High-energy turnover at low temperatures: recovery from exhaustive exercise in Antarctic and temperate eelpouts

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Hardewig, I., P. L. M. van Dijk, and H. O. Pörtner. High-energy turnover at low temperatures: recovery from exhaustive exercise in Antarctic and temperate eelpouts. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R1789–R1796, 1998.—Earlier work on Notothenioids led to the hypothesis that a reduced glycolytic capacity is a general adaptation to low temperatures in Antarctic fish. In our study this hypothesis was reinvestigated by comparing changes in the metabolic status of the white musculature in two related zoarcid species, the stenothermal Antarctic eelpout *Pachycara brachycephalum* and the eurythermal *Zoarces viviparus* during exercise and subsequent recovery at 0°C. In both species, strenuous exercise caused a similar increase in white muscle lactate, a drop in intracellular pH (pH_i) by about 0.5 pH units, and a 90% depletion of phosphocreatine. This is the first study on Antarctic fish that shows an increase in white muscle lactate concentrations. Thus the hypothesis that a reduced importance of the glycolytic pathway is characteristic for cold-adapted polar fish cannot hold. The recovery process, especially the clearance of white muscle lactate, is significantly faster in the Antarctic than in temperate eelpout. Based on metabolite data, we calculated that during the first hour of recovery aerobic metabolism is increased 6.6-fold compared with resting rates in *P. brachycephalum* vs. an only 2.9-fold increase in *Z. viviparus*. This strong stimulation of aerobic metabolism despite low temperatures may be caused by a pronounced increase of free ADP levels, in the context of higher levels of pH_i and ATP, which is observed in the Antarctic species. Although basal metabolic rates are identical in both species, the comparison of metabolic rates during situations of high-energy turnover reveals that the stenothermal *P. brachycephalum* shows a higher degree of metabolic cold compensation than the eurythermal *Z. viviparus*. Muscular fatigue after escape swimming may be caused by a drop of the free energy change of ATP hydrolysis, which is shown to fall below critical levels for cellular ATPases in exhausted animals of both species.

metabolic cold adaptation; free energy change of adenosine 5′-triphosphate hydrolysis; muscular fatigue; Zoarcidae

IN ECTOTHERMIC ORGANISMS exposure to cold environments requires special physiological adaptations to maintain physiological functions despite low temperatures. Early investigations on polar fish suggested that these animals show higher basal metabolic rates than temperate fish when compared at the same low temperatures (49). However, this hypothesis of metabolic cold adaptation has been critically discussed by Holeton (22) and Clarke (4). The current view is that some degree of metabolic cold adaptation does occur in Antarctic fish, but that complete compensation is not achieved (19, 40, 46). Histological and biochemical investigations show adaptive changes in the oxidative capacity of Antarctic fish. The locomotor muscles possess, for instance, higher mitochondrial volume densities than observed in temperate species (13). Crockett and Sidell (5) examined the activities of several glycolytic and oxidative enzymes in heart and skeletal muscle. They found that maximal activities of oxidative enzymes were 1.5–5 times enhanced compared with temperate species. These studies suggest that despite low resting rates of metabolism the maximal capacity for aerobic energy production is enhanced in Antarctic species. Therefore, it may be more meaningful to investigate situations of high-energy flux, when metabolic rate is reaching its maximum, to determine whether metabolic cold adaptation does occur in Antarctic fish (for a recent review see Ref. 43).

High-energy flux is observed during burst swimming activity and subsequent recovery. Exhaustive exercise in fish, in contrast to steady-state aerobic swimming, involves short bouts of high intensity swimming, primarily powered by white musculature and supported by anaerobic metabolism. As a result of high glycolytic rates, lactate accumulates in the white muscle. Peak levels of lactate are an indication of the capacity for strenuous exercise. Interestingly, in Notothenioids, the most common Antarctic fish group, lactate levels in white muscle remained constant or increased only slightly during exercise (7, 14). Based on measurements of the maximal activities of glycolytic enzymes and glycolytic metabolites Dunn and Johnston (14) concluded that Antarctic fish have a reduced glycolytic capacity, perhaps as a special adaptation to the cold Antarctic environment due to the difficulty of clearing lactate at low temperatures. Because the lack of lactate production has only been shown for Notothenioids, the question arises whether this is a general feature of cold adaptation or rather a particular phylogenetic trait of this family.

The fish family Zoarcidae form an ideal group for comparative investigations on Antarctic vs. non-Antarctic fish. Unlike Notothenioids, an endemic Antarctic fish family on which practically all previous research on Antarctic fish has been carried out, zoarcids are cosmopolites. This family therefore provides the unique opportunity to compare related fish species from polar and temperate waters. In the present study we investigated the effect of strenuous exercise and subsequent recovery on the metabolite status in the stenothermal Antarctic eelpout *P. brachycephalum* in comparison with the eurythermal temperate *Z. viviparus*. Both zoarcids have a benthic lifestyle and are sluggish in their movements. Moreover, both species were subjected to long-term acclimation to the experimental temperature of 0°C, making a meaningful comparison possible. The aim of our study was to investi-

gate whether the stenothermal Antarctic eelpout *P. brachycephalum* has developed an enhanced metabolic capacity to provide sufficient ATP for strenuous exercise at low temperature and to recover from exhaustion.

