# Expression pattern and histone acetylation of energy metabolic genes in Xenopus laevis liver in response to diet statuses

**Title Page**

# Expression pattern and histone acetylation of energy metabolic genes in *Xenopus laevis* liver in response to diet statuses

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Abbreviations: ChIP, chromatin immunoprecipitation; H3K9me3, trimethylated histone H3 lysine 9; H3K36me3, trimethylated histone H3 lysine 36; H4ac, acetylated histone H4; H3K9ac, acetylated histone H3 lysine 9; TBS, Tris-buffered saline; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; SDS, sodium dodecyl sulfate; SEM, standard error of the mean.

**Abstract** (245 words)

Amphibians can survive without food for relatively long periods by reducing locomotor activity and metabolic rate and can recover quickly with refeeding from a dormant state. To clarify the molecular mechanism underlying this survival strategy, we investigated serum biochemical parameters, the transcript levels of energy metabolic genes and global and gene-specific histone modifications in the liver of adult male *Xenopus laevis*, which were fed, fasted or refed after fasting. Glucose, triglyceride, cholesterol and free fatty acid levels in sera decreased with fasting for 22 days, with only glucose levels recovered with one day of refeeding. The transcript levels of two-thirds of energy metabolic genes tested decreased with fasting for 22 days and partially recovered with one day of refeeding. The transcript levels of gluconeogenesis and lipid catabolism genes did not increase with fasting for 22 days. Western blot analysis revealed no significant differences in the amounts of acetylated and methylated histones in the liver among the three groups on Day 22. The amounts of acetylated histone H4 did not changed in diet-response genes, although the transcript levels of these genes quickly responded to fasting and refeeding. Our results indicate that *Xenopus* liver may respond to fasting toward an overall decrease in transcriptional activity and to refeeding toward quick recovery, despite no significant changes in histone acetylation level. This unusual unresponsiveness of histone acetylation to diet conditions may serve as an effective adaptation strategy to minimize energy demands during fasting and to quickly respond to refeeding.

**Keywords**: fasting, refeeding, metabolism, transcript, histone modification, *Xenopus laveis*

**Text**

## 1. Introduction

Ectotherm vertebrates such as amphibians can survive for relatively long periods without food. Frogs inhabiting the northern high latitudes do not feed at all or feed only to a small extent during long winter. Aquatic frogs or toads inhabiting these latitudes usually hibernate on land or underwater (Tattersall & Ultsch, 2008). Alternatively, tropical frogs or toads, like the African clawed frog *Xenopus laevis*, can estivate in mud during hot or dry seasons (Pinder, Storey, & Ultsch, 1992). Amphibians conserve energy during hibernation and estivation by depressing their metabolic rate (McCue, 2010; Storey & Storey, 2004). Although the growing body of evidence suggests that hypometabolism induced by various environmental stresses, such as anoxia, extreme temperatures and dryness, in various vertebrates, is accompanied by epigenetic changes (Storey, 2015), experimental evidence as to how frogs modulate metabolism transcriptionally to ensure survival in response to food deprivation is still poor.

The liver plays a central role in metabolic responses to fluctuations in food availability and controls nutrient homeostasis by sensing levels of circulating hormones. In mammals, fasting for several days decreased glucose and increased free fatty acid and glycerol levels in plasma (Stout, Henry, & Buchanan, 1976), suggesting a switch from carbohydrate to lipid metabolism as the main source of energy (Wang, Hung, & Randall, 2006). In the liver, glycogenolysis, fatty acid oxidation, ketogenesis and gluconeogenesis are sequentially activated in a partial overlapping fashion during

fasting (Goldstein & Hager, 2015). Similar metabolic changes were observed in the liver of juvenile male chickens during fasting (Desert et al., 2008). In contrast, transcriptome analyses of fish liver after 21 days of fasting revealed overall depression of metabolic activity including lipid metabolism, protein biosynthesis, proteolysis, cellular respiration, and responses to oxidative stress (Antonopoulou et al., 2013; Drew et al., 2008; Salem, Silverstein, Rexroad, & Yao, 2007). However, it is unclear whether these species-specific metabolic changes in liver are due to different metabolic responses to the feeding status of animals.

In this study, we first investigated the biochemical parameters in serum to understand an overview of metabolic changes of *X. laevis* that were fed, fasted for three weeks, or refed for one day after fasting. We next examined the transcript levels of energy metabolic genes in the liver by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Finally, to clarify whether epigenetic histone modifications are involved in the transcriptional changes, the amounts of acetylated or methylated histones, which are known to be positively or negatively associated with transcriptional activity (Zhang, Cooper, & Brockdorff, 2015), were investigated by western blotting and chromatin immunoprecipitation (ChIP) assay.

