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BRIEF COMMUNICATION

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Dichloroacetate reveals the presence of metabolic inertia at the start of exercise in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792)

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Abstract

A lag in the increase in oxygen consumption (MO_2) occurs at the start of sustainable exercise in trout. Waterborne dichloroacetate (0.58 and 3.49 mmol l^{-1}), a compound which activates pyruvate dehydrogenase (PDH) by inhibiting PDH kinase in muscle, accelerates the increase in MO_2 during the first 10 min of sustainable exercise when velocity is elevated to 75% critical swimming speed in a swim tunnel. There are no effects on MO_2 thereafter or at rest. This indicates that a delay in PDH activation ("metabolic inertia") contributes to the lag phenomenon.

KEYWORDS

lactate, oxygen consumption, pyruvate dehydrogenase, swimming

1 | INTRODUCTION

Traditionally, "anaerobic exercise" at the start of exercise occurs when pyruvate is reduced to lactate by lactate dehydrogenase (*i.e.*, increased glycolytic flux) rather than oxidized to acetyl CoA (coenzyme A) by pyruvate dehydrogenase (PDH), and this is attributed to a delay in ramping up O_2 supply to serve as the terminal electron acceptor for oxidative metabolism (Katz & Sahlin, 1988; Margaria *et al.*, 1933; Wasserman *et al.*, 1973). Nonetheless, an alternate theory ("metabolic inertia") postulates that O_2 supply is not the limiting factor; rather, there is a delay in the activation of PDH by dephosphorylation (reviewed by Greenhaff *et al.*, 2004). In mammalian systems, evidence for this theory has been obtained by the use of dichloroacetate (DCA), a drug which is known to activate PDH indirectly by inhibiting the enzyme (PDH kinase) which phosphorylates PDH (McAllister *et al.*, 1973; Stacpoole, 1989; Whitehouse *et al.*, 1974). In a number of reports on mammalian systems, DCA treatment reduced lactate accumulation and/or increased O_2 consumption (MO_2) at the onset of exercise (*e.g.*, Calvert *et al.*, 2008; Howlett *et al.*, 1999; Ludvik *et al.*, 1993; Parolin *et al.*, 2000; Timmons *et al.*, 1998), though some exceptions exist (*e.g.*, Grassi *et al.*, 2002; Jones *et al.*, 2004).

In fish, this question has never been investigated. Lactate accumulation at the end of exhaustive exercise has been well studied (reviewed by Kieffer, 2000), but there is much less work on lactate build-up at the start of sustainable exercise. Nonetheless, in salmonids, there is evidence that at the start of sustainable "aerobic" exercise there is an initial build-up of lactate in both red and white muscles over the first 10–20 min. This occurred at 75% Ucrit (Wokoma & Johnston, 1981) and 90% Ucrit but not at 60% Ucrit (Richards *et al.*, 2002). Wokoma and Johnston (1981) also reported that lactate accumulation progressively decreased as swimming continued for 24 h. Nonetheless, there were no time-matched measurements of MO_2 in either of these studies.

Over the past two decades, DCA has received attention as an aquatic toxicant and potential human carcinogen that is present in groundwater due to legacy contamination of industrial sites and in drinking water as a product of chlorination (Stacpoole, 2011). Information on fish is limited, with evidence of hepatotoxicity (McHugh-Law *et al.*, 1998), as well as oxidative stress and developmental abnormalities (Hassoun *et al.*, 2005) resulting from waterborne exposures. Interestingly, tests on trout have shown that DCA exposure reduces resting blood lactate concentration (Fitzsimmons *et al.*, 2009),

suggesting that it activates PDH as in mammals. Nonetheless, no studies have been reported of its effects on exercise metabolism in fish. The present investigation employed DCA exposure to test whether metabolic inertia occurs at the start of sustainable exercise in juvenile rainbow trout. The possible effects on whole animal O_2 consumption at rest, at the onset of exercise and during continuing steady-state swimming at the same speed were examined.

2 | MATERIALS AND METHODS

The care and use of experimental animals complied with the guidelines of the Canada Council of Animal Care as approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Permit 02-10-61). Juvenile rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792, N = 90) were obtained from Humber Springs Trout Hatchery (Orangeville, ON, Canada) and acclimated to flowing dechlorinated Hamilton tap water in 200 l tanks (50 per tank) for 4 months at 15°C. Water composition (in mmol l^{-1}) was Ca = 1.0, Mg = 0.2, Na = 0.6, Cl = 0.8, SO_4 = 0.25, with titratable alkalinity to pH 4.0 = 1.9 mmol l^{-1}, dissolved organic carbon = 3 mg l^{-1}, total hardness (as $CaCO_3$) = 140 mg l^{-1} and pH = 7.9–8.0. During this period, they were fed a 1% ration of commercial pellets every second day, and food was withheld for 2 days before the experiment. At the time the experiments were performed, the fish weighed *c.* 16 g with a fork length of *c.* 10.5 cm (see Figure 1 for details).

