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Hepatic fibroblast growth factor 21 is required for curcumin or resveratrol in exerting their metabolic beneficial effect in male mice

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BACKGROUND: Our mechanistic understanding on metabolic beneficial effects of dietary polyphenols has been hampered for decades due to the lack of functional receptors for those compounds and their extremely low plasma concentrations. Recent studies by our team and others suggest that those dietary polyphenols target gut microbiome, and gut-liver axis and that hepatic fibroblast factor 21 (FGF21) serves as a common target for various dietary interventions.
METHODS: Utilizing liver-specific FGF21 null mice (*lFgf21*$^{-/-}$), we are asking a straightforward question: Is hepatic FGF21 required for curcumin or resveratrol, two typical dietary polyphenols, in exerting their metabolic beneficial effect in obesogenic diet-induced obesity mouse models.
RESULTS: On low-fat diet feeding, no appreciable defect on glucose disposal was observed in male or female *lFgf21*$^{-/-}$ mice, while fat tolerance was moderately impaired in male but not in female *lFgf21*$^{-/-}$ mice, associated with elevated random and fasting serum triglyceride (TG) levels, and reduced hepatic expression of *Ehhadh* and *Ppargc1a*, which encodes the two downstream effectors of FGF21. On high-fat-high-fructose (HFHF) diet challenge, *Fgf21*$^{fl/fl}$ but not *lFgf21*$^{-/-}$ mice exhibited response to curcumin intervention on reducing fasting serum TG, and on improving fat tolerance. Resveratrol intervention also affected FGF21 expression or its downstream effectors. Metabolic beneficial effects of resveratrol intervention observed in HFHF diet-challenged *Fgf21*$^{fl/fl}$ mice were either absent or attenuated in *lFgf21*$^{-/-}$ mice.
CONCLUSION AND SIGNIFICANCE: We conclude that hepatic FGF21 is required for curcumin or resveratrol in exerting their major metabolic beneficial effect. The recognition that FGF21 as the common target of dietary intervention, demonstrated in current as well as previous investigations, brings us a novel angle in understanding metabolic disease treatment and prevention. It remains to be further explored how various dietary interventions regulate FGF21 expression and function, via certain common or unique gut-liver or gut-brain-liver axis.

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INTRODUCTION

Except for prescribed drugs and physical exercise, various dietary interventions were also shown to improve metabolic homeostasis [1, 2]. One type of dietary intervention is the change of dietary behaviors, such as nutritional restriction and intermittent fasting [3], while another type is the addition of chemical compounds from edible plants into the diet [4]. One category of those compounds is dietary polyphenols, with the curry compound curcumin and resveratrol, mostly found in red grapes, as two typical examples [1, 5]. Interestingly, previous observations made by our team and others have shown that both dietary polyphenol intervention and amino acid restriction target the hepatic hormone fibroblast growth factor 21 (FGF21) [2, 3, 6, 7].

Members of the fibroblast growth factor (FGF) family interact with FGF receptors (FGFRs) including FGFR1, leading to complicated downstream signaling events. Due to the lack of a heparin binding domain, FGF21, FGF19 (FGF15 in rodents) and FGF23 can be released freely into the bloodstream, serving as endocrine hormones [8]. In addition to FGFRs, the obligatory co-receptor β-klotho (KLB) is also required for FGF21 to exert its metabolic functions [8]. It is generally accepted that plasma FGF21 is liver driven, although *FGF21/Fgf21* mRNA expression can be detected in adipose tissues, pancreas, and elsewhere [8–10]. Various FGF21 analogues have been tested in pre-clinical and clinical trials for treating metabolic disorders including diabetes and fatty liver disorders [8]. The most promising effects of those "pre-drugs" are

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the attenuation of hyperlipidemia and the improvement of insulin sensitivity. Other investigators and our group have also reported that hepatic FGF21 expression can be regulated by glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs), including both exenatide and liraglutide [11–14]. Utilizing a liver-specific *Fgf21* knockout mouse model, we have demonstrated that hepatic FGF21 is required for liraglutide in improving energy homeostasis in male mice with obesogenic diet challenge, making hepatic FGF21 as a "central molecule" for both GLP-1R-based drugs and diet interventions [11]. Liraglutide treatment or curcumin intervention was also shown to attenuate high-fat-diet (HFD)-induced repression on *Fgfr1*, which encodes the FGF21 receptor FGFR1, or *Klb*, which encodes the obligatory co-receptor KLB [6, 11]. These regulatory events are commonly interpreted as the improvement of FGF21 sensitivity [6, 11].

Utilizing the hepatic FGF21 deficient mice, here we have asked a straightforward question: Whether FGF21 is also required for curcumin or resveratrol, two typical dietary polyphenols, in exerting their metabolic beneficial effects?

MATERIALS AND METHODS

The source of dietary polyphenols and the experimental diet

The curry compound curcumin was purchased from Organika Health Products (Richmond, BC, Canada; a 95% standardize curcumin extract) while resveratrol was purchased from Combi-Blocks (Catalog #: OR-1053, San Diego, CA, USA), as we have reported previously [5, 6]. Methods for curcumin and resveratrol intervention have been described in our previous studies [5, 6, 15–18]. Contents of experimental diets utilized in this study are shown in Supporting Table 1.

Animals and animal experimental design

Six-week-old wild type C57BL/6J mice, liver-specific *Fgf21* knockout (*lFgf21*$^{-/-}$) mice and the wild type littermate controls (*Fgf21*$^{fl/fl}$) were utilized in this study. *lFgf21*$^{-/-}$ mice were generated by mating *Fgf21*^{loxP} (Strain #: 022361, Jackson lab) with *Alb-Cre* mice (Strain #: 003574, Jackson lab) as we have reported previously [11] (Figure S1A). Mice were maintained at ambient room temperature and relative humidity of 50%, with free access to food and water under a 12 h light:12 h darkness cycle ($n = 4$–5 per cage). At the end of the experimental period, mice were fasted overnight before being euthanized with CO_2 treatment followed by cervical dislocation. The animal experiments and protocol were approved by the University Health Network Animal Care Committee (AUP 2949.18) and were performed in accordance with the guidelines of the Canadian Council of Animal Care. For curcumin intervention, 6-week-old male mice were fed an high fat high fructose diet (60% HFD with 20% fructose, HFHF, $n = 5$) with or without curcumin (4 g/kg diet, $n = 5$) for 15 weeks, as we have reported previously [6]. For resveratrol intervention, 6-week-old male mice were fed on an LFD ($n = 5$ for C57BL/6J; $n = 4$ for *Fgf21*$^{fl/fl}$), obesogenic diet (HFD [$n = 5$ for C57BL/6J] or HFHF diet [$n = 5$ for *Fgf21*$^{fl/fl}$, and $n = 6$ for *lFgf21*$^{-/-}$]) with or without resveratrol (0.5% of the diet [$n = 5$ for *Fgf21*$^{fl/fl}$, and $n = 6$ for *lFgf21*$^{-/-}$]) for indicated period of time [5]. Mice were randomly assigned to either receive or not receive dietary intervention. Based on our animal protocol, mouse with serious body weight loss or shown "sickness" symptoms will be excluded from the study. For the current study, no mice or data points were excluded.

The generation of *Fgf21*$^{fl/fl}$ *and lFgf21*$^{-/-}$ mice were verified by genotyping. For data presented in Figs. 1–2, male and female *Fgf21*$^{fl/fl}$ and *lFgf21*$^{-/-}$ littermates were fed with LFD. Metabolic tolerance tests were conducted at the week of 8th, 10th, 12nd and 15th for glucose tolerance test (GTT), pyruvate tolerance test (PTT), insulin tolerance test (ITT) and fat tolerance test (FTT), respectively. Prior to fat tolerance test (FTT), the blood triglyceride (TG) levels at random or fast state were assessed at the week of 14th. For data presented in Figs. 3–6, only male *Fgf21*$^{fl/fl}$ and *lFgf21*$^{-/-}$ littermates were examined.

Metabolic tolerance tests and TG measurement

Methods for GTT, PTT and ITT have been previously presented [19]. For intraperitoneal GTTs and PTTs, both male and female mice were fasted for 16 h prior to the intraperitoneal injection of glucose (2 g/kg body weight) or pyruvate (2 g/kg body weight). For ITTs, male and female mice were fasted for 4 h prior to the injection of insulin (0.5 U/kg body weight). Blood glucose levels were determined at 0, 15, 30, 60, 90, and 120 min. We adopted the method by Gniuli et al. for conducting FTT [20]. Briefly, mice were fasted overnight prior to oral gavage of 1% olive oil of body weight. Blood was collected from tail vein at 0, 1, 2 and 4 h for TG measurement. To determine TG produced by the liver (lipid tolerance test), mice fasted overnight were injected intraperitoneally with poloxamer 407 (Sigma-Aldrich, Catalog #: P2443) to block lipolysis, and blood was collected from tail vein at indicated hours to measure TG levels [5].

qRT-PCR and western blotting

Methods for qRT-PCR and Western blotting have been described previously [5, 19], with oligo nucleotide primers and antibodies listed in Supporting Table 2 and Supporting Table 3, respectively.

