LRH-1 heterozygous knockout mice are prone to mild obesity

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Abstract. Obesity is a global health problem that increases the risk of several common diseases. Liver receptor homologue-1 (LRH-1) has an important role in steroid hormone metabolism, which influences body weight. Whether LRH-1 gene deletion causes obesity is yet to be clarified. In this study using LRH-1 heterozygous knockout (LRH-1+/-) mice, we investigated the role of LRH-1 on body weight gain and glucose and lipid metabolism. LRH-1+/- mice showed mild but significant body weight gains compared with wild-type littermate mice after being fed a high-fat diet. We performed glucose tolerance tests and insulin tolerance tests and did not find any significant differences between wild-type and LRH-1+/- mice. To clarify how LRH-1 gene deletion affects body weight gain, we measured food intake, oxygen consumption, respiratory quotient, spontaneous activity and rectal temperature, and found no significant differences between wild-type and LRH-1+/- mice fed a normal diet and a high-fat diet. The results suggest that heterozygous gene deletion of LRH-1 causes body weight gains without any apparent worsening of glucose and lipid metabolism. Identifying the effects of LRH-1 on body weight will aid in understanding the pathogenesis of obesity.

Key words: Liver receptor homologue-1, Obesity, CYP7A1, CYP8B1, Small heterodimer partner

OBESITY is an increasing clinical and economic problem worldwide [1]. Obese patients have multiple risk factors for cardiovascular diseases, such as glucose intolerance, dyslipidemia, hypertension and hepatic steatosis [2]. The pathogenesis of obesity, including genetic disorders (gene mutation and epigenetics), over-nutrition (fructose and a high-fat diet), and hormonal disorders are known; however, they are as yet not fully clarified [1–5].

We previously reported that single nucleotide polymorphisms in a small heterodimer partner (SHP), NR0B2, are associated with mild obesity in Japanese people [6]. SHP is a member of the nuclear receptor superfamily, and has many functions in the development of cancer and the control of metabolism, including bile acid synthesis, and cholesterol, lipid and glucose homeostasis [7]. These functions are mediated through interactions with other nuclear receptors, such as the liver receptor homologue-1 (LRH-1), NR5A2 [7].

LRH-1 belongs to the NR5A subfamily of the nuclear receptor superfamily [8]. LRH-1 is expressed in endoderm-derived tissues such as the liver, pancreas, and intestine in adults and the developing embryo [8]. LRH-1 plays important roles in cholesterol and steroid hormone metabolism in the liver [8], and regulates bile acid synthesis through a feedback system between bile acid and FXR-SHP-LRH-1 pathways [9–10]. LRH-1 by itself promotes bile acid production through the induction of CYP7A1 and CYP8B1 gene expression. However, bile acid activates FXR and in turn FXR induces SHP gene expression. Consequently, SHP inhibits LRH-1-induced CYP7A1 gene expression. Thus, LRH-1 regulates bile acid metabolism. A study has reported that bile acid induced energy expenditure and prevented obesity in diet-induced obese mice by activating G protein-coupled bile acid receptor 1, TGR5 [11]. Another study reported that LRH-1 is expressed only in preadipocytes and LRH-1 inhibits adipogenesis [12]. However, whether gene deletion of LRH-1 in vivo affects body weight is unclear.

In the present study, using LRH-1 heterozygous knockout mice, we tried to clarify whether LRH-1 in vivo affected mild obesity. Identifying the effects of
LRH-1 on body weight will be beneficial for understanding the pathogenesis of obesity.

**Materials and Methods**

**Mice**

All animal care was approved by the Animal Care Committee of the University of Gifu. Mice were housed at 23°C on a 12-h light/dark cycle. Male mice were used for all studies and all experiments were performed using littermates. Mice had free access to water and were fed an autoclaved CE-2 diet (CLEA Japan, Tokyo, Japan) as the normal chow diet. Quick Fat diet (46.7% carbohydrate, 24.8% protein and 14.4% fat) was purchased from CLEA Japan.

