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ABSTRACT

Aim. – In hepatocyte, PPARα and insulin receptor (IR) are critical for the transcriptional responses to fasting and feeding, respectively. Here we analyzed the effects of the nutritional status (fasting *vs* feeding) on the expression of a large panel of hepatokines in hepatocyte-specific PPARα ($Ppar\alpha^{hep-/-}$) and IR ($IR^{hep-/-}$) null mice.

Methods. – $Ppar\alpha^{hep-/-}$, and $IR^{hep-/-}$ mice and their wild-type littermate were subjected to fasting or feeding metabolic challenges, and then analyzed for hepatokine gene expression. Experiments were conducted in mice of both sexes.

Results. – Our data confirmed that PPARα is critical for regulating fasting-induced *Fgf21* and *Angptl4* expression. In mice lacking PPARα, fasting led to an increased *Igfbp1* and *Gdf15* expression. In the absence of hepatic IR, feeding induced overexpression of *Igfbp1*, follistatin (*Fst*), and adropin (*Enho*). We also observed reduced activin E (*Inhbe*) expression in $IR^{hep-/-}$ mice. Sex only had a modest influence on hepatokine gene expression in the liver.
Conclusion. – The present results highlight the potential roles of hepatokines as a class of hormones that substantially influence nutritional regulation in both females and males.

Keywords: Hepatokines, PPARα, Insulin receptor, Nutritional regulations

Introduction

The liver exhibits highly flexible metabolic activity, which requires major transcriptional regulation. During fasting, hepatocytes show elevated expression of thousands of genes, including those involved in fatty acid catabolism and ketogenesis. This catabolic adaptation to starvation strongly relies on transcriptional control by the nuclear receptor peroxisome proliferator-activated receptor α (PPARα) [1]. In response to feeding, insulin stimulates hepatic synthesis of glycogen and fatty acids. This anabolic response to insulin also includes extensive changes in the expression of various genes, including transcription factors such as forkhead box protein O1 (FoxO1) and sterol regulatory element binding protein 1c (Srebp1c) [2].

In addition to altering the expression of rate-limiting metabolic enzymes, fasting and feeding also influence the synthesis and secretion of numerous proteins, including hepatokines. Hepatokines are proteins secreted from the hepatocytes, which act as hormones and are involved in autocrine, paracrine, and endocrine signaling. The first discovered and most well-known hepatokine is fibroblast growth factor 21 (Fgf21), which is strongly induced in response to fasting through a mechanism involving transcriptional control via PPARα [3]. Since the discovery of Fgf21, several other hepatokines have been identified and shown to be involved in many endocrine regulations. Some of these hepatokines are related to the pathogeny of type 2 diabetes and other metabolic disorders, such as non-alcoholic liver fatty diseases [4]. Such hepatokines include not only Fgf21 [5] but also adropin [6], Angptl4 [7], fetuin-A [8], fetuin-B [9], hepassocin [10], Lect2 [11], follistatin [12], Gdf15 [13], Rbp4 [14] and selenoprotein P [15]. The transcriptional control of Fgf21 expression in hepatocytes has been extensively studied [3] and shown to be highly dependent on nutritional regulations [16,17].

In the present study, we analyzed the respective effects of fasting and feeding on the hepatic expression of a large panel of hepatokines identified to date. Since PPARα and the insulin receptor (IR) are critical for the hepatic transcriptional responses to fasting and feeding, respectively, we analyzed hepatokine gene expression in liver tissue from mice with hepatocyte-specific deletion of either PPARα ($Ppar\alpha^{hep-/-}$) or IR ($IR^{hep-/-}$). Given the well-established sexual dimorphism in liver and metabolic homeostasis [18], we performed these analysis in both sexes. Our data thus provide an overview of hepatokine regulation *via* PPARα and IR signaling, according to the nutritional status.

Materials and methods

Mice

In vivo studies were conducted following the EU guidelines for the use and care of laboratory animals, and protocols were approved by an independent local ethics committee. *Pparα*$^{hep-/-}$ animals were created at INRA's rodent facility (Toulouse, France) as previously described by Montagner et al. [19]. To generate the hepatocyte-specific IR knockout mouse line [20], mice carrying the LoxP sites flanking the fourth exon of the *IR* gene (*IR*$^{lox/lox}$ stock number: 006955; Jackson Laboratory, USA) were intercrossed with C57BL/6J mice, which specifically express the Cre recombinase in the liver under the transthyretin promoter (TTR-Cre^{Tam} mice), as previously described by Nemazanyy et al. [20]. Mice were fed a standard rodent diet (Safe 04 U8220G10R). Mice were housed with controlled temperature (21–23°C) and light (12-h light/12-h dark). The lights were turned on at Zeitgeber time (ZT) 0, and turned off at ZT12. Twelve-week-old male and female mice were sacrificed at ZT16 either fed *ad libitum* or fasted for 20 h with free access to water. Female estrus cycle was synchronized using male urine, so that all females were in proestrus or estrus at the time of sacrifice.

Immunoblotting

Frozen liver, white and brown adipose tissue samples were homogenized in lysis buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1% NP-40, 0.25% Sodium Deoxycholate, 0.1% SDS, 2 mM EDTA and 1 mM PMSF) supplemented with 2 µg/mL apropotinin, 5 µg/mL leupeptin and 1 mM sodium orthovanadate for 30 min at 4°C. Samples were then centrifuged at 13000 rpm for 30 minutes at 4°C. Supernatants were collected and protein concentration in each lysate was measured using BC Assay Protein Quantitation Kit (Interchim). Proteins (50 µg) were separated by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were incubated with primary antibodies against insulin receptor β (sc-57342, Santa Cruz Biotechnology) and β-actin (4970, Cell Signaling) overnight at 4°C and then with horseradish peroxidase-conjugated secondary antibodies (anti-mouse and anti-rabbit, respectively) for 1h at room temperature. Immunoreactive proteins were detected with Clarity Western ECL (Bio-Rad) according to the manufacturer's instructions and signals were acquired using Chemidoc touch imaging system (Bio-Rad).

