Effects of hypoxia and low temperature on substrate fluxes in fish: plasma metabolite concentrations are misleading

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Haman, François, Georges Zwinglestein, and Jean-Michel Weber. Effects of hypoxia and low temperature on substrate fluxes in fish: plasma metabolite concentrations are misleading. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R2046–R2054, 1997.—Oxygen levels and temperature can fluctuate rapidly in aquatic environments. Even though the effects of environmental stresses on fish metabolism have been studied extensively, information on fuel kinetics is extremely limited because it relies almost exclusively on changes in substrate concentrations. The turnover rate of nonesterified fatty acids (NEFA) has never been measured in fish. Therefore, our goal was to quantify glucose and NEFA fluxes in rainbow trout acutely exposed to severe hypoxia (25% O2 saturation) or low temperature (6°C for fish acclimated to 15°C) by performing continuous infusions of 6-[3H]glucose and 1-[14C]palmitate in vivo. Results show that hypoxia causes a 53% decrease in NEFA turnover rate, together with a transient increase in hepatic glucose production, whereas a rapid drop in temperature induces equivalent declines in glucose, NEFA, and oxygen fluxes [temperature coefficient \( T_c \approx 2 \)]. More importantly, kinetic changes in glucose and NEFA fluxes are not accompanied by interpretable changes in the plasma concentrations of these metabolites. Thus using concentration changes to draw conclusions about fluxes must be avoided.

Hypoxia and temperature stresses can frequently occur in aquatic environments (6, 14, 30), and their effects on fish metabolism have been the subject of many investigations. Reduced oxygen availability is known to have profound metabolic consequences, particularly for hypoxia-sensitive teleosts such as rainbow trout (2, 7, 8, 31, 36). Even though this species is able to increase oxygen extraction when exposed to prolonged hypoxia (9, 22), aerobic pathways cannot maintain normal ATP turnover on their own, and anaerobic metabolism is stimulated (2, 18, 30). Acute exposure to low environmental temperature can compromise the normal function and the structural integrity of fish cells (16) via changes in membrane fluidity (13), catalytic rates of enzymes (10, 17), and diffusive processes (26, 37). Unfortunately, our present understanding of the integrative response to these stresses at the whole animal level is still very primitive because it is based almost entirely on measurements of metabolite concentrations. Changes in concentration do not provide reliable inference about metabolite fluxes because they merely reflect transient differences between rates of appearance (\( R_a \)) and disappearance (\( R_d \)) (33, 39). Therefore, the same change in concentration can be elicited by altering \( R_a \), \( R_d \), or both simultaneously, and large changes in flux can also occur while concentration stays perfectly steady. Furthermore, the effects of hypoxia on plasma metabolite concentrations of fish are unclear, and rapid temperature changes have not been investigated. Some authors report that hypoxia causes an increase in plasma glucose (15, 31) and nonesterified fatty acids (NEFA) levels (23), whereas others show no change in glucose (8) or a decrease in NEFA (31). It is clear that the metabolic response of teleosts to environmental stresses can only be captured if underlying changes in the turnover rates of energy substrates are quantified directly. Glucose flux has only been measured by bolus injection before and after exposure of trout to hypoxia (8), and NEFA fluxes have never been measured in fish (33). Therefore, the goal of this study is to quantify the turnover rates of glucose and NEFA in rainbow trout acutely exposed to hypoxia or low temperature, using modern methods of continuous isotope infusion (12) to obtain a time course of changes in metabolic fuel kinetics.

METHODS

Animals

Rainbow trout (Oncorhynchus mykiss, Walbaum) of both sexes were purchased from Linwood Acres Trout Farm (Campbellcroft, Ontario, Canada) and held in 550-liter flow-through tanks. They were kept in dechloraminated well-oxygenated water under a 12:12-h light-dark photoperiod and were divided in two groups. The first group was kept at 10°C and used for hypoxia experiments (937 ± 53 g, n = 8), whereas the second group was kept at 15°C and used for cold exposure experiments (714 ± 44 g, n = 8). The animals were acclimated to these conditions for at least 1 mo before experiments were run, and they were fed Purina trout chow three times a week until satiation.