Furthermore, we were interested to define the metabolic status at which the fish were exhausted. What causes the inability to perform muscular work at this state? An answer that would seem logical is that the energy stores of the white musculature are depleted. In rainbow trout both phosphocreatine and ATP tend to decline during exercise, although the extent of depletion can vary between 40 and 90% at exhaustion (28). Therefore, ATP availability is obviously not limiting. It has been proposed that the elongation of the relaxation time observed in fatiguing muscle is correlated with a drop of the Gibbs free energy change of ATP hydrolysis ($dG/d\xi$), which represents the energy content of ATP available to cellular ATPases (8). In the present study, we have measured metabolites and white muscle intracellular pH (pH_i) to be able to calculate the free energy change of ATP in resting and exhausted fish and tested the hypothesis that a drop of $dG/d\xi$ below a certain threshold is correlated with muscular fatigue. Although ATP may still be present in sufficient quantities at exhaustion, its energy content may be too low to drive the relevant cellular processes.

MATERIAL AND METHODS

Animals. Benthic eelpout *P. brachycephalum* were caught in traps at a depth of 500 m in the vicinity of King George Island (61° 43.3′ S, 59° 12.5′ W) in December 1996. Traps were exposed for 36 h and then raised slowly (<0.5 m/s) to allow the animals to adjust to the decreasing pressure. To obtain healthy Antarctic eelpout living at intermediate depths the use of traps was far more successful than bottom trawling. Specimens of this fish family are rarely found in bottom trawl catches, and, more importantly, fish caught in traps are in much better physiological condition. Set out at a favorable position one trap yielded up to 40 healthy specimens of *P. brachycephalum*. All specimens used in this experiment were caught in one haul. Fish (length 27.7 ± 2.4 cm) were kept in well-aerated water of 0.0 ± 0.5°C for at least 1 wk before experimentation under permanent dim light. The animals appeared to have been feeding well before capture but they were starved during captivity. Experiments were performed aboard the research vessel "Polarstern."

Z. viviparus, eelpout from temperate waters and with a comparable lifestyle as *P. brachycephalum* were caught in trawls in the German Wadden Sea during the winter of 1996–1997. Fish (length 14.5 ± 1.0 cm) were acclimated to 0.0 ± 0.5°C for at least 2 mo. Ad libitum feeding with shrimp was terminated 1 wk before experimentation. Pregnant females were not used in this study.

Experimental protocol. Fish were chased manually in a shallow rectangular tank until exhaustion. Exhausted fish were decapitated (0 h) or allowed to recover for 1, 3, 10, or 24 h. Recovering animals were kept individually in darkened, plastic containers containing 3 liters of aerated seawater at 0.0 ± 0.5°C. Control (unexercised) fish were kept in the same kind of containers for 24 h. Fish were anesthetized by adding 0.3 g/l MS-222 (unneutralized) before samples of epaxial white muscle and blood were taken. Sampling was performed in a cool room at 0–2°C. The muscle samples were freeze-clamped and stored in liquid nitrogen until analysis. Plasma samples were kept at −80°C.

Tissue preparation and analysis. pH_i in white muscle tissue was measured according to Pörtner et al. (34) as described in van Dijk et al. (42). The remaining muscle tissue was ground under liquid nitrogen, extracted in ice-cold perchloric acid, and neutralized with KOH. Extracts were stored at −80°C until analysis. Concentrations of lactate, creatine phosphate, creatine, and ATP were determined enzymatically according to Bergmeyer (3).

Calculations and statistics. Levels of free ADP and AMP were calculated on the basis of the equilibrium of creatine kinase (CK) and myokinase (MK). Values for K_{eqCK} and K_{eqMK} were taken from Teague and Dobson (38) and Tewari et al. (39) and corrected to 0°C. The $dG/d\xi$ was calculated on the basis of the determined metabolite concentrations and pH_i values considering the pH-dependent concentrations of the reactive species of ATP, ADP, and P_i at a constant free cellular Mg^{2+} concentration of 1 mmol/l as outlined by Pörtner et al. (35). Free P_i was assumed to be 1 mmol/l in resting animals (G. van den Thillart, personal communication). A maximum estimate of the increase of P_i during exercise was derived from changes in creatine phosphate and ATP concentrations ($\Delta P_i = \Delta CP + 2\Delta ATP$), assuming that ATP is degraded to IMP. Data were checked for outliers beyond the $r(95)$ limits of an r distribution [$r_A < r(95)$] using Nalimov's test (32). Statistical significance was tested at the $P < 0.05$ level using ANOVA and the post hoc Student-Newman-Keuls test for independent samples. Data are given as means ± SD.