Blood and tissue samples

Prior to sacrifice, the submandibular vein was lanced, and blood was collected into EDTA-coated tubes (BD Microtainer, K2E tubes). Plasma was prepared by centrifugation (1500 × *g*, 10 min, 4°C) and stored at −80°C. Mice were killed by cervical dislocation, and organs were removed, weighed, dissected (when necessary), and prepared for histological analysis or snap-frozen in liquid nitrogen and stored at −80°C.

Gene expression analysis

Total cellular RNA was extracted using Tri reagent (Molecular Research Center). Total RNA samples (2 μg) were reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) for real-time quantitative polymerase chain reaction (qPCR) analyses. Supplementary Table 1 presents the primers used for SYBR Green assays. Amplifications were performed using a Stratagene Mx3005P (Agilent Technology). The qPCR data were normalized to the level of *TATA-box binding protein* (*Tbp*) transcripts, and analyzed using LinRegPCR.22.

Biochemical analysis

Plasma samples were assayed for free fatty acid, triglyceride, total cholesterol, and low-density lipoprotein (LDL) cholesterol levels using a COBAS-MIRA + biochemical analyzer (Anexplo facility).

Measurement of blood glucose and ketone bodies

Blood glucose was measured using an Accu-Chek Go glucometer (Roche Diagnostics). β-hydroxybutyrate content was measured using Optium β-ketone test strips that carried Optium Xceed sensors (Abbott Diabetes Care).

Statistical analysis

Data were analyzed using GraphPad Prism 7. Differential effects were assessed on natural logarithm (ln)-transformed data by performing analyses of variance, followed by Student's *t*-tests with a pooled variance estimate. *P*-values from *t*-tests were adjusted with Sidak's correction. A *P*-value of <0.05 was considered significant.

Results

Male and female *Pparα*$^{hep-/-}$ mice and their *Pparα*$^{hep+/+}$ littermates were sacrificed after being fasted for 20 hours or fed *ad libitum*. First, we confirmed the deletion of *Pparα*

and its reduced activity in the liver of *Pparα*$^{hep-/-}$ mice by measuring *Pparα* mRNA and the expression of *Cyp4a10,* one of the most specific PPARα target genes (Fig. 1A). Body and liver weights, presented in Fig. 1B were not dependent on genotype. As expected, fasted *Pparα*$^{hep-/-}$ mice of both sexes exhibited significantly reduced ketone body levels compared to fasted *Pparα*$^{hep+/+}$ mice (Fig. 1C). Except for LDL-cholesterol in males, plasma lipids were not significantly different between genotypes (Fig. 1C). We then confirmed that *Fgf21* expression was induced by fasting, through a mechanism dependent on hepatocyte PPARα (Fig. 2A and B). Similar results were observed for the hepatokines *Igfbp2*, *Angptl4* and *Inhbe* (Fig. 2A, 2B, S1). Interestingly, the expression of *Gdf15* and *Igfbp1* was upregulated in liver of fasted *Pparα*$^{hep-/-}$ mice (Fig. 2B), irrespective of sex. We identified *Serpinb1a* as upregulated in the liver of fed *Pparα*$^{hep-/-}$ mice, most significantly in females (Fig. S1). Fig. S1 presents the detailed relative expression levels of other hepatokines.

Male and female *IR*$^{hep-/-}$ mice and their *IR*$^{hep+/+}$ littermates were also sacrificed after being fasted for 20 hours or fed *ad libitum*. We first confirmed the specific deletion of *IR* in hepatocytes by assessing its reduced expression in the liver but not in white and brown adipose tissues (Fig. 3A). We also evaluated the expression of *Fasn,* a well-known insulin sensitive gene, in the liver of *IR*$^{hep+/+}$ and *IR*$^{hep-/-}$ mice and observed a profile consistent with the deletion of IR (Fig. 3B). Body and liver weights are presented in Fig. 3C. Fed male *IR*$^{hep-/-}$ mice exhibited significant hyperglycemia compared to *IR*$^{hep+/+}$ mice (Fig. 3D). In contrast, no difference in fasting and/or fed blood glucose was observed in female mice from both genotypes. Total plasma cholesterol concentration was reduced in both male and female *IR*$^{hep-/-}$ mice as compared to their respective *IR*$^{hep+/+}$ littermates (Fig. 3D). Moreover, we found that the expression of *Pparα* itself and one of its specific target gene *Cyp4a10* was not altered upon deletion of *IR* in the liver (Fig. 3E). We also analyzed hepatokine gene expression in response to fasting in both sexes (Fig. 4A). The fasting-mediated induction of *Fgf21* was not dependent on hepatic IR. Upon fasting, *Igfbp1* was upregulated in *IR*$^{hep-/-}$ mice from both sexes (Fig. 4B), while *Igfbp2* expression was only upregulated in *IR*$^{hep+/+}$ mice (Fig. 4A). Under fed condition, hepatocyte IR deletion led to an increased expression of *Igfbp1, Igfbp2, Enho* (adropin), *Fst* (follistatin), *Fetuin-a*, *Fetuin-b*, *Fgl* (hepassocin), *Postn* (periostin), *Rbp4*, *Sepp1* (selenoprotein) and *Serpinb1a* and to a reduced expression of *Inhbe* (activin E), *Angptl8, Lect2* and *Tsku* (tsukushi) (Fig. 4B, S2). Fig. S2 presents the detailed relative expression levels of other hepatokines.

Discussion

During fasting and feeding, the liver adjusts its metabolism via extensive regulation of gene expression. Fasting and feeding also strongly impact on protein expression and secretion by hepatocytes. The affected proteins include hepatokines, which act as hepatic hormones with systemic effects on metabolism, growth, behavior, and fertility [4]. Here we investigated the potential regulation of hepatokines by two receptors that play critical roles in metabolic homeostasis, namely PPARα and IR.