RNAseq sample preparation and data analysis

Mice were subjected to a 16-week feeding with LFD, HFHF diet, or HFHF diet plus resveratrol (HFR). By the end of the experiment, mice liver tissues were collected for RNA isolation. Total RNA was harvested using RNeasy Mini kit (QIAGEN) and further quantified and analyzed using Nanodrop spectrophotometer and Bioanalyzer. One microgram of total RNA was utilized and sent to Center of Applied Genomics (Sickkids Hospital, Canada) for sequencing library construction, as we have described previously [11]. Data processing and analyzing were conducted through a standardized pipeline called RNAseq IMmune Analysis Pipeline, per literature description [21]. Briefly, unprocessed FASTQ files containing raw data were downloaded and transferred. The RNAseq reads were aligned against the mm10 reference genome assembly (Genome Reference Consortium Mouse Build 38) obtained from the NCI Genome Data Commons (GDC) using STAR (version 2.4.2a). Quality control was performed on aligned BAM files using RSeQC and then expression levels were quantified using SALMON (V.0.14.0). After converting Ensemble IDs to mouse gene symbols (GRCm38.p6), the reads per gene were normalized and differentially expressed genes were analyzed with DESeq2 (V1.22.2). Raw data and processed data have been submitted to the Gene Expression Omnibus (GEO) database (GSE241713).

Statistical analysis

Results are expressed as mean ± SEM. Normality test was performed using Shapiro-Wilk test and for normally distributed data with similar variance, student's unpaired one-tailed or two-tailed t test was performed. For multiple comparisons, one-way ANOVA followed by Sidak post-hoc correction was applied for calculating the statistical significance. Statistical details are presented in the figure legends and n values means biological replicates in all experiments. In the current study, the sample size was based on previous studies that employed mice, no power analysis and blinding was performed. In all cases, $p < 0.05$ is considered statistically significant. Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Male but not female *lFgf21*$^{-/-}$ mice on LFD feeding show impaired fat tolerance

We have generated *lFgf21*$^{-/-}$ mice and the littermate controls (*Fgf21*$^{fl/fl}$) (C57BL/6J genetic background) for current study by mating *Alb-Cre* with *Fgf21*$^{fl/fl}$, as illustrated in Fig. S1A. *lFgf21*$^{-/-}$ mouse liver showed barely detectable FGF21 (Fig. S1B–D), while FGF21 expression in both brown adipose tissue and hypothalamus were virtually unaffected (Fig. S1E, F).

Six-week-old male and female *lFgf21*$^{-/-}$ mice and the control *Fgf21*$^{fl/fl}$ mice were fed with LFD for 12 weeks, while three metabolic tolerance tests (GTT, ITT, and PTT) were conducted at indicated time for all mice (Fig. 1A). On LFD feeding, male and female *lFgf21*$^{-/-}$ mice exhibited comparable glucose, pyruvate, and insulin tolerance when compared with age and sex-matched control littermates *Fgf21*$^{fl/fl}$ mice (Fig. 1B–G). There were no appreciable differences on body weight with hepatic FGF21 knockout in both sexes (Fig. 1H, I).

The above mice were then fed with LFD for an additional 3-week period (Fig. 1A), followed by collecting random blood

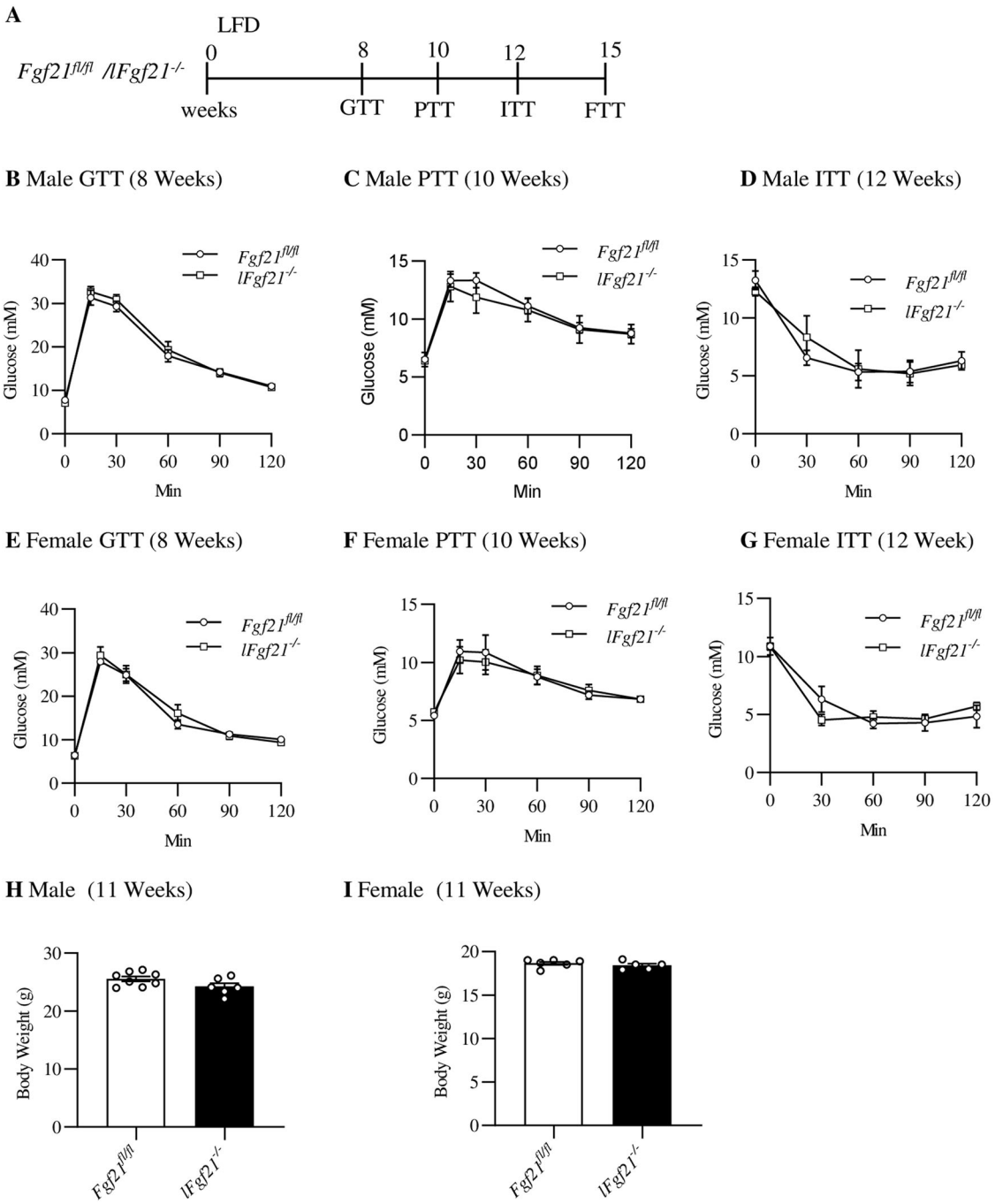

Fig. 1 No appreciable defect on glucose disposal in male or female *lFgf21*$^{-/-}$ mice when fed with LFD. A Illustration of the animal experimental timeline. **B**–**D** Glucose level during tolerance test in adult male mice, Glucose tolerance test (GTT) at the age of 8 weeks ($n = 6$ for both *Fgf21*$^{fl/fl}$ and *lFgf21*$^{-/-}$) (**B**). Pyruvate tolerance test (PTT) at the age of 10 weeks ($n = 4$) (**C**). Insulin tolerance test (ITT) at the age of 12 weeks ($n = 4$) (**D**). **E**–**G** Glucose level during GTT in adult female mice, GTT at the age of 8 weeks ($n = 6$ for *Fgf21*$^{fl/fl}$, $n = 5$ for *lFgf21*$^{-/-}$) (**E**). PTT at the age of 10 weeks ($n = 3$) (**F**). ITT at the age of 12 weeks ($n = 3$) (**G**). Body weight in both male (**H**) and female mice (**I**). Data are shown as the mean ± SEM.

samples and conducting FTT. Mice were then fasted overnight before they were sacrificed for tissue sample collections. As shown, male *lFgf21*$^{-/-}$ mice exhibited elevated random and fasting plasma TG levels, along with a trend toward impaired fat tolerance ($p = 0.0519$ for the area under the curve of the FTT) when compared with corresponding littermate controls (Fig. 2A, B). Such defects were not observed in female *lFgf21*$^{-/-}$ mice (Fig. 2C, D). We then assessed the expression of a battery of hepatic genes that are related to the FGF21 cellular signaling. Expression levels of genes encoding FGFR1 (*Fgfr1*) showed a trend toward reduction; while those encoding KLB (*Klb*) and two FGF21 downstream mediators (*Ehhadh*, which encodes enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase; and *Ppargc1a*, which encodes PPARG coactivator 1 alpha) were significantly attenuated in male *lFgf21*$^{-/-}$ mice (Fig. 2E). In female *lFgf21*$^{-/-}$ mice, only *Ppargc1a* level was reduced. Interestingly, unlike male *lFgf21*$^{-/-}$, *Klb* expression was significantly elevated in female *lFgf21*$^{-/-}$ mice, potentially due to an unidentified sex-dependent compensatory mechanism (Fig. 2F).