RESULTS

Burst exercise of 3–5 min was sufficient to fully exhaust the Antarctic *P. brachycephalum*, whereas it took 7–10 min to fatigue *Z. viviparus*. No mortality was observed during the recovery period.

Exhaustive exercise caused a marked rise in muscle lactate concentration in both species (Fig. 1). Although resting lactate levels were significantly higher in eelpout from North Sea (3.1 ± 0.7 vs. 0.3 ± 0.2 μmol/g wet wt), the exercise-induced increase was about the same in both groups. In *P. brachycephalum*, lactate levels peaked immediately after the exercise regimen at t equals 0 h and fell to control levels within 10 h. In *Z. viviparus* the concentration of lactate increased further during the first hour of recovery and remained elevated for >10 h. Plasma lactate levels were generally lower in *P. brachycephalum* (0.10 ± 0.09 mM) than in *Z. viviparus* (1.68 ± 1.11 mM) but did not change significantly during the exercise regimen in either group (data not shown).

The pH_i of the white musculature under control conditions was significantly higher in *P. brachycephalum* (7.51 ± 0.06 vs. 7.26 ± 0.08). During exhaustive exercise, pH_i dropped by about 0.5 pH units in both species (Fig. 1). Intracellular realkalization occurred at similar rates during the first 3 h of recovery after which it was delayed in *Z. viviparus*.

Resting levels of phosphocreatine and creatine were similar in both species (see Figs. 2 and 3). Exhaustive exercise caused a depletion by 84 and 91% of the phosphagen pool in Antarctic and North Sea eelpout, respectively. Rephosphorylation occurred at similar rates in both groups. Alterations in phosphocreatine concentrations were mirrored by stoichiometric changes of creatine.

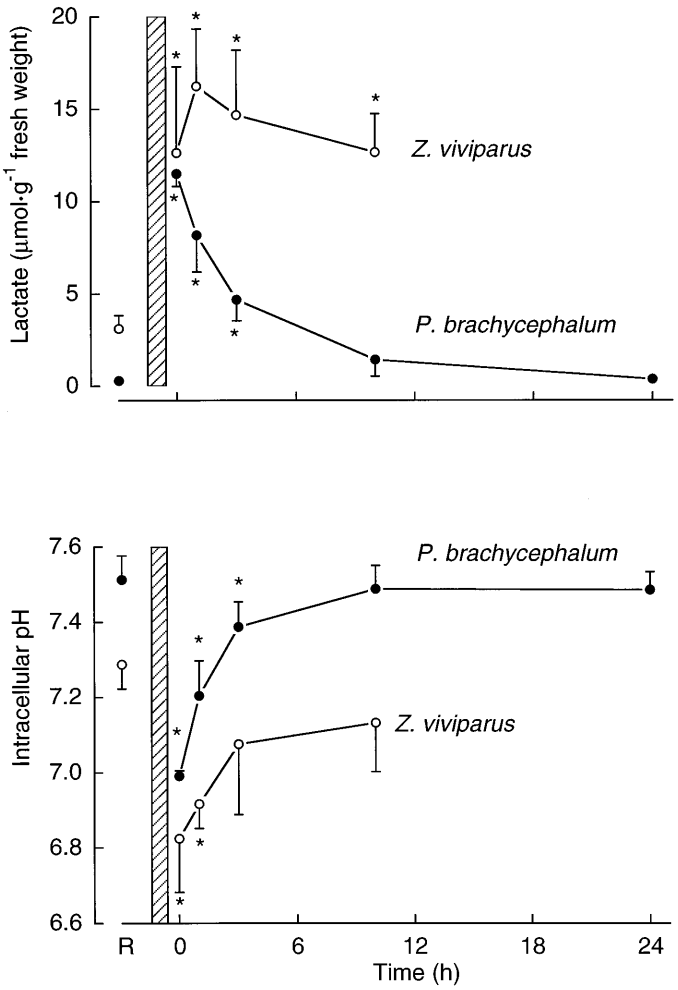

Fig. 1. Changes in white muscle lactate content (*top*) and intracellular pH (*bottom*) during exhaustive exercise and subsequent recovery in common and Antarctic eelpout. Values are means ± SD, $n = 5$, except for *P. brachycephalum* at $t = 0$, where $n = 3$. *Significant difference from resting value ($P \leq 0.05$).

The total adenylate pool was higher in Antarctic than in temperate eelpout (Fig. 4). Exhaustive exercise caused a drop of ATP levels to 44% of the control value in *Z. viviparus*. In contrast, ATP levels remained constant during exercise in *P. brachycephalum*, despite a pronounced exhaustion of the phosphagen stores. Surprisingly, ATP levels even increased above control values during the first hours of recovery, although ATP turnover was presumably still enhanced owing to the replenishment of phosphagen and glycogen stores.

Although levels of free ADP increased about sixfold during exercise in *P. brachycephalum*, only a threefold rise was observed in *Z. viviparus*. In this species free ADP levels returned to control levels within the first hour of recovery but remained elevated for about 3 h in *P. brachycephalum*. Levels of free AMP showed the same trend as free ADP in both species.

