# Diminished diet-induced hyperglycemia and dyslipidemia and enhanced expression of PPARα and FGF21 in mice with hepatic ablation of brain-derived neurotropic factor

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## Abstract

Brain-derived neurotropic factor (BDNF) mediates many aspects of neuronal function, and plays a chief role in the central regulation of energy balance. In the periphery, it is expressed in organs involved in energy, lipid, and glucose homeostasis, including the liver, but its role there remains unclear. Here, we describe studies examining the effect of selectively depleting hepatic BDNF. Liver-specific mutant mice exhibited normal food intake and body weights when fed standard chow or high-fat diets (HFDs). However, whereas HFD intake induced mild hyperglycemia and hyperinsulinemia in wild-types (WTs), liver-specific BDNF mutants were protected from these effects. Serum levels of cholesterol and triglycerides were also elevated in HFD-fed WTs, but they were normal or slightly increased in BDNF mutants. Furthermore, whereas WTs fed HFD exhibited elevated levels of circulating alanine aminotransferase and aspartate aminotransferase, BDNF mutant males fed a similar diet had a normal content of both enzymes. Molecular analysis indicated that the livers of BDNF mutants fed HFD contained elevated levels of peroxisome proliferator-activated receptor α (*Pparα* or *Ppara* as listed in the MGI Database) and fibroblast growth factor 21 (*Fgf21*) transcripts compared with WTs. This is a notable finding as this pathway has anti-diabetic and lipid clearance effects. Accordingly, genes involved in lipid and glucose handling and targets of PPARα and FGF21 were upregulated in the BDNF mutant livers. The collective data indicate that hepatic BDNF might facilitate the emergence of insulin resistance, dyslipidemia, and liver disease following HFD challenge by suppressing PPARα and FGF21.
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## Introduction

Brain-derived neurotropic factor (BDNF) signals through the TrkB (or NTRK2) receptor to mediate neuronal survival, differentiation, and plasticity (Lewin & Barde 1996, McAllister *et al.* 1999), and its effects in the brain are pivotal for the control of appetite (Rios *et al.* 2001). Perturbing BDNF and TRKB signaling in the brains of mice results in hyperphagic behavior, dramatic obesity, hyperleptinemia, hyperinsulinemia, and hyperglycemia (Lyons *et al.* 1999, Kernie *et al.* 2000, Rios *et al.* 2001, Xu *et al.* 2003, Unger *et al.* 2007). Because expression of BDNF is not limited to the brain, it is possible that it also influences ingestive behavior and metabolic activity by acting in the periphery. Indeed, this neurotropin is expressed in peripheral tissues with known roles in energy, glucose, and lipid homeostasis, including adipose and muscle tissues and the liver (Lommatzsch *et al.* 1999, Cassiman *et al.* 2001, Mousavi & Jasmin 2006, Ukropec *et al.* 2008). Here, we investigated the necessity of its expression in hepatic tissue for these physiological processes.

The liver is a site of lipid and glucose uptake, storage, and secretion. It acts as an energy sensor and conveys information regarding nutritional status to the brain via afferent vagal nerve pathways (Thorens & Larsen 2004). In response to hepatic input, food intake and metabolic changes are initiated by central mechanisms to preserve energy and glucose homeostasis (Langhans 2003). The liver is highly susceptible to chronic increases in dietary fat intake, which elicits hepatic steatosis, reduced hepatic insulin sensitivity, and a concomitant failure to suppress liver glucose output. These deficits are thought to result in lipoperoxidative stress and hepatic injury and inflammation (Musso *et al.* 2003). However, mechanisms underlying these detrimental effects or mediating their prevention are not completely understood. This is an important area of research as hepatic steatosis increases susceptibility to type 2 diabetes, liver cirrhosis, and cardiovascular disease. Recently, the transcription factor peroxisome proliferator-activated receptor α (Pparα or *Ppara* as listed in the MGI Database) and its downstream target, fibroblast growth factor 21 (*Fgf21*), were shown to be essential for hepatic lipid oxidation, triglyceride clearance, and improved insulin sensitivity following high-fat (HF) dietary intake (Kharitonenkov *et al.* 2005, 2007, Badman *et al.* 2007, Xu *et al.* 2009), indicating the potential of these genes as drug targets for the metabolic syndrome.