**Genotyping and identification of LRH-1+/− mice**

LRH-1+/− mice were purchased from Lexicon Genetics Inc. (The Woodlands, TX, USA). LRH-1+/− mice were backcrossed for at least 10 generations onto the C57BL/6J background. Genotyping was performed using PCR and was performed on genomic DNA extracted from tail biopsies using a GenElute Mammalian Genomic DNA Miniprep kit (Sigma, Tokyo, Japan). LRH-1+/− mice were detected by PCR analysis using the following primers: F1: 5′-TATCTGGCCACCAACCACATCAGC-3′; R1: 5′-ACGTGAGGAGACCGTAATGGTACC-3′; and R2: 5′-TCCCCTGTAGTATGTTAACATGTT-3′. Primers F1 and R1 predict a 217-bp product for the wild-type allele. Primers F1 and R2 predict a 158-bp product for the null allele. Amplification conditions were: 94°C for 2 min, then 40 cycles of 94°C for 30 s, and 64°C for 30 s, and a final incubation of 72°C for 3 min.

**High-fat diet feeding and tissue preparation**

After genotyping, LRH-1+/+ and LRH-1+/− mice were separated and housed three mice per cage. Body weight was measured weekly between the ages of 5 and 30 weeks. At the start of 15 weeks of age, the normal chow diet was discontinued and mice were fed the Quick Fat diet. Mice were sacrificed at 32 weeks of age by cervical dislocation. Several tissues, including liver, kidney, epididymal fat and brown adipose tissue, were collected and weighed. All tissue samples were immediately placed into liquid nitrogen and stored at −80°C until further analysis by quantitative PCR and for hepatic triglyceride and cholesterol content.

**RNA isolation and quantitative real-time PCR**

To evaluate LRH-1 mRNA expression, LRH-1+/+ and LRH-1+/− mice (n=4) at 10 or 21 weeks old were sacrificed, and collected tissue samples were immediately stored in RNAlater solution (Invitrogen, Tokyo, Japan). We also used livers of mice (n=6) at 32 weeks of age that had been frozen in liquid nitrogen. Total RNA was isolated using an RNeasy Plus Universal Mini kit (Qiagen, Tokyo, Japan). RNA integrity was checked by electrophoresis using E-Gel EX gels (Invitrogen). Total RNA (500 ng) was reverse transcribed to cDNA using a SuperScript III First-Strand Synthesis System for RT-PCR kit (Invitrogen) primed with random hexamers. The cDNA reaction mixture was diluted to a total volume of 500 μL. We designed Taqman primers for mouse LRH-1, SHP, CYP7A1, CYP8B1 and RNA polymerase II. LRH-1 forward and reverse primers included the regions from exons 5 to 8. Primer and probe sequences used were: SHP F: 5′-TGAGCTGGGTCCCAAAGGAG-3′, probe: 5′-TCCTCTTCAACCCAGATGTGCCAGGC-3′ R: 5′-CCAGTGAGCCTCCTGTTGC-3′; LRH-1; F: 5′-TCTCCTCAACCCAGATGTGCCAGGC-3′, probe: 5′-CGCAGAGAAAACGATGTCCCTACTGTCG-3′ R: 5′-GCATGCGGTCGGCTCTTA-3′; CYP7A1; F: 5′-GGATCAAGAGCAACTAAACA-3′, probe: 5′-CATCATCAAGGAGGCTCTGCGGCTCT-3′ R: 5′-CCATCCTCAAGGTGTAAGTGAAG-3′; CYP8B1; F: 5′-AAGGTGGCTCTCTTCCCCTGGTAGATGTTAACATGTT-3′; RNA polymerase II; F: 5′-CGGGAAGTGCCTAGGGTCA-3′, probe: 5′-CAACTGGTGACAGCAGGTGGTCCTCC-3′ R: 5′-GAGGGAGAGCGGAGTGA-3′. Taqman quantitative PCR analysis was performed using an Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems, Tokyo, Japan) in a 384-well plate. The reaction mixture consisted of 2 × TaqMan Universal PCR Master Mix (Applied Biosystems, Tokyo, Japan), forward and reverse primers, Taqman probe and 5 μL of cDNA in a final volume of 20 μL. PCR amplification was: 2 min incubation at 50°C and 10 min incubation at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. All amplifications were performed in triplicate. The relative amounts of mRNA were calculated using the comparative Ct method. Expression of RNA polymerase II was used as an internal control.
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**Plasma profile of LRH-1+/- mice**