DISCUSSION

Lactate production during exercise. This is the first study that shows the production of significant amounts of lactate in an Antarctic fish species during strenuous exercise. Neither in *Pagothenia borchgrevinki* nor in

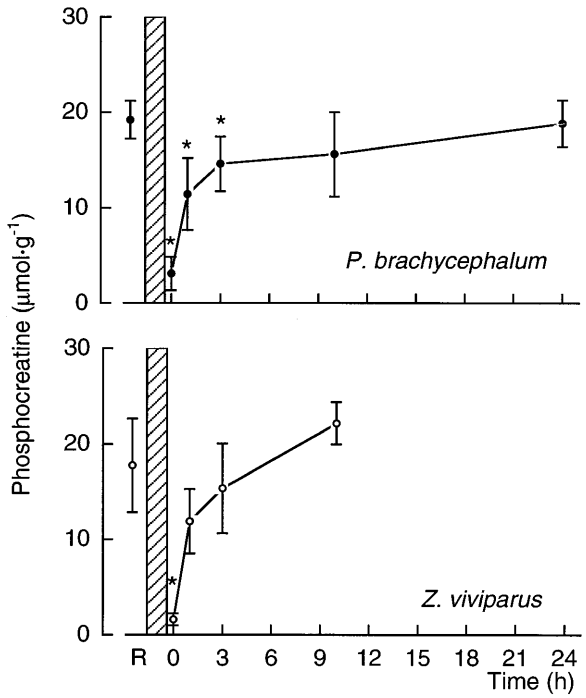

Fig. 2. Phosphocreatine content during exhaustive exercise and subsequent recovery in white musculature of common eelpout *Z. viviparus* and Antarctic eelpout *P. brachycephalum*. Values are means ± SD, $n = 5$, except for *P. brachycephalum* at $t = 0$, where $n = 3$. *Significant difference from resting value ($P \leq 0.05$).

Notothenia coriiceps, both Notothenioids from the Southern Ocean, did exhaustive exercise cause a significant increase of lactate concentrations in the white musculature (7, 14). Several studies on the

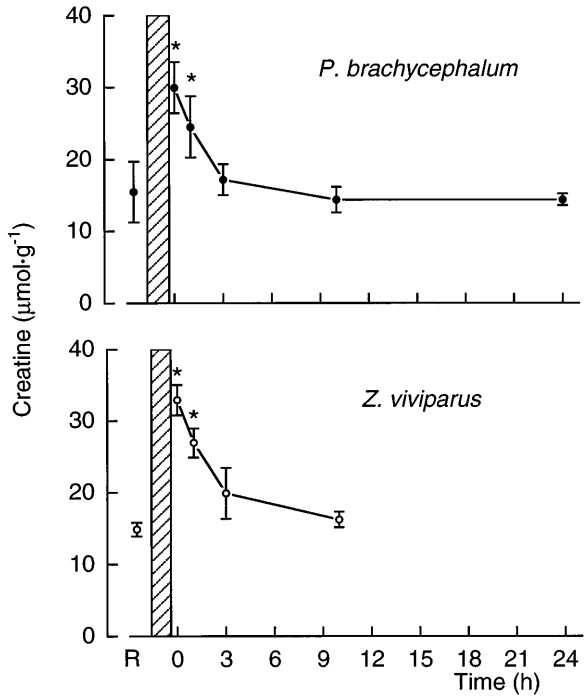

Fig. 3. Changes in white muscle creatine content during exhaustive exercise and subsequent recovery. Values are means ± SD, $n = 5$, except for *P. brachycephalum* at $t = 0$, where $n = 3$. *Significant difference from resting value ($P \leq 0.05$).

