© 2017. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2017) 220, 3686-3694 doi:10.1242/jeb.161406

RESEARCH ARTICLE

Long-term programming effect of embryonic hypoxia exposure and high-carbohydrate diet at first feeding on glucose metabolism in juvenile rainbow trout

Jingwei Liu, Karine Dias, Elisabeth Plagnes-Juan, Vincent Veron, Stéphane Panserat and Lucie Marandel*

ABSTRACT

Environmental conditions experienced during early life play an important role in the long-term metabolic status of individuals. The present study investigated whether hypoxia exposure [for 24 h: 2.5 mg O_2 l^{-1} (20% dissolved O_2)] during the embryonic stage alone (hypoxic history) or combined with a 5-day high-carbohydrate (60%) diet stimulus at first feeding (HC dietary history) can affect glucose metabolism later in life, i.e. in juvenile fish. After 19 weeks of growth, we observed a decrease in final body mass in fish with an HC dietary history. Feed efficiency was significantly affected by both hypoxic and HC dietary histories. After a short challenge test (5 days) performed with a 30% carbohydrate diet in juvenile trout, our results also showed that, in trout that experienced hypoxic history, mRNA levels of gluconeogenic genes in liver and glucose transport genes in both liver and muscle were significantly increased at the juvenile stage. Besides, mRNA levels of glycolytic genes were decreased in fish with an HC dietary history. Both hypoxic and dietary histories barely affected plasma metabolites or global epigenetic modifications in juvenile fish after the challenge test. In conclusion, our results demonstrated that an acute hypoxic stimulus during early development alone or combined with a hyperglucidic stimulus at first feeding can modify growth performance and glucose metabolism at the molecular level in juvenile trout.

KEY WORDS: Teleost, Hyperglycaemia, Metabolic programming, Growth, Gene expression, Epigenetics

INTRODUCTION

It is widely recognised that environmental modifications (nutritional or non-nutritional) experienced at critical periods of an organism's life (for instance during early development) can cause long-term changes in metabolism and physiology, which is termed developmental programming, or metabolic programming when modifying metabolism (Lucas, 1998). Such adaptations often manifest through persistent modifications in gene expression patterns or metabolic signalling pathways, and may persist later in life in the absence of the environmental stimulus that initiated them (George et al., 2012; Kongsted et al., 2014; Patel and Srinivasan, 2002).

Metabolic programming studies have attracted broad attention in aquaculture in recent years and shed light on how modulations in early nutrition may confer adaptive advantages to organisms to better cope with their future nutritional environment (Balasubramanian et al., 2016; Fang et al., 2014; Panserat et al., 2017; Rocha et al., 2015; Vagner et al., 2009). In particular, in order to better understand and improve the glucose-intolerant phenotype in rainbow trout (*Oncorhynchus mykiss*), researchers have examined metabolic programming strategy using a strict nutritional stimulus in this species (Geurden et al., 2007, 2014). Promising results were obtained in some recent studies in trout. For instance, early nutritional stimulus at the first-feeding stage, such as high carbohydrate (HC) intake, was shown to induce persistent modifications in the mRNA levels of glucose-metabolism-related genes at the juvenile stage (Geurden et al., 2014). However, such a long-term programming effect was only noted in the muscle, and no persistent programming effect was observed in the liver of juvenile fish, despite the central role of this organ in glucose metabolism. These facts highlighted the necessity to optimise and review the programming conditions.

With this aim, a short-term metabolic programming assay was conducted with a strict non-nutritional stimulus, i.e. through embryonic hypoxia exposure (Liu et al., 2017). Indeed, hypoxia is a favourable stimulus candidate when investigating glucose-metabolism programming because hypoxia is known to affect hepatic glucose utilisation (Osumek et al., 2014; Zhong and Mostoslavsky, 2010). Moreover, such a stimulus can be easily applied before first feeding. Finally, effects of hypoxia are at least in part mediated by epigenetic mechanisms (Shmakova et al., 2014; Zhong and Mostoslavsky, 2010) such as DNA methylation or histone modifications (H3K4me3, H3K9me3, H3K36me3 and H3K9ac). Epigenetic modifications are considered an important mechanism mediating the long-term metabolic programming effect because they are sensitive to environmental changes and they constitute, by definition, the horizontal cell memory that is transmissible from one cell generation to another (Feil and Fraga, 2012; Szyf, 2009). In addition, the epigenetic modifications at the global level are known to affect chromatin stability, thereby leading to activation or repression of gene transcription (Klose and Bird, 2006; Kurdistani et al., 2004). Abnormal global DNA methylation or histone modification patterns are associated with metabolic disorders; thus, they were considered important biomarkers for health (Robertson, 2005; Seligson et al., 2005).

Considering hypoxia as a promising stimulus in the context of metabolic programming in trout, Liu and collaborators (2017) demonstrated that an acute hypoxic exposure during the embryonic stage affected glucose-metabolism-related gene expression in whole embryos at first feeding (Liu et al., 2017). For instance, when fed an HC diet at first feeding, significant increases in mRNA levels of genes involved in glycolytic and glucose transport pathways were noted in fish previously subjected to hypoxia. However, questions remain to be elucidated: (i) can such programming effects of an early hypoxic stimulus persist at the juvenile stage in trout; (ii) can a

INRA, Université de Pau et des pays de l'Adour, UMR1419 Nutrition Metabolism and Aquaculture, Aquapôle, F-64310 Saint-Pée-sur-Nivelle, France.

*Author for correspondence (lucie.marandel@inra.fr)

L.M., 0000-0001-9395-6041

Received 19 April 2017; Accepted 7 August 2017

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 3686-3694 doi:10.1242/jeb.161406

hypoxic stimulus applied during the embryonic stage combined with a nutritional stimulus at first feeding more profoundly affect glucose metabolism in trout later in life than a single stimulus; and (iii) if so, do these early stimuli modify the global epigenome in the long term?

The main objective of the present study was to investigate the above questions in juvenile trout that encountered an acute hypoxic stimulus (20% dissolved O_2 for 24 h) at the embryonic stage (152 degree days) alone (hypoxic history) or combined with an HC-diet stimulus at first feeding (HC dietary history) when challenged again with a 5-day high carbohydrate diet (30%) at the juvenile stage. The growth performance, main plasma metabolites, mRNA levels of glucose-metabolism-related genes and global epigenetic modifications were analysed in juvenile trout in the present study.

MATERIALS AND METHODS

Ethical issues and approval

Investigations were conducted according to the guiding principles for the use and care of laboratory animals and in compliance with French and European regulations on animal welfare (Décret 2001-464, 29 May 2001 and Directive 2010/63/EU, respectively). This protocol and the project as a whole were approved by the French National Consultative Ethics Committee (reference numbers 2015112018112159 and 201511201756973).

Experimental design

Embryonic hypoxia and first-feeding stimuli were previously described and analysed by Liu and collaborators (Liu et al., 2017). Briefly, rainbow trout [*Oncorhynchus mykiss* (Walbaum 1792)] embryos at 152 degree days [corresponding to setting up of the primitive liver (Vernier, 1969)] were initially subjected to a 24-h acute hypoxic stimulus [20% dissolved O_2 (2.5 mg l^{-1}); 8°C] or maintained at normoxic conditions (11.0 mg l^{-1} dissolved oxygen; 8°C) (Fig. 1, phase 1, stimulus 1). This first stimulus will be referred to as 'oxygen history' hereafter: either hypoxic history or normoxic history, respectively. Hypoxia was obtained and maintained by bubbling nitrogen in the water as previously described by Liu and collaborators (Liu et al., 2017). After the stimulus, embryos were kept in normoxic conditions. After hatching, fish alevins were reared in a flow-through rearing system supplied with natural spring water (18°C) under a natural photoperiod. At first feeding, fish were fed either an HC (~60% carbohydrate) diet or a no carbohydrate (NC; 0% carbohydrate) diet for 5 days (Fig. 1, phase 1, stimulus 2, and Table S1). This second stimulus will be referred as 'dietary history' hereafter. Each group was conducted in triplicate.