Blood plasma was collected from the retro-orbital venous plexus *ad libitum* or after a 6-h fast. Blood glucose was measured using a FreeStyle Freedom monitoring system (Nipro, Osaka, Japan). Plasma insulin, free fatty acid, triglyceride, total cholesterol, adiponectin, leptin and testosterone levels were determined using commercial assay kits. These were: Glazyme Insulin-EIA test (Wako Pure Chemicals, Osaka, Japan), mouse insulin ELISA kit (H type) (Sibayagi, Gunma, Japan), NEFA C-test (Wako Pure Chemicals, Tokyo, Japan), Triglyceride E-test (Wako Pure Chemicals, Osaka, Japan), Cholesterol E-test (Wako Pure Chemicals, Osaka, Japan), mouse/rat adiponectin ELISA kit (Otsuka Pharmacy, Tokyo, Japan), mouse leptin ELISA kit (Morinaga, Tokyo, Japan), and testosterone ELISA kit (EIA-1559) (DRG Instruments, Marburg, Germany).

**Measurement of liver triglyceride and cholesterol content**

Liver lipids were extracted using the Folch method [13]. Frozen liver tissue samples (150 mg) were homogenized in chloroform-methanol (2:1 vol/vol). After a 24-h incubation at 4°C, the extract was centrifuged and the liquid phase collected. Water was added and the mixture was mixed sufficiently and centrifuged at 1000 × g for 10 min. An aliquot of the organic phase was collected and dried under nitrogen gas. The dry residue was dissolved in tert-butanol, methanol and Triton X-100 (3:1:1 vol/vol/vol), and the triglyceride and cholesterol contents were assessed.

**Glucose tolerance tests and insulin tolerance tests**

For the oral glucose tolerance test, after a 24-h fast, mice were given an oral bolus of glucose (1 g/kg body weight), and both blood glucose and plasma insulin levels were measured in samples taken at 0, 15, 30, 60 and 120 min after glucose bolus administration. The insulin tolerance test was performed *ad libitum* by an intraperitoneal injection of 1 unit/kg body weight human insulin (HumalinR, Eli Lilly, Kobe, Japan). Blood glucose levels were determined at 0, 20, 40, 60, 80 and 100 min after insulin administration.

**Physiological measurements**

Mice were housed individually from 19 weeks. At 20 weeks, mice were maintained in a cFDM-300AS (Melquest, Toyama, Japan), a transparent plastic cage (15.5 × 20.5 × 19 cm) with a food intake monitor, which was equipped with an AS-10 (Melquest), an infrared activity sensor, above the cage. Mice were allowed to acclimatize to the cage for 3 days and then food intake and locomotor activity was continuously recorded every 10 min for the following 4 days. At 21 weeks, rectal temperature and oxygen consumption were assessed. Rectal temperature was determined with a D717 digital thermometer (Tateyama Kagaku Industry, Tokyo, Japan) between 14:00 and 16:00 by inserting a SXN-54 probe sensor (Tateyama Kagaku Industry) for a length of 2 cm into the rectum. Oxygen consumption and respiratory quotient were measured for 24 h using an Eco Oxymax Indirect Calorimetry System (Columbus Instruments, Columbus, OH, USA). At 22 weeks, mice were housed in plastic cages (22.5 × 34 × 21.5 cm) equipped with a running wheel (14-cm diameter, SW-15, Melquest). Each wheel revolution was registered by a contact switch and the number of revolutions was recorded every 1 min for 7 days.

**Statistical analysis**

All values are presented as mean ± standard deviation. Statistical analysis was carried out between LRH-1+/- and LRH-1+/- mice on the same diet with a GraphPad Prism program version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). Data from body weight curves and tolerance studies were analyzed using two-way repeated-measures ANOVA, followed by a post hoc Holm–Sidak multiple comparison test. The other data were analyzed using Student's *t*-tests. A *p*-value <0.05 was considered statistically significant.