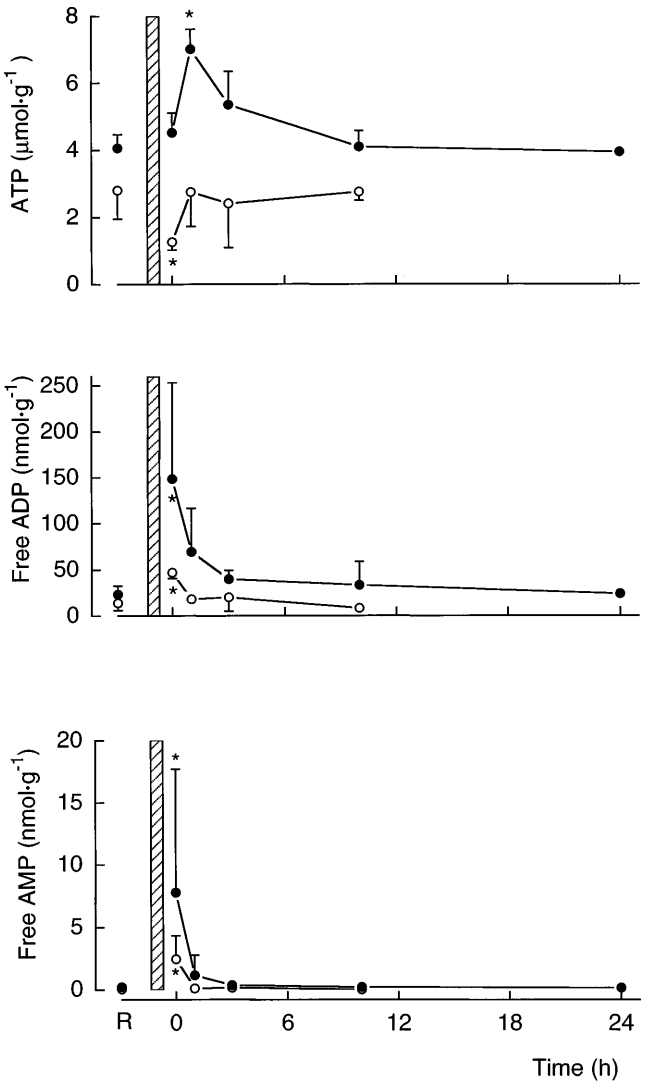

Fig. 4. Levels of ATP (*top*), calculated values of free ADP (*middle*), and free AMP (*bottom*) in white musculature at rest, during exhaustion, and subsequent recovery. ○, Common eelpout *Z. viviparus*; ●, Antarctic eelpout *P. brachycephalum*. Values are means ± SD, $n = 5$, except for *P. brachycephalum* at $t = 0$, where $n = 3$. *Significant difference from resting value ($P \leq 0.05$).

effects of exercise stress in Antarctic fish determined only blood or plasma levels of lactate. In all cases lactate concentrations remained close to or even below 1 mmol/l (7, 15, 17). Based on measurements of the specific activities of key glycolytic enzymes in the white muscle of *N. coriiceps*, Dunn and Johnston (14) concluded that the absence of lactate formation is due to a reduced glycolytic capacity in these animals. The authors speculated that this phenomenon may have an adaptive value, because the clearance of lactate may require long recovery periods due to low metabolic rates in the cold. However, all species so far investigated, lacking lactate formation, belong to the Notothenioids. Because *P. brachycephalum*, a zoarcid from the Antarctic, does produce large amounts of lactate, we conclude that the reduced glycolytic capacity observed in Notothenioids is a specific phylogenetic trait of this family rather than a special adaptation to cold temperatures.

Resting levels of lactate in the white musculature were significantly lower in *P. brachycephalum* than in *Z. viviparus*, but exhaustive exercise caused a similar increase in both species by 11.2 and 13.1 μmol/g wet wt, respectively. The extent of lactate formation seems to correlate with the overall metabolic rate and the ability for burst swimming. Although the benthic flounder, *Platichthys stellatus*, accumulates only about 7.6 μmol lactate/g wet wt at 11°C (30), postexercise lactate levels of up to 40 μmol/g have been reported in trout at 10°C (37; see Table 1). Because lactate accumulation in both zoarcid species at 0°C is well in the range of that observed in temperate species at higher temperatures, we conclude that anaerobic glycolysis is not compromised by acclimation to low temperature nor by living in the permanent cold of polar environments. Because Antarctic eelpout fatigued more rapidly (3–5 min vs. 7–10 min) but accumulated almost the same amount of lactate in a shorter period the glycolytic rate during the exercise regimen was even higher in these animals. It would be revealing to compare the rate of lactate formation in *Z. viviparus* at low and high acclimation temperatures to see if indeed perfect cold compensation of glycolytic capacity does occur. For comparison, trout showed complete cold compensation with respect to anaerobic glycolytic rate between 5 and 18°C (26, 48), whereas roach produced only one-half as much lactate when acclimated to 4°C compared with 20°C acclimated fish (6, 47).

Although exhaustive exercise resulted in substantial lactate production in the white muscle of both zoarcids, there was no significant increase of lactate in the

Table 1. *Comparison of amount of lactate produced during exhaustive exercise and maximal lactate clearance rates of various fish species*

Species	T(°C)	Δ Lactate, μmol/g	Recovery Period, h	Clearance Rate μmol · g⁻¹ · h⁻¹	Source (Ref.)
Prachycara brachycephalum	0	11.2	10	3.3	Present study
Zoarces viviparus	0	13.1	≫10	0.79	Present study
Platichthys stellatus (flounder)	11	6.7	12	0.62	30
Oncorhynchus mykiss (trout)	5	30	8	3.9	26
	10	38	24	3.6	37
	18	29	8	6.4	26
Semotilus atromaculatus (creek chub)	22	15.8	3	4.6	20
Umbra limi (mud minnow)	22	22	6	4.0	20
Katsuwonus pelamis (tuna)	25	100	1.3–1.7	78	1

Recovery period is time required for lactate levels to reach control values. T, temperature.