Catheterizations

Two PE-50 catheters (Intramedic, Clay-Adams) were implanted in the dorsal aorta under ethyl-N-aminobenzoate sulfonic acid anesthesia (MS-222) as described previously (12). After surgery, the animals were allowed to recover overnight in opaque Plexiglas chambers (60 × 16 × 18 cm) supplied with the same quality water as in their respective acclimation tanks at a flow rate of 5–6 l/min. Continuous infusions of radiolabeled metabolites were carried out in the same chambers, and only the animals showing hematocrits higher than 20% after recovery from surgery were used in experiments.

Metabolic Rate Measurements

During hypoxia and cold exposure experiments, oxygen consumption (\( M_{O2} \)) was measured by stopping external water supply for periods of 10 min and by recycling the same water within a 15-liter closed system. Particular care was taken to eliminate air bubbles and to avoid exchange between recircu-
lipid-binding proteins. 1-[14C]palmitate supplied commer-
cially (Amersham 1.1 TBq/mmol). Trout plasma was collected from donor
1-[14C]palmitate (Amersham 1.85–2.2 GBq/mmol) and 6-
[3H]glucose (New England Nuclear 1.6 TBq/mmol or Amer-
sham 1.1 TBq/mmol). Trout plasma was collected from donor
individuals of the same batch of fish and used as a source of
lipid-binding proteins. 1-[14C]palmitate supplied commer-
cially in ethanol was mixed with 600 µl plasma and well
agitated before addition of 6-[3H]glucose dissolved in Cort-
land saline (38). After overnight recovery from surgery and
while the animal was at rest, a priming dose of 6-[3H]glucose
equivalent to 90 min of infusion was injected before the
infusion of the 1-[14C]palmitate/6-[3H]glucose mixture with a
calibrated syringe pump (Harvard Apparatus, South Natick,
MA) at 1 ml/h was started. Infusion rates ranged between
~155,000 and 410,000 disintegrations per minute
(dpm)·kg⁻¹·min⁻¹ for each isotope. In all experiments, base-
line glucose and fatty acid kinetics were determined by
drawing 400-µl blood samples 40, 50, and 60 min after the
infusion was started. Five additional samples (400 µl each)
were then taken during hypoxia or cold exposure. All samples
were centrifuged immediately, and the plasma was separated
and stored at −20°C until analysis.

Hypoxia Experiments

After infusing the radiotracers for 1 h under normoxic
conditions, the oxygen content of the chamber was gradually
decreased from 100 to 25% saturation in 20 min by bubbling
nitrogen through a column containing glass beads. Blood
samples were collected when water PO₂ reached 60 Torr (38% 
saturation) and 40 Torr (25% saturation) as well as 30, 60, 
and 90 min after reaching 25% saturation. Mo₂ was measured
under normoxic conditions and 1 h after reaching 25% 
saturation.

Cold Exposure Experiments

After infusing the isotopes for 1 h at 15°C, water tempera-
ture was gradually lowered to 6°C over 20 min. Blood samples
were collected when the temperature reached 10 and 6°C, as
well as 30, 60, and 90 min after reaching 6°C. Mo₂ was
measured three times under baseline conditions (15°C) and
times three times during the 6°C exposure period.

Plasma Sample Analyses

Glucose and lactate concentrations were measured at 340
nm on a Beckman DU 640 spectrophotometer (1). Total NEFA
concentration was determined with an analytic test-kit (NEFA
C, Wako Chemicals, Osaka, J apan). Measurement of stan-
dards for all the NEFA present in trout plasma revealed that
this test kit underestimates the concentration of 22:6 by 25%,
and total NEFA concentration was corrected accordingly. The
fractional distribution of individual fatty acids to total NEFA
concentration was measured on a Hewlett-Packard 5890
series II gas chromatograph with HP 7673 autosampler and
flame ionization detector after extraction and methylation as
described previously (19, 29). Following plasma extraction
with heptane-isopropanol (3:2 vol/vol) and centrifugation, 1 ml
supernatant was counted to measure total 14C activity and
partially [3H]glucose activity. Tritium activity remaining in the
pellet was measured by dissolving it in 1 ml NaOH (1 N) and
10 ml water. Each vial was then acidified with glacial acetic
acid (17.5 M) before counting to avoid chemiluminescence.