**Results**

**LRH-1 target gene expression decreased in livers of LRH-1+/- mice**

We first examined whether LRH-1 mRNA expression decreased in all tissue samples of 10-week-old male heterozygous LRH-1 knockout mice. LRH-1 mRNA showed high expression in the liver, pancreas, small intestine and colon, and low expression in the hypothalamus, thymus, heart, lung, stomach, kidney, epididymal fat, brown adipose tissue and lower limb muscle (Fig. 1A). LRH-1 mRNA levels in LRH-1+/- mice were lower than those in wild-type mice (Fig. 1A). We then examined hepatic LRH-1 levels in LRH-1+/- mice. At 32 weeks old, LRH-1 mRNA expression levels in LRH-1+/- mice were approximately 50% less than
Hattori et al. vs. 29.7 ± 2.3 g at 30 weeks, *p* < 0.05) (Table 1, Fig. 2). Similarly, body weight of LRH-1+/- mice fed the Quick Fat diet was significantly greater than that of wild-type mice (41.3 ± 4.3 g vs. 37.4 ± 4.3 g at 30 weeks, *p* < 0.05) (Table 1, Fig. 2). Liver and epididymal fat weights of LRH-1+/- mice were similar to that of LRH-1+/+ mice (Table 1). Compatible with these findings was the observation that liver cholesterol and triglyceride contents of LRH-1+/- mice were not significantly different from those of wild-type mice (Table 1). Blood glucose levels of LRH-1+/- mice were similar to those of wild-type mice (110 ± 32 mg/dL (wild-type fed normal diet), 107 ± 13 mg/dL (LRH-1+/- fed normal diet), 119 ± 25 mg/dL (wild-type fed high-fat diet) and 123 ± 22 mg/dL (LRH-1+/- fed high-fat diet)). However, plasma insulin levels of LRH-1+/- mice fed the high-fat diet were greater than those of wild-type mice fed that of LRH-1+/- mice fed a normal chow or a high-fat diet (Fig. 1B). We then tested whether LRH-1 in vivo regulated mRNA expression of LRH-1 target genes, such as SHP, CYP8B1 and CYP7A1. SHP and CYP8B1 mRNA levels showed lower expression levels in LRH-1+/- mice compared with LRH-1+/+ mice (Fig. 1C, 1E). CYP7A1 mRNA levels decreased with a normal chow diet and increased with a high-fat diet (Fig. 1D). mRNA levels of LRH-1 target genes decreased in the livers of LRH-1 heterozygous knockout mice.

**Phenotypic comparison between wild-type and LRH-1 heterozygous knockout mice**

We examined the in vivo effects of LRH-1 gene deletion on body weight. Body weight of LRH-1+/- mice fed the normal chow diet was modestly but significantly greater than that of wild-type mice (31.6 ± 1.6 g vs. 29.7 ± 2.3 g at 30 weeks, *p* < 0.05) (Table 1, Fig. 2). Similarly, body weight of LRH-1+/- mice fed the Quick Fat diet was significantly greater than that of wild-type mice (41.3 ± 4.3 g vs. 37.4 ± 4.3 g at 30 weeks, *p* < 0.05) (Table 1, Fig. 2). Liver and epididymal fat weights of LRH-1+/- mice were similar to that of LRH-1+/- mice (Table 1). Compatible with these findings was the observation that liver cholesterol and triglyceride contents of LRH-1+/- mice were not significantly different from those of wild-type mice (Table 1). Blood glucose levels of LRH-1+/- mice were similar to those of wild-type mice (110 ± 32 mg/dL (wild-type fed normal diet), 107 ± 13 mg/dL (LRH-1+/- fed normal diet), 119 ± 25 mg/dL (wild-type fed high-fat diet) and 123 ± 22 mg/dL (LRH-1+/- fed high-fat diet)). However, plasma insulin levels of LRH-1+/- mice fed the high-fat diet were greater than those of wild-type mice fed...
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Table 1 Phenotypic comparison between LRH-1+/+ and LRH-1+/- mice fed a normal and high-fat diet