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Much remains to be unraveled regarding the effects of neurotrophins on hepatic function under basal or dietary stress conditions. The patterns of BDNF and TRKB expression suggest neurotrophic support of the autonomic innervation of the liver. In adult mice, whereas *Bdnf* mRNA is expressed in mouse hepatocytes (Lommatzsch *et al.* 1999), TRKB is expressed by periductal nerve fibers innervating the liver (Garcia-Suarez *et al.* 2006). As liver and portal vein afferents serve as sensors for amino acids, glucose, insulin, and glucagon, their expression of TRKB could facilitate the transmission of metabolic signals to the brain. BDNF was previously reported to enhance insulin signaling in livers of leptin receptor (*db/db*) mutant mice that were obese by facilitating phosphatidylinositol 3-kinase (PI3K) activity (Tsuchida *et al.* 2001). BDNF treatment normalized liver weights and glycogen content of *db/db* mutants, and these changes were not entirely associated with reduced food intake (Tonra *et al.* 1999). It remains to be elucidated whether endogenous BDNF is required for the regulation of energy and glucose homeostasis and for metabolic adaptations triggered by increased fat intake.

To address the role of hepatic BDNF, we examined mutant mice lacking expression of this neurotrophin in the liver. Mutant animals exhibited normal food intake behavior, body weight, and circulating levels of glucose, insulin, and lipids when fed normal chow. However, depletion of hepatic BDNF was protective against deficits in lipid and glucose homeostasis and liver damage induced by increased dietary fat intake. Additionally, the absence of BDNF in the liver facilitated a robust induction of local expression of PPARα and FGF21 by HF diet (HFD). These studies demonstrate that in contrast to its appetite-inhibiting effects in the brain, BDNF expression in the liver might mediate the detrimental effects of dietary stress by suppressing hepatic PPARα/FGF21 signaling and its anti-diabetic and lipid clearance effects.

## Materials and Methods

### *Animals*

BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice were generated by crossing mice carrying floxed BDNF alleles with transgenic mice in which liver-specific expression of cre recombinase was directed by the albumin promoter (Postic & Magnuson 2000). All mice were in a hybrid C57Bl6/129 background, and littermate wild-type (WT) controls were used for these studies. Mice were housed in a 12 h light:12 h darkness cycle, and were given free access to water and standard lab chow (5% fat, 18% protein, and 57% carbohydrate; 2018; Harlan Teklad, Madison, WI, USA). At 8 weeks of age, animals in the WT and BDNF$^{2L/2LAlb\text{-}cre}$ HF groups were switched from a standard chow (SC) to a HFD (45% fat, 20% protein, and 35% carbohydrate; D12451; Research Diets, New Brunswick, NJ, USA) for 12 weeks. All of the following procedures were approved by the Institutional Animal Care and Use Committee at Tufts University, and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

### *Body weight and food intake measurements*

Body weight measurements were taken from BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice fed standard or HF chow and their age- and sex-matched WT controls. To measure food intake, mice were housed individually and given a premeasured amount of food. One week later, the amount of food remaining was weighed and subtracted from the initial amount given.

### *Liver tissue analysis*

Livers were extracted from mice immediately following euthanasia, and their wet weight was obtained for analysis. For the histological examination, livers were immersion fixed in 4% paraformaldehyde for 16 h at 4 °C, cryoprotected in a 30% sucrose solution, and frozen in mounting media (Tissue-Tek, Torrance, CA, USA) until further use. Ten-micrometer-thick cryostat liver sections were stained with hematoxylin and eosin for the gross morphology analysis. For the liver lipid analysis, livers were extracted from fed mice or mice fasted for 16 h, and snap frozen in liquid nitrogen. Analysis was conducted at the Vanderbilt MMPC Lipid Core, where levels of total cholesterol and triglycerides were determined.

