles), and ducklings (black triangles). Inset of Panel B: Mitochondrial efficiency channel factor (mtEC; details in text). Values represent means ± s.e.m. from (n) independent mitochondrial preparations; (n) is provided alongside the species name. Data with different superscript letters are significantly different at $P<0.05$; In panels A, lower case and upper case letters compare rates of oxygen consumption and ATP synthesis, respectively. †$P<0.05$, indicates significant overall differences in kinetics (see text for details).

Figure 3: ANT content in liver mitochondria from crocodiles (grey diamonds), rats (white circles), and ducklings (black triangles). (A) Phosphorylating respiration was titrated by successive additions of carboxyatractyloside (CAT) until maximal inhibition of mitochondrial respiration was achieved. ANT content was determined by the amount of CAT required for complete inhibition of phosphorylating respiration. (B) ANT contents measured through CAT titration. Data with different superscript letters are significantly different at $P<0.05$. Values

represent means ± s.e.m. from (n) independent mitochondrial preparations; (n) is indicated alongside the species name.

Figure 4: Mitochondrial oxidative phosphorylation efficiency in liver mitochondria from crocodiles (grey diamonds), rats (white circles), and ducklings (black triangles). The rates of ATP synthesis and oxygen consumption from Fig. 1A were normalized by the corresponding ANT contents of mitochondria from Fig. 2B. Values represent means ± s.e.m. from (n) independent mitochondrial preparations; (n) is indicated alongside the species name. * and † indicate that the maximal rates of ATP synthesis and oxygen consumption in rats were significantly different from crocodiles and ducklings (P<0.05).

Supplemental Figure 1: Basal proton conductance of liver mitochondria from crocodiles (grey diamonds), rats (white circles), and ducklings (black triangles). Proton conductance was assessed in medium containing rotenone, succinate, oligomycin and nigericin, and membrane potential and respiration rate were titrated with malonate as described in the "Materials and Methods" section. Proton leakage rate was calculated from respiration rate by assuming a H^+/O ratio of 6 for succinate oxidation. Values represent means ± s.e.m. from (n) independent mitochondrial preparations; (n) is given alongside the species name.

Table 1: Body weight, liver mass and properties of liver mitochondria

	Crocodile (5)	Rat (6)	Duckling (6)
Body weight (g)	143 ± 13[a]	194 ± 9[b]	189 ± 14[b]
Liver (g)	2.9 ± 0.3[a]	7.4 ± 0.7[b]	8.5 ± 0.9[b]
Liver (g/100 BW)	2.0 ± 0.1[a]	3.8 ± 0.3[b]	4.6 ± 0.4[b]
Mitochondrial oxygen consumption (nmol O/min.mg protein)			
Non-phosphorylating rate	10.6 ± 2.3[a]	19.8 ± 2.7[b]	30.1 ± 3.6[c]
CAT-insensitive non-phosphorylating rate[1]	7.5 ± 1.6[a]	7.9 ± 1.7[a]	22.3 ± 2.4[b]
CAT-sensitive respiration (% of non-phosphorylating state)[1]	15 ± 3[a]	54 ± 7[b]	21 ± 5[a]

Values are means ± s.em. from (n) independent mitochondrial preparations; (n) is provided alongside the species name. Data with different superscript letters are significantly different at $P<0.05$.