<table>
<thead>
<tr>
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<th>Normal chow diet</th>
<th>High fat diet</th>
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<tbody>
<tr>
<td></td>
<td>LRH-1+/+</td>
<td>LRH-1+/-</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>30.4±2.9 (21)</td>
<td>32.4±1.9* (19)</td>
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<tr>
<td>Liver (g)</td>
<td>1.59±0.20 (21)</td>
<td>1.64±0.17 (19)</td>
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<tr>
<td>Epydidymal fat (g)</td>
<td>0.49±0.15 (21)</td>
<td>0.54±0.19 (19)</td>
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<tr>
<td>Brown adipose tissue (g)</td>
<td>0.12±0.02 (21)</td>
<td>0.13±0.04 (19)</td>
</tr>
<tr>
<td>Liver cholesterol (mg/gliver)</td>
<td>2.01±0.16 (9)</td>
<td>1.97±0.21 (9)</td>
</tr>
<tr>
<td>Liver triglyceride (mg/gliver)</td>
<td>7.1±3.1 (9)</td>
<td>8.7±3.9 (9)</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>110±32 (9)</td>
<td>107±13 (9)</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.57±0.35 (9)</td>
<td>0.43±0.16 (9)</td>
</tr>
<tr>
<td>Free fatty acid (mEq/L)</td>
<td>0.93±0.30 (9)</td>
<td>0.78±0.17 (9)</td>
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<tr>
<td>Triglyceride (mg/dL)</td>
<td>61.2±9.1 (9)</td>
<td>57.6±7.9 (9)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>92.1±14.9 (9)</td>
<td>103.5±5.7* (9)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>6.5±0.9 (9)</td>
<td>6.2±2.1 (9)</td>
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<tr>
<td>Adiponectin (μg/mL)</td>
<td>13.5±1.4 (9)</td>
<td>13.8±2.0 (9)</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>5.86±5.83 (17)</td>
<td>3.35±4.07 (23)</td>
</tr>
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</table>

High-fat diet groups were fed a normal chow diet between 5 and 15 weeks and then fed a high-fat diet after 15 weeks old. We collected blood and plasma samples of 30-week-old mice from the retro-orbital venous plexus ad libitum or after a 6-h fast. Mice were sacrificed at 32 weeks and tissue weights were measured. All values are mean ± SD (n). *p<0.05 between LRH-1+/+ vs. LRH-1+/- groups.

Fig. 2 Reduction of LRH-1 gene expression leads to weight gain

Body weights of mice were assessed at indicated time points between the ages of 5 and 30 weeks. (A) LRH-1+/+ and LRH-1+/- mice were fed a normal chow diet (NCD) (n=17–18). (B) LRH-1+/+ and LRH-1+/- mice were fed a normal chow diet for 15 weeks, and then fed a high-fat diet (HFD) (n=20–21). All values are mean ± SD. * p<0.05 between LRH-1+/+ and LRH-1+/- groups.

The high-fat diet (0.85 ± 0.63 ng/mL vs. 3.48 ± 2.44 ng/mL, respectively) (Table 1). Plasma cholesterol, triglyceride and free fatty acid concentrations of LRH-1+/- mice were similar to those of wild-type mice (Table 1). Plasma leptin levels of LRH-1+/- mice fed the high-fat diet were greater than those of wild-type mice fed the high-fat diet; a finding that is compatible with body weight differences (Table 1). To evaluate leptin sensitivity, intraperitoneal injections of either phosphate-buffered saline or leptin were administered to LRH-1+/+ and LRH-1+/- mice at 15 weeks, and no significant differences between LRH-1+/+ and LRH-1+/- mice with regard to decreases in body weight and food intake were observed (Supplementary Fig. 1). Plasma adiponectin levels of LRH-1+/- mice fed the normal or high-fat diet were similar to those of wild-type mice fed the normal
or high-fat diet (Table 1). Plasma testosterone levels of LRH-1+/− mice fed the normal or high-fat diet were also similar (Table 1). LRH-1+/− mice showed modest but significant body weight gains without apparent changes in glucose and lipid metabolism.