### *I.p. glucose tolerance*

Following 24 h of fasting, baseline (0) blood glucose concentrations were measured from tail bleeds using the TheraSense freestyle blood glucose meter. Next, D-glucose, 2 mg/kg body weight, was administered by i.p. injection. Subsequent blood glucose measurements were taken at 15, 30, 60, and 120 min after glucose administration.

### *Serum analysis*

Blood was collected by retro-orbital bleeding from mice under basal feeding conditions and spun down in a microcentrifuge, and serum was stored at −80 °C. Cholesterol levels were measured using the Cholesterol E kit (Wako Chemicals USA, Inc., Richmond, VA, USA). Levels of triglycerides were measured using the serum triglyceride determination kit (Sigma). Serum content of insulin was determined using the rat/mouse insulin ELISA kit (Linco Research, St Charles, MO, USA). Serum samples were sent to IDEXX Laboratories (North Grafton, MA, USA) where levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured.

### *Quantitative reverse transcription-PCR analysis*

RNA extracted from liver tissue was treated with 4 units of RQ1 Rnase-free DNAse (Fisher Scientific FP2231,

Pittsburg, PA, USA), and was tested for genomic DNA contamination in PCRs. Reverse transcription (RT) to generate cDNA was conducted with 2 μg of RNA and by using 200 units of Superscript II reverse transcriptase (Invitrogen) and 150 ng of random primers (Invitrogen) in a 20-μl reaction mixture. Real-time PCR amplification was performed using a MX-3000P Stratagene cycler and SYBR green PCR master mix (Qiagen); the final primer concentration in a 20-μl reaction mixture was 500 nm. For each primer set, the specificity of the product amplification was confirmed by dissociation curve analysis and agarose gel electrophoresis. Furthermore, standard curves were created using serial dilutions, and the efficiencies for each primer set were calculated. The amplification efficiency for all the primers used in this study was >90. Forty-five cycles of a two-step protocol were used: 95 °C for 10 min; 45 cycles with 95 °C 30 s, 55 °C 30 s, and 72 °C 30 s. The actin gene was used as the normalizer. The primers used for *Bdnf* measurements were as follows: forward: 5′-GAAAGTCCC-GGTATCCAAAG-3′, reverse: 5′-CCAGCCAATTCT-CTTTTT-3′. Primers sequences for the other genes were obtained from http://pga.mgh.harvard.edu/primerbank/index.html: actin (ID# 6671509a1), *Pparα* (ID #31543500a1), *Pparγ* (ID# 6755138a1), *Fgf21* (ID# 9910218a1), *Sirt1* (ID# 9790229a1), glucose-6-phosphatase (*G6Pase*; ID# 31982353a1), long-chain acyl-coenzyme A dehydrogenase (*Acadl*; ID# 31982520a1), acyl-coenzyme A dehydrogenase medium-chain (*Acadm*; ID# 6680618a), cluster of differentiation 36 (*Cd36*; ID# 31982474a1), and glucose transporter isoform 1 (*Glut1*; ID# 22094111a1) (Wang & Seed 2003, Spandidos *et al*. 2008). All samples were analyzed in triplicates, and nontemplate controls were included to ascertain any level of contamination. Amplification products ranged from 100 to 173 bp. Data obtained were analyzed using the comparative $C_t$ method. For each primer set, a validation experiment was performed to demonstrate that the PCR efficiencies were approximately equal.

### *Statistical analysis*

Statistical significance was determined using two-way ANOVA analysis followed by Fisher's protected least significant difference (PLSD) post-test, and values represent mean ± S.E.M. $P<0{\cdot}05$ was considered statistically significant.