## 2. Materials and Methods

### *2.1. Animals and experimental design*

Adult male *X. laevis* (one year old, 41–58 g) were commercially obtained from Watanabe Breeding (Hyogo, Japan). They were maintained in aerated and dechlorinated tap water at 22–24°C under natural lighting conditions and were fed four crickets (each approximately 0.3 g in weight) per frog every morning. After acclimation to laboratory conditions for one or two weeks, frogs were divided into three groups (eight individuals/group) and placed in 60 L-aquaria with 15 L of dechlorinated tap water at 22–24°C. In the previous experiment using the small intestine of the same species (Tamaoki et al., 2016), only 2 out of 27 genes responded significantly to one-week fasting, whereas 59 out of 74 genes responded significantly to three-weeks fasting. Therefore, we chose three weeks as a time period in this experiment like the previous study. For the fed group, frogs were fed ad libitum four crickets per frog every morning for 22 days. For the fasted group, frogs were reared without food for 22 days. For the refed group, frogs were reared without food and then fed four crickets/frog on Day 21. Aquaria water was changed three times per week. On Day 22, frogs were weighed, anesthetized by immersion in a 0.5% tricaine methanesulfonate, and their blood and liver collected. The serum was separated from blood cells by centrifugation at 3,000 × *g* for 15 min at 4°. The serum and liver was stored at -35°C and -84°C, respectively, for later use.

All housing and experimental procedures were approved by the Animal Welfare Committee of Shizuoka University (permit #29F-8) under the international guideline “Act on Welfare and Management of Animals” (Ministry of Environment of Japan).

*2.2. Measurements of plasma parameters*

The concentration of glucose in plasma was determined according to the mutarotase-glucose oxidase method (Miwa, Okudo, Maeda, & Okuda, 1972) using a kit (Glucose C II-testWako), triglycerides according to the glycerol-3-phosphate oxidase method (Spayd et al., 1978) using a kit (Triglyceride E-testWako), cholesterol according to the cholesterol oxidase method (Allain, Poon, Chan, Richmond, & Fu, 1974) using a kit (Cholesterol E-testWako), and free fatty acids according to the acyl-CoA synthetase and acyl-CoA oxidase method (Shimizu, Yasui, Tani, & Yamada, 1979) using a kit (NEFA C-testWako). All kits were used following the manufacturer's directions.

*2.3. RT-qPCR analysis*

Total RNA was extracted from liver (~0.1 g) by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski & Sacchi, 1987), and its integrity was confirmed by agarose gel electrophoresis containing 2.0 M formaldehyde. RNA (200 ng) was reverse transcribed in 10 μL of 1× Taqman RT buffer using Taqman RT reagent kit (Applied Biosystems, Foster City, CA, USA) for 30 min at 48°C and then for 5 min at 95°C according to the manufacturer's instructions. Sequence data of *X. laevis* genes were obtained from public databases. Detailed information of primer sets is shown in Supplementary Table 1. We included controls lacking cDNA templates to determine the specificity of target cDNA amplification and to assess the contamination of cDNA samples. To avoid amplification of genomic DNA, we specified forward and reverse PCR primers at neighboring exons. The

expression of genes of interest was estimated in triplicate using Power SYBR Green Master Mix and ABI Prism 7000 sequence detection System (Applied Biosystems) with a specific primer set (each 200 nM), using the following protocol: one cycle of 50°C for 2 min and 95°C for 10 min, and then 40 cycles of 95°C for 15 sec and 60°C for 1 min, as described previously (Tamaoki et al., 2016). All assays gave unique dissociation curves. Quantification was determined by applying the $2^{-Cq}$ formula and calculating the average of the values obtained for each sample. Eligibility of this formula was verified by qPCR using RT-qPCR or RT product of total RNA as a template at different concentrations that covered three to five orders of magnitude. PCR efficiency was 95.3 ± 9.6%. Relative quantification of transcript amounts was calculated with the $2^{-\Delta\Delta Cq}$ method (Livak & Schmittgen, 2001). We tested three candidates as reference genes: *ribosomal protein L8* (*rpl8*), *eukaryotic translation elongation factor 1 α 1* (*eef1a1*) and *yy1 transcription factor* (*yy1*). As the Cq values for the *rpl8* transcript were more invariable among the three experimental groups than those for the *eef1a1* and *yy1* transcripts, we used *rpl8* as a reference gene.

*2.4. Western blotting analysis*

Frozen liver (approximately 20 mg) was homogenized in 500 μL of homogenization buffer (50 mM Tris-HCl, pH 7.5, 25 mM KCl, 250 mM sucrose, 10 mM sodium butyrate, 1 mM sodium orthovanadate, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM leupeptin, 1.4 mM E-64) on ice (Rumbaugh & Miller, 2011), by a Polytron homogenizer. Homogenate was immediately mixed with

2× sodium dodecyl sulfate (SDS) sample buffer (140 mM Tris-HCl, pH 6.8, 22.4% glycerol, 6% SDS, 0.02% bromophenol blue, 10% mercaptoethanol) and then boiled for 5 min.