After the second stimulus (first feeding), all groups (initial average wet body mass=0.1 g) were subsequently subjected to a 24-week growth trial (Fig. 1, phase 2) with a commercial diet (Skrettring, Fontaine-lès-Vervins, France) followed by a 5-day challenge test (Fig. 1, phase 3) with a challenge diet containing 30% carbohydrate (Table S1). Fish were fed eight times per day at the beginning and the feeding frequency gradually reduced to two times per day (from 18 weeks and thereafter). Each meal, the fish were fed to apparent satiation. Dry feed intake was calculated as the amount of feed supplied minus the amount of unconsumed feed. Residual feed pellets were counted and the corresponding mass was deducted from feed intake. The parameters for growth performance were monitored every 3 weeks and the following variables were calculated: (1) survival (%)=100×final fish number/initial fish number; (2) specific growth rate (SGR; % day^{-1})=100×($\ln M_2$–$\ln M_1$)/d; (3) feed intake (% day^{-1})=100×{dry feed intake (g)/[(M_2+M_1)/2]}/d; and (4) feed efficiency (FE)=(M_2+M_3–M_1)/dry feed intake, where M_1 and M_2 are the initial and final average wet body mass, respectively, M_3 is the mass for dead fish (Li et al., 2010; Silva-Carrillo et al., 2012; Thodesen et al., 1999), and d is the experimental period in days.

At the end of the 5-day challenge test, fish were first anaesthetized in a benzocaine bath at 30 mg l^{-1} and then killed in a benzocaine bath at 60 mg l^{-1} 6 h after the last meal. Blood was collected from the caudal vasculature of nine fish per condition (three fish per tank) and centrifuged (3000 ***g***, 5 min). The recovered plasma was frozen and stored at −20°C until analysis. Liver and white muscle tissues of six fish per condition (two fish per tank) were dissected and immediately frozen in liquid nitrogen, and stored at −80°C for further analysis.

Chemical composition of the diets

The approximate composition of the diets was analysed as follows: dry matter was determined after drying to constant mass at 105°C; crude protein (N×6.25) was determined by the Kjeldahl method

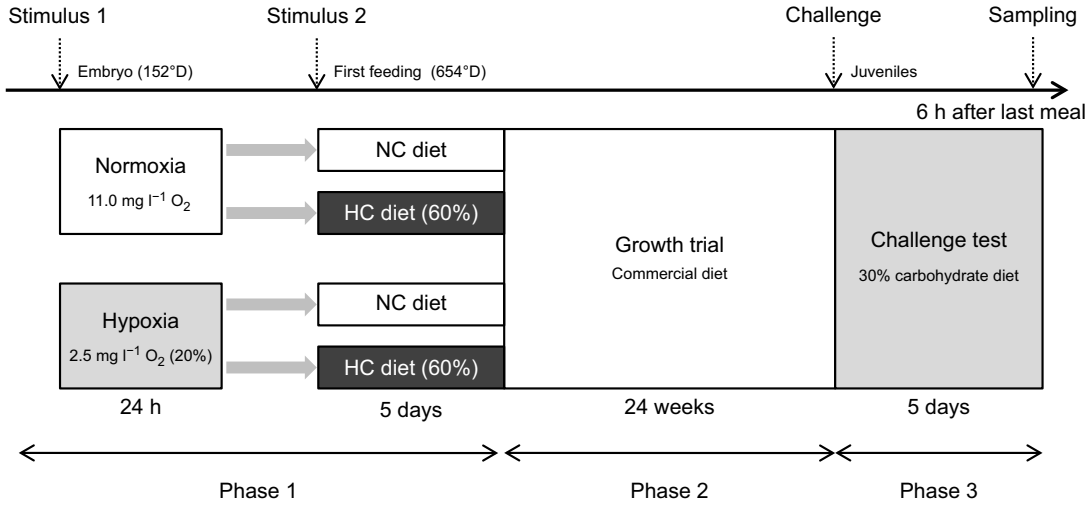

Fig. 1. Experimental design. A 24-h hypoxic stimulus was applied at the embryonic stage [152 degree day (152°D)] and a 5-day dietary stimulus (60% carbohydrate) was performed at first-feeding stage (654°D) in rainbow trout. After a 24-week growth trial with a commercial diet, fish were subjected to a 5-day challenge test with a 30% carbohydrate diet and sampled 6 h after the last meal. NC, no carbohydrate; HC, high carbohydrate.

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 3686-3694 doi:10.1242/jeb.161406

after acid digestion; crude lipid was determined by petroleum ether extraction (Soxtherm); gross energy was measured in an adiabatic bomb calorimeter (IKA, Heitersheim Gribeimer, Germany); ash was estimated through incinerating in a muffle furnace for 6 h at 600°C.

Plasma metabolites

Plasma glucose, triglycerides, lactate and NEFA (non-esterified fatty acid) were analysed with Glucose RTU (BioMerieux, Marcy-l'Étoile, France), PAP 150 (BioMerieux), Lactate PAP (BioMerieux) and NEFA C (Wako Chemicals GmbH, Neuss, Germany) kits, respectively, according to the recommendations of each manufacturer.

Analysis of mRNA levels: quantitative real-time PCR

Total RNA extraction and cDNA synthesis

The analysis of mRNA levels was conducted in liver and white muscle tissues of juvenile fish. Samples were homogenised in Trizol reagent (Invitrogen, Carlsbad, CA, USA) with Precellys®24 (Bertin Technologies, Montigny-le-Bretonneux, France), and total RNA was then extracted according to the Trizol manufacturer's instructions. Total RNA (1 µg) was subsequently reverse-transcribed to cDNA in duplicate using the SuperScript III RNase H-Reverse Transcriptase kit (Invitrogen) with random primers (Promega, Charbonnières-les-Bains, France).

Quantitative real-time PCR assays

The primers used in quantitative real-time PCR (qPCR) assays for glucose-metabolism-related genes have previously been published, and the primer sequences used in the present study can be found in the previous studies (Marandel et al., 2015, 2016b; Liu et al., 2017). qPCR assays were performed with the Roche Lightcycler 480 system (Roche Diagnostics, Neuilly-sur-Seine, France). The reaction mix was 6 µl per sample, including 2 µl of diluted cDNA template (1:76), 0.12 µl of each primer (10 µmol l^{-1}), 3 µl of Light Cycler 480 SYBR® Green I Master mix and 0.76 µl of DNase/RNase-free water (5 Prime GmbH, Hamburg, Germany). The qPCR protocol was initiated at 95°C for 10 min for the initial denaturation of the cDNA and hot-start Taq-polymerase activation, followed by 45 cycles of a two-step amplification programme (15 s at 95°C; 10 s at 60°C). Melting curves were monitored systematically (temperature gradient 0.11°C s^{-1} from 65 to 97°C) at the end of the last amplification cycle to confirm the specificity of the amplification reaction. Each qPCR assay included replicate samples (duplicate of reverse transcription and PCR amplification) and negative controls (reverse-transcriptase- and cDNA-template-free samples). The relative quantification of mRNA levels of target genes were normalised to the transcript abundance of reference genes (β-actin for liver: forward 5′-GATG-GGCCAGAAAGACAGCTA-3′, reverse 5′-TCGTCCCAGTTGG-TGACGAT-3′; *ef1α* for muscle: forward 5′-TCCTCTTGGTCG-TTTCGCTG-3′, reverse-5′-ACCCGAGGGACATCCTGTG-3′) using the E-method on Light Cycler software.