## Results

### *Food intake and body weights of BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice are normal*

To determine how BDNF content in the liver influences hepatic function and body weight regulation, we generated mice with liver-specific targeting of the *Bdnf* gene, hereafter referred to as BDNF$^{2L/2LAlb\text{-}cre}$ mutants. For this, we crossed our previously reported line of floxed BDNF mice (Rios *et al*. 2001) with transgenic mice expressing cre recombinase under the control of the albumin promoter (Postic & Magnuson 2000). In the albumin-cre line of mice, cre recombinase is exclusively expressed in hepatocytes, and it mediates extensive recombination of floxed alleles in the liver (Postic & Magnuson 2000, Chen *et al*. 2007, Wallace *et al*. 2007).

Quantitative RT-PCR analysis showed that *Bdnf* mRNA was virtually depleted in the livers of BDNF$^{2L/2LAlb\text{-}cre}$ mice (Fig. 1). Furthermore, normal levels of *Bdnf* transcripts were detected in white adipose and pancreatic tissue in BDNF$^{2L/2LAlb\text{-}cre}$ mice (Fig. 1), consistent with previous reports indicating that cre recombinase is not expressed outside the liver of mice carrying the albumin-cre allele (Postic & Magnuson 2000). We further assessed cre-mediated recombination by crossing albumin-cre mice with animals carrying a LacZ reporter allele that is activated by cre-mediated recombination (Soriano 1999). We detected extensive LacZ activity in livers but not in hypothalamus, kidney, pancreas, heart, or testis of LacZ/albumin-cre reporter mice (Supplementary Figure 1, see section on supplementary data given at the end of this article). These data show that the albumin-cre allele drives selective recombination of floxed alleles in the liver, and that BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice have liver-specific depletion of BDNF.

To determine whether reduced expression of *Bdnf* in the liver had any effects on energy balance, we monitored food intake of BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice and WT controls fed a SC or a HFD. Female and male BDNF$^{2L/2LAlb\text{-}cre}$ mutants ate comparable amounts of food compared to their sex- and diet-matched WT controls (Table 1). Moreover, both WT and mutant mice fed HFD limited their caloric intake by eating significantly less than animals fed SC (Table 1).

BDNF mutant males and females fed SC or HFD exhibited body weights comparable to those of WT controls fed similar diets (Table 1). Consistent with their reduced food intake, mice fed HFD exhibited weights that were similar to those of the mice fed SC. These results indicate that in contrast to its actions in the brain, *Bdnf* expression in the liver is not required for the regulation of food intake and body weight.

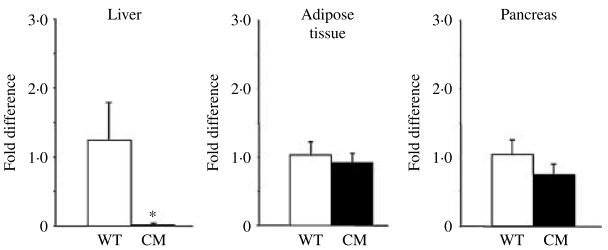


**Figure 1** Measurement of *Bdnf* mRNA content in the livers of BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice. Quantitative RT-PCR analysis of hepatic, adipose, and pancreatic contents of *Bdnf* mRNA in wild-type (WT) and BDNF$^{2L/2LAlb\text{-}cre}$ conditional mutant (CM) mice ($n=4$ per group). Quantitative RT-PCR results from wild-types and BDNF$^{2L/2LAlb\text{-}cre}$ conditional mutants were analyzed using the comparative $C_t$ method. *$P=0{\cdot}002$.