Figure 1

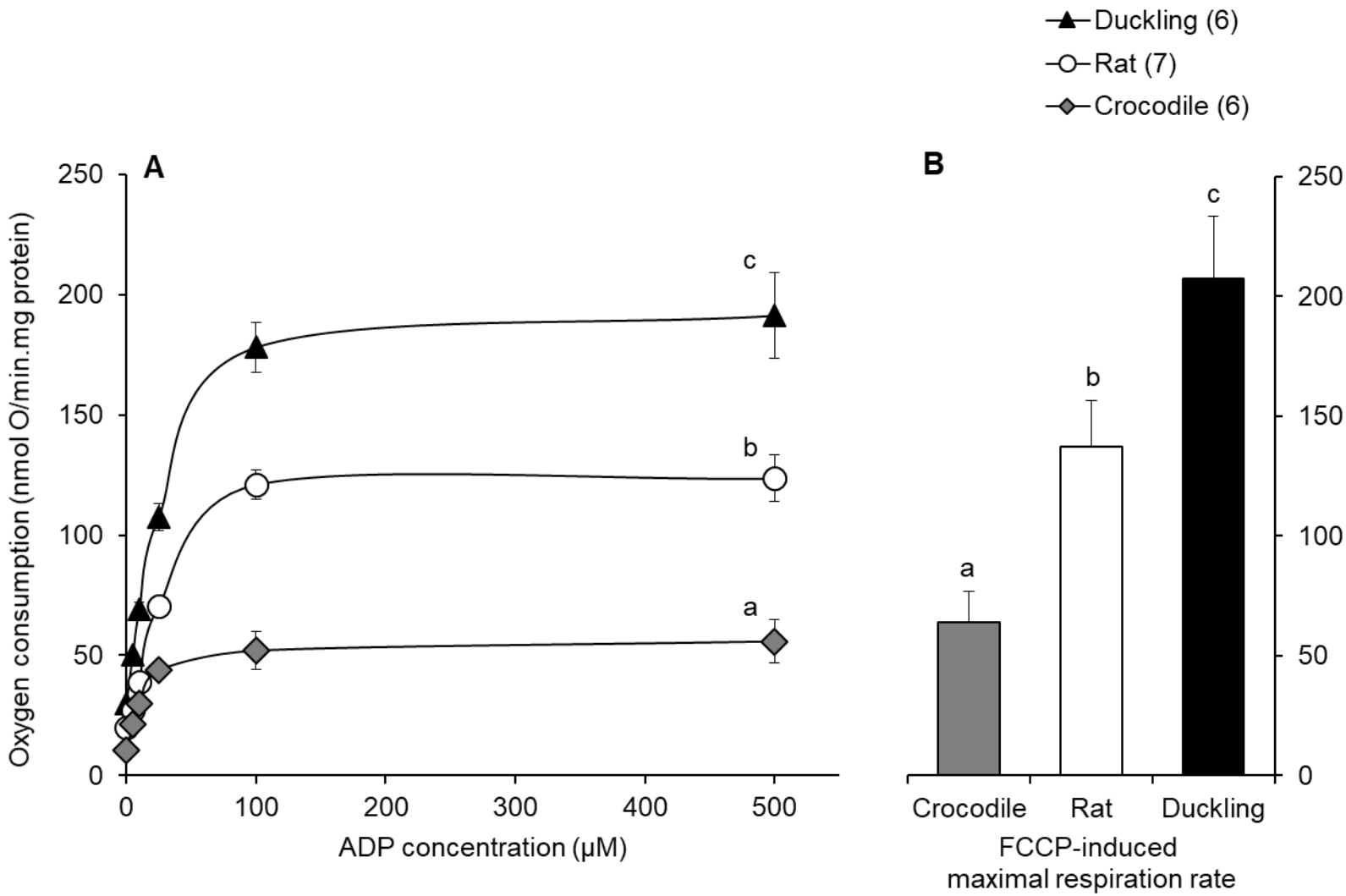

Duckling (6)
Rat (7)
Crocodile (6)
A
B
Oxygen consumption (nmol O/min.mg protein)
ADP concentration (μM)
0
50
100
150
200
250
0
100
200
300
400
500
a
b
c
Crocodile
Rat
Duckling
FCCP-induced
maximal respiration rate