Calculations and Statistics

Turnover rates were calculated with the equations of Steele
(27), assuming a volume of distribution of 50 ml/kg for glucose
when the non-steady state equation was used. Throughout
this article, we have used the term “flux rate” as a synonym
for turnover rate, Ra, or R.d. The turnover rate of total NEFA
was calculated by dividing palmitate turnover rate by the
fractional contribution of palmitate to total NEFA concentra-
tion. Changes in metabolite concentrations, specific activi-
ties, and flux rates over time were assessed by two-way
analysis of variance (ANOVA), and differences between Ra
and Rd values at individual sampling times were evaluated by
the Bonferroni t-test. In the cold exposure experiments,
temperature coefficient (Q10) values were calculated with the
equation of Van’t Hoff using average 15°C measurements
(baseline) and the last values measured at low temperature.
All values presented are means ± SE (n = 8) unless indicated
otherwise.

RESULTS

Hypoxia Experiments

Water PO₂, metabolic rate, and plasma lactate. Figure
1 shows changes in water PO₂ (line) and metabolic rate
(histogram) before and after 90 min of exposure to
hypoxia at 25% oxygen saturation. Water PO₂ de-
creased from 153 Torr (96% saturation) to 39 Torr (25%
saturation) in 20 min, but this change did not cause a
significant decline in metabolic rate (P = 0.29). Mo₂
averaged 41.8 ± 6.1 µmol · kg⁻¹ · min⁻¹ throughout the
experiments.

Observed activity levels were minimal during the
measurements, except for a few seconds when water
PO₂ reached ∼80 Torr. After this brief episode, each
animal stopped moving and started hyperventilating
until the end of the experiment. Mean plasma lactate
concentration was 1.1 ± 0.11 mM under normoxic
conditions and increased progressively during hypoxia
to reach a maximum of 5.8 ± 0.53 mM (Fig. 2A, \( P < 0.001 \)).

**Hypoxia and glucose metabolism.** Glucose concentration, specific activity, and glucose flux throughout the hypoxia experiments are plotted in Fig. 2. Normoxic values for plasma glucose concentration and glucose turnover rate were 5.5 ± 0.7 mM and 5.4 ± 0.5 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), respectively. Concentration increased after 30 and 60 min of hypoxia (\( P < 0.001 \)), but returned to a lower value that was not significantly different from normoxic control after 90 min. \( R_a \) and \( R_d \) values of glucose were only different from each other at 15 min after reaching 25% oxygen saturation (\( P < 0.05 \)). At that time, \( R_a \) glucose was transiently higher than baseline values. At all other times, \( R_a \) and \( R_d \) values were the same and not significantly different from control values measured under normoxic conditions.

**Hypoxia and fatty acid metabolism.** Concentrations of individual NEFA and their percent contribution to total plasma fatty acids before and after exposure to hypoxia are presented in Table 1. Fatty acid concentrations and their fractional contribution were not affected by the change in water PO2 (\( P > 0.05 \)). Percent palmitate (16:0) and percent docosahexaenoate (22:6) had the lowest coefficients of variation of all individual fatty acids (11.9 and 10.1, respectively). Figure 3 plots the effect of hypoxia on plasma palmitate concentration, specific activity, and on the fractional contribution of this acid to total NEFA. Palmitate concentration remained at its normoxic level of 0.21 ± 0.02 mM throughout hypoxia (n = 7, \( P > 0.05 \)). After separation of the different plasma lipids by TLC, 64.6 ± 0.03% of total \(^{14}\text{C} \) activity was found in the NEFA fraction, and this percentage did not change over time (\( P > 0.05 \)). Palmitate specific activity was increased during hypoxia (\( P < 0.05 \)), but percent palmitate was not affected by the change in oxygen availability (\( P > 0.05 \)). In normoxic fish, total NEFA concentration and NEFA turnover rate averaged 0.98 ± 0.07 mM and 5.9 ± 1.4 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) (n = 7), respectively (Fig. 4). Total NEFA concentration was not affected by changes in water PO2 (\( P > 0.05 \)), but the turnover rate of NEFA decreased significantly after 60 min of hypoxia and reached 53% of normoxic levels at the end of the experiment (\( P < 0.001 \)).