**Table 1** Food intake, body weights and liver weights and content of lipids in wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice. Wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice fed HFD ate less than animals fed standard chow. Moreover, BDNF$^{2L/2LAlb\text{-}cre}$ mutant females fed HFD trended toward a significant reduction in liver weights when compared with wild-type females fed HFD

| | Standard chow | | High-fat diet | |
|---|---|---|---|---|
| **Parameter** | WT | BDNF$^{2L/2LAlb\text{-}cre}$ | WT | BDNF$^{2L/2LAlb\text{-}cre}$ |
| Males | | | | |
| Food intake/week (g) | 29·6±1·2 | 30·5±1·1 | 17·9±0·4* | 17·8±0·6* |
| Body weight (g) | 27·3±1·3 | 28·0±1·0 | 29·7±1·5 | 27·9±1·3 |
| Liver weight (g) | 1·1±0·1 | 1·1±0·1 | 1·0±0·1 | 1·2±0·1 |
| Liver chol. (μg/mg) | 2·0±0·2 | 1·9±0·1 | 3·4±0·4* | 3·5±0·6* |
| Liver trig. (μg/mg) | 10·4±2·4 | 14·9±2·9 | 45·0±10·0* | 51·0±10·0* |
| Females | | | | |
| Food intake/week (g) | 26·6±1·0 | 27·3±0·4 | 17·2±0·8* | 15·6±0·4* |
| Body weight (g) | 22·0±0·7 | 21·2±0·8 | 21·5±0·8 | 21·3±0·8 |
| Liver weight (g) | 1·0±0·1 | 0·9±0·03 | 1·1±0·1 | 0·9±0·02† |
| Liver chol. (μg/mg) | 2·2±0·1 | 1·9±0·1 | 4·1±0·8* | 4·1±0·4* |
| Liver trig. (μg/mg) | 13·6±0·9 | 13·1±2·3 | 51·0±4·8* | 47·0±5·4* |

*$P<0·05$ compared with genotype- and sex-matched SC-fed mice; †$P=0·08$ compared with wild-type females fed HFD. Chol., cholesterol; Trig., triglycerides; WT, wild-types.

### *The effects of a HFD are attenuated in BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice*

We sought to ascertain the effects of deleting *Bdnf* on the morphology and general architecture of the liver under SC feeding conditions and following administration of a HFD. Gross liver morphology of female and male BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice was normal compared with that of WTs fed SC (Fig. 2A, C, E and G). Moreover, livers of both WT and mutant mice fed a HFD contained larger, vacuolated cells, representative of lipid-laden cells, compared with animals fed SC (Fig. 2B, D, F and H). There was a trend toward an effect of genotype on liver weights ($F=3·4$; $P=0·07$) in females. Whereas the liver weights of WT and mutant females fed SC were not significantly different, there was a trend toward decreased liver weight in BDNF$^{2L/2LAlb\text{-}cre}$ mutant females fed HFD relative to WT females fed a similar diet (Table 1).

To determine whether an excess of lipid deposits contributed to the observed cellular pathology in livers of animals fed HFD, liver lipid content was assessed. There were no significant differences in the levels of liver cholesterol or triglycerides in WTs and BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed SC (Table 1). Moreover, HFD administration elicited similar elevations in liver cholesterol and triglyceride content in WTs and BDNF$^{2L/2LAlb\text{-}cre}$ mutants (Table 1). Together, the data show that depletion of BDNF content in the liver does not alter liver morphology under SC conditions. Moreover, they demonstrate that WTs and BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed a HFD exhibited similar levels of liver steatosis, but that mutant females trended toward reduced liver weight.

To further interrogate the effect of dietary stress on liver status, we measured the levels of ALT and AST. Elevated levels of serum ALT and AST are indicative of a damaged or diseased liver, and are often a feature of patients and mice with nonalcoholic fatty liver disease (Adams *et al.* 2004). There were no significant differences in ALT content among any of the female experimental groups (Fig. 3A). In the males, there was a significant interaction between genotype and diet ($F=7·9$; $P=0·01$) on the levels of ALT. Whereas HFD induced a 180% increase in ALT levels in WT mice relative to WTs fed SC ($P=0·005$), BDNF$^{2L/2LAlb\text{-}cre}$ mice fed HFD contained normal serum levels of this enzyme (Fig. 3B). Accordingly, ALT levels in HFD WTs were 156% higher than those of BDNF$^{2L/2LAlb\text{-}cre}$ mice fed the same diet (Fig. 3B) (WT HFD: 350±64·9; BDNF$^{2L/2LAlb\text{-}cre}$ HFD: 137·3±41·6; $P=0·005$).