PPARα is a nuclear receptor that orchestrates a broad range of fasting-induced changes in hepatic gene expression, notably, playing a critical role in regulating the expression of the hepatokines Angptl4 [7] and Fgf21 [3,19]. Our present data confirm that fasting induces *Fgf21* and *Angptl4* expression in a PPARα-dependent manner. Importantly, we found that fasting also induced *Gdf15* and *Igfbp1* expression in $Ppar\alpha^{hep-/-}$ mice. Previous studies reported the fasting-mediated induction of *Fgf21*, *Gdf15*, and *Igfbp1* expression in mice with defective fatty acid oxidation in hepatocytes resulting from carnitine palmitoyltransferase 2 (CPT2) deficiency [21]. Our current data support these observations and reveal that, unlike *Fgf21*, both *Gdf15* and *Igfbp1* can be induced in the absence of hepatic PPARα. *Igfbp1* [22,23] and *Gdf15* [24] are known to be induced by amino acid deprivation. Therefore, their PPARα-independent increase in response to fasting may occur as a consequence of amino acid restriction. Moreover, this response was enhanced when PPARα was inactive. In the fasting state, this suggests the existence of at least one PPARα-independent mechanism in hepatocytes, such that the overexpression of *Gdf15* and *Igfbp1* triggers a systemic hormetic response in the setting of defective liver fatty acid oxidation. This response might be involved in the critical dialogue between the liver and the white adipose tissue during fasting [19]. Our data also first reveal that the absence of hepatocyte PPARα up-regulates serpinB1a which was identified as an insulin sensitive hepatokine regulating pancreatic β-cell proliferation [25], which may contribute to the effect of PPARα on glucose homeostasis [26].

While fasting-induced catabolic pathways are driven by PPARα in hepatocytes, feeding-induced anabolic pathways are triggered by insulin signals through IR, which activates the PI3K-AKT pathway, thereby inhibiting gluconeogenesis while promoting glycogen and lipid synthesis. Here we investigated whether feeding regulates hepatokine expression in hepatocytes through IR-dependent signaling. Igfbp1 is a well-known target of FoxO1 [27], a transcription factor that translocates from the nucleus to the cytoplasm upon

insulin-induced activation of PI3K-AKT signaling. As expected, we found increased *Igfbp1* expression in the absence of IR. Our data also confirm that hepatocyte IR deficiency increases *Serpinb1a* expression [25]. Similarly, the lack of IR in hepatocytes promoted the expression of follistatin (*Fst*), another FoxO1-regulated hepatokine, which was recently identified as a regulator of glucose homeostasis through its effect on adipose tissue [12]. We also provide evidence that hepatic IR influenced the expression of two other hepatokines. Adropin (*Enho*), a hepatokine linked to macronutrient intake [6], was upregulated in fed $IR^{hep-/-}$ mice, while activin E (*Inhbe*) was downregulated in liver of these mice. Activin E was recently identified as an important modulator of adipocyte browning and insulin sensitivity [28]. Our observations suggest that increased adropin (*Enho*) and reduced activin E (*Inhbe*) expression in the context of defective hepatic IR signaling may represent, together with elevated follistatin (*Fst*), an additional mechanism through which hepatic insulin resistance promotes peripheral defects in glucose homeostasis. In this study performed during the night, when mice are active and feeding, we observed that IR deficiency in hepatocytes promotes hyperglycemia in males but not in females. This might reflect sexually dimorphic glucose homeostasis [29] but further investigations are required to define the mechanisms underlying this difference. However, in the fed state, hepatokine expression profiles in mice lacking hepatocyte IR are not sexually dimorphic, with the exception of *Igfbp1*. Low levels of IGFBP1 have been associated with incidence of type 2 diabetes [30], suggesting that, in our experimental setting, *Igfbp1* may account, at least in part, for the difference in glycemia in fed $IR^{hep-/-}$ mice between sexes. Finally, fed mice lacking IR in hepatocytes did not show any significant changes in the expression of *Fgf21* and *Angptl4,* suggesting that fasting-induced expression of these hepatokines dependent on PPARα [7,16] is not under direct control by insulin signaling in hepatocytes.

Altogether our data support and underline the potential roles of hepatokines as a class of hormones that strongly influence nutritional regulations in both males and females. Our findings in mouse models lacking hepatocytic PPARα or IR signaling elucidate changes in hepatokine expression under conditions of defective hepatic homeostasis. Hepatokines are potential biomarkers for NAFLD and insulin resistance [4]. Our work suggests that in the fasted state, reduced *Fgf21* combined with *Gdf15* overexpression may reflect impaired hepatocyte PPARα activity, which predisposes to steatosis. While in the fed state, follistatin [12] but also adropin and activin E levels may be associated with hepatic insulin resistance. Further research is needed to test whether hepatic expression profile correlates with the

circulating level of these hormones and to investigate the clinical relevance of these observations.

Legends

Fig. 1. Male and female wild-type (PPARα$^{hep+/+}$) and hepatocyte-specific knockout (PPARα$^{hep-/-}$) mice were fed *ad libitum* or fasted for 20 h and then sacrificed. In female mice, the estrus cycle was synchronized in proestrus or estrus at the time of sacrifice. **A.** Relative gene expression of *Pparα* and of a specific PPARα target gene *Cyp4a10* from liver samples of PPARα$^{hep+/+}$ and PPARα$^{hep-/-}$ mice. **B.** Body weight and relative liver weight measured at the time of sacrifice. **C.** Quantification of plasma glucose (glycemia), ketone bodies (ketonemia), free fatty acids (FFA), triglycerides (TG), total cholesterol, and low-density lipoprotein (LDL) cholesterol. Data shown are mean ± SEM. # significant nutritional status effect, * genotype effect, # <0.05, ## <0.01, ### <0.001.

Fig. 2. Male and female wild-type (PPARα$^{hep+/+}$) and hepatocyte-specific knockout (PPARα$^{hep-/-}$) mice were fed *ad libitum* or fasted for 20 h, and then sacrificed (n=6/group). In females, the estrus cycle was synchronized in proestrus or estrus at the time of sacrifice. **A.** Heat map representing qRT-PCR data (Z-scores) for hepatokine genes from liver samples, according to genotype (PPARα$^{hep-/-}$ or PPARα$^{hep+/+}$) and nutritional condition in males and females. **B.** Mean ± SEM of the relative gene expressions of *Fgf21, Angptl4, Gdf15*, and *Igfbp1* measured by qRT-PCR in PPARα$^{hep-/-}$ or PPARα$^{hep+/+}$ mice. # significant nutritional status effect, * genotype effect, # <0.05, ## <0.01, ### <0.001.