**Cold Exposure Experiments**

Water temperature and \( \text{MO}_2 \). Changes in environmental temperature and in metabolic rate during cold exposure are presented in Fig. 5. Mean temperature was decreased from 15.0 to 5.8°C in 20 min, and the water remained saturated with oxygen throughout the experiments. The 9°C change in temperature caused a progressive decrease in \( \text{MO}_2 \) from 41.2 ± 2.3 to 21.7 ± 1.4 \( \mu \text{mol} \cdot \text{O}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) by the end of the experiment (\( P < 0.001 \)). Observed activity levels remained minimal

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Fig. 1. Partial pressure of oxygen (PO2) in water during hypoxia experiments given as mean values (thick line) ± SE (thin lines) (n = 8). Also shown as histogram are mean rates of oxygen consumption (\( \text{MO}_2 \) ± SE in rainbow trout under normoxic and hypoxic conditions (n = 8).

Fig. 2. Plasma glucose (○) and lactate (■) concentrations (A), glucose specific activity (B), and glucose flux (C) in rainbow trout under normoxic (96% \( \text{O}_2 \) saturation) and hypoxic conditions (25% \( \text{O}_2 \) saturation). Shaded area indicates transition from normoxia to hypoxia. Values are means ± SE (n = 8). dpm, disintegrations per minute. *Significant differences from normoxic values (\( P < 0.05 \)). ** Rates of appearance and disappearance were significantly different (\( P < 0.05 \)). C: ○ and ■ represent rates of glucose appearance and disappearance, respectively, at the only time when the animals were out of steady state. ** Rates of appearance and disappearance were significantly different (\( P < 0.05 \)). At all other times (○), rates of appearance and disappearance were not different from each other, and the values given are turnover rates.
during the measurements, except for ~1 min, when water temperature reached 10°C.

Cold exposure and glucose metabolism. Glucose concentration, glucose specific activity, and glucose flux throughout the cold exposure experiments are plotted in Fig. 6. Control values measured at 15°C for plasma glucose concentration and turnover rate were 7.5 ± 1.1 mM and 8.6 ± 1.3 µmol·kg⁻¹·min⁻¹, respectively. Glucose concentration was not significantly altered during the experiments, but specific activity was increased after 30 min at 6°C (P < 0.001). Rₐ and R₅ values were never different from each other; therefore, Table 1. Plasma NEFA concentration and percent contribution to total NEFA concentration in rainbow trout during normoxia and hypoxia

<table>
<thead>
<tr>
<th>NEFA</th>
<th>Normoxia</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration, nmol/ml</td>
<td>%</td>
</tr>
<tr>
<td>16:0</td>
<td>208 ± 19</td>
<td>21.2 ± 0.2</td>
</tr>
<tr>
<td>16:1</td>
<td>23 ± 3</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>18:0</td>
<td>120 ± 10</td>
<td>12.4 ± 0.3</td>
</tr>
<tr>
<td>18:1</td>
<td>149 ± 15</td>
<td>15.3 ± 0.2</td>
</tr>
<tr>
<td>18:2</td>
<td>25 ± 3</td>
<td>2.5 ± 0.0</td>
</tr>
<tr>
<td>18:3+20:1</td>
<td>61 ± 5</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>20:2</td>
<td>16 ± 2</td>
<td>1.6 ± 0.0</td>
</tr>
<tr>
<td>20:3</td>
<td>27 ± 4</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>20:4</td>
<td>58 ± 7</td>
<td>5.8 ± 0.0</td>
</tr>
<tr>
<td>20:5</td>
<td>24 ± 2</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>22:5</td>
<td>28 ± 4</td>
<td>2.7 ± 0.0</td>
</tr>
<tr>
<td>22:6</td>
<td>247 ± 25</td>
<td>24.9 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 experiments. NEFA, nonesterified fatty acids.

Fig. 3. Plasma palmitate concentration (A), specific activity (B), and fractional contribution to total nonesterified fatty acids (NEFA) concentration (C) in rainbow trout during the hypoxia experiments. Shaded area indicates transition from normoxia to hypoxia. Values are means ± SE (n = 7).

Fig. 4. Plasma NEFA concentration (A) and turnover rate (B) in rainbow trout during exposure to hypoxia. Values are means ± SE (n = 7). *Significant differences from normoxic levels (P < 0.05).