DNA extraction and global DNA methylation analysis

DNA extraction was performed on liver and muscle tissues of six fish per experimental treatment. Ten mg liver or muscle samples were subjected to 37°C overnight digestion under agitation (250 rpm) in 1 ml TNES buffer (pH=8; 125 mmol l^{-1} NaCl, 10 mmol l^{-1} EDTA, 0.5% SDS, 4 mol l^{-1} urea, 10 mmol l^{-1} Tris-HCl) supplemented with 100 µg proteinase K (P6556, Sigma-Aldrich, St Louis, MO, USA). One ml phenol–chloroform–isoamyl-alcohol (25:24:1) was added to each sample and mixed gently for 15 min followed by a 10,000 ***g*** centrifuge at room temperature for 15 min. A total of 100 µl 5 mol l^{-1} NaCl and 1 ml 100% ice-cold ethanol was added to the supernatant and centrifuged for 15 min at 10,000 ***g***, 4°C. The pellets were washed in 1 ml 75% ice-cold ethanol and centrifuged for 15 min at 10,000 ***g***, 4°C again. Finally, the pellets were dried and re-suspended in DNase-free water and treated with 2.4 µg RNase (A7973, Promega) for 1 h at 37°C. DNA was quantified by NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) and the quality (high molecular weight) was verified on 1% agarose gel. DNA global methylation pattern [5-methylcytosine (5-mC)] was assessed using the MethylFlash Methylated DNA Quantification kit (EpiGentek, Farmingdale, NY, USA). Each analysis was performed in duplicate with 100 ng liver DNA or 200 ng muscle DNA samples following the manufacturer's instructions.

Histone extraction and global histone modification analysis

Histone extraction was performed on liver and muscle tissues of six fish per experimental treatment. One hundred mg tissue was homogenised using Precellys®24 (Bertin Technologies) in 2 ml tubes containing 1 ml of TEB buffer [1×PBS, 0.5% Triton X-100, 5 mmol l^{-1} NaBu and one tablet of protease inhibitor (Roche, cat. no. 04693116001)] and four 2.8 mm ceramic beads. The grinding programme was set as 2×10 s (15 s off) at 5000 rpm for liver, and 2×20 s (15 s off) at 5500 rpm for muscle samples. The homogenates were kept on ice for 20 min, transferred to a new 1.5 ml tube and centrifuged for 10 min at 2000 rpm, 4°C. Pellets were then re-suspended in 0.5 N HCl containing 10% glycerol (volume between 100 µl and 1200 µl depending on the size of the pellet) and incubated on ice for 30 min (vortex every 10 min). Samples were then centrifuged for 5 min at 12,000 rpm, 4°C. Three volumes of iced acetone were then added to the supernatant and precipitation was performed at −20°C overnight. Samples were centrifuged at 12,000 rpm, 4°C for 5 min and pellets were washed three times with 100% ice-cold acetone. Pellets were re-suspended in distilled water and incubated at 60°C for 1 h. Total protein concentration was determined using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Munich, Germany). Lysate (5 µg and 14 µg of total protein for liver and muscle samples, respectively) were subjected to SDS-PAGE and western blotting using the appropriate antibody. Samples were loaded on AnykD™ Criterion™ TGX™ Precast Midi Protein Gel for the analysis of H3K4me3, H3K9me3, H3K36me3 and H3K9ac marks. Western blotting was performed with the Trans-Blot® Turbo™ Transfer Starter System (Bio-Rad Laboratories) using the pre-programmed protocol Mixed MW (Turbo) 5–150 kDa and 7 min for transfer. Anti-H3K4me3 (C15410003), anti-H3K9me3 (C15410056), anti-H3K36me3 (C15410192) and anti-H3K9ac (C15410004) were purchased from Diagenode (Liège, Belgium); anti-H3 (ab1791) was purchased from Abcam (Cambridge, UK).

Statistical analysis

The results are presented as means±s.d. The effects of oxygen, diet and oxygen×diet on different parameters were tested using R software (v3.1.0)/R Commander by two-way ANOVA with oxygen and diet as independent variables. When oxygen×diet interaction was significant, means of all treatments were compared by a Tukey's *post hoc* analysis.

RESULTS

Survival and growth performance of trout from stimuli to challenge

In general, there were few significant differences in survival and growth performance among different groups during the 24-week

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 3686-3694 doi:10.1242/jeb.161406

Table 1. Effects of embryonic hypoxia and high carbohydrate diet stimuli on growth performance

	NC dietary history		HC dietary history		*P*-value		
Index	Normoxic history	Hypoxic history	Normoxic history	Hypoxic history	Oxygen history	Dietary history	Interaction
Weeks 0–18 of growth trial							
Survival (%)	78.89±4.28	80.28±3.85	79.44±4.19	81.94±1.73	0.385	0.614	0.799
Final body mass (g)	33.45±0.62	33.87±2.30	32.90±1.34	31.41±0.69	0.532	0.101	0.274
0- to 18-week SGR (% day^{-1})	4.61±0.01	4.62±0.05	4.60±0.03	4.56±0.02	0.498	0.066	0.318
0- to 18-week feed intake (% day^{-1})	1.10±0.01	1.08±0.02	1.09±0.02	1.09±0.01	0.148	1.000	0.540
0- to 18-week feed efficiency	1.43±0.02	1.46±0.04	1.44±0.03	1.46±0.02	0.145	0.823	0.657
Weeks 19–24 of growth trial							
Survival (%)	78.89±4.28	80.28±3.85	79.44±4.19	81.94±1.73	0.385	0.614	0.799
Final body mass (g)	100.77±0.96	102.93±0.28	90.87±2.95	93.46±7.92	0.362	**0.004****	0.932
19- to 24-week SGR (% day^{-1})	2.69±0.04	2.71±0.16	2.48±0.09	2.65±0.16	0.208	0.098	0.345
19- to 24-week feed intake (% day^{-1})	2.19±0.03	2.16±0.12	2.14±0.09	2.18±0.05	0.855	0.855	0.411
19- to 24-week feed efficiency	1.11±0.01	1.13±0.01	1.09±0.01	1.11±0.01	**0.012***	**0.012***	0.608

Data are presented as means±s.d. (triplicate tank per condition, n=3); statistical differences were evaluated by two-way ANOVA followed by Tukey's *post hoc* analysis. Statistical significance is shown in bold: *P≤0.05, ** P≤0.01. NC, no carbohydrate; HC, high carbohydrate; SGR, specific growth rate.

growth trial (Fig. 1, phase 2; Table 1). However, during the last 6 weeks (19–24 weeks), juveniles with the HC dietary history showed significantly lower final body mass and feed efficiency compared with those receiving the NC diet at first feeding. Additionally, fish with a hypoxic history showed a significantly higher feed efficiency compared with those kept under normoxia during early development.

Plasma metabolites of juvenile trout at the end of the 5-day challenge test

The major plasma metabolites (Table 2) related to hypoxia and carbohydrate intake (glucose, triglycerides, lactate and NEFA) were measured in juveniles at the end of the 5-day challenge test (6 h after the last meal; Fig. 1, phase 3). There was no significant effect of oxygen or dietary history on plasma metabolites.

mRNA levels of glucose-metabolism-related genes in liver and muscle of juvenile trout at the end of the 5-day challenge test

We then analysed mRNA levels of glucose-metabolism-related genes in both liver and muscle of juvenile trout after the 5-day challenge test (6 h after the last meal; Fig. 1, phase 3). No interaction effect between oxygen and dietary histories were observed on the mRNA levels of all target genes in juvenile fish.