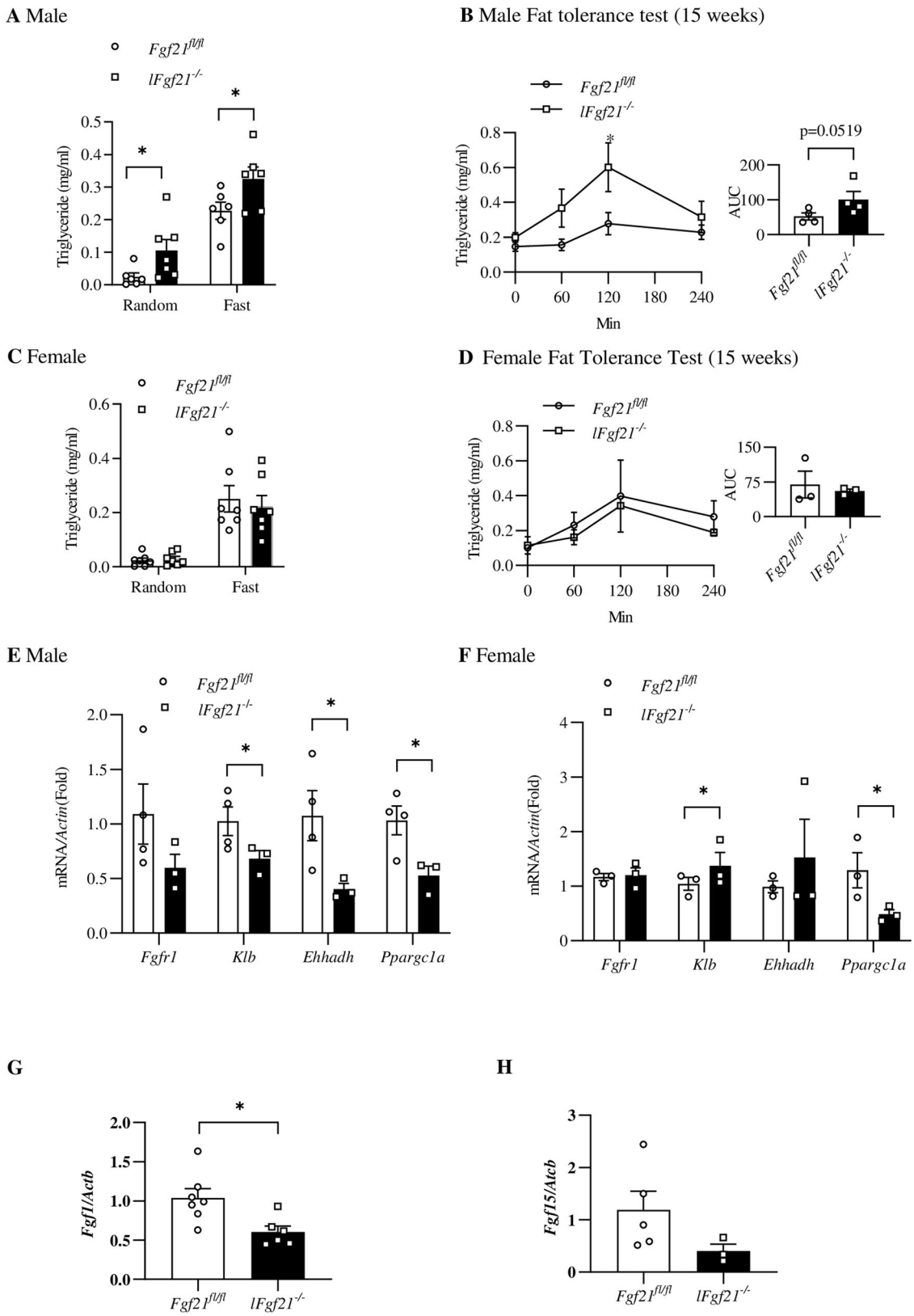

Fig. 2 Male but not female *lFgf21*$^{-/-}$ mice show impaired fat tolerance when fed on LFD. A Random and fasting serum TG levels in indicated adult (8 weeks) male mice ($n = 6$ for both groups). **B** Postprandial TG levels during fat tolerance test (FTT) in male mice at the age of 15 weeks (oral gavage 1% olive oil). **C** Random and fasting serum TG levels in adult female mice ($n = 7$ for both groups, 8 weeks). **D** Postprandial TG levels during FTT in female mice at the age of 15 weeks. Comparison of expression levels of hepatic genes that encode FGFR1 (*Fgfr1*) and KLB (*Klb*), as well as two FGF21 downstream effectors, peroxisomal L-bifunctional enzyme (*Ehhadh*) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Ppargc1a*) in male (**E**) and female mice (**F**). *Fgf1* (**G**) and *Fgf15* (**H**) expression levels in the liver of *Fgf21*$^{fl/fl}$ ($n = 4$–7) and *lFgf21*$^{-/-}$ ($n = 3$–6). mice. AUC, area under the curve. *$p < 0.05$ and **$p < 0.01$.