Fig. 5. Changes in water temperature during the cold exposure experiments (line). Values are means ± SE (n = 8). Histogram shows VO₂ in 15°C-acclimated rainbow trout rapidly brought to 6°C. *Significant differences from the 15°C control values (P < 0.05).
The turnover rates presented in Fig. 6C were all calculated with the steady-state equation of Steele (27). Cold exposure caused a large decrease in glucose turnover rate to a mean value of 4.6 ± 0.7 µmol·kg⁻¹·min⁻¹ (P < 0.001).

Cold exposure and fatty acid metabolism. Concentrations of individual NEFA and their percent contribution to total plasma fatty acids before and after cold exposure are presented in Table 2. Fatty acid concentrations were significantly increased after water temperature reached 6°C (P < 0.05), but their fractional contributions to total NEFA were not affected by this change (P > 0.05). Percent palmitate (16:0) and percent docosahexaenoate (22:6) had the lowest coefficients of variation of all individual fatty acids (8.7 and 11.6, respectively). Palmitate concentration, specific activity, and percent palmitate in total plasma NEFA throughout the cold exposure experiments are plotted in Fig. 7. Under control conditions at 15°C, palmitate concentration and percent palmitate averaged 0.18 ± 0.02 mM and 23.6 ± 0.1%. Acute exposure to 6°C caused a 40% increase in palmitate concentration (P < 0.001), but had no effect on percent palmitate (P = 0.25). After separation of the different plasma lipids by TLC, 79.4 ± 0.7% of total ¹⁴C activity was found in the NEFA fraction, and this percentage did not change over time (P > 0.05). Palmitate specific activity was increased 30 min after reaching 6°C and stayed elevated until the end of the experiment (P < 0.001). Total plasma NEFA

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Table 2. Plasma NEFA concentration and percent contribution to total NEFA concentration at 15°C and after 90 min at 6°C in rainbow trout acclimated to 15°C

<table>
<thead>
<tr>
<th>NEFA</th>
<th>Concentration, nmol/ml</th>
<th>%</th>
<th>Concentration, nmol/ml</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>182 ± 4</td>
<td>23.6 ± 0.1</td>
<td>248 ± 22*</td>
<td>23.8 ± 0.1</td>
</tr>
<tr>
<td>16:1</td>
<td>13 ± 0</td>
<td>1.7 ± 0.0</td>
<td>24 ± 2*</td>
<td>1.6 ± 0.0</td>
</tr>
<tr>
<td>18:0</td>
<td>113 ± 2</td>
<td>15.4 ± 0.2</td>
<td>153 ± 12*</td>
<td>15.2 ± 0.3</td>
</tr>
<tr>
<td>18:1</td>
<td>88 ± 2</td>
<td>11.3 ± 0.1</td>
<td>132 ± 15*</td>
<td>11.5 ± 0.1</td>
</tr>
<tr>
<td>18:2</td>
<td>20 ± 1</td>
<td>2.5 ± 0.0</td>
<td>33 ± 4*</td>
<td>2.6 ± 0.0</td>
</tr>
<tr>
<td>18:3 + 20:1</td>
<td>36 ± 1</td>
<td>4.6 ± 0.1</td>
<td>62 ± 8*</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>20:3</td>
<td>25 ± 1</td>
<td>3.1 ± 0.1</td>
<td>42 ± 8*</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>20:4</td>
<td>43 ± 1</td>
<td>5.6 ± 0.1</td>
<td>63 ± 5*</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>20:5</td>
<td>46 ± 1</td>
<td>6.2 ± 0.2</td>
<td>64 ± 7*</td>
<td>6.1 ± 0.1</td>
</tr>
<tr>
<td>22:5</td>
<td>16 ± 0</td>
<td>2.2 ± 0.2</td>
<td>24 ± 3*</td>
<td>2.1 ± 0.0</td>
</tr>
<tr>
<td>22:6</td>
<td>168 ± 4</td>
<td>21.9 ± 0.4</td>
<td>229 ± 22*</td>
<td>21.6 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 experiments. *Significant increments in concentration after exposure to cold water (P < 0.05).
concentration went from a control value of 0.76 ± 0.09 to 1.07 ± 0.09 mM after 2 h at 6°C (P < 0.0001, Fig. 8A). The turnover rate of plasma NEFA averaged 4.8 ± 0.7 µmol·kg⁻¹·min⁻¹ under 15°C control conditions, and it was significantly decreased after exposure to 6°C (P < 0.001, Fig. 8B).