Regarding the effect of dietary history in liver tissue (Table 3), *gcka* (glucokinase a) and *gckb* (glucokinase b) mRNA levels were significantly lower in fish that received the HC-diet stimulus at first feeding compared with those fed the NC diet. Concerning the effect of oxygen history, fish subjected to a hypoxic stimulus during embryogenesis displayed significantly higher mRNA levels of gluconeogenic *fbp1a* (fructose 1,6-bisphosphatase-1a), *g6pcb1b* (glucose 6-phosphatase-b1 b) and *g6pcb2a* (glucose 6-phosphatase-b2 a) compared with their normoxic controls. Similarly higher mRNA levels of glucose transporters *glut1ba* (glucose transporter-1b a) and *glut2b* (glucose transporter-2b) were found in juvenile trout with the hypoxic history than in those kept under normoxia during early life.

In muscle (Table 4), no significant effect of dietary history was observed in juvenile fish except for the mRNA level of *pfkmbb* [phosphofructokinase (muscle)-bb], which was significantly lower in fish with the HC dietary history than in fish with the NC dietary history. With respect to the effect of oxygen history (Fig. 1, phase 1, stimulus 1), significantly higher mRNA levels of glucose transport genes *glut4a* (glucose transporter-4a) and *glut4b* (glucose transporter-4b) and glycolytic *pkmaa* [pyruvate kinase (muscle)-aa] were observed in juvenile fish with hypoxic history compared with their controls.

Global DNA methylation and global histone modifications in liver and muscle of juvenile trout at the end of the 5-day challenge test

The 5-methyl-cytosine methylation status was analysed in liver and muscle tissues of juvenile trout to test the global DNA methylation level (Table 5). No significant differences in global DNA methylation level in either liver or muscle of juvenile trout were observed regardless of the oxygen or dietary history.

Global levels of selected histone modifications (H3K4me3, H3K9me3, H3K36me3 and H3K4ac) were also measured in both liver and muscle tissues of juvenile trout (Fig. 2). In liver (Fig. 2A), a significant interaction between the effects of oxygen and dietary histories (Fig. 1, phase 1, stimuli 1 and 2, respectively) was observed on H3K9me3 level even though no significant independent effect of oxygen or nutritional history was monitored. By contrast, the global

Table 2. Effects of embryonic hypoxia and HC-diet stimuli on postprandial plasma metabolite levels of juvenile trout subjected to a 5-day challenge test

	NC dietary history		HC dietary history		*P*-value		
Plasma metabolite	Normoxic history	Hypoxic history	Normoxic history	Hypoxic history	Oxygen history	Dietary history	Interaction
Glucose	8.31±1.75	6.62±2.28	7.41±1.14	7.10±2.29	0.128	0.745	0.288
Triglycerides	5.11±2.03	4.73±2.03	4.62±1.61	4.13±3.66	0.617	0.532	0.948
Lactate	5.32±1.93	6.28±2.37	5.39±1.28	6.29±2.81	0.209	0.963	0.969
NEFA	0.16±0.05	0.16±0.03	0.13±0.07	0.17±0.04	0.263	0.706	0.324

Data are mmol l^{-1} and are presented as means±s.d. (n=9 fish); statistical differences were evaluated by two-way ANOVA followed by Tukey's *post hoc* analysis. NEFA, non-esterified fatty acid.

Journal of Experimental Biology (2017) 220, 3686-3694 doi:10.1242/jeb.161406

Table 3. Effects of embryonic hypoxia and HC-diet stimuli on the mRNA level of glucose-metabolism-related genes in liver of juvenile trout subjected to a 5-day challenge test

	NC dietary history		HC dietary history		*P*-value		
Target gene	Normoxic history	Hypoxic history	Normoxic history	Hypoxic history	Oxygen history	Dietary history	Interaction
gcka	1.24±0.36	1.26±0.59	0.66±0.59	0.66±0.56	0.949	**0.014***	0.961
gckb	1.20±0.54	1.41±0.49	0.67±0.67	0.64±0.58	0.716	**0.012***	0.600
pfkla	0.93±0.26	1.18±0.40	0.86±0.46	0.90±0.26	0.315	0.243	0.453
pfklb	0.93±0.22	1.22±0.48	1.06±0.43	0.98±0.26	0.479	0.712	0.215
pklr	0.87±0.15	0.82±0.28	0.90±0.29	0.84±0.32	0.641	0.830	0.994
pck1	0.58±0.41	1.49±1.29	0.84±1.08	0.62±0.46	0.353	0.420	0.141
pck2	0.52±0.16	0.85±0.32	0.77±0.35	0.79±0.35	0.881	0.336	0.249
fbp1a	0.84±0.59	1.43±0.75	0.62±0.38	1.38±0.79	**0.019***	0.599	0.743
fbp1b1	0.86±0.23	1.01±0.33	1.17±0.49	1.20±0.53	0.618	0.160	0.745
fbp1b2	0.81±0.54	1.20±0.74	1.11±0.45	1.04±0.41	0.487	0.739	0.313
g6pca	0.88±0.13	1.12±0.50	1.11±0.37	1.04±0.42	0.619	0.619	0.335
g6pcb1a	0.68±0.52	0.76±1.04	1.65±2.85	0.97±0.86	0.651	0.377	0.569
g6pcb1b	0.67±0.14	1.11±0.84	0.87±0.38	1.75±1.21	**0.047***	0.200	0.489
g6pc b2a	1.11±0.83	1.94±1.61	0.59±0.42	1.44±0.47	**0.044***	0.208	0.971
g6pcb2b	0.98±1.15	2.42±1.74	1.00±1.58	1.47±1.73	0.150	0.474	0.454
glut1ba	0.62±0.12	0.97±0.36	0.75±0.14	1.02±0.41	**0.018***	0.442	0.743
glut1bb	0.87±0.51	0.94±0.32	1.02±0.44	1.11±0.38	0.638	0.341	0.962
glut2a	0.81±0.12	0.91±0.36	0.87±0.20	0.98±0.24	0.300	0.524	1.000
glut2b	0.83±0.16	1.07±0.20	0.86±0.15	1.07±0.29	**0.016***	0.832	0.847

Data show the relative quantification of mRNA levels of target genes normalised to the transcript abundance of β-actin and are presented as means±s.d. (n=6 fish); statistical differences were evaluated by two-way ANOVA followed by Tukey's *post hoc* analysis. Statistical significance is shown in bold: *P≤0.05. *fbp*, fructose 1,6-bisphosphatase; *g6pc*, glucose 6-phosphatase; *gck*, glucokinase; *glut1* (*slc2a1*), solute carrier family 2 (facilitated glucose transporter), member 1; *glut2* (*slc2a2*), solute carrier family 2 (facilitated glucose transporter), member 2; *pklr*, pyruvate kinase (liver and red blood cell); *pck*, phosphoenol pyruvate carboxykinase (cytosolic pck1 and mitochondrial pck2); *pfkl*, phosphofructokinase (liver).

level of all these four histone modifications remained stable in the muscle of juvenile fish (Fig. 2B), whatever the historical stimuli conditions.

DISCUSSION

Previous studies in mammals extensively documented that early-life history may profoundly affect the metabolic adaptability and risk of developing disease in adults, making metabolic programming an important topic in scientific research (Dunn and Bale, 2011; Hochberg et al., 2010; Lucas, 1998; Romani-Perez et al., 2016; Taylor and Poston, 2007; Vickers et al., 2000). This type of study was introduced into aquaculture a decade ago, raising the prospect of tailoring the metabolic capacities in fish through the manipulation of early nutrition (Fang et al., 2014; Geurden et al., 2007, 2014; Gong et al., 2015; Izquierdo et al., 2015; Lazzarotto et al., 2015; Rocha et al., 2015). Recently, we reported that an acute non-nutritional hypoxic stimulus during early developmental stages can affect glucose metabolism in rainbow trout alevins in the short term (Liu et al., 2017). With the aim of investigating the long-term effects in juvenile fish induced by such an early hypoxic stimulus, the present study further tested the effects of an acute hypoxic stimulus alone or combined with an HC diet at first feeding on the growth performance, levels of plasma metabolites, mRNA level of glucose-metabolism-related genes and epigenetic modifications in juvenile trout.