Figure 2

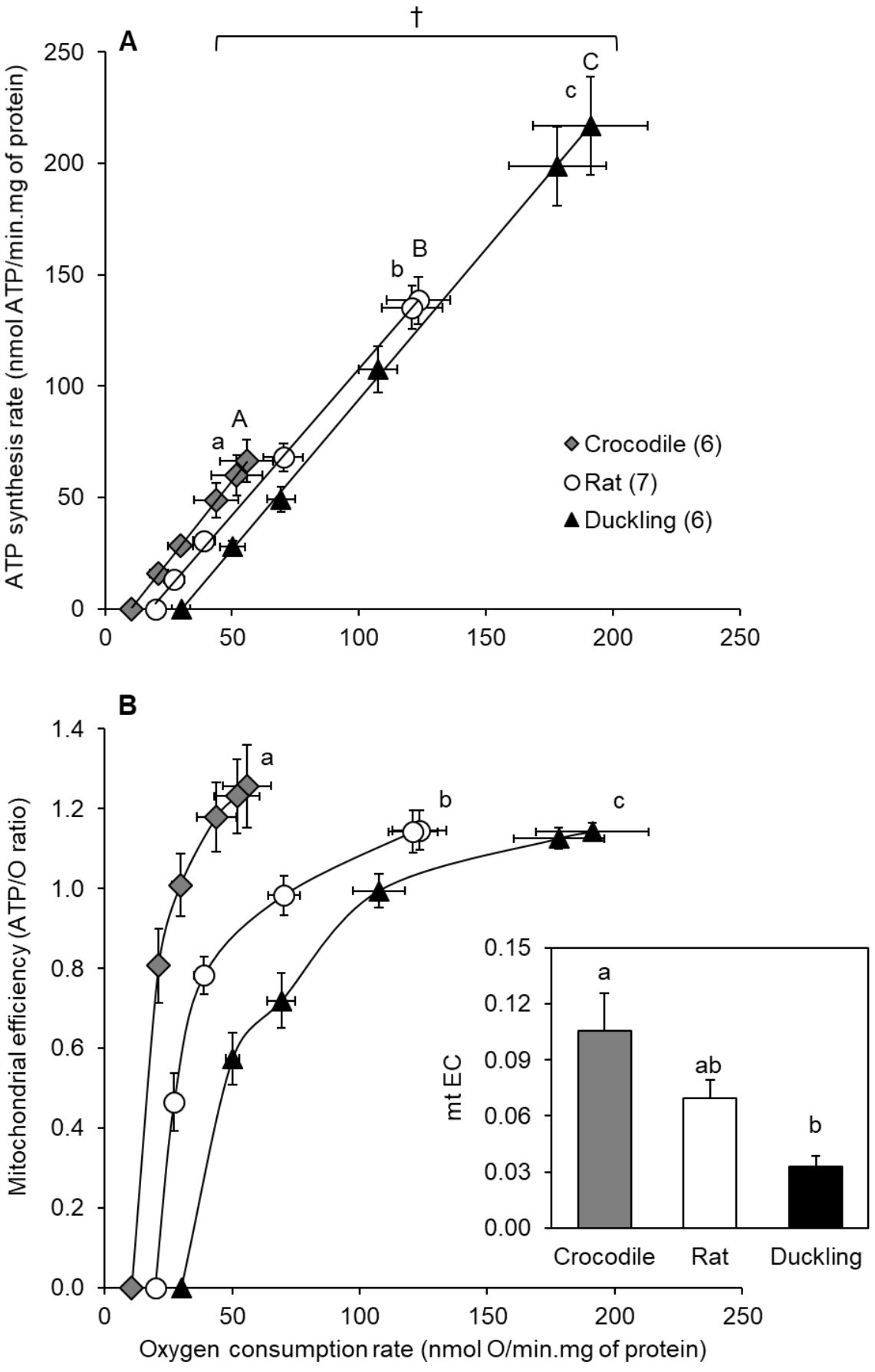

A
†
C
c
B
b
A
a
ATP synthesis rate (nmol ATP/min.mg of protein)
Crocodile (6)
Rat (7)
Duckling (6)
B
a
b
c
Mitochondrial efficiency (ATP/O ratio)
mt EC
a
ab
b
Crocodile
Rat
Duckling
Oxygen consumption rate (nmol O/min.mg of protein)