Western blotting was performed as previously shown (Tamaoki, Ishihara, & Yamauchi, 2018). In brief, aliquots containing 20–50 μg protein were loaded into a 15% SDS-polyacrylamide gel. After electrophoresis at 100 V for 3 h, the resolved proteins were transferred onto a polyvinylidene difluoride membrane (0.22 μm, FluoroTrans®; PALL, Port Washington, New York, USA) at 1.2 $mA/cm^2$ for 1 h. The membrane was blocked in Tris-buffered saline (TBS, 50 mM Tris-HCl, pH, 7.6, and 140 mM NaCl) containing 10% of skim milk overnight at 4°C. The membrane was probed for 1 h at room temperature with rabbit antibodies against trimethylated histone H3 at lysine 36 (H3K36me3, 1:1,000) and at lysine 9 (H3K9me3, 1:500), and acetylated histone H3 at lysine 9 (H3K9ac, 1:20,000) and H4 (H4ac, 1:500), human histone H3 pan (1:25,000) or histone H4 pan (1:30,000) in TBS containing 1% skim milk. Rabbit antibody against H3K36me3 (ab9050) was obtained from Abcam (Tokyo, Japan), and those against H3K9me3 (07-442), H3K9ac (17-615), H4ac (06-598), human histone H3 pan (07-690) and histone H4 pan (05-858) were from Merck Millipore (Darmstadt, Germany). After incubation, the membrane was rinsed three times with TBS containing 0.1% Tween-20 and then incubated with the secondary antibody (1:2,500, alkaline phosphatase-linked anti-rabbit immunoglobulin, from goat) in TBS containing 1% skim milk for 30 min at room temperature. Immunoblots were developed using a detection kit for alkaline phosphatase activity (ProtoBlot, Promega, Madison, WI, USA). Band intensity was quantified using an image analyzer (LAS-4000, GE

Healthcare Life Sciences, Chicago, IL, USA). To control for loading in western blots, the intensity of bands immunoreactive to antibodies against modified histones in each lane was normalized to the intensity of bands immunoreactive to antibodies against histone H3 pan or H4 pan in the same lane.

*2.5. ChIP assay*

ChIP assay was performed as described previously (Tamaoki et al., 2016). Formaldehyde was added to give a final concentration of 1% in 10 mL of ChIP lysis buffer (4.5 mM HEPES, pH 8.0, 9 mM NaCl, 0.09 mM EDTA, 0.04 mM EGTA), and the liver (approximately 0.5 g) from fed, fasted and refed frogs was homogenized with a Potter Elvehjem homogenizer (5,000 rpm, 10 strokes). After the homogenate was incubated for 30 min at 37°C, the cross-linking reaction was stopped by addition of 1 mL of 1.5 M glycine. Crude chromatin was washed with 10 mL of FACS solution (1× phosphate buffered saline, 2% bovine serum and 0.05% sodium azide), and the pellet was re-suspended in lysis buffer containing 50 mM Tris, pH 8.0, 10 mM EDTA, 1% SDS. Chromatin sample was sonicated with a sonicator (max 50W, 20khz; VP-5S ULTRAS, Taitec; Saitama, Japan) using the following settings: middle power output, 12 cycles of 30 sec ON/30 sec OFF pulses, to yield DNA fragments of sizes ranging from 200 to 700 bp. ChIP assays were performed using monoclonal antibody against histone H4 pan, polyclonal antibodies against histone H4ac (0.3−0.6 μg) or normal IgG (3.0 μg). The immunoprecipitated chromatin was reverse crosslinked by incubating overnight at 65°C with 50 μg/mL proteinase K and 10 μg/mL ribonuclease A, and the DNA was purified by phenol-chloroform extraction. The recovered

DNA was quantified by qPCR using primers specific to the 5' transcribed regions of the *X. laevis* acox2, *cyp7a1* and *g6pc2* genes (Supplementary Table 1). The relative enrichment of histone H4ac in the chromatin was measured according to the $2^{-\Delta\Delta Cq}$ method (Livak & Schmittgen, 2001), which was expressed as a percentage of the ChIP signal for the input DNA. We also used the ChIP signal for histone H4 pan as a reference to exclude the possibility that the apparent changes in amount of modified histones result from nucleosomal removal that occurs in 5' regions of actively transcribed genes (Fujimoto, Matsuura, Das, Fu, & Shi, 2012). Detailed information of primer sets is shown in Supplementary Table1.

*2.6. Statistical analysis*

All assay data are presented as means ± standard errors of the means (SEM). Data were analyzed using one-way analysis of variance with the Fisher's test for multiple comparisons to show significant differences. $p < 0.05$ was considered statistically significant.