Table 4. Effects of embryonic hypoxia and HC-diet stimuli on the mRNA level of glucose-metabolism-related genes in muscle of juvenile trout subjected to a 5-day challenge test

	NC dietary history		HC dietary history		*P*-value		
Target gene	Normoxic history	Hypoxic history	Normoxic history	Hypoxic history	Oxygen history	Dietary history	Interaction
pfkmaa	0.97±0.36	1.10±0.30	1.02±0.23	1.22±0.37	0.230	0.562	0.788
pfkmab	0.73±0.42	1.12±0.30	1.05±0.35	1.09±0.39	0.176	0.365	0.251
pfkmba	0.84±0.41	1.23±0.43	1.06±0.18	1.17±0.57	0.161	0.640	0.436
pfkmbb	0.92±0.29	1.15±0.20	0.69±0.21	0.86±0.28	0.064	**0.018***	0.733
pkmaa	0.90±0.22	1.30±0.60	0.87±0.21	1.07±0.13	**0.048***	0.366	0.493
pkmab	0.86±0.36	1.21±0.23	1.06±0.18	1.12±0.26	0.075	0.593	0.200
pkmba	0.83±0.33	1.13±0.23	1.01±0.17	1.02±0.24	0.148	0.715	0.180
pkmbb	0.79±0.34	1.09±0.26	0.98±0.17	0.93±0.16	0.238	0.856	0.104
glut1ba	0.71±0.26	0.99±0.35	0.89±0.43	0.95±0.42	0.279	0.670	0.494
glut1bb	0.73±0.26	1.10±0.17	1.04±0.61	1.05±0.46	0.277	0.450	0.294
glut4a	0.75±0.30	1.14±0.37	0.60±0.19	1.04±0.47	**0.008****	0.400	0.852
glut4b	0.71±0.22	1.20±0.23	0.95±0.32	1.05±0.38	**0.025***	0.673	0.119
slc16a3a	0.87±0.61	1.18±0.61	0.98±0.28	0.80±0.44	0.744	0.531	0.243
slc16a3b	0.72±0.56	1.01±0.53	0.59±0.30	1.17±1.03	0.119	0.961	0.588

Data show the relative quantification of mRNA levels of target genes normalised to the transcript abundance of *ef1α* and are presented as means±s.d. (n=6 fish); statistical differences were evaluated by two-way ANOVA followed by a Tukey's *post hoc* test. Statistical significance is shown in bold: *P≤0.05, **P≤0.01. *glut1* (*slc2a1*), solute carrier family 2 (facilitated glucose transporter), member 1; *glut4* (*slc2a4*), solute carrier family 2 (facilitated glucose transporter), member 4; *pfkm*, phosphofructokinase (muscle); *pkm*, pyruvate kinase (muscle); *slc16a3*, solute carrier family 16 (monocarboxylate transporter), member 3.

Journal of Experimental Biology (2017) 220, 3686-3694 doi:10.1242/jeb.161406

Table 5. Effects of embryonic hypoxia and HC-diet stimuli on global DNA methylation of juvenile trout subjected to a 5-day challenge test

	NC dietary history		HC dietary history		*P*-value		
Tissue	Normoxic history	Hypoxic history	Normoxic history	Hypoxic history	Oxygen history	Dietary history	Interaction
Liver	1.62±0.48	1.81±0.70	1.92±0.57	1.66±0.60	0.895	0.747	0.373
Muscle	0.44±0.27	0.39±0.12	0.36±0.23	0.48±0.19	0.676	0.909	0.356

Data show the levels of 5-methylcytosine (5-mC%: 5-mC/total DNA) in DNA and are presented as means±s.d. (n=6 fish individuals); statistical differences were evaluated by two-way ANOVA followed by Tukey's *post hoc* analysis.

HC dietary history decreases growth performance and affects glycolysis-related genes in juvenile trout

In the present study, long-term programming effects of an HC stimulus applied at first feeding was tested in juvenile trout after the 24-week growth trial. Significantly lower body mass and feed efficiency were observed at the end of the growth trial in fish with an HC dietary history compared with those fed the NC diet at first feeding. Results indicated that an early HC dietary stimulus may lead to some detrimental effects in long-term growth performance in trout. However, such effects were not reported in the study previously performed by Geurden and collaborators (2014), in which a 5-day HC dietary stimulus at first feeding did not affect growth performance in juvenile trout. This difference can possibly be explained by the different durations of the growth trial and of the challenge period in these two studies. In the present study, the inferior growth performance of fish with the HC dietary history was not observed until the end of the growth trial (19–24 weeks; 133–168 days). In comparison, the corresponding pre-challenge period in the study of Geurden and collaborators was done over 105 days, which may be too short to monitor a significant difference in growth.

Durable adaptive changes in gene expression patterns are one of the most important biological mechanisms that mediate the persistence of programming effects (Lucas, 1998). At the molecular level, results obtained from the present study showed that dietary history significantly affected mRNA levels of some glycolytic genes in juvenile trout. Indeed, the HC dietary stimulus at first feeding seems to decrease glycolysis in juvenile trout, i.e. mRNA levels of hepatic *gcka* and *gckb* and muscular *pfkmbb* were lower in fish with an HC dietary history than those with an NC dietary history. These findings were opposite to what was reported for the short-term effect (i.e. at first feeding) (Liu et al., 2017). Indeed, at first feeding, *gcka* and *gckb* mRNA levels were increased in fish that received the HC dietary stimulus compared with the control fish (Liu et al., 2017). Additionally, the lower mRNA level of *pfkmbb* in the muscle of juvenile fish with the HC dietary history was not detected previously in the short term. All these results revealed the presumable existence of indirect programming regulations (i.e. through altered hormonal levels) of the long-term expression of metabolic genes by early dietary stimulus. In comparison, the decrease in glycolytic genes in juvenile fish fed an HC diet at first feeding was consistent with the previous study performed by Geurden and collaborators (2014), which showed a decrease in *hk1* (hexokinase 1) and *pkm* mRNA levels in the muscle of juvenile trout with an HC dietary history (Geurden et al., 2014).

It should be noted that, similar to previous studies in mammals (Bellinger and Langley-Evans, 2005; de Maredsous et al., 2016; Devaskar and Thamotharan, 2007), the present study balanced the dietary carbohydrate proportion against protein content to keep the same energy level in diets. Therefore, cautions should be taken when considering the exact causal factor for the long-term effect of dietary history: high dietary carbohydrates, low dietary protein, or both.

Hypoxic history enhances the mRNA level of gluconeogenic and glucose-transport-related genes in juvenile trout

Apart from perinatal nutrition, fetal hypoxia is one of the most extensively studied environmental factors able to induce long-term metabolic changes in mammals (Myatt, 2006; Seckl and Holmes, 2007). Several studies in both mammals and teleost species showed that hypoxia exposure during early life can affect growth and glucose metabolism in adulthood (Camm et al., 2011; Osumek et al., 2014; Vanderplancke et al., 2015). In the present study, a subtle but significant increase in feed efficiency in weeks 19–24 was found in juveniles with a hypoxic history compared with those with a normoxic history. Such an increase in feed efficiency by early hypoxic stimulus has never been reported in trout, although the detailed mechanism behind this long-term effect is still not clear.