Trout were placed in individual Blazka-type swimming respirometers (3.114 l) similar to those described by Beamish *et al.* (1989) and allowed to settle overnight (*c.* 12 h) at a speed of 15 cm s^{-1} (*c.* 1.4 lengths s^{-1}). At this speed, the fish would maintain orientation and generally not swim but would immediately start swimming smoothly when the velocity was increased. Water was re-circulated through each respirometer at 150 ml min^{-1} from a central 200 l temperature-controlled (15°C) aerated reservoir serving all six to seven respirometers in a treatment. The reservoir water was dosed with DCA (sodium dichloroacetate, $C_2HCl_2NaO_2$, >97% purity) from TCI America (Portland, OR, USA) at a dichloroacetate concentration of either 450 mg l^{-1} (3.49 mmol l^{-1}; Series 1) or 75 mg l^{-1} (0.58 mmol l^{-1}; Series 2). The reservoir for the parallel control treatments received no DCA. Oxygen consumption (MO_2) was measured over individual

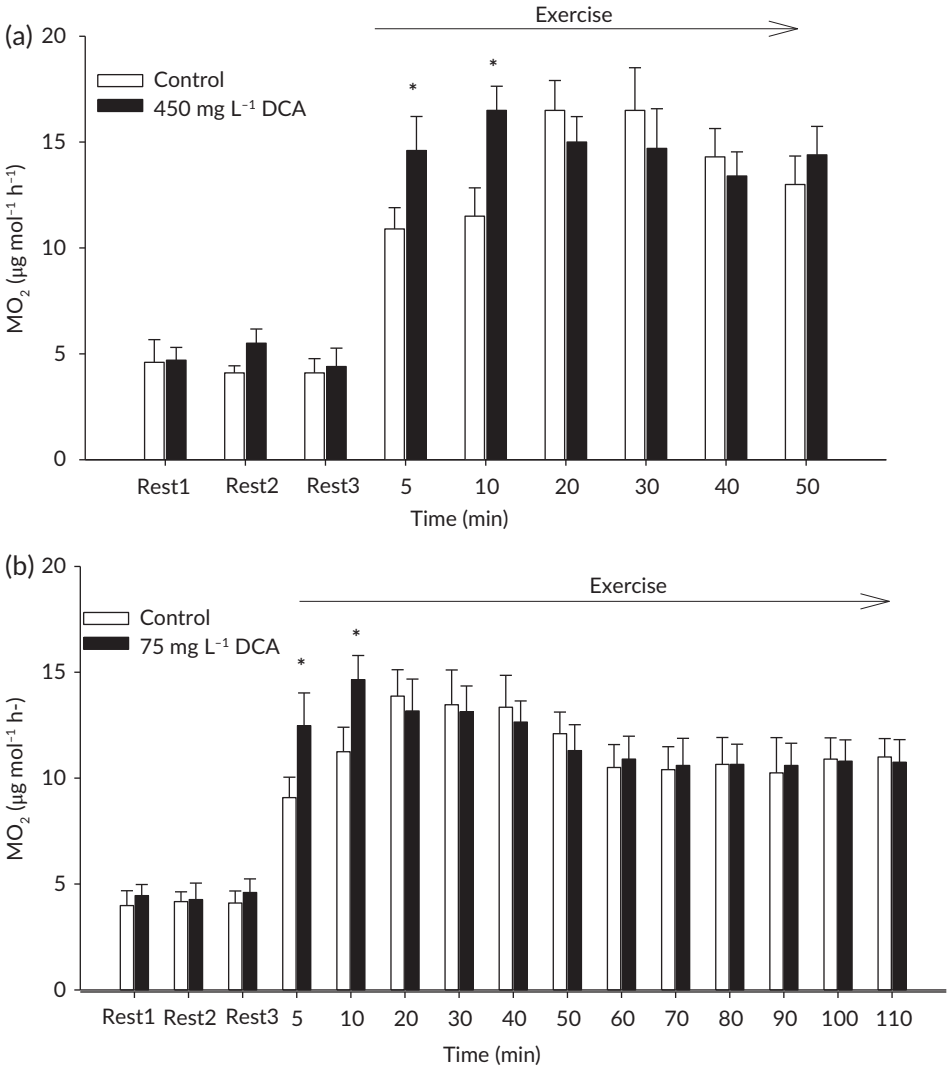

FIGURE 1 (a) The influence of exposure to 450 mg l^{-1} dichloroacetate (DCA) or control conditions (no DCA) on O_2 consumption (MO_2) during three periods at rest (15 cm s^{-1}, Rest1, Rest2 and Rest3) and at 5, 10, 20, 30, 40 and 50 min of swimming at 40 cm s^{-1} in juvenile rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). (▭) Control, (▬) 450 mg l^{-1} DCA. Mean ± S.E. (N = 7 in both treatments throughout; controls: 14.0 ± 0.9 g, 10.4 ± 0.7 cm; 450 mg l^{-1} DCA 15.1 ± 1.8 g, 10.6 ± 0.5 cm). Asterisk indicates significant difference by *t*-test (one-tailed: P = 0.037 at 5 min, P = 0.007 at 10 min; two-tailed: P = 0.073 at 5 min, P = 0.014 at 10 min). (b) The influence of exposure to 75 mg l^{-1} DCA or control conditions (no DCA) on MO_2 during three periods at rest (15 cm s^{-1}, Rest1, Rest2 and Rest3, N = 37) and at 5 min (N = 31), 10 min (N = 25), 20 min (N = 19), 30 min (N = 19), 40, 50, 60, 70, 80, 90, 100 and 110 min (all N = 13 in both groups) of swimming at 40 cm s^{-1}. (▭) Control, (▬) 75 mg l^{-1} DCA. Asterisk indicates significant difference by *t*-test (one-tailed: P = 0.035 at 5 min, P = 0.027 at 10 min; two-tailed: P = 0.070 at 5 min, P = 0.055 at 10 min)