Fig. 3. Male and female wild-type (IR$^{hep+/+}$) and hepatocyte-specific knockout (IR$^{hep-/-}$) mice were fed *ad libitum* or fasted for 24 h and then sacrificed. In female mice, the estrus cycle was synchronized in proestrus or estrus at the time of sacrifice. **A.** IR levels was determined by Western blot analysis from liver, white and brown adipose tissue (WAT and BAT) samples. B. Relative gene expression of *Fasn* from liver samples of IR$^{hep+/+}$ and IR$^{hep-/-}$ mice. **C.** Body weight and relative liver weight measured at time of sacrifice. **D.** Quantification of plasma glucose (glycemia), ketone bodies (ketonemia), free fatty acids (FFA), triglycerides (TG), total cholesterol, and low-density lipoprotein (LDL) cholesterol. **E.** Relative gene expression of *Pparα* and of a specific PPARα target gene *Cyp4a10* from liver samples of IR$^{hep+/+}$ and

$IR^{hep-/-}$ mice. Data shown are mean ± SEM. # significant nutritional status effect, * genotype effect, # <0.05, ## <0.01, ### <0.001.

Fig. 4. Male and female wild-type ($IR^{hep+/+}$) and hepatocyte-specific knockout ($IR^{hep-/-}$) mice were fed *ad libitum* or fasted for 20 h, and then sacrificed (n=6/group). In females, the estrus cycle was synchronized in proestrus or estrus at the time of sacrifice. **A.** Heat map representing qRT-PCR data (Z-scores) for hepatokine genes from liver samples, according to genotype ($IR^{hep-/-}$ or $IR^{hep+/+}$) and nutritional condition in males and females. **B.** Mean ± SEM of the relative gene expressions of *Igfbp1*, *Fst, Enho,* and *Inhbe* measured by qRT-PCR in $IR^{hep-/-}$ or $IR^{hep+/+}$ mice. # significant nutritional status effect, * genotype effect, # <0.05, ## <0.01, ### <0.001.

Supplementary data Fig. S1. Relative gene expression of *Angptl6, Angptl8, Ctsd, Enho, Fetuin a, Fetuin b, Fgl1, Fst, Igf1, Igfbp2, Igfbp3, Inhbe, Lect2, Postn, Rbp4, Sepp1, Serpinb1a* and *Tsku* measured by qRT-PCR in $PPAR\alpha^{hep+/+}$ and $PPAR\alpha^{hep-/-}$ mice. Data shown are mean ± SEM. # significant nutritional status effect, * genotype effect, # <0.05, ## <0.01, ### <0.001.

Supplementary data Fig. S2. Relative gene expression of *Angptl4, Angptl6, Angptl8, Ctsd, Fetuin a, Fetuin b, Fgf21, Fgl1, Gdf15, Igf1, Igfbp2, Igfbp3, Lect2, Postn, Rbp4, Sepp1, Serpinb1a* and *Tsku* measured by qRT-PCR in $IR^{hep+/+}$ and $IR^{hep-/-}$ mice. Data shown are mean ± SEM. # significant nutritional status effect, * genotype effect, # <0.05, ## <0.01, ### <0.001.