extracellular space. Similar results were obtained in other sluggish benthic marine fish from temperate waters, the plaice, *Pleuronectes platessa*, and the flounder, *Platichthys stellatus* (30, 45). Although some lactate was seen to accumulate in the blood space in these species, the bulk of lactate is retained in the muscle for metabolism in situ. Obviously, the distribution of lactate between intra- and extracellular compartments is far from electrochemical equilibrium during and after exercise (51, 52). pH-dependent equilibrium distribution with higher extra- than intracellular concentrations is reached only during long-term hypoxia as observed in some fish species (for review see Ref. 33). Lactate release may be limited by perfusion of the musculature, although blood flow to the white muscle is known to increase substantially after exhaustive exercise also in Antarctic fish (16, 45). According to several authors (2, 41, 51) lactate retention involves active transport via an anion exchanger. Using labeled lactate, Milligan and McDonald (29) found that the release of lactate to the extracellular space was increased threefold during the first 2 h of recovery after exercise. A recent study by Wood and Wang (51) even found an 8- to 10-fold increase of lactate efflux from 200 to 1,500–2,000 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ in isolated trunk from exercised trout. Given that the uptake of lactate by the white musculature against the electrochemical gradient involves active transport, it would be surprising should Antarctic fish use this energy-consuming mechanism to such an extent that lactate is quantitatively retained in the white muscle. Lactate efflux rates, however, may be reduced by low temperatures. It has been shown that the muscle-to-blood gradient of lactate is dependent on the acclimation temperature in trout. Lower blood lactate concentrations were found at 5°C than at 18°C, even though intracellular levels were similar in both groups (26).

Little is known about the control mechanisms of lactate fluxes. Wardle (45) demonstrated that β-adrenoreceptor blockade increased plasma lactate levels in exercised plaice, suggesting that catecholamines were involved in lactate retention in muscle. However, attempts by others to obtain similar results were unsuccessful. Neither Wood and Milligan (50) in starry flounder nor van Dijk and Wood (44) in rainbow trout, were able to demonstrate a regulatory role of catecholamines in postexercise blood lactate dynamics. Data relating to this subject are scarce for Antarctic fish. Egginton (15) found that induced exercise did not cause any catecholamine response in three Antarctic teleosts, *N. coriiceps*, *N. rossii*, and *Chaenocephalus aceratus*. As a corollary, it seems unlikely that catecholamines play a regulatory role in lactate retention in *P. brachycephalum*.

The accumulation of lactate coincides with a large drop in pH_i in the white musculature of both zoarcid species. Surprisingly, the resting values of pH_i are significantly higher in Antarctic eelpout, although both species were kept at the same temperature. This difference is not due to the absence of alpha-stat pH regulation in *Z. viviparus* (42) but reflects a higher set point of pH_i regulation in the Antarctic eelpout.

Muscular fatigue. Metabolic status of white musculature during fatigue was different in both species. The most striking difference is that ATP levels had dropped by 66% in *Z. viviparus*, whereas they remained constant in *P. brachycephalum*. These fish are obviously exhausted even though ATP is still available. The reasons for muscular fatigue are not yet understood. Several factors are discussed in the literature as potential causes for fatigue. The intracellular acidification and the increase of free P_i have been shown to be correlated with prolonged relaxation times and decreased force production (18). These parameters, however, may not only have a direct effect on muscular function but may also act through their effect on the chemical potential of ATP, the dG/dξ (8, 25). dG/dξ is reduced during strenuous muscular activity and may drop to critical levels at which they are insufficient to drive relevant cellular ATPases (8, 23, 35). The values of the free energy change of ATPase reactions have not directly been determined, but have been estimated based on cellular conditions. Values for Ca^{2+}-ATPase in the sarcoplasmatic reticulum range between 39 kJ/mol (in frog sartorius muscle; 8) and 52 kJ/mol (in rat myocardium; 25). The energy requirement of rat myocardium actomyosin ATPase was estimated to be 45–50 kJ/mol (25). As discussed by Pörtner et al. (35) these values may differ between species and tissues.

We calculated the free energy change values of ATP hydrolysis for the white muscle of eelpouts and trout using metabolite data from Schulte and co-workers (37; see Fig. 5). We found that levels of dG/dξ in both resting as well as fatigued animals were remarkably similar in all three species, although the concentrations of the individual metabolites affecting the chemical potential of ATP (like ATP, ADP, and H^+) differed largely between them (see Fig. 6). The dG/dξ in exhausted animals was -46.6 ± 0.54 kJ/mol for *Z. viviparus* and -48.0 ± 1.61 kJ/mol for *P. brachycephalum*, which is in the limiting range for vertebrate Ca^{2+}-ATPase and actomyosin ATPase. These data suggest that the reduction in dG/dξ during exhaustive exercise in fish white muscle may be the reason for muscular fatigue.