7–12 min periods by sealing the respirometer and measuring the decline in PO_2 over the period, after which re-circulatory flow was re-established until the next measurement. In all tests, three resting measurements (12 min periods) were made at rest over a 45 min interval, and then the speed was immediately increased to 40 cm sec^{-1} (*c.* 3.8 lengths s^{-1}), which corresponds to about 75% of Ucrit in trout of comparable size from the same source, of the same water quality, temperature and respirometers (Gregory & Wood, 1998). MO_2 was measured across 7 min periods spanning 5, 10, 20, 30, 40 and 50 min in Series 1 (N = 7 in control, N = 7 in 450 mg l^{-1} DCA) and at the same times but continuing at 10 min intervals up to 110 min of exercise in Series 2 (starting with N = 37 in control, N = 37 in 75 mg l^{-1} DCA). Thus, repeated MO_2 measurements were made in each fish. The internal pump which provided the flow in the Blazka-type respirometers provided excellent mixing. At the end of the experiment, all fish were euthanized by an overdose of neutralized MS-222 (Syndel Laboratories, Parksville, BC, Canada) and measured for terminal weight and fork length. As the original plan was to assay muscle metabolites at selected times in Series 2, groups of fish were sequentially killed at these times such that N declined in both groups (rest = 37, 5 min = 31, 10 min = 25, 20–30 min = 19, 40–110 min = 13). Unfortunately, all these muscle samples were lost due to a procedural error.

MO_2 measurements were made by drawing water samples from the respirometer at the start and end of each period into a 1 ml gas-tight syringe (Hamilton, Reno, NV, USA) fitted with a three-way Teflon Luerlock to vent the dead space. The sample was injected into the thermostatted chamber of an E5046 O_2 electrode attached to a pHM72 blood-gas meter (Radiometer, Copenhagen, Denmark). MO_2 was calculated by factoring the decline in PO_2 by time, respirometer volume, terminal weight and O_2 solubility coefficient at 15°C in fresh water (Boutilier *et al*., 1984). PO_2 remained above 85% saturation in all trials. The respirometers were regularly treated with bleach, and blank tests showed that there was no detectable background MO_2. Data are expressed as mean ± S.E. (N). In each series, experimental and control MO_2 values were compared using independent *t*-tests each time. As the authors were testing the hypothesis that MO_2 would increase more rapidly in the DCA-treated fish, they accepted significance at $P < 0.05$ for one-tailed comparisons, but they have also reported *P*-values for two-tailed comparisons in the Figure 1.