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Figure 1

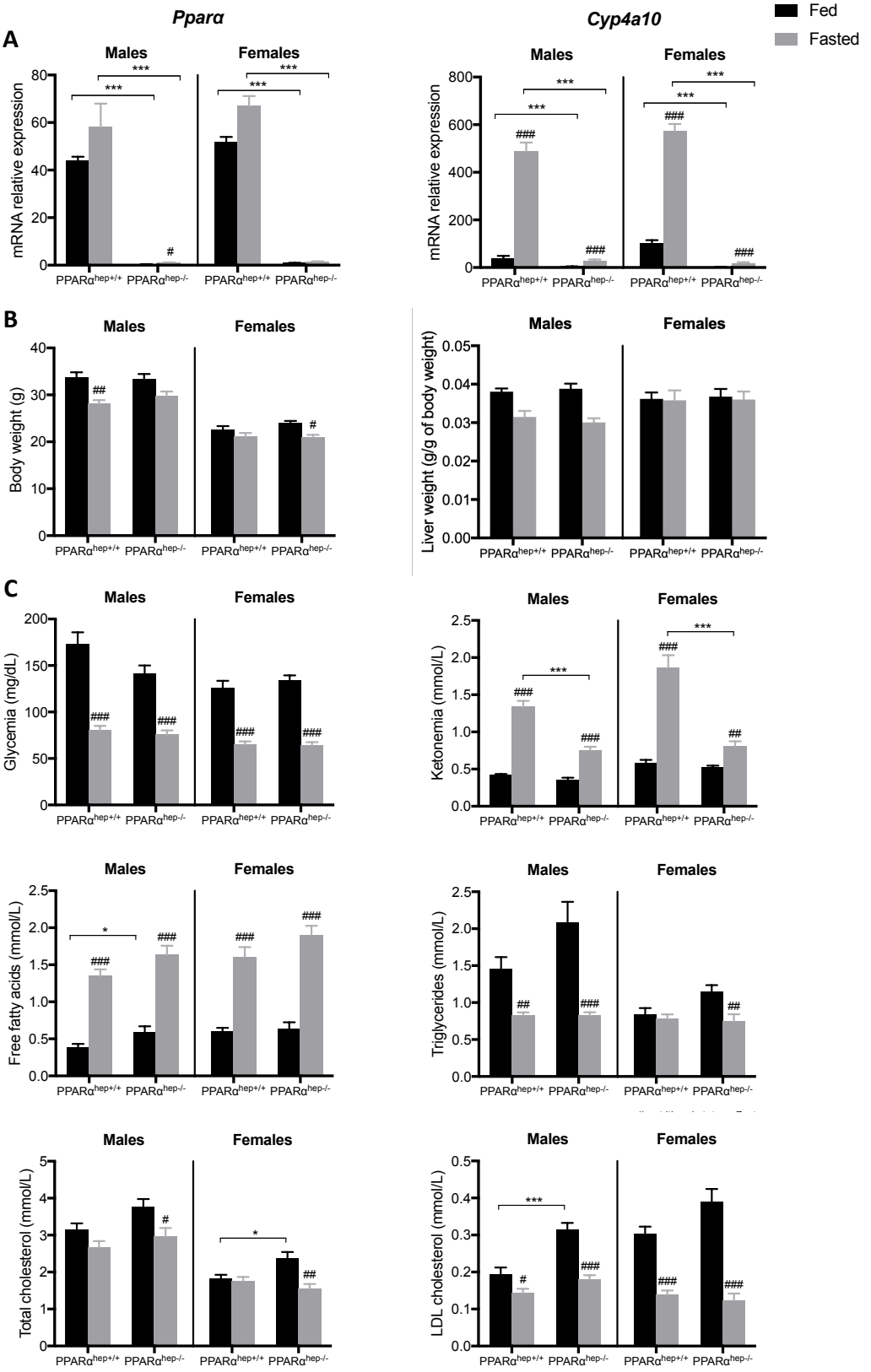
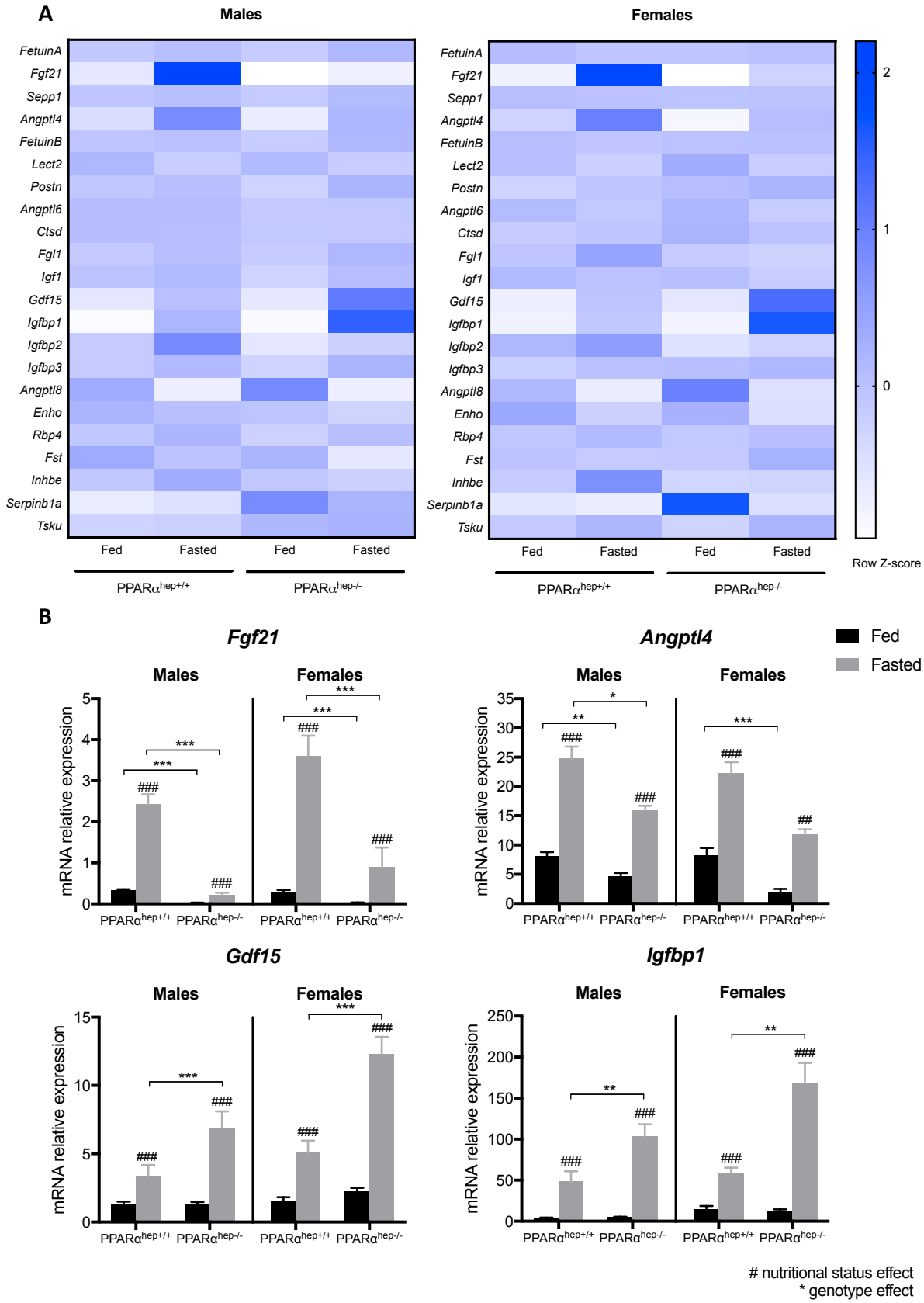

Ppara
Cyp4a10
Fed
Fasted
A
Males
Females
mRNA relative expression
B
Body weight (g)
Liver weight (g/g of body weight)
C
Glycemia (mg/dL)
Ketonemia (mmol/L)
Free fatty acids (mmol/L)
Triglycerides (mmol/L)
Total cholesterol (mmol/L)
LDL cholesterol (mmol/L)
PPARα hep+/+
PPARα hep-/-
nutritional status effect
* genotype effect