In support of this conclusion, fatigue has been observed in three squid species at different concentra-

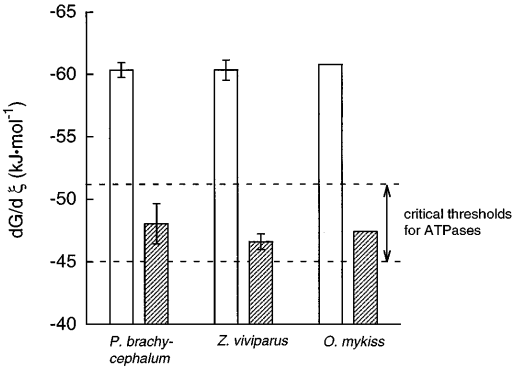

Fig. 5. Gibbs free energy change of ATP hydrolysis (dG/dξ) in white musculature at rest (open bars) and after exhaustive exercise (hatched bars) of *P. brachycephalum*, *Z. viviparus*, and *Oncorhynchus mykiss* (calculated from metabolite data from Ref. 37).

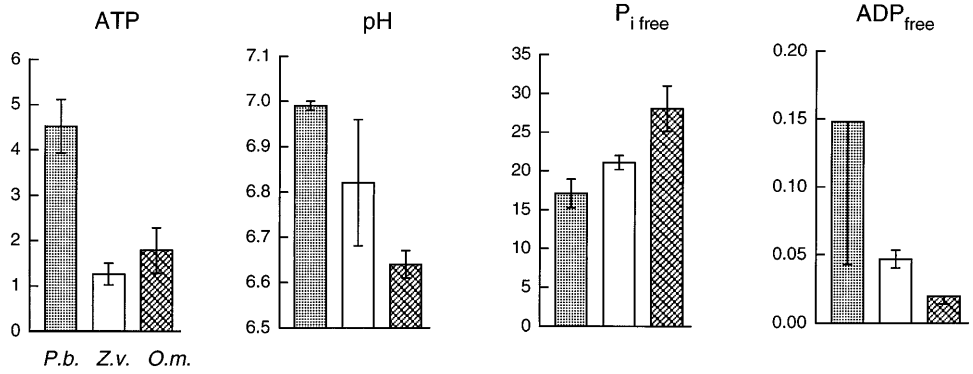

Fig. 6. Concentrations (μmol/g white muscle) of metabolites that affect free energy change of ATP hydrolysis in white musculature of exhausted fish. Stippled bars, *P. brachycephalum*; open bars, *Z. viviparus*; crosshatched bars, *Oncorhynchus mykiss* values from Ref. 37.

tions of ATP, ADP, P_i, and pH_i but at the same levels of dG/dξ. The ATP free energy change in fatigued mantle musculature ranged between −42.3 and −44.7 kJ/mol for all species (35). These low values may reflect lower energy requirements of the cellular ATPases in squid mantle tissue. Again, the differences between ATP free energy change at exhaustion in the same tissue of related species are strikingly small.

However, the causality between dG/dξ and fatigue does not seem to be universal. It appears that hypoxia-tolerant animals show only moderate rates of anaerobic metabolism during exercise and protect the dG/dξ at higher levels (H. O. Pörtner and W. R. Ellington, personal communication).

Recovery from fatigue. In both species phosphocreatine stores, which had been depleted to the same extent during exercise, were replenished at similar rates and reached control values within the first few hours of recovery. Levels of ATP were generally higher in Antarctic than in temperate eelpout. The reasons for that are not clear. There may be a positive correlation between pH_i and steady-state ATP levels as has been found in shrimp (36). In *Z. viviparus* ATP pools were recharged before phosphagen stores were replenished. A similar temporal decoupling of the replenishment of ATP and phosphocreatine pools has been observed in trout (9). Interestingly, in *P. brachycephalum* ATP levels remained unchanged during the exercise period and even increased above control values during the first hour of recovery. The rise in ATP levels before phosphagen replenishment may be necessary to drive the following reaction

$$ATP + Cr \rightarrow H^+ + PCr + ADP \qquad (1)$$

because during the early phase of recovery intracellular proton concentrations and levels of free ADP are still high.

Lactate clearance rates were considerably higher in Antarctic eelpout. Lactate concentrations were almost back to control levels within 10 h, whereas no significant decrease was observed during this period in animals from the North Sea. A comparison of lactate production and clearance rates of a variety of fish from different habitats shows that the value for Antarctic eelpout is well in the range of those observed in temperate fish at their respective acclimation temperature, whereas *Z. viviparus* shows very low clearance rates at 0°C (see Table 1). In accordance with high lactate clearance rates pH_i recovered rapidly in *P. brachycephalum*, but remained below control levels in *Z. viviparus* (see Fig. 1). The fast early phase of realkalinization in the latter species is probably due to recovery from respiratory rather than metabolic acidosis.