Q₁₀ effects on M˙O₂ and fluxes of oxidative fuels. Changes in metabolic rate, glucose flux, and fatty acid flux during cold exposure are presented in Fig. 9 as a percent of the 15°C control values. The three parameters responded similarly to the 9°C drop in water temperature and their Q₁₀ values were not different from each other (P > 0.05). Metabolic rate, glucose turnover, and fatty acid turnover had Q₁₀ values of 2.2 ± 0.2, 1.9 ± 0.1, and 2.1 ± 0.1, respectively.

DISCUSSION

Effects of hypoxia

Under severe hypoxia, rainbow trout are able to maintain normoxic metabolic rates (Fig. 1) (24) by increasing oxygen extraction through neuronal and hormonal mechanisms (9, 22). Anaerobic glycolysis is stimulated, as indicated by a steady accumulation of lactate in plasma (Fig. 2A) (2, 7, 8), and hypoxia is thought to produce a general shift in oxidative fuel preference, from predominantly lipids and proteins to carbohydrates (30, 31). Here, we report the first fatty acid turnover rates in fish and show that the importance of this fuel decreases throughout hypoxia (Fig. 4B). At the same time, hepatic glucose production is briefly stimulated at the onset of the hypoxic stress, but this increase is not accompanied by a change in glucose utilization (Fig. 2C), and a temporary imbalance between the rates of production and utilization leads to hyperglycemia (Fig. 2A). This change in blood glucose concentration was also noticed in the only other study where glucose turnover rate was measured in hypoxic fish (8). However, the transient increase in glucose production could not be detected at the time, because bolus injection was used and this technique does not allow continuous measurements under non-steady-state conditions (12). Severe hypoxia is known to produce the release of catecholamines and other stress hormones that are probably responsible for the hyperglycemic response through stimulation of glycogenolysis and/or gluconeogenesis (31, 34, 41).

The lower NEFA flux and the steady NEFA concentration observed in hypoxic fish can be explained by a parallel decrease in the rates of NEFA oxidation and mobilization. The actual signal for lowering NEFA flux may have been high plasma glucose concentration. It was suggested long ago that hyperglycemia could inhibit NEFA mobilization independently from hormonal control (25). More recently, this inhibition of lipolysis has been demonstrated in humans (4, 40), and our results suggest that the same reciprocal interaction between plasma glucose concentration and fatty acid mobilization is also present in fish. However, the Rₐ of glycerol was not quantified in our experiments, and, consequently, the possibility that the decrease in NEFA flux was caused by a stimulation of reesterification cannot be totally ruled out. Future development of adequate techniques to quantify the turnover rate of glycerol in fish will be necessary to measure reesterification and to resolve this issue. Finally, the fact that glucose production returns to normoxic levels after 1 h of hypoxia suggests that the observed increase in anaerobic glycolysis is mainly fueled by glycogen reserves rather than by circulating glucose.
Effects of Temperature

Compensatory mechanisms associated with temperature acclimation have been investigated thoroughly (5, 10, 13, 17), but the effects of acute temperature changes have not received much attention. This study quantifies the impact of a rapid temperature change on the metabolic fuel kinetics of rainbow trout. A 9°C drop in water temperature causes a 50% decrease in M˙O2 (Fig. 5), and this decrease is accompanied by equivalent changes in the turnover rates of glucose (Fig. 6C) and NEFA (Fig. 8B). No preferential inhibition of glucose or fatty acid mobilization is taking place when water temperature decreases (Fig. 9) and, therefore, Q10 values have also been reported for trout M˙O2 after temperature acclimation (3), and this suggests that glucose and NEFA fluxes would remain at the low levels measured at the end of our experiments, even after complete acclimation to 6°C. The changes in substrate turnover rates reported here probably reflect how rates of substrate oxidation are affected. However, further studies will be necessary to quantify fuel oxidation directly, thereby providing a clearer understanding of the effects of temperature on energy metabolism.

Cold exposure elicited a large increase in plasma NEFA concentration (Fig. 8A), but the difference between the rates of fatty acid release and uptake responsible for it was too small to be detectable, and we can only speculate on the mechanisms involved in this change. We can say with certainty that the rapid decrease in water temperature did not have exactly the same effect on release and uptake. The resulting imbalance may have been caused by differential temperature effects on membrane fluidity or on the capacity of various lipid-binding proteins to transport fatty acids.