Concerning the long-term effect of oxygen history on glucose metabolism in juvenile fish challenged with an HC diet, our results highlighted a significant activating effect of hypoxic history on mRNA levels of genes involved in gluconeogenesis (*fbp1a*, *g6pcb1b*, *g6pcb2a*) and glucose transport (*glut1ba* and *glut2b* in liver, and *glut4a*, *glut4b* in muscle) pathways. Meanwhile, the hypoxic stimulus rarely induced long-term modifications in mRNA levels of glycolytic genes, except for *pkmaa* in the muscle of juvenile fish.

These results were quite in accordance with the modifications induced by hypoxic history in the short term described in the previous study (Liu et al., 2017). Indeed, mRNA levels of *fbp1a*, *glut1ba*, *glut4a* and *pkmaa* in fish with a hypoxic history were consistently higher compared with those with a normoxic history in both the short (first-feeding alevins) (Liu et al., 2017) and long (juveniles) term. The persistent alteration in the expression pattern of these genes demonstrated that an acute hypoxic stimulus applied during early development can quickly modify the transcription of glucose-metabolism-related genes in trout, and these modifications may remain until the juvenile stage.

It is to be noted that, both in the short-term (Liu et al., 2017) and long-term experiments, we observed a strong and consistent activating effect of hypoxic history on glucose-transport genes in trout. These results were in line with a previous study in mammals that showed that prenatal hypoxia can cause persistent alterations in the expression of glucose-transport genes in adult rat offspring (Camm et al., 2011). However, contradictory results were obtained in a previous study in European sea bass, which reported that the mRNA level of *glut2* was increased after a moderate hypoxic episode during the larval stage but, concerning the long-term effect, decreased in the liver of juvenile fish (Vanderplancke et al., 2015). The differences between this study and the present study can be attributed to the different hypoxic intensity and duration, and also the different physiological responses to hypoxia within these two species. Nevertheless, the increased mRNA levels of glucose-transport genes in both liver and muscle of juvenile fish with a hypoxic history in the present study may to some extent prove a better functioning peripheral glucose disposal. Although, after the

Journal of Experimental Biology (2017) 220, 3686-3694 doi:10.1242/jeb.161406

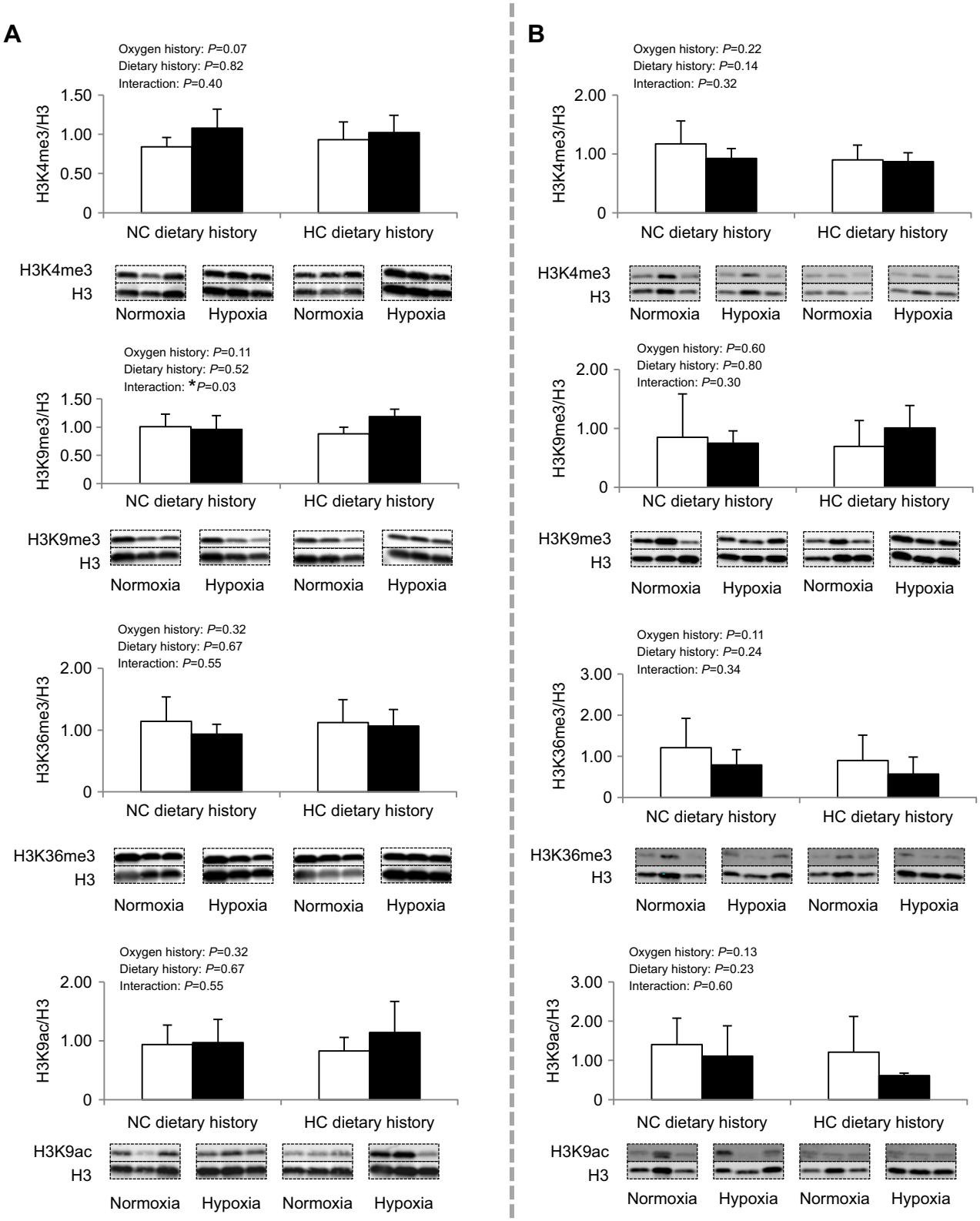

Fig. 2. Effects of embryonic hypoxia and an HC diet at first feeding on global histone modifications in liver and muscle of trout juveniles. (A) Liver. (B) Muscle. White bars and black bars represent fish subjected to normoxia or hypoxia at the embryonic stage, respectively. Data are presented as means±s.d. (n=6); statistical differences were evaluated by two-way ANOVA. When oxygen×diet interaction was significant (*), means of all treatments were compared by Tukey's *post hoc* analysis.

challenge test, the glycaemic level was similar and ranged between 6.6 and 8.3 mmol l^{-1} among different groups, which corresponded to the HC-diet-induced hyperglycaemia (>4 mmol l^{-1}) in rainbow trout, it could possibly be explained by the concomitant enhanced gluconeogenesis (*fbp1a*, *g6pcb1b* and *g6pcb2a*) in the liver of fish that experienced hypoxia during development. Globally, we

Journal of Experimental Biology

hypothesised that glucose transport activated by hypoxic history might provide some clues to ameliorate the glucose-intolerant phenotype in trout, which was known to be related to low glucose transport in muscle of this species (Blasco et al., 1996; Kamalam et al., 2017; Polakof et al., 2012; West et al., 1993).

Oxygen and dietary histories affected global epigenetic modifications in juvenile trout

Remodelling of the epigenetic landscape is an important mechanism relating to the persistent metabolic adaptations by early environmental stimuli. DNA methylation and histone modifications are the most comprehensively studied forms of epigenetic markers, which are known to affect gene transcription (Klose and Bird, 2006; Kurdistani et al., 2004). Previous studies showed that both dietary carbohydrate levels and a hypoxic stimulus can alter global DNA methylation and histone modification (Craig and Moon, 2013; Marandel et al., 2016a; Chen et al., 2006; Hancock et al., 2015; Honma et al., 2007; Perez-Perri et al., 2011; Tausendschön et al., 2011; Watson et al., 2010). However, results obtained in the present study showed that the global DNA methylation level and histone modifications were barely affected by oxygen and dietary histories in juvenile fish after the challenge test, except for an interactive effect of oxygen and dietary histories on the global H3K9me3 level observed in the liver of juvenile fish. Despite this interaction, no statistically significant difference was found within groups.