Taken together, our data show that Antarctic *P. brachycephalum* is able to recover faster from exhaustive exercise than the eurythermal eelpout *Z. viviparus* at low temperature. This is not reflected in the replenishment of phosphagen pools, which occurs at similar rates in both species. However, *Z. viviparus* is obviously not able to provide sufficient energy for this process by aerobic oxidation but is still relying on anaerobic metabolism as reflected in a continued increase of lactate concentrations during the first hour of recovery. Accordingly, lactate clearance is significantly slower in this species compared with Antarctic eelpout. Based on the metabolite data, we calculated the aerobic ATP turnover and the additional oxygen consumption necessary for gluconeogenesis, rephosphorylation of phosphocreatine, and ATP synthesis during the first hour of recovery in both species. While *Z. viviparus* only uses an additional 1.1 μmol $O_2 \cdot g^{-1} \cdot h^{-1}$ during this period the oxygen consumption of *P. brachycephalum* has to be increased by 2.5 μmol $O_2 \cdot g^{-1} \cdot h^{-1}$. Because resting metabolism is similar in both species at 0°C (about 0.38 μmol $O_2 \cdot g^{-1} \cdot h^{-1}$; I. Hardewig, P. L. M. van Dijk, C. Tesch, and H. O. Pörtner, unpublished results), the factorial increase of oxygen consumption during recovery is much higher in Antarctic eelpout (6.6 vs. 2.9). Obviously, the ventilatory and circulatory system of the two species is able to deliver sufficient oxygen to meet this increase in oxygen demand despite low temperatures. Cold-induced adaptional changes in heart performance and metabolism have been shown for several fish, including Antarctic species (10).

The strong enhancement of the oxidative metabolism in *P. brachycephalum* may be induced by the sixfold increase of free ADP levels during exercise from 23.4 ± 9.0 to 148.1 ± 105.2 nmol/g, which corresponds to an intracellular concentration of 33 and 211 μM, respectively (see Fig. 4). This increase is exceptionally high. *Z. viviparus* only shows a threefold increase, whereas in trout free ADP even decreases during exhaustive exercise (37). ADP is known to stimulate oxidative phosphorylation (12, 27) with an apparent Michaelis

constant value in the physiological range between 40 and 100 μM determined in isolated mitochondria of the short-horned sculpin *Myoxocephalus scorpius* (21). A decrease of free ADP levels or the resulting increase of the ATP/free ADP ratio observed in recovering rainbow trout has been shown to strongly inhibit mitochondrial respiration (31). In this species the high ATP/free ADP ratios may be necessary to favor the reversal of the pyruvate kinase reaction, which is speculated to be involved in gluconeogenesis (31, 37). The dephosphorylation of phospho*enol*pyruvate to pyruvate is generally believed to be irreversible under cellular conditions. Schulte and co-workers (37) suggested, however, that in trout white muscle high ATP/free ADP levels aided by elevated pyruvate concentrations may drive the pyruvate kinase reaction in the reverse direction during the early phase of recovery. The authors speculate that there is a trade-off between the effect of ATP/free ADP on the rate of gluconeogenesis and oxidative phosphorylation. However, in both *Z. viviparus* and *P. brachycephalum* ATP/free ADP levels decrease during recovery, thus favoring mitochondrial respiration rather than the reversal of the pyruvate kinase reaction resulting in high aerobic metabolic rates during postexercise recovery.

The sensitivity of respiratory control toward changes of ADP levels may be enhanced by increased mitochondrial density, as has been suggested by Dudley and co-workers (12). Because increased proportions of mitochondria are frequently observed in cold-adapted fish, reflecting higher aerobic capacity (e.g., Ref. 24), the stimulation of oxidative phosphorylation by increasing ADP levels may be even more pronounced in Antarctic organisms. Therefore, high levels of free ADP may be the key to the strong increase in aerobic postexercise metabolism observed in *P. brachycephalum*, which enables this species to recover quickly from exhaustive exercise despite low resting metabolic rates. It remains to be investigated if this is a universal phenomenon in polar species.

How does *P. brachycephalum* achieve the pronounced increase of free ADP levels during exercise and early recovery? Because ADP participates in the creatine kinase reaction, which is assumed to be in equilibrium in the musculature, the concentration of free ADP is determined by the ratio of the reactants (see *Eq. 1*). The concentrations of creatine and phosphocreatine do not differ between the two species. Therefore, higher levels of ATP (see Fig. 4) as well as lower intracellular proton concentrations (see Fig. 1) are responsible for the more pronounced increase of free ADP in *P. brachycephalum*. This may be the explanation why in this species ATP concentrations have to be kept at control levels during exhaustive exercise. This can be achieved by blockage of the purine nucleotide cycle at the site of AMP deaminase, to avoid ATP degradation to IMP. This is in accordance with the about 40-fold increase of free AMP levels during exercise (see Fig. 4). We suggest that *P. brachycephalum* shows only low specific activity of AMP deaminase or that AMP deaminase is inhibited by the high pH_i (11) to maintain high ATP concentrations and thereby high levels of free ADP.

Perspectives

Our data strongly support that Antarctic fish show metabolic cold compensation to a higher extent than temperate zone eelpout at 0°C. This is, however, not reflected in elevated resting metabolic rates but becomes evident during situations of high-energy turnover. The enhanced aerobic capacity of the Antarctic species is likely to be correlated with higher mitochondrial densities and higher specific activities of oxidative enzymes, as has been shown for Notothenioids (43). The genetic basis for the adjustments of the metabolic machinery to low temperatures, such as mitochondrial proliferation and qualitative or quantitative adaptations of enzymes, remains to be investigated.

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