Relationship Between Substrate Concentration and Flux

This study shows that variation in plasma metabolite levels do not reflect changes in flux, and, therefore, that concentration cannot be used to speculate on potential dynamic changes in flux. The relative effects of hypoxia and low temperature on the concentrations and fluxes of glucose and NEFA are summarized in Fig. 10. Concentration and flux never changed in parallel during our experiments, and it would have been impossible to predict the effects of environmental changes on substrate fluxes simply by examining plasma metabolite concentrations. Hypoxia caused a large increase in glucose concentration, but had no effect on glucose flux; in contrast, it did not increase NEFA concentration significantly, but caused a sharp decrease in NEFA flux. Lowering water temperature caused a parallel decrease in glucose and NEFA fluxes, but glucose concentration was maintained steady, whereas NEFA concentration increased by more than 40%. The experimental manipulations performed in this study illustrate clearly that flux and concentration can change independently. For example, it is known from the mammalian literature that plasma NEFA concentration and flux are strongly correlated during exercise (11, 21), and, therefore, NEFA concentration is commonly used as an index of the rate of NEFA utilization in this context. Present results warn us that such a correlation is only valid under specific circumstances and should never be viewed as a universal phenomenon. In our experiments, and contrary to expectation, a strong increase in NEFA concentration was accompanied by a large decrease in NEFA flux when water temperature was lowered. In future studies, metabolite fluxes will have to be measured directly to investigate the impact of environmental, hormonal, and other changes on fish metabolism. Furthermore, published findings from experiments where plasma concentrations were used to draw conclusions about fluxes should be reevaluated with the above concerns in mind (e.g., see Refs. 20, 23, 32).

Plasma NEFA Composition and Use of Palmitate as a Tracer

The fractional contribution of individual fatty acids to total plasma NEFA was not affected by exposure to hypoxia (Table 1) or low temperature (Table 2). Palmitate (16:0) and docosahexaenoate (22:6) were the most abundant fatty acids in rainbow trout plasma as reported previously (31). Palmitate is commonly used to measure NEFA kinetics in mammals because its fractional contribution to total NEFA shows the lowest coefficient of variation in this group of vertebrates (19). In trout, we found that 16:0 and 22:6 had the lowest coefficient of variation in this group of vertebrates (19). The fractional contribution to total NEFA was not affected by exposure to hypoxia (Table 1) or low temperature (Table 2). Palmitate (16:0) and docosahexaenoate (22:6) were the most abundant fatty acids in rainbow trout plasma as reported previously (31). Palmitate is commonly used to measure NEFA kinetics in mammals because its fractional contribution to total NEFA shows the lowest coefficient of variation in this group of vertebrates (19). In trout, we found that 16:0 and 22:6 had the lowest coefficient of variation in this group of vertebrates (19).
ent study, we have selected 16:0 because it is more readily available commercially, but also because we were more specifically interested in energy metabolism, and 16:0 is more likely to be used as an oxidative fuel than 22:6 (33). However, polyunsaturated fatty acids (PUFA) are known to play an important role in the reorganization of phospholipids to maintain adequate membrane fluidity during temperature changes. The substitution of saturated with unsaturated fatty acids in membrane phospholipids is known to occur rapidly in fish exposed to cold water (16, 35). Consequently, the changes in NEFA turnover rates measured in our cold exposure experiments with palmitate as a tracer may have underestimated temperature effects on PUFA fluxes.

Conclusions

This study provides the first continuous measurements of oxidative substrate kinetics in fish exposed to a rapid decrease in oxygen availability or in temperature. It shows that acute hypoxia causes a progressive decrease in the turnover rate of NEFA, together with a transient increase in hepatic glucose production, whereas a rapid drop in water temperature induces a rapid decrease in oxygen availability or in temperature. The reorganization of phospholipids to maintain adequate membrane fluidity has been observed both in vitro and in vivo metabolite turnover rates in trout (40, 41). The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout (14) is well known. The effects of hypoxia upon carbohydrate energy stores and metabolism in two species of freshwater fish. Physiol. Zool. 38: 325–334, 1965.


White, A., and T. C. Fletcher. The effect of physical disturbance, hypoxia and stress hormones on serum components of the

REFERENCES