Figure 2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Females
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Fgf21
Sepp1
Angptl4
FetuinB
Lect2
Postn
Angptl6
Ctsd
Fgl1
Igf1
Gdf15
Igfbp1
Igfbp2
Igfbp3
Angptl8
Enho
Rbp4
Fst
Inhbe
Serpinb1a
Tsku
Fed
Fasted
PPARα hep+/+
PPARα hep-/-
2
1
0
Row Z-score
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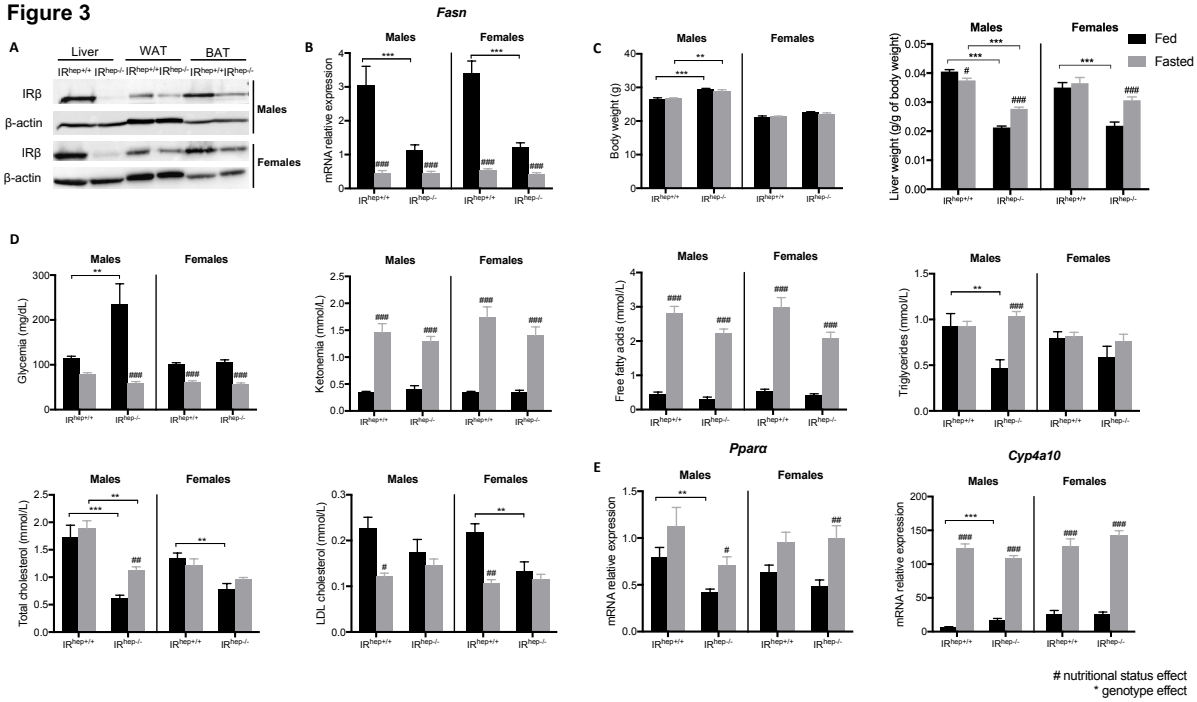
Gdf15
Igfbp1
Fed
Fasted
mRNA relative expression
nutritional status effect
* genotype effect

Figure 3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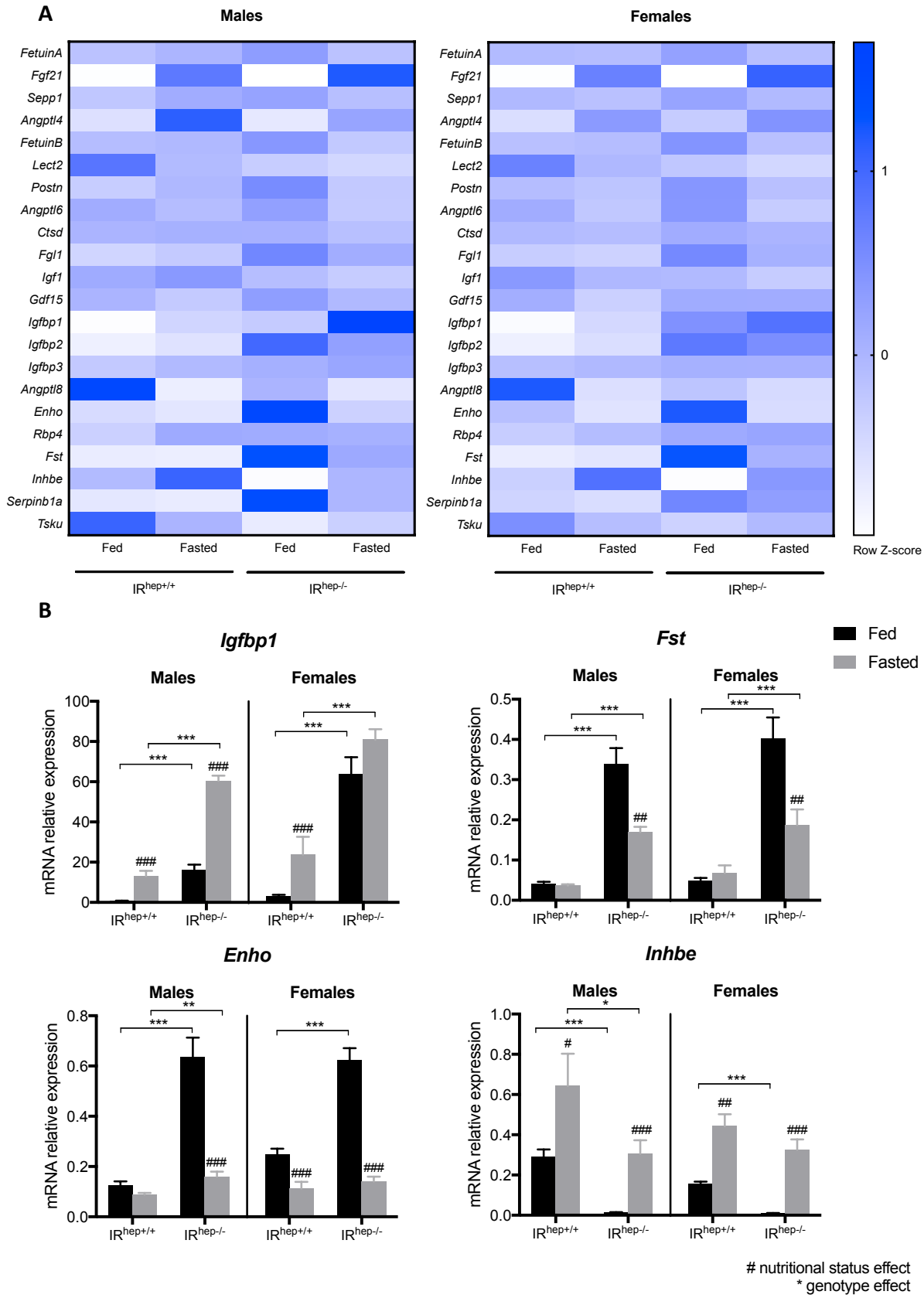
Liver
WAT
BAT
IRβ
β-actin
Males
Females
B
Fasn
mRNA relative expression
C
Body weight (g)
Liver weight (g/g of body weight)
Fed
Fasted
D
Glycemia (mg/dL)
Ketonemia (mmol/L)
Free fatty acids (mmol/L)
Triglycerides (mmol/L)
Total cholesterol (mmol/L)
LDL cholesterol (mmol/L)
E
Pparα
Cyp4a10
mRNA relative expression
nutritional status effect
* genotype effect