**3. Results**

The mean liver weight of the fasted group on Day 22 was 75% of that of the fed group (control) and recovered to 90% in the refed group, although there was no significant difference in body weight among the groups (Table 1). The hepatosomatic index, which is the ratio of liver weight to body

weight (%), also decreased with fasting and recovered with refeeding. The concentration of all serum biochemical parameters tested decreased with fasting. The decreases in serum glucose (65% of control) and cholesterol (64%) were less than the decreases in serum triglyceride (36%) and free fatty acid (40%). In the refed group, serum glucose levels recovered partially (81% of control) whereas the lipid parameters remained significantly low (triglyceride, 55%; cholesterol, 60%; free fatty acid, 49%).

The most striking feature of the transcriptional analysis was an overall suppression in transcript levels of two-thirds of genes examined on Day 22 of fasting and partial recovery to the levels of fed frogs with one day of refeeding (Fig. 1). Responses of the transcript levels to fasting and refeeding were similar between genes involved in the fasting signaling pathways (gluconeogenesis and fatty acid metabolism) and in the feeding signaling pathways (fatty acid synthesis, glycogen synthesis and cholesterol synthesis/metabolism).

To clarify whether the global transcriptional changes found by fasting and refeeding are associated with epigenetic histone modifications, we investigated the amounts of H3K9ac, H3K9me3, H3K36me3 and H4ac in the liver homogenates from frogs on Day 22 by western blotting (Fig. 2A). Quantitative analysis revealed no significant differences in the amounts of the four histone modifications among the groups (Fig. 2B). Finally, we investigated the effect of fasting and refeeding on the amounts of histone H4ac in the livers on Day 22 by ChIP assay. Figure 3 shows the ChIP signals for histone H4ac (*panel B*) and H4 pan *(panel C)* in the diet-response genes *acox2*, *cyp7a1* and *g6pc2* with the transcript levels of these genes (*panel A*). These transcripts responded quickly to our

diet-conditions. The transcript levels were down-regulated to 20−60 % of those of the fed frogs by fasting and were partially recovered by refeeding, whereas their ChIP signals for histone H4ac, as well as those for H4 pan, were not altered by the diet-conditions.

## 4. Discussion

The morphological and biochemical changes with fasting and refeeding were in agreement with those reported in previous studies using the same species (S. Merkle & W. Hanke, 1988); however, decreases in the adiposomatic and intestinal-somatic indices (approximately 70% and 60%, respectively, of the fed group) were greater than that in the hepatosomatic index (77% in this study) with fasting. An increased concentration of serum free fatty acids, reported in fasted endotherms including humans where they serve as substrates for β-oxidation (McCue, 2010; Stout et al., 1976), was not observed in the fasted frogs. These observations suggest that although fasting for 22 days mobilizes nutrients, such as glucose and simple lipids, from the adipose tissue, intestine or liver, it also diminishes the demand for these nutrients as reflected by decreased serum concentrations. This assumption is compatible with a drop in mean oxygen consumption by 30% with fasting for the same period (S Merkle & W Hanke, 1988).

An overall suppression in transcript levels with fasting for three weeks was also observed in the livers of zebrafish (Drew et al., 2008) and rainbow trout (Salem et al., 2007), where more than

80% of the genes whose transcript levels were altered were down-regulated, reflecting metabolic rate depression. However, this magnitude of down-regulation was not observed in the transcriptome analyses of the livers of fasted chickens (for 16 and 48 h) (Désert et al., 2008) and mice (for 24 h and 48 h) (Bauer et al., 2004).

In higher vertebrates, multiple lines of evidence indicate that the transcriptional responses to fasting and refeeding in the liver are principally regulated by transcription factors, some of which are controlled by hormones and/or nutrients (Goldstein & Hager, 2015). Fasting signals are input via the glucagon and glucocorticoid signaling pathways, which activate the genes for gluconeogenesis, fatty acid oxidation and ketogenesis through transcription factors such as Creb1, Hnf4α, Pparα, Foxo1 and Pgc1α (full names of the protein abbreviations are shown in Supplementary Table 1) (Bauer et al., 2004; Desert et al., 2008). In fed states, glucose and insulin activate the genes for lipogenesis, cholesterol metabolism and bile acid metabolism, through transcription factors such as Srebp, Lxr and Fxr (full names of the protein abbreviations are shown in Supplementary Table 1) (Bauer et al., 2004; Desert et al., 2008). Our data suggest that feeding signaling pathways in *X. laevis,* like in higher vertebrates, were suppressed by fasting to conserve energy and partially recovered with one day of refeeding. The down-regulation of fatty acid synthesis and cholesterol synthesis/metabolism genes in the liver and decreased concentration of serum lipid parameters were in agreement with the findings reported in the livers of chicken and mammals (Bauer et al., 2004; Desert et al., 2008). Contrary to what we expected, the transcript levels of the genes on the fasting signaling pathways

[gluconeogenesis and fatty acid metabolism (β-oxidation)] were not increased but rather decreased with fasting for 22 days. It is likely that that the energy metabolism of the *X. laevis* liver may not be in the fasting state on Day 22 of fasting, despite the concentration of serum glucose decreasing with fasting, which is a common response to fasting in various vertebrates from fish to mammals (McCue, 2010; Rodgers & Puigserver, 2007; Stout et al., 1976).