Examination of serum levels of AST revealed additional differences between WTs and BDNF$^{2L/2LAlb\text{-}cre}$ mutants. BDNF$^{2L/2LAlb\text{-}cre}$ females fed HFD exhibited an 86% increase ($P=0·01$) in AST levels compared with their counterparts fed SC (Fig. 3C). There was a significant interaction of genotype and diet on levels of AST ($F=13$; $P=0·002$) in the males. Chronic administration of a HFD dramatically elevated serum levels of AST in WT males (WT SC: 123·8±14·4; WT HFD: 643·8±105·2; $P<0·0001$; Fig. 3D). In contrast, HFD administration did not affect the levels of AST in BDNF$^{2L/2LAlb\text{-}cre}$ mutant males (BDNF$^{2L/2LAlb\text{-}cre}$ SC: 211±38·8; BDNF$^{2L/2LAlb\text{-}cre}$ HFD: 249±44·9; $P=$NS; Fig. 3D). Accordingly, WT mice fed HFD contained 158% more serum AST than BDNF$^{2L/2LAlb\text{-}cre}$ mutant males fed a similar diet ($P=0·0003$; Fig. 3D). The collective data suggest that lack of hepatic BDNF confers protection against liver damage induced by dietary stress.

### *Deficits in glucose homeostasis elicited by HFD administration are diminished in BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice*

Deficits in glucose homeostasis are elicited by chronic HFD intake and are linked to the emergence of liver disease (Browning & Horton 2004, Festi *et al.* 2004, Angelico *et al.* 2005). We sought to evaluate glucose homeostasis in the absence of hepatic BDNF under SC and HFD conditions.

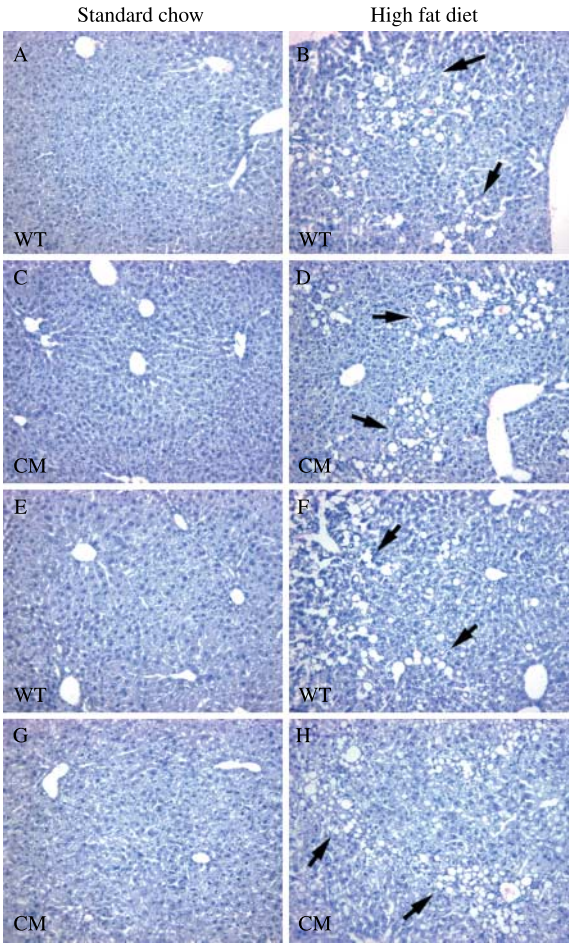


**Figure 2** Wild-type and BDNF mutant mice have comparable liver morphology. Representative hematoxylin and eosin-stained liver sections from wild-type (WT) and BDNF$^{2L/2LAlb\text{-}cre}$ conditional mutant (CM) mice fed standard chow or a high-fat diet ($n$=4). No gross differences were observed in females (A–D) or males (E–H). Arrows indicate lipid-laden cells in tissue obtained from mice fed HFD.