Conclusions

In summary, our study showed that an acute hypoxic stimulus during embryonic development alone or an HC-diet stimulus at first feeding can affect growth performance in the long term in rainbow trout. Both stimuli also have a long-term programming effect on the mRNA levels of glucose-metabolism-related genes in juvenile trout.

Acknowledgements

We thank F. Terrier, P. Maunas, N. Turonnent and F. Vallée for animal care and preparation of diets. We thank I. Geurden for the design of the diet formulae. Thanks to A. Surget, L. Larroquet and E. Arbenoits for their help with sampling and their technical assistance in the laboratory.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: S.P., L.M.; Methodology: K.D., E.P.-J., V.V.; Formal analysis: J.L., K.D., E.P.-J., V.V.; Investigation: J.L., K.D., E.P.-J., V.V.; Data curation: J.L.; Writing - original draft: J.L.; Writing - review & editing: S.P., L.M.; Visualization: J.L.; Supervision: S.P., L.M.; Project administration: S.P., L.M.; Funding acquisition: S.P., L.M.

Funding

J.L. received a doctoral fellowship from the China Scholarship Council (file no. 201506330063). This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 652831 [AQUAEXCEL^{2020} (aquaculture infrastructures for excellence in European fish research towards 2020), 'WP6 early life history impact on later experimental fish performance'].

Supplementary information

Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.161406.supplemental

References

Balasubramanian, M. N., Panserat, S., Dupont-Nivet, M., Quillet, E., Montfort, J., Le Cam, A., Medale, F., Kaushik, S. J. and Geurden, I. (2016). Molecular pathways associated with the nutritional programming of plant-based diet acceptance in rainbow trout following an early feeding exposure. *BMC Genomics* **17**, 449.

Bellinger, L. and Langley-Evans, S. C. (2005). Fetal programming of appetite by exposure to a maternal low-protein diet in the rat. *Clin. Sci.* **109**, 413-420.

Blasco, J., Fernandez-Borras, J., Marimon, I. and Requena, A. (1996). Plasma glucose kinetics and tissue uptake in brown trout in vivo: effect of an intravascular glucose load. *J. Comp. Physiol. B* **165**, 534-541.

Camm, E. J., Martin-Gronert, M. S., Wright, N. L., Hansell, J. A., Ozanne, S. E. and Giussani, D. A. (2011). Prenatal hypoxia independent of undernutrition promotes molecular markers of insulin resistance in adult offspring. *FASEB J.* **25**, 420-427.

Chen, H., Yan, Y., Davidson, T. L., Shinkai, Y. and Costa, M. (2006). Hypoxic stress induces dimethylated histone H3 lysine 9 through histone methyltransferase G9a in mammalian cells. *Cancer Res.* **66**, 9009-9016.

Craig, P. M. and Moon, T. W. (2013). Methionine restriction affects the phenotypic and transcriptional response of rainbow trout (Oncorhynchus mykiss) to carbohydrate-enriched diets. *Br. J. Nutr.* **109**, 402-412.

de Maredsous, C. D., Oozeer, R., Barbillon, P., Mary-Huard, T., Delteil, C., Blachier, F., Tomé, D., van der Beek, E. M. and Davila, A.-M. (2016). High-protein exposure during gestation or lactation or after weaning has a period-specific signature on rat pup weight, adiposity, food intake, and glucose homeostasis up to 6 weeks of age. *J. Nutr.* **146**, 21-29.

Devaskar, S. U. and Thamotharan, M. (2007). Metabolic programming in the pathogenesis of insulin resistance. *Rev. Endocr. Metab. Disord.* **8**, 105-113.

Dunn, G. A. and Bale, T. L. (2011). Maternal high-fat diet effects on third-generation female body size via the paternal lineage. *Endocrinology* **152**, 2228-2236.

Fang, L., Liang, X.-F., Zhou, Y., Guo, X.-Z., He, Y., Yi, T.-L., Liu, L.-W., Yuan, X.-C. and Tao, Y.-X. (2014). Programming effects of high-carbohydrate feeding of larvae on adult glucose metabolism in zebrafish, Danio rerio. *Br. J. Nutr.* **111**, 808-818.

Feil, R. and Fraga, M. F. (2012). Epigenetics and the environment: emerging patterns and implications. *Nat. Rev. Genet.* **13**, 97-109.

George, L. A., Zhang, L., Tuersunjiang, N., Ma, Y., Long, N. M., Uthlaut, A. B., Smith, D. T., Nathanielsz, P. W. and Ford, S. P. (2012). Early maternal undernutrition programs increased feed intake, altered glucose metabolism and insulin secretion, and liver function in aged female offspring. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **302**, R795-R804.

Geurden, I., Aramendi, M., Zambonino-Infante, J. and Panserat, S. (2007). Early feeding of carnivorous rainbow trout (Oncorhynchus mykiss) with a hyperglucidic diet during a short period: effect on dietary glucose utilization in juveniles. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292**, R2275-R2283.

Geurden, I., Mennigen, J., Plagnes-Juan, E., Veron, V., Cerezo, T., Mazurais, D., Zambonino-Infante, J., Gatesoupe, J., Skiba-Cassy, S. and Panserat, S. (2014). High or low dietary carbohydrate: protein ratios during first-feeding affect glucose metabolism and intestinal microbiota in juvenile rainbow trout. *J. Exp. Biol.* **217**, 3396-3406.

Gong, G., Xue, M., Wang, J., Wu, X.-F., Zheng, Y.-H., Han, F., Liang, X.-F. and Su, X.-O. (2015). The regulation of gluconeogenesis in the Siberian sturgeon (Acipenser baerii) affected later in life by a short-term high-glucose programming during early life. *Aquaculture* **436**, 127-136.

Hancock, R. L., Dunne, K., Walport, L. J., Flashman, E. and Kawamura, A. (2015). Epigenetic regulation by histone demethylases in hypoxia. *Epigenomics* **7**, 791-811.

Hochberg, Z. E., Feil, R., Constancia, M., Fraga, M., Junien, C., Carel, J.-C., Boileau, P., Le Bouc, Y., Deal, C. and Lillycrop, K. (2010). Child health, developmental plasticity, and epigenetic programming. *Endocr. Rev.* **32**, 159-224.

Honma, K., Mochizuki, K. and Goda, T. (2007). Carbohydrate/fat ratio in the diet alters histone acetylation on the sucrase–isomaltase gene and its expression in mouse small intestine. *Biochem. Biophys. Res. Commun.* **357**, 1124-1129.

Izquierdo, M., Turkmen, S., Montero, D., Zamorano, M. J., Afonso, J. M., Karalazos, V. and Fernández-Palacios, H. (2015). Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream. *Aquaculture* **449**, 18-26.

Kamalam, B. S., Medale, F. and Panserat, S. (2017). Utilisation of dietary carbohydrates in farmed fishes: new insights on influencing factors, biological limitations and future strategies. *Aquaculture* **467**, 3-27.

Klose, R. J. and Bird, A. P. (2006). Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* **31**, 89-97.

Kongsted, A. H., Tygesen, M., Husted, S. V., Oliver, M., Tolver, A., Christensen, V. G., Nielsen, J. H. and Nielsen, M. O. (2014). Programming of glucose–insulin homoeostasis: long-term consequences of pre-natal versus early post-natal nutrition insults. Evidence from a sheep model. *Acta Physiol.* **210**, 84-98.