Figure 4

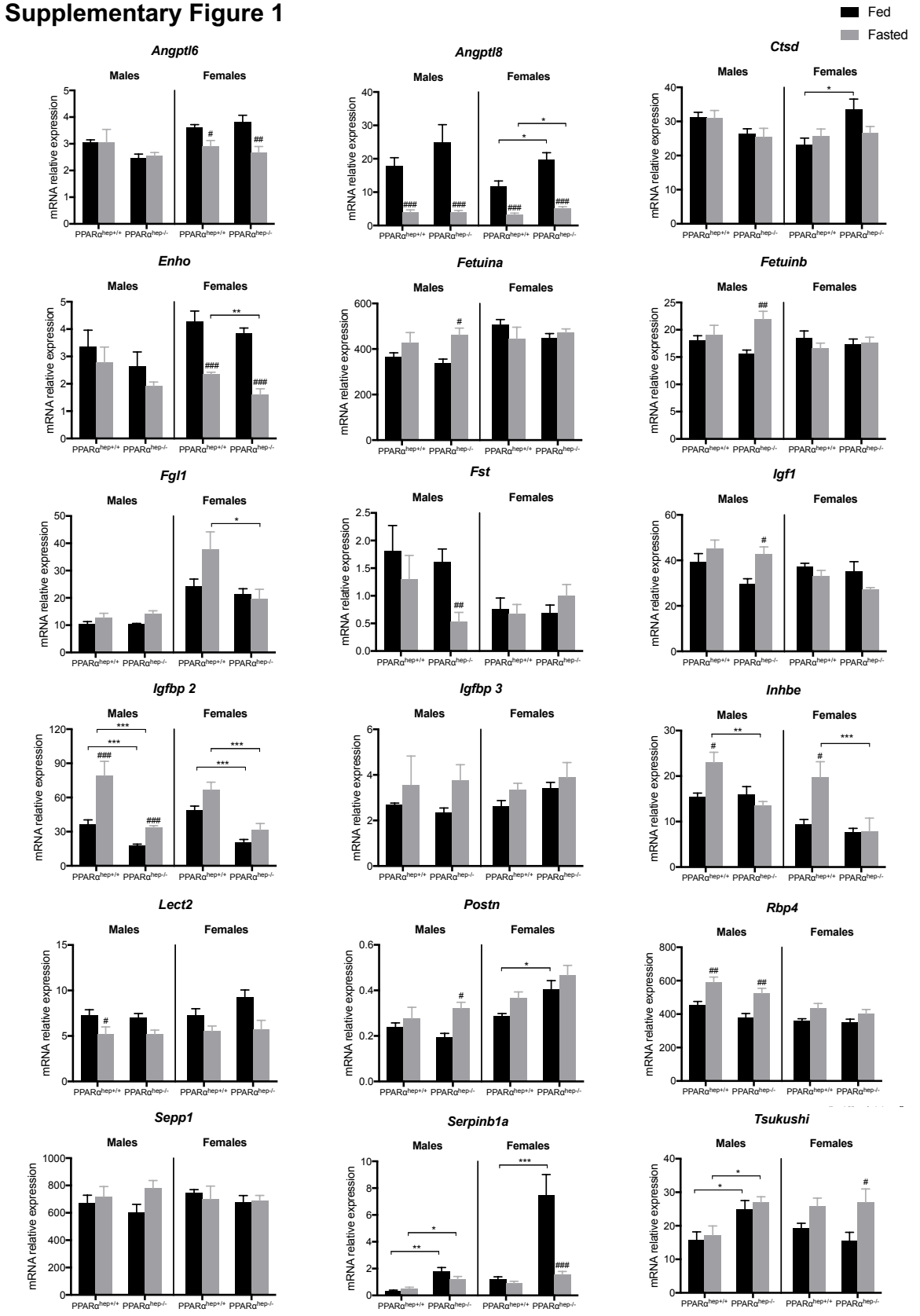

A
Males
Females
FetuinA
Fgf21
Sepp1
Angptl4
FetuinB
Lect2
Postn
Angptl6
Ctsd
Fgl1
Igf1
Gdf15
Igfbp1
Igfbp2
Igfbp3
Angptl8
Enho
Rbp4
Fst
Inhbe
Serpinb1a
Tsku
Fed
Fasted
IRhep+/+
IRhep-/-
1
0
Row Z-score
B
Igfbp1
Fst
Enho
Inhbe
Fed
Fasted
mRNA relative expression
nutritional status effect
* genotype effect