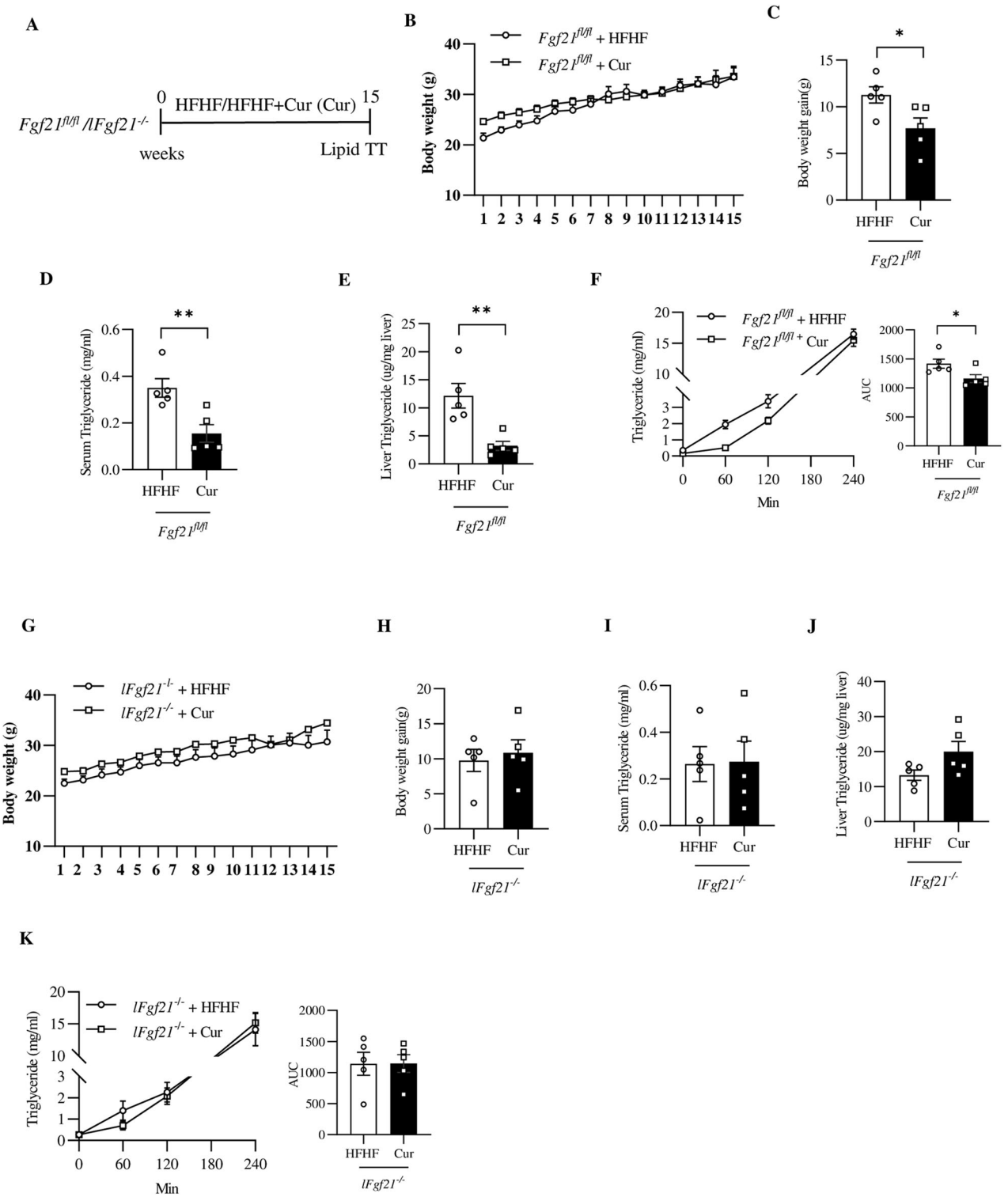

Fig. 3 Dietary curcumin intervention exhibits metabolic beneficial effects on obesogenic diet challenged male *Fgf21*$^{fl/fl}$ mice but not male *lFgf21*$^{-/-}$ mice. A Illustration of the animal experimental timeline. **B** Body weight of *Fgf21*$^{fl/fl}$ mice during the experimental period. **C** Body weight gain of *Fgf21*$^{fl/fl}$ at the end of the 15th week after overnight fasting. Serum (**D**) and hepatic (**E**) TG levels in *Fgf21*$^{fl/fl}$ mice. **F** FTT and AUC. Overnight fasted *Fgf21*$^{fl/fl}$ mice were (intraperitoneal, *i.p*) injected with 1 g/kg poloxamer 407 to block lipolysis. Blood samples were then collected from tail vein at indicated time for TG level measurement. **G** Body weight of *lFgf21*$^{-/-}$ mice during the experimental period. **H** Body weight gain of *lFgf21*$^{-/-}$ mice at the end of the 15th week after overnight fasting. **I** Serum TG level of *lFgf21*$^{-/-}$ mice. **J** Hepatic TG level in the *lFgf21*$^{-/-}$ mice. **K** FTT and AUC for overnight fasted *lFgf21*$^{-/-}$ mice. Data are shown as the mean ± SEM ($n = 5$ each group). *$p < 0.05$ and **$p < 0.01$.

We also assessed expressions of liver *Fgf1* and gut (ileum) *Fgf15*, asking whether hepatic FGF21 deficiency results in elevated *Fgf1 or Fgf15* expression for compensation. No such compensatory elevation was observed in *lFgf21*$^{-/-}$ mice (Fig. 2G, H). Together, we conclude that male but not female *lFgf21*$^{-/-}$ mice showed impaired lipid homeostasis in the absence of obesogenic dietary challenge, associated with reduced hepatic expression of *Fgfr1*, *Klb*, and *Ehhadh*. Our following investigations were then performed on male mice only.

Curcumin intervention improves lipid homeostasis in control but not *lFgf21*$^{-/-}$ mice with the obesogenic dietary challenge

We reported previously that in HFD-challenged male mice, curcumin or anthocyanin intervention regulated hepatic FGF21 production and improved FGF21 sensitivity in hepatocytes [6, 22]. Curcumin intervention stimulated hepatic FGF21 expression in mice on LFD feeding and attenuated HFD-induced hepatic FGF21 over-expression and FGF21 resistance [6]. Here we aim to determine whether hepatic FGF21 is required for curcumin in exerting its major metabolic beneficial effect, especially lipid homeostatic effect in mice on obesogenic dietary challenge. As shown, male *lFgf21*$^{-/-}$ mice or the control littermate *Fgf21*$^{fl/fl}$ mice were fed with HFHF diet without or with curcumin intervention for 15 weeks (Fig. 3A). In control littermates, curcumin intervention moderately attenuated HFHF diet-induced body weight gain (Fig. 3B, C), reduced fasting serum and hepatic TG contents (Fig. 3D-E), and improved lipid tolerance (Fig. 3F). Curcumin intervention also reduced hepatic expression of ChREBP, as well as expression of genes that encode ChREBP (*Mlxipl*) and fatty acid synthase (*Fasn*) (Fig. S2A, B). In *lFgf21*$^{-/-}$ mice, none of the above regulatory effects of dietary curcumin intervention were observable (Fig. 3G–K and Fig. S2C, D). We hence conclude that liver FGF21 is required for curcumin intervention in exerting its metabolic beneficial effect, especially the improvement of lipid homeostasis.

In obesogenic diet-challenged male mice, resveratrol intervention also regulates hepatic FGF21

In 2014, Li and colleagues reported that resveratrol treatment increased the transcriptional activity of *FGF21* promoter [7]. We hence ask whether in vivo resveratrol intervention in wild type mice with an obesogenic dietary challenge affects hepatic FGF21 expression, FGF21 sensitivity, or FGF21 mediated cellular signaling events. Here we conducted such assessments in two different sets of mice. In the first set, wild type C57BL/6J mice were fed with LFD, HFD, or HFD with resveratrol intervention (HFD+Res) for 8 weeks (Fig. 4A). In such experimental settings, hepatic *Fgf21* expression was reduced by HFD feeding, and the reduction was reversed by resveratrol intervention (Fig. 4B). Hepatic FGF21 protein expression was not significantly affected by HFD while concomitant resveratrol intervention exhibited a stimulatory effect on hepatic FGF21 expression (Fig. 4C). Importantly, resveratrol intervention increased the expression of *Fgfr1*, which was reduced by HFD challenge (Fig. 4D). *Klb* level was not significantly affected by 8-week HFD challenge, while resveratrol intervention exhibited a stimulatory effect on hepatic *Klb* expression (Fig. 4E). Among the four downstream effectors of FGF21 we have assessed, expression of *Ehhadh* was inhibited by HFD and the inhibition was effectively reversed by concomitant resveratrol intervention. *Acox1* (encodes Acyl-CoA Oxidase 1) expression was not affected by HFD challenge while resveratrol intervention elevated its expression level. HFD challenge significantly reduced expression of *Ppargc1a*, while resveratrol intervention generated no appreciable reversing effect in the current experimental settings. Finally, hepatic *Pdk4* (which encodes pyruvate dehydrogenase lipoamide kinase isozyme 4) level was not affected by HFD challenge or resveratrol intervention in our current experimental settings (Fig. 4F). The above observations suggest that like curcumin intervention reported previously by our team and by others [6, 23, 24], resveratrol intervention also targets hepatic FGF21 or its downstream signaling events.

Fructose consumption is known to up-regulated hepatic FGF21 expression, associated with the development of insulin resistance [25]. In the second set of mice, we challenged *Fgf21*$^{fl/fl}$ mice with HFHF diet without or with resveratrol intervention (HFR) for an extended period, as indicated in Fig. 5A. As shown, 16-week HFHF-diet challenge significantly increased hepatic *Fgf21* levels, while resveratrol intervention attenuated the elevation effectively (Fig. 5B). At FGF21 protein level, elevation was observed in mice with HFHF challenge, without or with 16-week resveratrol intervention (Fig. 5C).

We then collected liver tissues from those mice for RNAseq analysis. As anticipated (Fig. 5D, Fig. S3, and Supporting Table 4), HFHF-diet challenge increased hepatic expression of *Fgf21* but reduced the expression of *Klb*, while those effects were reciprocally reversed by 16-week dietary resveratrol intervention. The attenuation effect of HFHF-diet and reversible effect of resveratrol intervention was also observed on certain downstream effectors of FGF21 signaling, which were then further verified by qRT-PCR analyses (Fig. 5E–G). As shown in Fig. S3, HFHF diet feeding most significantly repressed expression of genes including *Eif4ebp3* (which encodes eukaryotic translation initiation factor 4E binding protein 3) and *Zbtb16* (which encodes zinc finger and BTB domain-containing protein 16), and those genes were also significantly restored by resveratrol intervention. Exact metabolic functions of these two genes remain to be further explored. Additional information on the effect of HFHF diet challenge and resveratrol intervention on hepatic gene expression are presented in Figs. S4 and S5.