WTs and BDNF$^{2L/2LAlb\text{-}cre}$ mice fed normal chow exhibited similar fasted levels of serum glucose (Fig. 4A and B). In contrast, whereas WT mice fed an HFD exhibited a mild increase in fasting levels of glucose compared with their SC-fed counterparts, increased dietary fat intake did not induce hyperglycemia in BDNF$^{2L/2LAlb\text{-}cre}$ mutant males or females (Fig. 4A and B).

To further assess glucose homeostasis, we measured circulating levels of insulin. WT and BDNF mutant mice fed SC had similar levels of serum insulin (Fig. 4C and D). However, whereas HFD administration induced dramatic elevations in serum levels of insulin in WT females (2·5-fold, $P$=0·009) and males (2·8-fold, $P$=0·01), it had no significant effect on BDNF mutants, which exhibited normal levels after the diet challenge (Fig. 4C and D). Indeed, levels of insulin were significantly higher in WT animals fed HFD compared with BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed a similar diet (Fig. 4C and D).

Next, we performed glucose tolerance tests. BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed SC responded normally to the glucose challenge when compared with WTs fed a similar diet (Fig. 4E–H). Moreover, both mutant and WT mice fed a HFD exhibited compromised glucose metabolism when compared with their SC-fed counterparts (Fig. 4E–H). The cumulative data indicate that BDNF depletion in the liver prevents HFD-induced hyperglycemia and hyperinsulinemia in both males and females, but not against compromised responses to a glucose challenge elicited by HFD consumption.

### *HFD-induced hyperlipidemia is attenuated by depletion of BDNF in the liver*

To further evaluate liver function in the absence of BDNF, we measured serum levels of cholesterol and triglycerides. There was a significant interaction between genotype and diet on

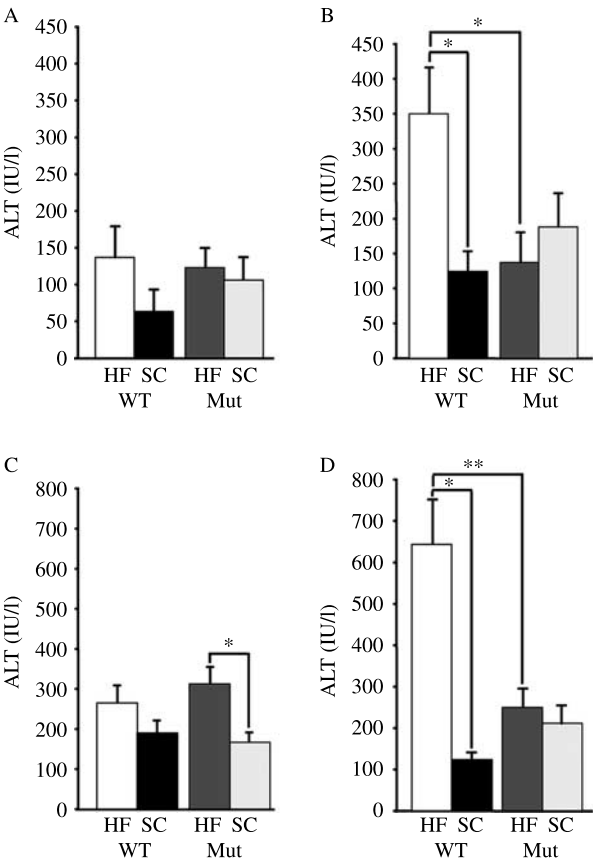


**Figure 3** Serum levels of ALT and AST in wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice. ALT levels in female (A) and male (B) wild-type (WT) and BDNF$^{2L/2LAlb\text{-}cre}$ mutant (Mut) mice fed standard chow (SC) or a high-fat (HF) diet for 3 months ($n$=5 or 6). *$P$=0·005; **$n$=5–6). (C) AST levels in wild-type and BDNF mutant females (*$P$=0·01, $n$=5–6). (D) AST levels in WT and BDNF$^{2L/2LAlb\text{-}cre}$ mutant males (*$P$<0·0001; **$P$=0·0003; $n$=5–6).