Metabolic rate depression observed in ectotherms may be a powerful survival strategy during food deprivation and may minimize the consumption of metabolic energy sources (Storey & Storey, 1990), by which a sequential transition of metabolic changes may be delayed over several months. Early findings that fat bodies disappeared and the liver consumed glycogen in *X. laevis* until six months of fasting (S. Merkle & W. Hanke, 1988), and that nitrogen excretion rate increased after six months (Merkle, 1989) can be explained by a decreased consumption rate of lipids and carbohydrates, which is an adaptation to prolonged fasting that occurs often in their natural habitats (Wang et al., 2006).

Positive relationships are well accepted between the transcript levels and the amounts of euchromatin-associated epigenetic marks such as histone acetylation (Zhang et al., 2015), even in diet-response genes in rodents (Honma, Mochizuki, & Goda, 2013; Suzuki, Douard, Mochizuki, Goda, & Ferraris, 2011). However, in this study, we detected no significant changes in the amount of acetylated histone H4 among the three diet-conditions, in the diet-response genes of the frog liver. The inconsistency between the transcript levels and the amounts of histone acetylation was also reported in

the other diet-response genes in the frog intestines, where acetylated histone levels were inversely correlated with the transcript levels (Tamaoki et al., 2016). Interestingly, these genes elicited a quick transcriptional response to refeeding within one day, suggesting a standby mode under fasting conditions to activate their transcription soon in response to refeeding. These unusual states of histone acetylation detected in fasted and refed frogs may be generated as an adaptation strategy to survive as much as possible under food-restricted conditions.

In various environmental stresses, such as hypoxia and extreme temperature, the amounts of epigenetically modified histones in cells or the activity of corresponding enzymes change dramatically in both endotherms and ectotherms (Storey, 2015) when examined by western blotting (Krivoruchko & Storey, 2010; Morin & Storey, 2009; Pinto et al., 2005) and enzyme assays for specific modifications (Rouble & Storey, 2015). However, we could not detect any changes in amounts of epigenetically modified histone marks in the liver of *X. laevis*. It is unclear whether the fasting-induced responses occur by the same mechanism as the stress responses to hypoxia and temperatures. More detailed analyses are necessary to elucidate the involvement of epigenetic regulation in the adaptation to high fluctuations of food availability.

In conclusion, *Xenopus* liver responded to fasting toward an overall decrease in transcriptional activity, probably due to metabolic rate depression, without any significant changes in the amounts of H4ac in diet-response genes. This response was quickly recovered with one day refeeding. Unusual epigenetic unresponsiveness to diet conditions may serve as an effective adaptation

strategy to minimize energy demands during fasting and to quickly respond to refeeding.

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## Table

Table 1. Changes of body weight, liver weight, and serum parameters in fed, fasted and refed *Xenopus laevis*.

| | Fed group | Fasted group | Refed group |
|---|---|---|---|
| | 22 days | 22 days | one day after 21 days fasting |
| Body weight (g) | | | |
| Start of experiment | 44.22 ± 0.74 [a] | 44.00 ± 0.89 [a] | 43.88 ± 0.58 [a] |
| End of experiment | 39.52 ± 0.78 [a] | 38.48 ± 1.19 [a] | 37.84 ± 0.58 [a] |
| Liver | | | |
| Wet weight (g) | 2.11 ± 0.14 [a] | 1.58 ± 0.09 [b] | 1.91 ± 0.13 [ab] |
| Hepatosomatic index (%) | 5.3 ± 0.3 [a] | 4.1 ± 0.3 [b] | 5.1 ± 0.3 [a] |
| Blood | | | |
| Glucose (mg/dL) | 48.25 ± 3.71 [a] | 31.02 ± 2.15 [b] | 39.09 ± 3.95 [ab] |
| Triglyceride (mg/dL) | 223.00 ± 28.64 [a] | 81.00 ± 9.16 [b] | 122.65 ± 12.02 [b] |
| Cholesterol (mg/dL) | 157.32 ± 19.28 [a] | 100.71 ± 5.89 [b] | 93.78 ± 9.36 [b] |
| Free fatty acid (mEq/L) | 1.22 ± 0.17 [a] | 0.49 ± 0.04 [b] | 0.60 ± 0.06 [b] |