**Glucose tolerance tests and insulin tolerance tests in LRH-1+/− mice**

Plasma insulin levels in LRH-1+/− mice fed the high-fat diet were greater than those of wild-type mice fed the high-fat diet (Table 1). We then considered whether insulin sensitivity in high-fat diet fed LRH-1+/− mice decreased when compared with insulin sensitivity in wild-type mice, and whether increased insulin secretion compensated for insulin resistance and consequently glucose tolerance was maintained. However, we did not detect significant differences between LRH-1+/+ and LRH-1+/− mice on a normal chow or a high-fat diet (Fig. 3A–3F). Compatible with the results shown in Fig. 3E–3F, was the observation that there was no apparent difference between LRH-1+/+ and LRH-1+/− mice with regard to insulin-induced Akt phosphorylation at Ser473 in the liver and white adipose tissue (Supplementary Fig. 2). Compatible with the observed high plasma insulin levels, baseline phosphorylation of liver Akt in high-fat diet-fed heterozygous mice tended to be elevated (Supplementary Fig. 2). Glucose tolerance and insulin sensitivity of LRH-1+/− mice were similar to that of wild-type mice (Fig. 3).

**Effect of LRH-1 reduction on physiological parameters**

Generally, body weight is influenced mainly by calorie intake and energy expenditure. To clarify the cause of weight gain by LRH-1 gene deletion, we assessed physiological parameters, which were categorized as calorie intake (food intake), energy expenditure (oxygen consumption, respiratory quotient, rectal temperature) and physical activities (locomotor activity, wheel activity). We observed no remarkable differences between LRH-1+/+ and LRH-1+/− mice on the normal chow or high-fat diet with regard to food consumption, oxygen consumption, respiratory quotient, rectal temperature, locomotor activity and wheel activity (Table 2).
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LRH-1 heterozygous knockout mice, CYP8B1 and SHP mRNA expression were reduced in LRH-1+/− knockout mice fed the normal and the high-fat diets. The body weight of LRH-1 heterozygous knockout mice fed the high-fat diet was significantly greater than wild-type mice fed the high-fat diet; however, glucose tolerance and plasma lipid levels in LRH-1+/− mice were similar to those in wild-type mice. LRH-1 induced body weight gains without worsening glucose and lipid metabolism. LRH-1 gene deletion may be prone to mild obesity.

LRH-1 regulates bile acid synthesis, and SHP, CYP7A1 and CYP8B1 are known to be LRH-1 target genes in the liver [8–10]. In both LRH-1 knockdown and hepatic-specific knockout mice, mRNA expression levels of SHP and CYP8B1 decreased compared with the control groups. Compatible with this result, in LRH-1+/− mice fed the normal or high-fat diet, CYP8B1 and SHP mRNA expression decreased. These results suggest that LRH-1 may be critical for the maintenance of CYP8B1 and SHP mRNA. We successfully established heterozygous LRH knockout mice.

The CYP7A1 mRNA process is very complicated. In primary hepatocytes, LRH-1 up-regulates CYP7A1 gene expression [14]. However, some studies have reported that in liver-specific LRH-1 knockout mice, CYP7A1 mRNA levels did not reduce [15–16], and in contrast, another study has reported that CYP7A1 mRNA levels in the liver of LRH-1 knockdown mice were much lower when compared with control mice [17]. Considering that shRNA targeted against LRH-1 may suppress unknown gene expression (except lrh-1), the discrepancy between cultured cell studies and in vivo studies has not yet been clarified. In the present study, CYP7A1 mRNA levels in LRH-1+/- mice decreased with the normal chow diet. Therefore, our data support the latter (LRH-1 knockdown mice) and suggest that LRH-1 in vivo also regulates CYP7A1 mRNA expression. In high-fat diet fed LRH-1+/- mice, CYP7A1 mRNA levels were similar to those of high-fat diet fed wild-type mice. Compatible with this finding is a study that reported that hyperglycaemia increased histone acetylation status on the CYP7A1 gene promoter, leading to elevated basal CYP7A1 expression and an enlarged bile acid pool with altered bile acid composition [18]. These results suggest that hyperglycaemia might mask the effect of LRH-1 on CYP7A1 mRNA regulation in high-fat diet-induced LRH-1+/− mice.