Figure 3

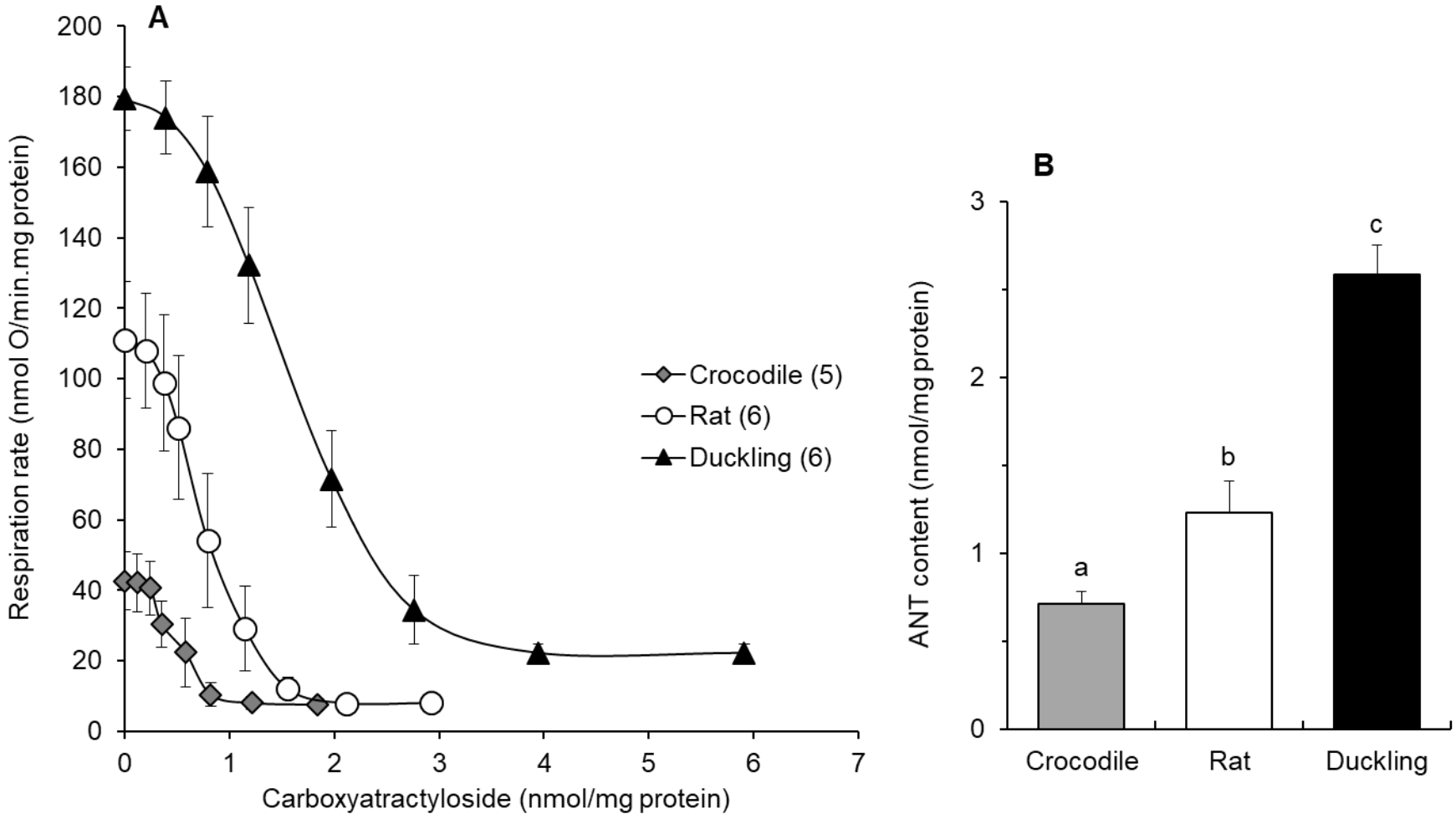
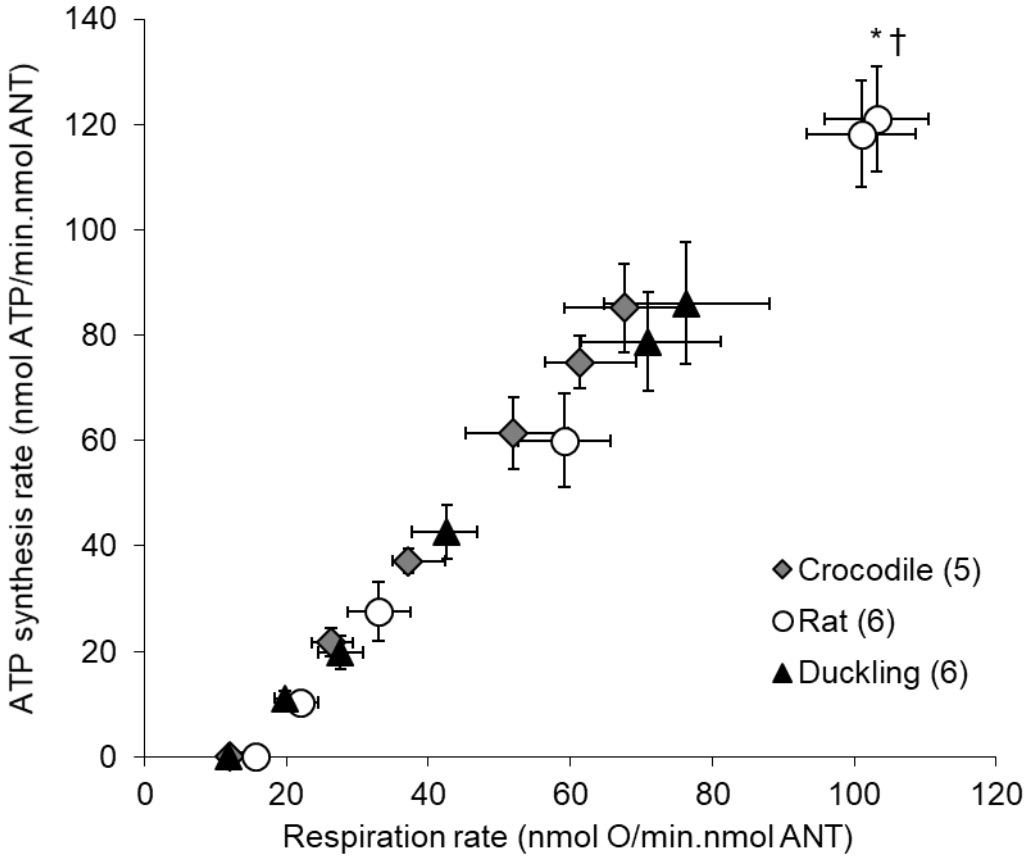

A
Respiration rate (nmol O/min.mg protein)
Carboxyatractyloside (nmol/mg protein)
Crocodile (5)
Rat (6)
Duckling (6)
B
ANT content (nmol/mg protein)
a
b
c
Crocodile
Rat
Duckling

Figure 4


ATP synthesis rate (nmol ATP/min.nmol ANT)
Respiration rate (nmol O/min.nmol ANT)
0
20
40
60
80
100
120
140
* †
Crocodile (5)
Rat (6)
Duckling (6)

Supplemental Figure 1S