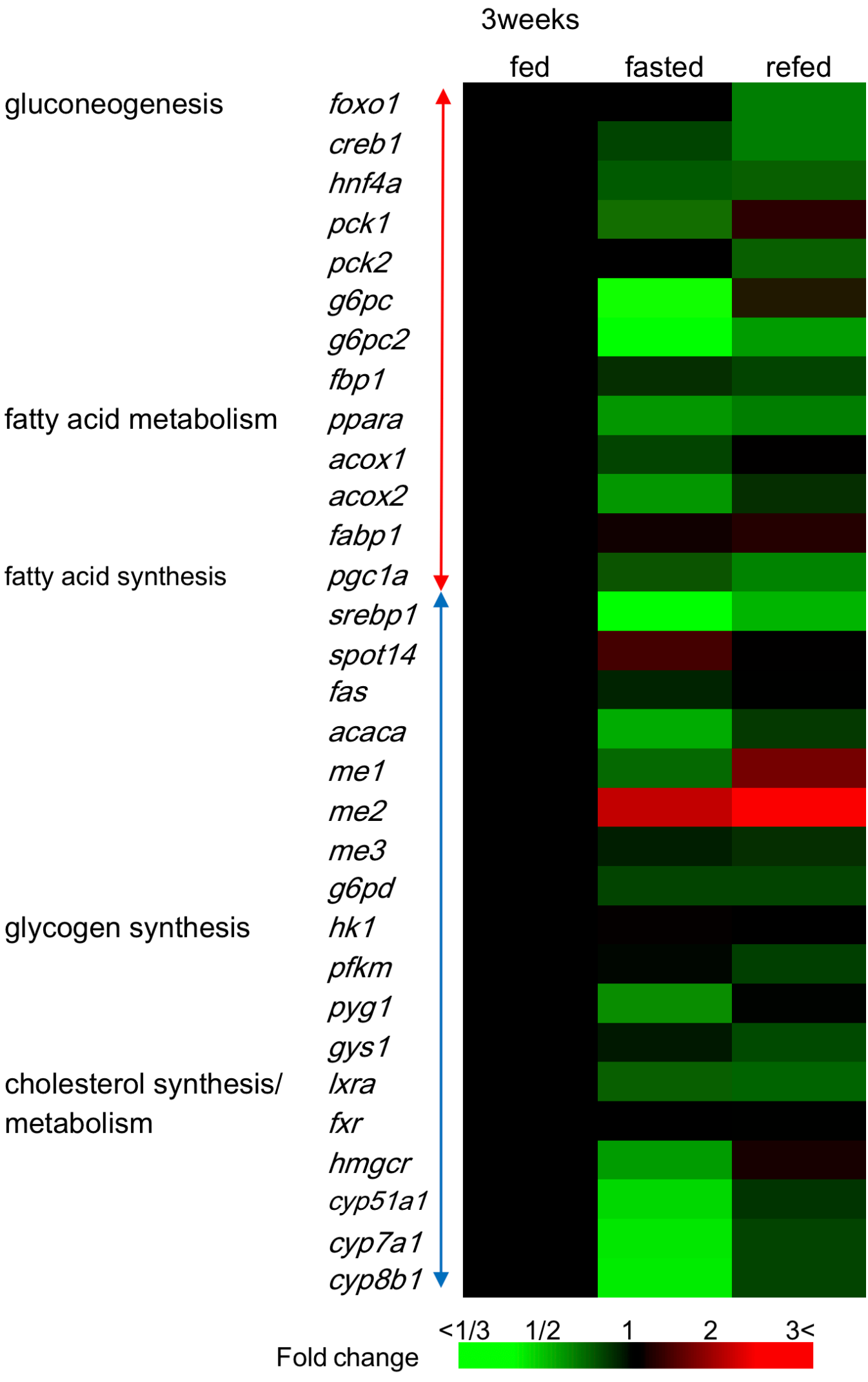
Values presented are means ± SEM ($n$ = 8). Different letters indicate significant differences between the groups ($p < 0.05$).

**Figure legends**

Fig. 1 Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of gene transcripts in the livers of fed, fasted and refed *X. laevi*s. RNAs were prepared from the liver of the frogs that were fed for 22 days (*fed, control*), fasted for 22 days (*fasted*), or fasted for 21 days and then refed for one day (*refed*), followed by RT-qPCR ($n$ = 8). These experiments were repeated at least two times. Transcripts of 31 genes were divided to the following categories: gluconeogenesis (eight genes), fatty acid metabolism (four genes), fatty acid synthesis (nine genes), glycogen synthesis/glycolysis (four genes) and cholesterol synthesis/metabolism (six genes). Color scale indicates fold changes of gene expression. Vertical red and blue arrows indicate genes mainly involved in fasting and feeding signaling pathways, respectively. Full names of the genes tested, the exact fold changes and SEM are shown in Supplementary Table 1.

Fig. 2 Whole cellular histone modifications in the livers of the fed, fasted and refed frogs. (*A*) Western blotting analyses of liver homogenates (three different samples/each group) with indicated antibodies. (*B*) Quantification of band intensities of western blotting analyses. Band intensities were analyzed and expressed relative to H4 pan or H3 pan. Each value is the mean ± SEM ($n$ = 3). Different letters denote significantly different means ($p < 0.05$). These experiments were repeated at least three times.

