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Full Length Research Paper

Evaluation of a fast and simple method for measuring plasma lactate levels in Atlantic cod, *Gadus morhua* (L.)

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The aim of the present study was to investigate the validity of measuring circulatory lactate levels in plasma from Atlantic cod using Lactate Pro™, a portable meter described for measuring whole blood lactate in this species. Atlantic cod were either unstressed or subjected to short- or long-term crowding stress. Whole blood was analyzed on site, and a strong positive relationship was evident between lactate concentrations measured in whole blood and 5 months later in frozen and thawed plasma. Our results indicate that the portable meter can be effectively used also to detect differences in plasma lactate in this species.

Key words: Blood, exercise, portable meter, stress.

INTRODUCTION

Measurement of circulating lactate is a commonly used tool for assessing the metabolic status of fish during swimming and fatigue (Kieffer, 2010) and the physiological response to a wide range of stressors (Barton and Iwama, 1991; Mommsen et al., 1999). As opposed to the analytical techniques used in research laboratories, the use of portable, handheld lactate meters has been shown as a useful tool for monitoring fish stress in the field (Iwama et al., 1995; Wells and Pankhurst, 1999). These lactate meters, primarily developed for monitoring training performance in human athletes, are simple and quick to use, typically requiring only one minute for measuring lactate concentration from a drop of fresh blood. Some of them have already been described for use in rainbow trout *Oncorhynchus mykiss* (Walbaum) (Wells and Pankhurst, 1999), catfish *Ictalurus punctatus* (Rafinesque) (Beecham et al., 2006) and Atlantic cod, *Gadus morhua* (L.) (Brown et al., 2008). However, their simplicity of use, the low initial investment required and the small amount of sample needed for analysis (5 to 25 µl) turn them into an attractive alternative for measuring lactate even off site, once the blood has been stored. Because stored blood samples often consist of frozen plasma, validating the use of these portable meters on frozen and thawed plasma samples is required. The aim of the present study was to investigate the validity of measuring lactate levels in thawed plasma from Atlantic cod using Lactate Pro™ (ARKRAY, Kyoto), a portable lactate meter recently validated for measuring whole-blood lactate in this species (Brown et al., 2008). The measurement is based on an amperometric method using an enzymatic reaction. It is fast (60 s) and easy to use; it requires only 5 µl of sample, and exhibits good correlation with laboratory analysis including for Atlantic cod (Brown et al. 2008; Pyne et al., 2000).

MATERIALS AND METHODS

In mid March 2008, sixty immature Atlantic cod (2 years old, mean weight 1054 ± 208 g) were distributed among six separate 300 L tanks and assigned to one of three treatments fourteen days later: control, short-term stress and long-term stress. Water temperature was 4°C and all fish were fasted for 96 h prior to treatment. Fish in both stress groups were first exposed to an abrupt drop of the water level (1/3 of normal), and then chased around the tank with a

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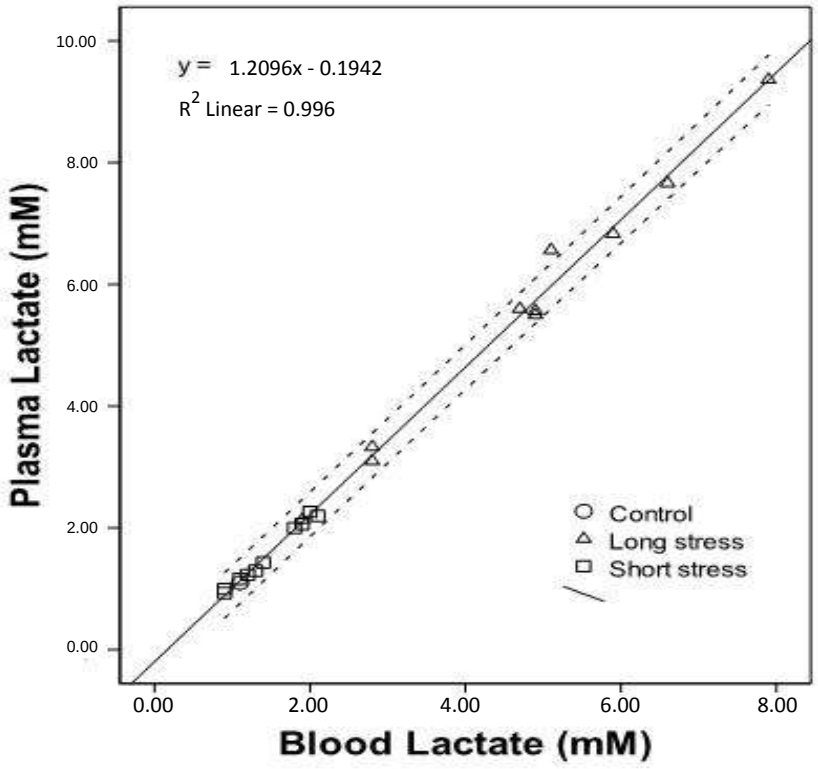

Figure 1. Regression analysis of whole blood and plasma lactate values obtained with Lactate Pro™ (n = 21). Fitted regression relationship (solid line) with 95% confidence intervals (dotted lines) is shown.

landing net for 10 s every minute during a 1 min (short-term) or a 60 min (long-term) stress period. Initial water level was then re-established and, 30 min post-stress, 10 fish per group were dip-netted into an anaesthetic bath (metomidate, 0.2 g L^{-1} in seawater). Control fish (n = 10) were moved directly into anaesthetic bath. Blood (1.5 ml) was collected from the caudal vasculature and a subsample immediately analyzed using Lactate Pro™, after which the remaining blood was centrifuged for 10 min at 2700 g (4°C). Plasma was transferred to eppendorf tubes, frozen and stored at -40°C for 5 months before being thawed and analyzed with Lactate Pro™.