In the present study, the glucose tolerance capacity of LRH-1+/− mice was similar to that of wild-type mice. The insulin tolerance capacity of LRH-1+/- mice was also similar to that of wild-type mice. Only baseline phosphorylation of liver Akt in high-fat diet-fed heterozygous mice appeared elevated, which may reflect the high plasma insulin levels of these mice. Compatible with this result is a study that reported that glucose tolerance in liver-specific LRH-1 knockout mice was similar to control mice [19]. That study also reported that hepatic glucokinase mRNA levels in liver-specific LRH-1 knockout mice decreased. However, glucokinase mRNA in our heterozygous LRH-1+/− mice was similar to that of wild-type mice, and both liver type pyruvate kinase and fatty acid synthase mRNA,
which are ChREBP target genes [20–21], decreased (Supplementary Fig. 3). These results suggest that the regulation of glucokinase mRNA may be dependent on the extent of LRH-1 suppression.

LRH-1 mRNA was observed to be highly expressed in the pancreas, liver, small intestine and colon, which are derived from the endoderm, and expressed in low levels in the hypothalamus, thymus, heart, lung, stomach, kidney, epididymal fat, brown adipose tissue and lower limb muscle in the present study. We did not detect LRH-1 mRNA expression in the testes. Compatible with these observations is a study that reported that LRH-1 plays important roles in the liver and intestine [22]. Considering that LRH-1 homozgyous mice were lethal, and that we could not establish LRH-1 homozygous knockout mice, it appears that LRH-1 has important roles in bile acid metabolism, as well as in the regulation of development [23].

Body weights of LRH-1+/− mice fed the normal and high-fat diets were greater than those of wild-type mice without showing a worsening of glucose and lipid disorders. To clarify the mechanism, we measured the long-term energy balance between energy intake and energy expenditure. We first considered the contribution of food intake to body weight gain because: (1) LRH-1 mRNA is expressed in the arcuate and periventricular nucleus of the hypothalamus; and (2) gene deletion of Steroidogenic Factor-1 (NR5A1; SF-1), which belongs to NR5 orphan members of the Ftz-F1 subfamily of nuclear receptors together with LRH-1 (NR5A2), exhibits obesity through abnormalities of the ventromedial hypothalamic nucleus and consequently increased food intake [24]. However, food intake did not show any significant differences between wild-type and LRH-1+/− mice fed the normal chow or high-fat diet in the present study. Moreover, we also checked food intake at 5, 7, 12, and 19 weeks, however we could detect no differences of food intake (Supplementary Table 1). We next considered the effect of energy expenditure on body weight gain because bile acid modulates thermogenesis in brown adipocytes through TGR5 activation [11]. However, we could not detect any differences in oxygen consumption and respiratory quotient to assess whole-body energy expenditure, and rectal temperature between wild-type and LRH-1+/− mice. Furthermore, we could not detect any change of activity, such as locomotor and wheel activity, to assess physical activity. Therefore, at present, we are not able to say why LRH-1+/− mice were prone to obesity. We suggest the following possible reasons: (1) LRH-1 is only modestly but significantly expressed in adipose tissues, and mainly in preadipocytes but not adipocytes [12]. During adipogenesis, LRH-1 mRNA levels decrease. A study reported that LRH-1 gene deletion induces adipogenesis [12, 25]. Thus, LRH-1 suppression may induce adipogenesis and body weight gain; and (2) LRH-1 regulates sex hormones. For example, for estrogen metabolism, LRH-1 up-regulates aromatase expression in several tissues [25]. Considering that aromatase deficiency in both male and female mice causes obesity [26], LRH-1 may affect body weight through local estrogen synthesis. In the present study, plasma testosterone levels did not change between LRH-1+/+ and LRH-1 +/- mice. Moreover, in our preliminary study, we did not detect any differences in body weights between female LRH-1+/+ and LRH-1 +/- mice (23.0 ±1.03 g (n=5) vs. 22.66 ± 0.63 g (n=5), respectively).