Dietary resveratrol intervention improves glucose tolerance and reduces serum and hepatic TG levels in HFHF challenged control but not *lFgf21*$^{-/-}$ mice

We then directly compared the effect of resveratrol intervention in *Fgf21*$^{fl/fl}$ mice and *lFgf21*$^{-/-}$ mice with HFHF diet challenge (Fig. 6A). As shown, glucose tolerance was improved by resveratrol intervention in the control littermate *Fgf21*$^{fl/fl}$ mice but not in *lFgf21*$^{-/-}$ mice (Fig. 6B). Dietary resveratrol intervention also attenuated HFHF diet-induced fasting hyperglycemia and hyperinsulinemia in *Fgf21*$^{fl/fl}$ mice but not in *lFgf21*$^{-/-}$ mice (Fig. 6C, D). Interestingly, in both *Fgf21*$^{fl/fl}$ and *lFgf21*$^{-/-}$ mice, 16-week dietary resveratrol intervention attenuated HFHF-diet induced body weight gain (Fig. 6E, F) and fat accumulation (Fig. S6), although the degree of the attenuation in *Fgf21*$^{fl/fl}$ mice appeared much stronger than that in *lFgf21*$^{-/-}$ mice (Fig. 6E, F and Fig. S6A–C). In addition, in both *Fgf21*$^{fl/fl}$ and *lFgf21*$^{-/-}$ mice, we observed that resveratrol intervention reduced *Fgf21* expression in epididymal white adipose tissue (eWAT) and brown adipose tissue (BAT) (Fig. S6D, E). We have also examined plasma FGF21 level in *Fgf21*$^{fl/fl}$ mice. As shown, resveratrol intervention attenuated plasma FGF21 level (Fig. S6F). Nevertheless, in *Fgf21*$^{fl/fl}$ but not *lFgf21*$^{-/-}$ mice, resveratrol intervention attenuated HFHF diet-induced elevation on serum as well as hepatic TG levels (Fig. 6G, H). Thus, although hepatic FGF21 is required for resveratrol intervention in exerting its metabolic beneficial effect on improving energy homeostasis, the body weight lowering effect of dietary resveratrol intervention does not completely rely on hepatic FGF21. Whether this involves FGF21 expressed in adipose tissues or elsewhere deserves further investigations.

DISCUSSION

Although native FGF21 and a few FGF21 analogues were shown to bring metabolic and other beneficial effects in various diseases models [8, 26, 27], individuals with obesity show elevated hepatic and plasma FGF21 levels, suggesting that obesity represents an FGF21 resistance state [8, 28, 29]. We and others have reported that dietary intervention with curcumin or anthocyanin, or other

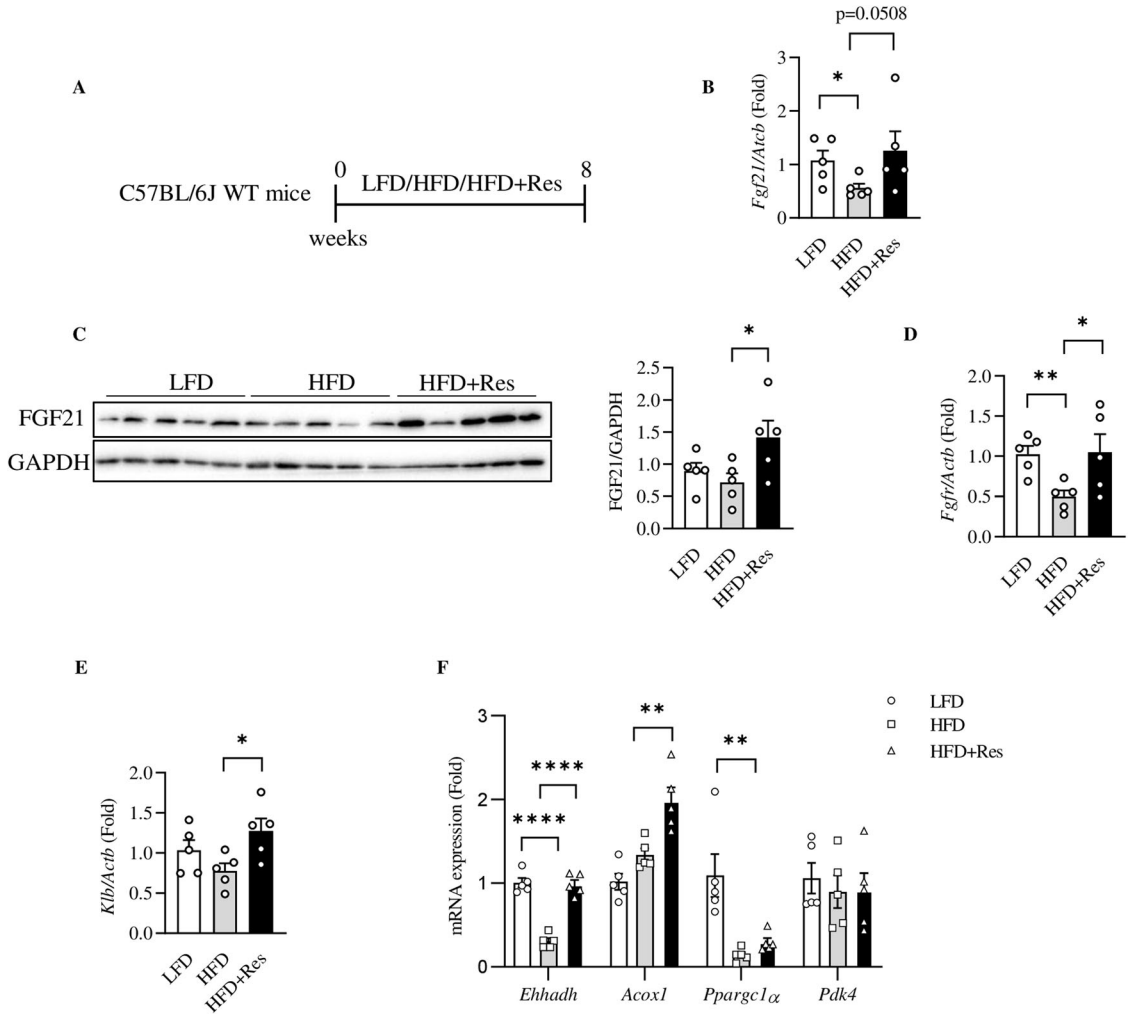

Fig. 4 Dietary resveratrol intervention targets hepatic FGF21 and FGF21 signaling in HFD challenged male wild type C57BL/6 J mice. **A** Illustration of the animal experimental timeline in male C57BL/6J mice. **B** Hepatic *Fgf21* level in indicated group of mice. **C** Hepatic FGF21 protein level. The right panel shows the densitometric analysis results. **D**, **E** Liver expression levels of *Fgfr1* and *Klb* in indicated group of mice. **F** Liver expression levels of *Ehhadh*, *Acox1*, *Ppargc1a* and *Pdk4* in indicated group of mice. Data are shown as the mean ± SEM ($n = 5$ per group). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{****}p < 0.0001$.

dietary polyphenols, regulate plasma and hepatic FGF21 levels, as well as FGF21 sensitivity [6, 17, 22, 23]. Other edible plant compounds, such betaine, were also shown to regulate hepatic FGF21 [30]. Importantly, in mice on LFD feeding, dietary curcumin or anthocyanin intervention stimulated hepatic FGF21 level; while in mice on HFD, the intervention attenuates HFD-induced FGF21 over-expression, associated with the reversal on HFD-induced repression on *Fgfr1* or *Klb* expression [6, 22, 24]. These regulatory events are commonly interpreted as the improvement of FGF21 sensitivity [6, 31].