Fig. 3 Relationship in the diet-response genes between the amounts of transcripts and histone acetylation marks. (*A*) Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis of gene transcripts (*acox2*, *cyp7a1* and *g6pc2*). RNAs were prepared from the liver of the frogs that were fed for 22 days (*fed, control*), fasted for 22 days (*fasted*), or fasted for 21 days and then refed for one day (*refed*), followed by RT-qPCR ($n = 8$). The results for each sample were normalized by the corresponding *rpl8* transcript amount. (*B*) Histone H4 acetylation of *acox2, cyp7a1* and *g6pc2* genes in the liver of fed, fasted and refed *X. laevi*s. (*C*) Histone H4 pan of *acox2, cyp7a1* and *g6pc2* genes in the liver of fed, fasted and refed *X. laevi*s. Signals of ChIP on *acox2, cyp7a1* and *g6pc2* were detected by qPCR ($n = 8$). Each signal was converted to the percentage of the signal for input DNA. These experiments were repeated at least three times, with similar results. Different letters denote significantly different means ($p < 0.05$).


3weeks
fed
fasted
refed
gluconeogenesis
foxo1
creb1
hnf4a
pck1
pck2
g6pc
g6pc2
fbp1
fatty acid metabolism
ppara
acox1
acox2
fabp1
fatty acid synthesis
pgc1a
srebp1
spot14
fas
acaca
me1
me2
me3
g6pd
glycogen synthesis
hk1
pfkm
pyg1
gys1
cholesterol synthesis/
metabolism
lxra
fxr
hmgcr
cyp51a1
cyp7a1
cyp8b1
<1/3
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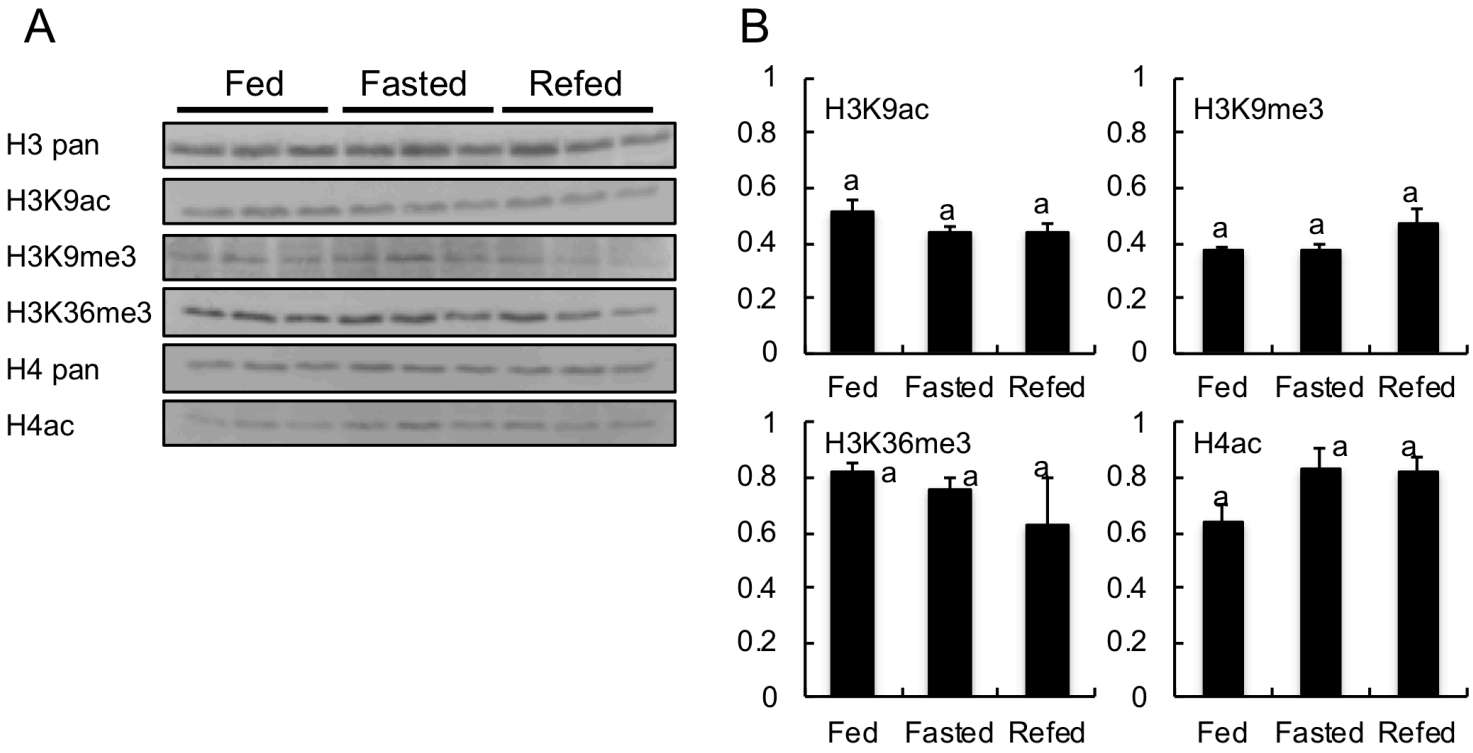
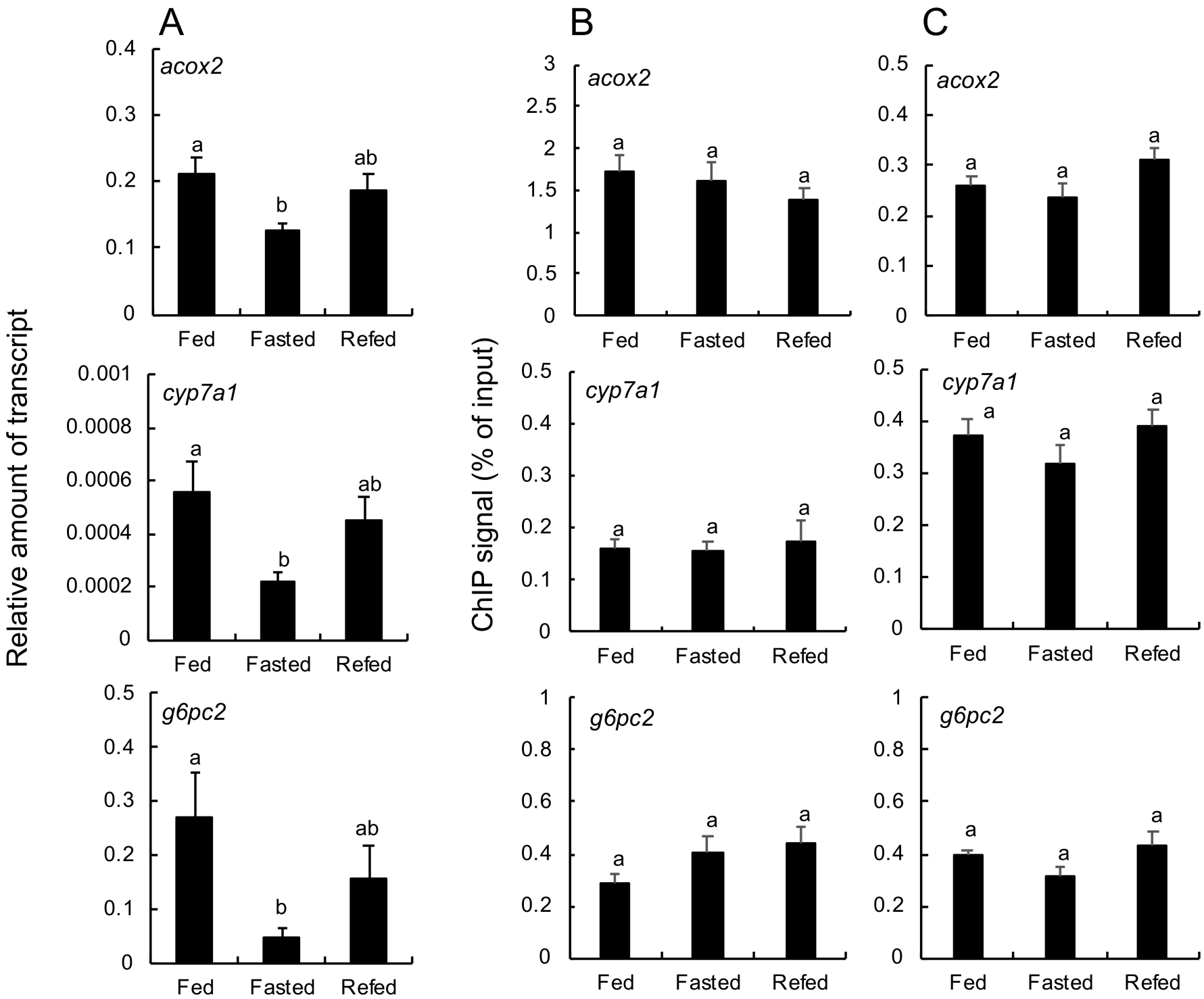
1
2
3<
Fold change


A
Fed
Fasted
Refed
H3 pan
H3K9ac
H3K9me3
H3K36me3
H4 pan
H4ac
B
H3K9ac
H3K9me3
H3K36me3
H4ac
1
0.8
0.6
0.4
0.2
0
a
Fed
Fasted
Refed


A
B
C
Relative amount of transcript
ChIP signal (% of input)
acox2
cyp7a1
g6pc2
Fed
Fasted
Refed
a
b
ab