Differences in body weights between wild-type and LRH-1+/− mice were modest. There were no significant changes in glucose and lipid metabolism between LRH-1+/+ and LRH-1+/− mice. Accordingly, further investigation is required to identify why suppression of LRH-1 transactivity causes mild obesity using intestine- or liver-specific LRH-1 knockout mice.

In summary, heterozygous LRH-1 knockout mice fed a high-fat diet were prone to mild obesity without showing worsening of glucose and lipid disorders. To clarify the mechanism, we measured the long-term energy balance between energy intake and energy expenditure. We first considered the contribution of food intake to body weight gain because: (1) LRH-1 mRNA is expressed in the arcuate and periventricular nucleus of the hypothalamus; and (2) gene deletion of Steroidogenic Factor-1 (NR5A1; SF-1), which belongs to NR5 orphan members of the Ftz-F1 subfamily of nuclear receptors together with LRH-1 (NR5A2), exhibits obesity through abnormalities of the ventromedial hypothalamic nucleus and consequently increased food intake [24]. However, food intake did not show any significant differences between wild-type and LRH-1+/− mice fed the normal chow or high-fat diet in the present study. Moreover, we also checked food intake at 5, 7, 12, and 19 weeks, however we could detect no differences of food intake (Supplementary Table 1). We next considered the effect of energy expenditure on body weight gain because bile acid modulates thermogenesis in brown adipocytes through TGR5 activation [11]. However, we could not detect any differences in oxygen consumption and respiratory quotient to assess whole-body energy expenditure, and rectal temperature between wild-type and LRH-1+/− mice. Furthermore, we could not detect any change of activity, such as locomotor and wheel activity, to assess physical activity. Therefore, at present, we are not able to say why LRH-1+/− mice were prone to obesity. We suggest the following possible reasons: (1) LRH-1 is only modestly but significantly expressed in adipose tissues, and mainly in preadipocytes but not adipocytes [12]. During adipogenesis, LRH-1 mRNA levels decrease. A study reported that LRH-1 gene deletion induces adipogenesis [12, 25]. Thus, LRH-1 suppression may induce adipogenesis and body weight gain; and (2) LRH-1 regulates sex hormones. For example, for estrogen metabolism, LRH-1 up-regulates aromatase expression in several tissues [25]. Considering that aromatase deficiency in both male and female mice causes obesity [26], LRH-1 may affect body weight through local estrogen synthesis. In the present study, plasma testosterone levels did not change between LRH-1+/+ and LRH-1 +/- mice. Moreover, in our preliminary study, we did not detect any differences in body weights between female LRH-1+/+ and LRH-1 +/- mice (23.0 ±1.03 g (n=5) vs. 22.66 ± 0.63 g (n=5), respectively).

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In summary, heterozygous LRH-1 knockout mice fed a high-fat diet were prone to mild obesity without showing worsening of glucose and lipid metabolism when compared with wild-type mice fed a high-fat diet. The results of this study suggest that LRH-1 may protect against obesity.

Acknowledgements

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Conflicts of Interest

None of the authors have any potential conflicts of interest associated with this research.
Supplementary Table 1 Body weights and Food intakes of normal chow diet fed LRH-1+/+ and LRH-1+/− mice at 5, 7, 12, and 19 weeks.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Food intake (g)</th>
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<tbody>
<tr>
<td></td>
<td>LRH-1+/+</td>
<td>LRH-1+/−</td>
</tr>
<tr>
<td>5 weeks</td>
<td>19.2±1.5</td>
<td>19.0±1.2</td>
</tr>
<tr>
<td>7 weeks</td>
<td>21.6±1.5</td>
<td>22.0±1.2</td>
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<tr>
<td>12 weeks</td>
<td>24.7±1.3</td>
<td>25.6±1.6</td>
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<td>19 weeks</td>
<td>29.6±1.9</td>
<td>32.6±3.0</td>
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References