We show here that on LFD feeding, male but not female *lFgf21*$^{-/-}$ mice exhibited a moderate impairment on fat tolerance, associated with a trend of reduced expression of *Fgfr1*, and significant attenuation on expression of genes including *Klb*, *Ehhadh*, and *Ppargc1a*. Interestingly, female *lFgf21*$^{-/-}$ mice exhibited elevated *Klb* expression compared to the littermate controls; whereas such effect was absent in male mice; suggesting the existence of a sex-dependent adaptive response. Previously, we and others have also shown that female hormone estradiol (E2) increased FGF21 production [28, 32]. How can female hormones including E2 compensate the lack of hepatic FGF21 on lipid homeostasis in the absence of obesogenic dietary challenge remains to be further investigated. In the absence of an obesogenic dietary challenge, extra-hepatic FGF21, including those generated in adipose tissues, brain, and elsewhere, might be sufficient for female mice in maintaining metabolic homeostasis. Nevertheless, we presented here our comprehensive observations in HFD-challenged mice on hepatic FGF21 regulation with resveratrol intervention and then further expanded our investigation with HFHF diet-induced obesity and insulin resistance mouse model, show that resveratrol intervention reversed the repression of HFHF-diet on expression of *Fgfr1* and *Klb*, as well as genes that encode FGF21 effectors. We further underpinned *Fgf21* gene expression in extra-hepatic organs including BAT and eWAT and determined that resveratrol intervention repressed *Fgf21* expression in both HFHF-challenged *lFgf21*$^{-/-}$ and its littermate controls. Adipose tissue FGF21 signaling has been determined to regulate browning in adaptive thermogenesis and our own investigation also showed that dietary curcumin intervention increased thermogenic capacity [15, 33, 34]. Whether resveratrol intervention-mediated FGF21 signaling also contribute to adaptive thermogenesis and whether FGF21 serves as an autocrine or paracrine to exert its effects require further investigation. Importantly, we demonstrated here that hepatic FGF21 is required for either curcumin or resveratrol intervention in exerting their metabolic beneficial effect in the obesogenic diet

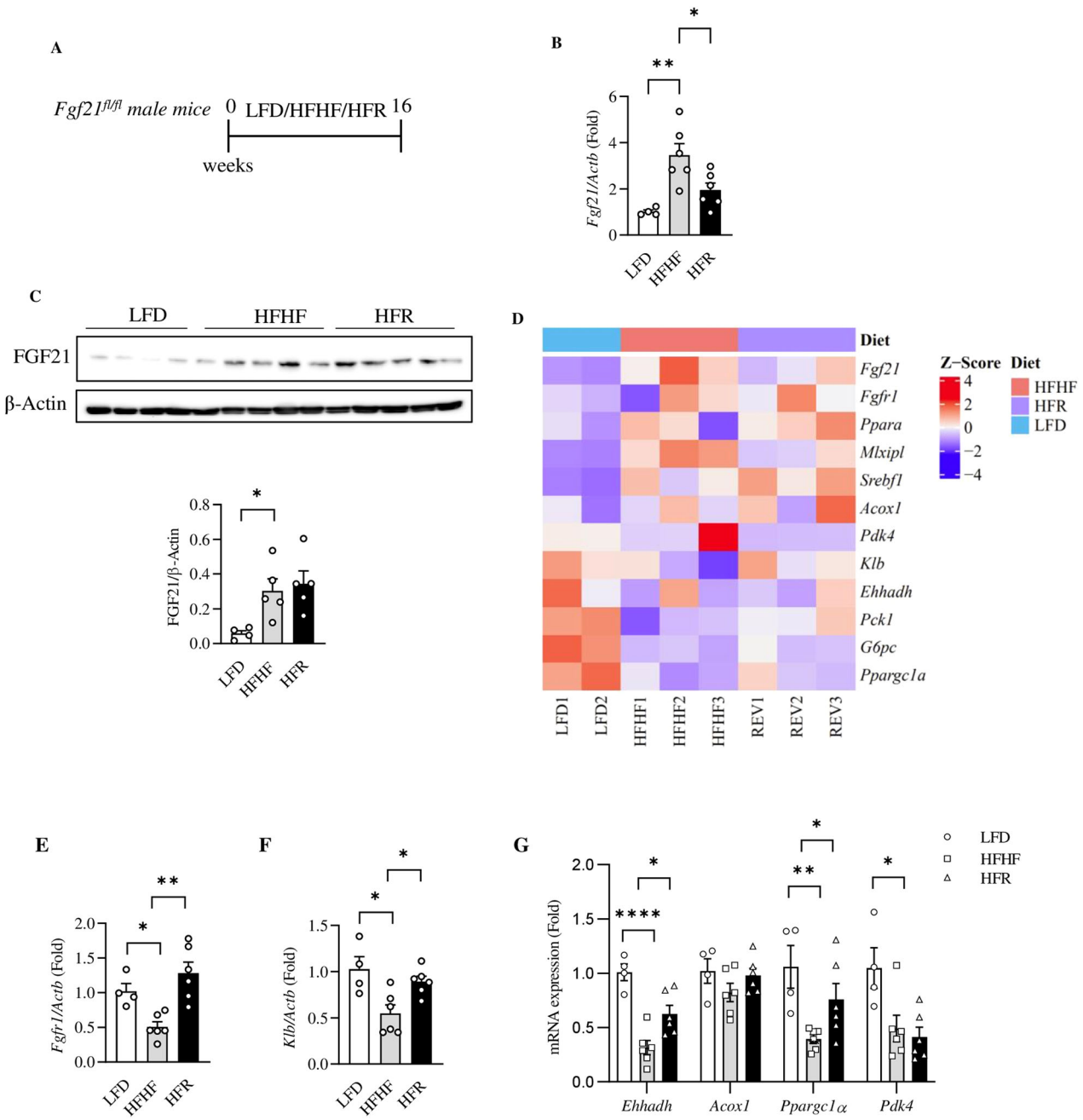

Fig. 5 Dietary resveratrol intervention targets hepatic FGF21 and FGF21 signaling in HFHF diet-challenged *Fgf21*$^{fl/fl}$ mice. A Illustration of the animal experimental timeline in *Fgf21*$^{fl/fl}$ mice [$n = 4$ for LFD group, and $n = 6$ for HFHF and HFR groups]. **B** Hepatic *Fgf21* level in indicated group of mice. **C** Hepatic FGF21 protein levels. The bottom panel shows the densitometric analysis results. **D** Heat map shows comparison of selected FGF21-related genes among mice fed with LFD, HFHF and HFR diet. **E–G** qRT-PCR assessment on hepatic expression of indicated genes in LFD ($n = 4$), HFHF ($n = 6$) and HFR ($n = 6$) mice. Data are shown as the mean ± SEM. *$p < 0.05$, **$p < 0.01$ and ****$p < 0.0001$.

challenged mouse models, although the body weight lowering effect of resveratrol or curcumin intervention may only partially rely on hepatic FGF21. In a previous investigation, we also noticed that hepatic FGF21 is not absolutely required for the GLP-1RA liraglutide in lowering the body weight in mice with HFD challenge [11].

How can hepatic FGF21 mediate metabolic beneficial effect of both nutrient restriction [3, 35] and dietary polyphenol interventions [6, 22, 24]? Why hepatic FGF21 is required for both the GLP-1R agonists (including liraglutide, semaglutide and exenatide) and various dietary interventions [2]? The diabetes drug metformin, which is also a chemical isolated from plant, was shown to induce GLP-1 secretion, and such function contributes to the actions of metformin in the treatment of type 2 diabetes [36]. As metformin has been shown to exert its function via "reshaping" gut microbiome, it remains to be determined whether gut microbiome is involved in regulating gut GLP-1 production or secretion [37]. A recent study by Martin and colleagues showed that in the absence of gut microbiome, FGF21 adaptive pathway is desensitized in response to dietary protein restriction [38]. We are aware of the fundamental role of gut microbiome in health and diseases for decades, and a few recent studies have demonstrated that beneficial effects of resveratrol intervention are strongly associated with alterations in gut microbiome [39–41]. Targeting gut microbiome or intestinal signaling