Gene	NCBI Refseq	Forward primer	Reverse primer
Angptl4 [7]	NM_020581.2	TTTCCCTGCCCTTCTCTACTTG	TACAGGTACCAAACCACCAGCC
Angptl6 [31]	NM_145154.2	GAATTGCCGCAAACCTCACT	ATGGCCGTCACCTCTCACAG
Angptl8 [32]	NM_001080940.1	CCCACCAAGAATTTGAGACCTT	ACTGTTGCTGCTCTGCCATCT
Ctsd [33]	NM_009983	CTTCGTCCTCCTTCGCGATTAT	GTCCGACGGATAGATGTGAACTT
Cyp4a10	NM_010011	TCCAGCAGTTCCCATCACCT	TTGCTTCCCCAGAACCATCT
Enho [6]	NM_027147	ACCGGGCTCAACTCAGGC	TGGCTGTCCTGTCCACACAC
Fasn	NM_007988	AGTCAGCTATGAAGCAATTGTGGA	CACCCAGACGCCAGTGTTC
Fetuina [8]	NM_013465	ATCGACAAAGTCAAGGTGTGGTCT	TGTCAACTTCCATCTCATACACCACT
Fetuinb [9]	NM_021564	CTCGTCAAAGTCACCAAGGCTAT	CACATAGTAAGCAGGGCCAGAC
Fgl1 [10]	NM_145594.2	TGCAAACCTGAACGGTGTTTAC	TTCAAGGAATACCACCACCCA
Fgf21 [34]	NM_020013.4	AAAGCCTCTAGGTTTCTTTGCCA	CCTCAGGATCAAAGTGAGGCG
Fst [12]	NM_008046.2	TGCTGCTACTCTGCCAGTTCAT	CACTCTTCCTTGCTCAGTTCTGTC
Gdf15 [35]	NM_011819	GCTGTCCGGATACTCAGTCCA	TTGACGCGGAGTAGCAGCT
Igf1 [36]	NM_0105124	GATCTGCCTCTGTGACTTCTTGAA	CAGGTAGAAGAGGTGTGAAGACGA
Igfbp1 [36]	NM_008341.4	CCTGCCAACGAGAACTCTAT	AGGGATTTTCTTTCCACTCC
Igfbp2 [36]	NM_008342	GCATGGCCGGTACAACCTTA	GCTGTCCGTTCAGAGACATCTT
Igfbp3 [36]	NM_008343	CAGGCAGCCTAAGCACCTAC	CTCCTCGGACTCACTGATGTTTC
Inhbe [28]	NM_008382.3	TCAGCTTTGCTACCATCATAGACA	CATGGAGCGGTAGGTTGAAGT
Lect2 [11]	NM_010702	GTGGACAGTACTCTGCTCAAA	TCCCAGTGAATGGTGCATAC
Pparα	NM_011144	CCCTGTTTGTGGCTGCTATAATTT	GGGAAGAGGAAGGTGTCATCTG
Pparα (genotyping)	NM_011144	GTACCACTACGGAGTTC	GAATAGTTCGCCGAAAG
Postn [37]	NM_015784	GAATGCTGCCCTGGCTATATGA	AATGCCCAGCGTGCCATAAA
Rbp4 [38]	NM_011255	GCCAAGTTCAAGATGAAGTACTGG	TGTCGTAGTCCGTGTCGATGA
Sepp1[39]	NM_009155	AAGATCGCTTACTGTGAGGAGAGG	GCTGAGGTCACAGTTTTACAGAAGTC
Serpinb1a [25]	NM_025429	GGACGAGTCCACGGGTCTTA	AGTTTGACGTGGACATCAATGAATTC
Tsku [40]	NM_001168541	CCGGTCTAACAGATTTCACGTGT	CAGCAGGAACAGAGAGCACAG
Tbp	NM_013684	ACTTCGTGCAAGAAATGCTGAA	GCAGTTGTCCGTGGCTCTCT

Supplementary Table 1

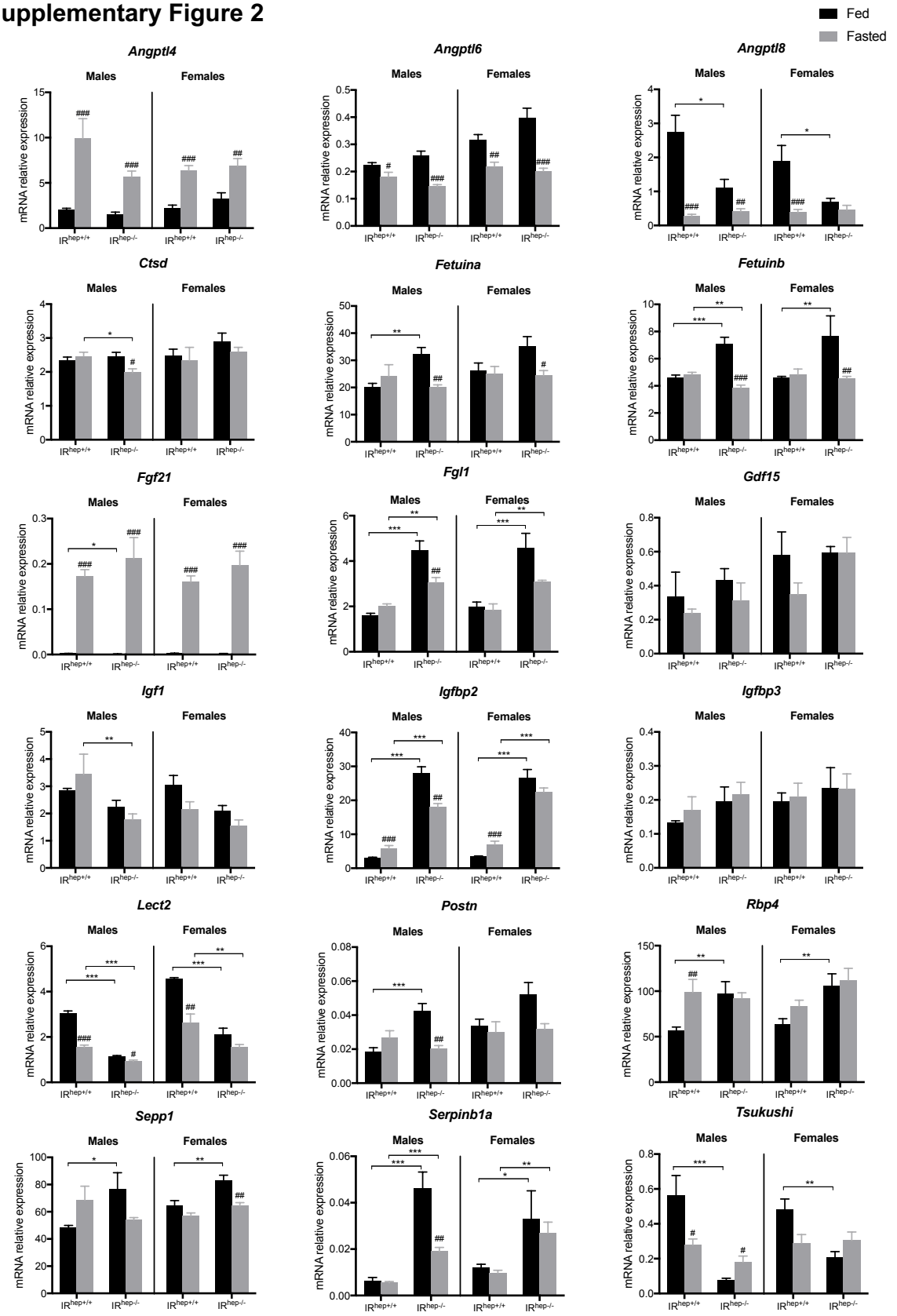
Oligonucleotide sequences for real-time PCR.

Supplementary Figure 1



nutritional status effect
* genotype effect

Supplementary Figure 2



nutritional status effect
* genotype effect