Kurdistani, S. K., Tavazoie, S. and Grunstein, M. (2004). Mapping global histone acetylation patterns to gene expression. *Cell* **117**, 721-733.

Lazzarotto, V., Corraze, G., Leprevost, A., Quillet, E., Dupont-Nivet, M. and Médale, F. (2015). Three-year breeding cycle of rainbow trout (Oncorhynchus mykiss) fed a plant-based diet, totally free of marine resources: consequences for reproduction, fatty acid composition and progeny survival. *PLoS ONE* **10**, e0117609.

Li, M. H., Robinson, E. H., Oberle, D. F. and Lucas, P. M. (2010). Effects of various corn distillers by-products on growth, feed efficiency, and body composition of channel catfish, Ictalurus punctatus. *Aquac. Nutr.* **16**, 188-193.

Liu, J., Plagnes-Juan, E., Geurden, I., Panserat, S. and Marandel, L. (2017). Exposure to an acute hypoxic stimulus during early life affects the expression of glucose metabolism-related genes at first-feeding in trout. *Sci. Rep.* **7**, 363.
Lucas, A. (1998). Programming by early nutrition: an experimental approach. *J. Nutr.* **128**, 401S-406S.
Marandel, L., Seiliez, I., Véron, V., Skiba-Cassy, S. and Panserat, S. (2015). New insights into the nutritional regulation of gluconeogenesis in carnivorous rainbow trout (Oncorhynchus mykiss): a gene duplication trail. *Physiol. Genomics* **47**, 253-263.
Marandel, L., Lepais, O., Arbenoits, E., Véron, V., Dias, K., Zion, M. and Panserat, S. (2016a). Remodelling of the hepatic epigenetic landscape of glucose-intolerant rainbow trout (Oncorhynchus mykiss) by nutritional status and dietary carbohydrates. *Sci. Rep.* **6**, 32187.
Marandel, L., Véron, V., Surget, A., Plagnes-Juan, É. and Panserat, S. (2016b). Glucose metabolism ontogenesis in rainbow trout (Oncorhynchus mykiss) in the light of the recently sequenced genome: new tools for intermediary metabolism programming. *J. Exp. Biol.* **219**, 734-743.
Myatt, L. (2006). Placental adaptive responses and fetal programming. *J. Physiol.* **572**, 25-30.
Osumek, J. E., Revesz, A., Morton, J. S., Davidge, S. T. and Hardy, D. B. (2014). Enhanced trimethylation of histone h3 mediates impaired expression of hepatic glucose 6-phosphatase expression in offspring from rat dams exposed to hypoxia during pregnancy. *Reprod. Sci.* **21**, 112-121.
Panserat, S., Marandel, L., Geurden, I., Veron, V., Dias, K., Plagnes-Juan, E., Pegourié, G., Arbenoits, E., Santigosa, E. and Weber, G. (2017). Muscle catabolic capacities and global hepatic epigenome are modified in juvenile rainbow trout fed different vitamin levels at first feeding. *Aquaculture* **468**, 515-523.
Patel, M. S. and Srinivasan, M. (2002). Metabolic programming: causes and consequences. *J. Biol. Chem.* **277**, 1629-1632.
Perez-Perri, J. I., Acevedo, J. M. and Wappner, P. (2011). Epigenetics: new questions on the response to hypoxia. *Int. J. Mol. Sci.* **12**, 4705-4721.
Polakof, S., Panserat, S., Soengas, J. L. and Moon, T. W. (2012). Glucose metabolism in fish: a review. *J. Comp. Physiol. B* **182**, 1015-1045.
Robertson, K. D. (2005). DNA methylation and human disease. *Nat. Rev. Genet.* **6**, 597-610.
Rocha, F., Dias, J., Engrola, S., Gavaia, P., Geurden, I., Dinis, M. T. and Panserat, S. (2015). Glucose metabolism and gene expression in juvenile zebrafish (Danio rerio) challenged with a high carbohydrate diet: effects of an acute glucose stimulus during late embryonic life. *Br. J. Nutr.* **113**, 403-413.
Romani-Perez, M., Lepinay, A. L., Alonso, L., Rincel, M., Xia, L., Fanet, H., Caille, S., Cador, M., Laye, S., Vancassel, S. et al. (2016). Impact of perinatal exposure to high-fat diet and stress on responses to nutritional challenges, food-motivated behaviour and mesolimbic dopamine function. *Int. J. Obes. (Lond)* **41**, 502-509.
Seckl, J. R. and Holmes, M. C. (2007). Mechanisms of disease: glucocorticoids, their placental metabolism and fetal'programming'of adult pathophysiology. *Nat. Rev. Endocrinol.* **3**, 479.
Seligson, D. B., Horvath, S., Shi, T., Yu, H., Tze, S., Grunstein, M. and Kurdistani, S. K. (2005). Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* **435**, 1262-1266.
Shmakova, A., Batie, M., Druker, J. and Rocha, S. (2014). Chromatin and oxygen sensing in the context of JmjC histone demethylases. *Biochem. J.* **462**, 385-395.
Silva-Carrillo, Y., Hernández, C., Hardy, R. W., González-Rodríguez, B. and Castillo-Vargasmachuca, S. (2012). The effect of substituting fish meal with soybean meal on growth, feed efficiency, body composition and blood chemistry in juvenile spotted rose snapper Lutjanus guttatus (Steindachner, 1869). *Aquaculture* **364**, 180-185.
Szyf, M. (2009). The early life environment and the epigenome. *Biochim. Biophys. Acta* **1790**, 878-885.
Tausendschön, M., Dehne, N. and Brüne, B. (2011). Hypoxia causes epigenetic gene regulation in macrophages by attenuating Jumonji histone demethylase activity. *Cytokine* **53**, 256-262.
Taylor, P. D. and Poston, L. (2007). Developmental programming of obesity in mammals. *Exp. Physiol.* **92**, 287-298.
Thodesen, J., Grisdale-Helland, B., Helland, S. J. and Gjerde, B. (1999). Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (Salmo salar). *Aquaculture* **180**, 237-246.
Vagner, M., Robin, J. H., Zambonino-Infante, J. L., Tocher, D. R. and Person-Le Ruyet, J. (2009). Ontogenic effects of early feeding of sea bass (Dicentrarchus labrax) larvae with a range of dietary n-3 highly unsaturated fatty acid levels on the functioning of polyunsaturated fatty acid desaturation pathways. *Br. J. Nutr.* **101**, 1452-1462.
Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferraresso, S., Sévère, A., Zambonino-Infante, J.-L. and Mazurais, D. (2015). Hypoxic episode during the larval period has long-term effects on European sea bass juveniles (Dicentrarchus labrax). *Mar. Biol.* **162**, 367-376.
Vernier, J.-M. (1969). Table chronologique du développement embryonnaire de la truite arc-en-ciel, *Salmo gairdneri* Rich. 1836. *Ann. Embryol. Morphog.* **2**, 495-520.
Vickers, M. H., Breier, B. H., Cutfield, W. S., Hofman, P. L. and Gluckman, P. D. (2000). Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am. J. Physiol. Endocrinol. Metab.* **279**, E83-E87.
Watson, J. A., Watson, C. J., McCann, A. and Baugh, J. (2010). Epigenetics: the epicenter of the hypoxic response. *Epigenetics* **5**, 293-296.
West, T. G., Arthur, P. G., Suarez, R. K., Doll, C. J. and Hochachka, P. W. (1993). In vivo utilization of glucose by heart and locomotory muscles of exercising rainbow trout (Oncorhynchus mykiss). *J. Exp. Biol.* **177**, 63-79.
Zhong, L. and Mostoslavsky, R. (2010). SIRT6: a master epigenetic gatekeeper of glucose metabolism. *Transcription* **1**, 17-21.