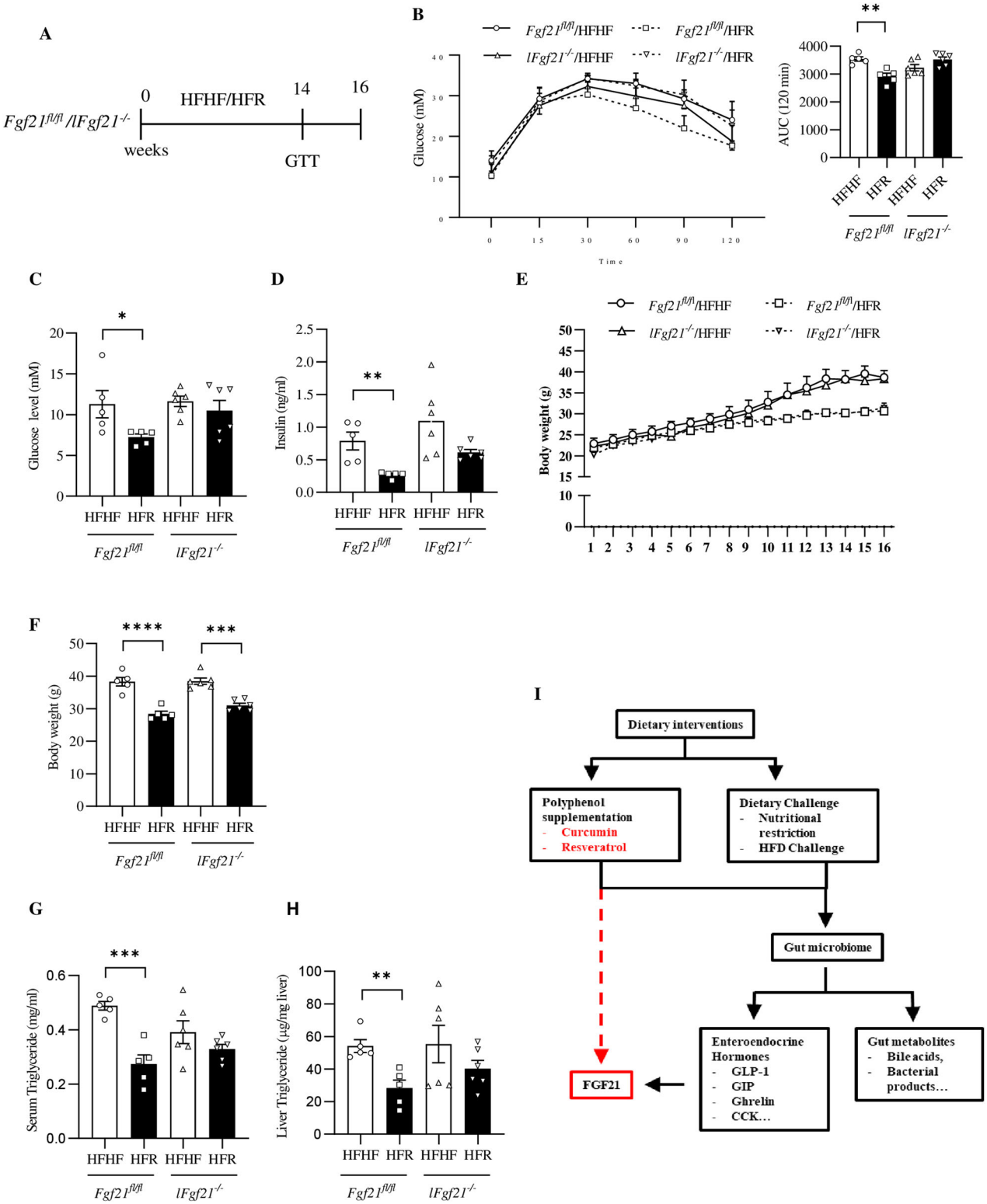

Fig. 6 Resveratrol intervention exhibits metabolic beneficial effects on obesogenic diet-challenged *Fgf21*$^{fl/fl}$ but not *lFgf21*$^{-/-}$ mice. **A** Illustration of the animal experimental timeline. **B** Blood glucose level and AUC during glucose tolerance test of *Fgf21*$^{fl/fl}$ and *lFgf21*$^{-/-}$ mice. **C** Fasting glucose level at the end of the study. **D** Serum Insulin level. **E** Body weight during the experimental period. **F** Body weight gain at the end of the 16th week after overnight fasting. **G** Serum TG level of indicated group of mice. **H** Hepatic TG content of indicated group of mice. **I** Diagram shows our view that it is gut microbiome that mediates functions of dietary interventions, involving the hepatic hormone FGF21. Dietary intervention including dietary polyphenol supplementation or dietary behavioral changes alter gut microbiome composition and diversity. This in term triggers organ-organ crosstalk involving the gut-brain, gut-liver or other axis, with altered entero-endocrine hormone (GLP-1, GIP, and others) production and function, and altered levels of gut metabolites (bile acids and others), leading to the alteration on hepatic FGF21 production and sensitivity. Hence, without hepatic FGF21, dietary intervention cannot exert its metabolic beneficial effect. Data are shown as the mean ± SEM ($n = 5$ for *Fgf21*$^{fl/fl}$, and $n = 6$ for *lFgf21*$^{-/-}$). *$p < 0.05$, **$p < 0.01$ and ****$p < 0.0001$.

cascades, including the gut hormone GLP-1, the intestinal Takeda G protein-coupled receptor 5 (TGR5) or Farnesoid X receptor (FXR) medicated bile acid signaling cascades, are also shared with the diabetes drug metformin, other phytomedicine including red ginseng extracts, theabrownin isolated from Pu-erh tea, blueberry and cranberry anthocyanin extracts [37, 42–45]. Prior to reporting our current investigation, we have reported very recently that resveratrol intervention target gut microbiome, leading to the attenuation of gut bile acid/FXR signaling and chylomicron secretion, and improved lipid homeostasis [5]. It is plausible to suggest that gut microbiome mediates functions of the two categories of dietary interventions (Fig. 6l), with the participation of gut metabolites and gut produced hormones, including GLP-1 and gastric inhibitory polypeptide (GIP), as well as gut/brain axis, gut/liver axis, or other axis that links gut and other peripheral organs, as we have reviewed recently [2].

Hepatic FGF21 is required for curcumin or resveratrol in exerting their major metabolic beneficial effects. The existence of common targets, such as FGF21, for GLP-1RAs and various types of dietary interventions, makes us to recognize the link between these two categories of "medicines", between these two lines of biomedical research, brings us a novel angle in understanding and further investigation of metabolic disease treatment and prevention, with prescribed drugs and various phytomedicine.

DATA AVAILABILITY

The raw data generated in the study are available upon reasonable request from the corresponding authors. Raw and processed RNAseq data have been submitted to the Gene Expression Omnibus (GEO) database (GSE241713).

CODE AVAILABILITY

All codes utilized in the manuscript are available upon reasonable request from the corresponding authors.