RESULTS AND DISCUSSION

In the control group, only one fish showed a blood lactate concentration above the lower detection limit of the meter (0.8 mM). After subjecting the fish to stress, blood lactate increased noticeably, being detected by Lactate Pro™ in all fish in both stress groups. These results match those obtained by Brown et al. (2008) for routinely active and stressed Atlantic cod using the same lactate meter. When measurements were performed in plasma, similar results were obtained, with lactate being detected only in one sample in the control group but in every sample from stressed fish. Whole blood and plasma values obtained with Lactate Pro™ were analyzed using linear regression and Pearson correlation (Figure 1). Results show a strong positive relationship ($r = 0.998$, $p<0.01$, $n = 21$) between lactate concentrations measured in whole blood and those obtained from thawed plasma, but with plasma values being significantly higher (Paired-samples t-test; $p<0.001$, $n = 21$). This difference between measurements increased with increasing lactate concentrations, with plasma values on average being 8% higher for blood lactate concentrations under 4 mM and 17.5% higher for blood lactate concentrations between 4 and 10 mM. A two-way ANOVA revealed a significant difference ($p<0.001$) between short- and long-term stress groups, regardless of the sample analyzed (Figure 2). There were no interactions between sample type and stress duration. Reproducibility of plasma lactate measurements was assessed by calculating the coefficient of variation (CV = standard deviation as a percentage of the mean) following five repeated measurements of six different plasma samples ranging from 1.1 to 9.5 mM plasma lactate. Calculated CVs were below 5% in every case, matching those referred in the user manual of the meter and those obtained for whole blood lactate measurements with the same meter by Brown et al. (2008).

Results from this study indicate that measurement of lactate in frozen and thawed plasma samples using Lactate Pro™ can effectively detect differences between unstressed and stressed Atlantic cod, and between groups subjected to different stress levels. Even though plasma

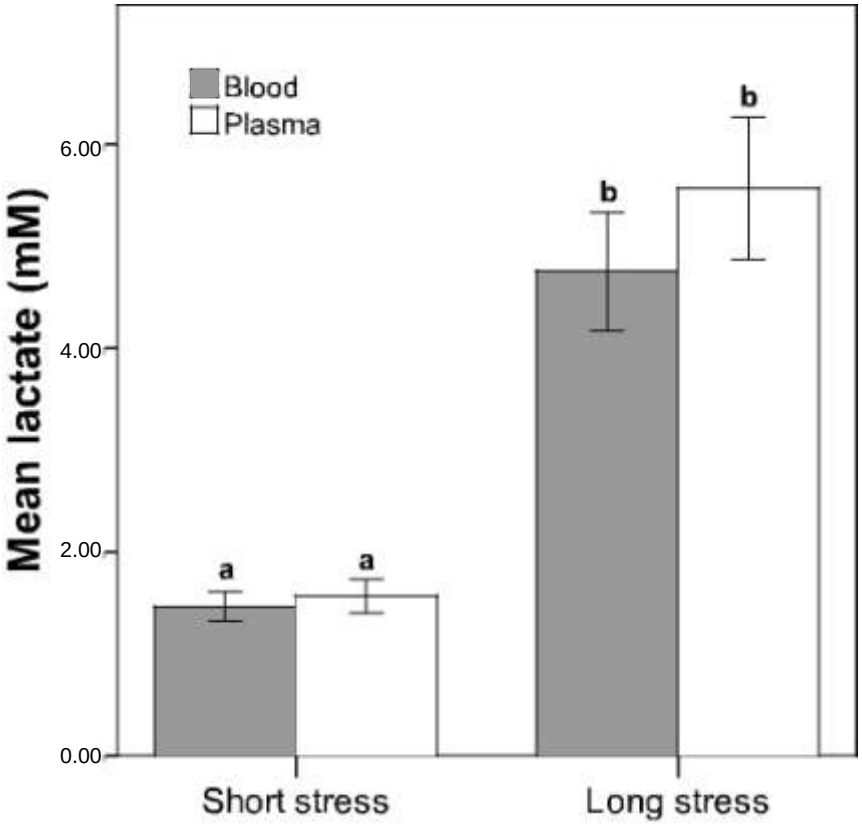

Figure 2. Mean (± S.E.) lactate concentrations obtained with Lactate Pro™ in blood and plasma samples from the short-term (N = 20) and long-term (N = 20) stress groups. Different letters represent significant differences ($p<0.001$) between groups.

lactate levels in unstressed cod tend to fall under the minimum detection limit of the meter, the '*Lo*' (low) reading output can be indicative of unstressed fish (Brown et al., 2008). Plasma lactate values below 0.8 mM in unstressed Atlantic cod have previously been reported using analytical methods (Butler et al., 1989; Larsen et al., 1997). Above the minimum detection limit, the meter can measure lactate concentrations both in whole blood and in plasma within the range evaluated here (up to 10 mM), but a correction could be made if concentrations obtained from whole blood and those obtained from thawed plasma are to be compared. Even though the lactate range evaluated here is limited, circulating lactate concentrations reported for Atlantic cod rarely exceed 10 mM, not even after exhaustive exercise (Nelson et al., 1996), exposure to hypercapnia (Larsen et al., 1997) or crowding stress (Brown et al., 2008).

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