3 | RESULTS

Series 1 compared the resting and exercise responses of trout treated with a relatively high concentration of DCA (450 mg l^{-1}) with those of a parallel control group (Figure 1a). Resting MO_2 was *c.* 4.5 μmol g^{-1} h^{-1} and similar in the two groups. Nonetheless, when the swimming speed was acutely elevated to 40 cm s^{-1}, MO_2 increased more quickly in the DCA group with significantly higher values at both 5 min (14.6 ± 1.6 *vs*. 10.9 ± 1.0 μmol g^{-1} h^{-1}, N = 7, P = 0.037) and 10 min (16.5 ± 1.1 *vs*. 11.5 ± 1.3 μmol g^{-1} h^{-1}, N = 7, P = 0.007). By 20 min, MO_2 in the control group had caught up to that in the DCA group, and there were no significant differences up to 50 min thereafter, though MO_2 declined slightly in both treatments with continued swimming. Series 2 examined a lower concentration of DCA (75 mg l^{-1}) over a longer swimming period (110 min) with a larger cohort of trout so as to increase the resolution and facilitate the intended metabolite measurements (Figure 1b). The overall results were similar to those of the 450 mg l^{-1} treatment. Resting MO_2 was unaffected by 75 mg l^{-1} DCA, with similar values (around 4 μmol g^{-1} h^{-1}) to those in the control group. Again MO_2 increased more rapidly at the start of the swimming period in the DCA treatment group at both 5 min (12.5 ± 1.5 *vs*. 9.9 ± 1.0 μmol g^{-1} h^{-1}, N = 31, P = 0.035) and 10 min (14.6 ± 1.2 *vs*. 11.2 ± 1.1 μmol g^{-1} h^{-1}, N = 25, P = 0.027). By 20 min, the control group had increased MO_2 to the same level as the DCA group, and thereafter there were no significant differences through 110 min of continuous swimming, though the rates declined slightly in both groups.

4 | DISCUSSION

Fitzsimmons *et al*. (2009) showed that as in mammals, injected DCA is subject to rapid metabolic clearance in trout (half-life < 4 h), whereas waterborne exposure results in greater persistence of DCA in the blood plasma. In that study, a waterborne concentration of 173 mg l^{-1} significantly decreased resting blood lactate levels, so the two exposures (75, 450 mg/l) of this study bracketed this concentration. Both were effective in significantly elevating MO_2 by 26%–43% during the first 10 min of sustainable exercise, with no effects thereafter. This result suggests that metabolic inertia contributes to the initial "anaerobic component" (substrate-level phosphorylation and lactate accumulation) documented by others at the start of the submaximal exercise (Richards *et al*., 2002; Wokoma & Johnston, 1981). The original intention of this study to assess muscle metabolites and the activation state of PDH in support of this conclusion should be pursued in future studies. Predictions would include increased levels of creatine phosphate and/or ATP, higher intracellular pH, lower lactate and inorganic phosphate levels, higher acetyl-CoA and acetylcarnitine concentrations and, of course, greater PDH activation in muscle during the early minutes of exercise (Greenhaff *et al*., 2004) in the DCA-treated fish. The lack of effect of DCA observed at later times (20 min onwards) is to be expected, as salmonids switch to burning mainly lipid rather than glycogen during prolonged swimming (Lauff & Wood, 1996; Richards *et al*., 2002). They also tend to swim more efficiently (Brett, 1995; Lauff & Wood, 1996), explaining the tendency for MO_2 to decrease during prolonged exercise. Nonetheless, it seems likely that both in nature and in aquaculture, most exercise is short lived, spontaneous and submaximal during the normal life of the fish such that long-term, steady-state swimming respirometry greatly underestimates true costs, because a large portion of those costs is incurred during short-term activities such as acceleration

(Boisclair & Tang, 1993; Krohn & Boisclair, 1994; Webb, 1991). The apparent limitation on carbon flux through PDH by metabolic inertia at the start of such exercise, which is relieved by DCA, and the higher costs associated with lactate clearance by oxidation or glycogen re-synthesis may then represent a significant portion of the fish's normal energy budget. Although this discrepancy between energy budgets obtained in lab swimming respirometry *vs*. the field has long been recognized, the present study is the first to show that metabolic inertia may represent an important part of its biochemical underpinning.

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AUTHOR CONTRIBUTIONS

C.M.W., E.F.P. and G.J.F.H. developed the concept; E.F.P. was involved in data collection; C.M.W., E.F.P. and G.J.F.H. assisted with analysis; C.M.W. prepared the first draft; C.M.W., E.F.P. and G.J.F.H. contributed to the revision of the manuscript.

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