REFERENCES

1. Jin T, Song Z, Weng J, Fantus IG. Curcumin and other dietary polyphenols: potential mechanisms of metabolic actions and therapy for diabetes and obesity. Am J Physiol Endocrinol Metab. 2018;314:E201–e5.
2. Jin T. Fibroblast growth factor 21 and dietary interventions: what we know and what we need to know next. Med Rev. 2022;2:524–30.
3. Laeger T, Henagan TM, Albarado DC, Redman LM, Bray GA, Noland RC, et al. FGF21 is an endocrine signal of protein restriction. J Clin Investig. 2014;124:3913–22.
4. Jin TR. Curcumin and dietary polyphenol research: beyond drug discovery. Acta Pharm Sin. 2018;39:779–86.
5. Pang J, Raka F, Heirali AA, Shao W, Liu D, Gu J, et al. Resveratrol intervention attenuates chylomicron secretion via repressing intestinal FXR-induced expression of scavenger receptor SR-B1. Nat Commun. 2023;14:2656.
6. Zeng K, Tian L, Patel R, Shao W, Song Z, Liu L, et al. Diet polyphenol curcumin stimulates hepatic Fgf21 production and restores its sensitivity in high-fat-diet-fed male mice. Endocrinology. 2017;158:277–92.
7. Li Y, Wong K, Giles A, Jiang J, Lee JW, Adams AC, et al. Hepatic SIRT1 attenuates hepatic steatosis and controls energy balance in mice by inducing fibroblast growth factor 21. Gastroenterology. 2014;146:539–49.e7.
8. Badakhshi Y, Jin T. Current understanding and controversies on the clinical implications of fibroblast growth factor 21. Crit Rev Clin Lab Sci. 2021;58:311–28.
9. Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, et al. Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. Diabetes. 2014;63:4057–63.
10. Shao W, Jin T. Hepatic hormone FGF21 and its analogues in clinical trials. Chronic Dis Transl Med. 2022;8:19–25.
11. Liu D, Pang J, Shao W, Gu J, Zeng Y, He HH, et al. Hepatic fibroblast growth factor 21 is involved in mediating functions of liraglutide in mice with dietary challenge. Hepatology. 2021;74:2154–69.
12. Le TDV, Fathi P, Watters AB, Ellis BJ, Besing GK, Bozadjieva-Kramer N, et al. Fibroblast growth factor-21 is required for weight loss induced by the glucagon-like peptide-1 receptor agonist liraglutide in male mice fed high carbohydrate diets. Mol Metab. 2023;72:101718.
13. Liu J, Yang K, Yang J, Xiao W, Le Y, Yu F, et al. Liver-derived fibroblast growth factor 21 mediates effects of glucagon-like peptide-1 in attenuating hepatic glucose output. EBioMedicine. 2019;41:73–84.
14. Lee J, Hong SW, Park SE, Rhee EJ, Park CY, Oh KW, et al. Exendin-4 regulates lipid metabolism and fibroblast growth factor 21 in hepatic steatosis. Metabolism. 2014;63:1041–8.
15. Song Z, Revelo X, Shao W, Tian L, Zeng K, Lei H, et al. Dietary curcumin intervention targets mouse white adipose tissue inflammation and brown adipose tissue UCP1 expression. Obesity. 2018;26:547–58.
16. Shao W, Yu Z, Chiang Y, Yang Y, Chai T, Foltz W, et al. Curcumin prevents high fat diet induced insulin resistance and obesity via attenuating lipogenesis in liver and inflammatory pathway in adipocytes. PLoS ONE. 2012;7:e28784.
17. Zeng K, Tian L, Sirek A, Shao W, Liu L, Chiang YT, et al. Pak1 mediates the stimulatory effect of insulin and curcumin on hepatic ChREBP expression. J Mol Cell Biol. 2017;9:384–94.
18. Tian L, Song Z, Shao W, Du WW, Zhao LR, Zeng K, et al. Curcumin represses mouse 3T3-L1 cell adipogenic differentiation via inhibiting miR-17-5p and stimulating the Wnt signalling pathway effector Tcf7l2. Cell Death Dis. 2017;8:e2559.
19. Ip W, Shao W, Song Z, Chen Z, Wheeler MB, Jin T. Liver-specific expression of dominant-negative transcription factor 7-like 2 causes progressive impairment in glucose homeostasis. Diabetes. 2015;64:1923–32.
20. Gniuli D, Calcagno A, Dalla Libera L, Calvani R, Leccesi L, Caristo ME, et al. High-fat feeding stimulates endocrine, glucose-dependent insulinotropic polypeptide (GIP)-expressing cell hyperplasia in the duodenum of Wistar rats. Diabetologia. 2010;53:2233–40.
21. Yang L, Wang J, Altreuter J, Jhaveri A, Wong CJ, Song L, et al. Tutorial: integrative computational analysis of bulk RNA-sequencing data to characterize tumor immunity using RIMA. Nat Protoc. 2023;18:2404–14.
22. Tian L, Ning H, Shao W, Song Z, Badakhshi Y, Ling W, et al. Dietary Cyanidin-3-Glucoside Attenuates High-Fat-diet-induced body-weight gain and impairment of glucose tolerance in mice via effects on the hepatic hormone FGF21. J Nutr. 2020;150:2101–11.
23. Cunningham RP, Moore MP, Moore AN, Healy JC, Roberts MD, Rector RS, et al. Curcumin supplementation mitigates NASH development and progression in female Wistar rats. Physiol Rep. 2018;6:e13789.
24. Tian L, Zeng K, Shao W, Yang BB, Fantus IG, Weng J, et al. Short-term curcumin gavage sensitizes insulin signaling in dexamethasone-treated C57BL/6 mice. J Nutr. 2015;145:2300–7.
25. Dushay JR, Toschi E, Mitten EK, Fisher FM, Herman MA, Maratos-Flier E. Fructose ingestion acutely stimulates circulating FGF21 levels in humans. Mol Metab. 2015;4:51–7.
26. Estall JL, Ruas JL, Choi CS, Laznik D, Badman M, Maratos-Flier E, et al. PGC-1alpha negatively regulates hepatic FGF21 expression by modulating the heme/Rev-Erb(alpha) axis. Proc Natl Acad Sci USA. 2009;106:22510–5.
27. Jin L, Geng L, Ying L, Shu L, Ye K, Yang R, et al. FGF21-Sirtuin 3 Axis confers the protective effects of exercise against diabetic cardiomyopathy by governing mitochondrial integrity. Circulation. 2022;146:1537–57.
28. Badakhshi Y, Shao W, Liu D, Tian L, Pang J, Gu J, et al. Estrogen-Wnt signaling cascade regulates expression of hepatic fibroblast growth factor 21. Am J Physiol Endocrinol Metab. 2021;321:E292–E304.
29. Fisher FM, Chui PC, Antonellis PJ, Bina HA, Kharitonenkov A, Flier JS, et al. Obesity is a fibroblast growth factor 21 (FGF21)-resistant state. Diabetes. 2010;59:2781–9.
30. Ejaz A, Martinez-Guino L, Goldfine AB, Ribas-Aulinas F, De Nigris V, Ribo S, et al. Dietary Betaine supplementation increases Fgf21 levels to improve glucose homeostasis and reduce hepatic lipid accumulation in mice. Diabetes. 2016;65:902–12.
31. Markan KR. Defining "FGF21 resistance" during obesity: controversy, criteria and unresolved questions. F1000Res. 2018;7:289.
32. Allard C, Bonnet F, Xu B, Coons L, Albarado D, Hill C, et al. Activation of hepatic estrogen receptor-alpha increases energy expenditure by stimulating the production of fibroblast growth factor 21 in female mice. Mol Metab. 2019;22:62–70.
33. Hondares E, Iglesias R, Giralt A, Gonzalez FJ, Giralt M, Mampel T, et al. Thermogenic activation induces FGF21 expression and release in brown adipose tissue. J Biol Chem. 2011;286:12983–90.
34. Potthoff MJ, Kliewer SA, Mangelsdorf DJ. Endocrine fibroblast growth factors 15/19 and 21: from feast to famine. Genes Dev. 2012;26:312–24.
35. Hill CM, Laeger T, Dehner M, Albarado DC, Clarke B, Wanders D, et al. FGF21 signals protein status to the brain and adaptively regulates food choice and metabolism. Cell Rep. 2019;27:2934–47.e3.
36. Bahne E, Sun EWL, Young RL, Hansen M, Sonne DP, Hansen JS, et al. Metformin-induced glucagon-like peptide-1 secretion contributes to the actions of metformin in type 2 diabetes. JCI Insight. 2018;3:e93936.
37. Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. Nat Med. 2018;24:1919–29.
38. Martin A, Ecklu-Mensah G, Ha CWY, Hendrick G, Layman DK, Gilbert J, et al. Gut microbiota mediate the FGF21 adaptive stress response to chronic dietary protein-restriction in mice. Nat Commun. 2021;12:3838.

39. Bird JK, Raederstorff D, Weber P, Steinert RE. Cardiovascular and antiobesity effects of resveratrol mediated through the gut microbiota. Adv Nutr. 2017;8:839–49.
40. Wang P, Gao J, Ke W, Wang J, Li D, Liu R, et al. Resveratrol reduces obesity in high-fat diet-fed mice via modulating the composition and metabolic function of the gut microbiota. Free Radic Biol Med. 2020;156:83–98.
41. Sung MM, Kim TT, Denou E, Soltys CM, Hamza SM, Byrne NJ, et al. Improved glucose homeostasis in obese mice treated with resveratrol is associated with alterations in the gut microbiome. Diabetes. 2017;66:418–25.
42. Huang F, Zheng X, Ma X, Jiang R, Zhou W, Zhou S, et al. Theabrownin from Pu-erh tea attenuates hypercholesterolemia via modulation of gut microbiota and bile acid metabolism. Nat Commun. 2019;10:4971.
43. Zhang E, Jin L, Wang Y, Tu J, Zheng R, Ding L, et al. Intestinal AMPK modulation of microbiota mediates crosstalk with brown fat to control thermogenesis. Nat Commun. 2022;13:1135.
44. Li W, Zhuang T, Wang Z, Wang X, Liu L, Luo Y, et al. Red ginseng extracts ameliorate high-fat diet-induced obesity and insulin resistance by activating the intestinal TGR5-mediated bile acids signaling pathway. Phytomedicine. 2023;119:154982.
45. Liu J, Hao W, He Z, Kwek E, Zhu H, Ma N, et al. Blueberry and cranberry anthocyanin extracts reduce bodyweight and modulate gut microbiota in C57BL/6 J mice fed with a high-fat diet. Eur J Nutr. 2021;60:2735–46.

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AUTHOR CONTRIBUTIONS

Jia Nuo Feng: Conceptualization, Methodology, Investigation, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. Weijuan Shao: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft, Writing – review & editing. Lin Yang: Data curation, Formal Analysis, Methodologies, Software. Juan Pang: Investigation, Formal analysis, Visualization. Wenhua Ling: Writing – review & editing. Dinghui Liu: Investigation, Formal analysis, Visualization. Michael B Wheeler: Writing – review & editing. Housheng Hansen He: Formal Analysis, Methodologies, Software, Writing – review & editing. Tianru Jin: Conceptualization, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

All animal experiments were approved by the University Health Network Animal Care Committee (AUP 2949.18) and were performed in accordance with the guidelines of the Canadian Council of Animal Care.

ADDITIONAL INFORMATION

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