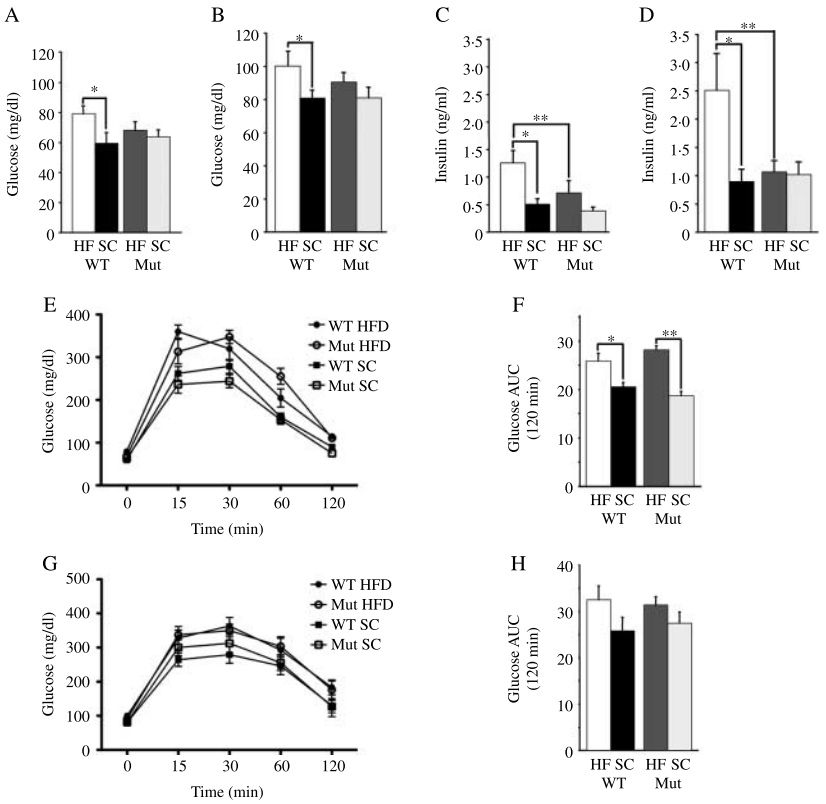


**Figure 4** Effects of hepatic depletion of BDNF on glucose homeostasis. (A) Fasting levels of serum glucose in wild-type (WT) and BDNF$^{2L/2LAlb\text{-}cre}$ mutant (Mut) female mice fed a high-fat (HF) diet or standard chow (SC) for 3 months (*$P$=0·02; $n$=8–11). (B) Fasting levels of serum glucose in wild-type (WT) and BDNF$^{2L/2LAlb\text{-}cre}$ mutant (Mut) male mice fed a high-fat (HF) diet or standard chow (SC) for 3 months (*$P$=0·05; $n$=7–10). (C) Serum levels of insulin in female wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice (*$P$=0·009; **$P$=0·04; $n$=5–7). (D) Serum levels of insulin in wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant male mice fed a high-fat (HF) diet or standard chow (SC) for 3 months (*$P$=0·01; **$P$=0·03; $n$=6–7). Glucose tolerance test in wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant females (E and F) and males (G and H) fed a high-fat diet or standard chow for 3 months. Area under the curve (AUC) for serum content of glucose during 120 min of glucose tolerance testing for WT and Mut females (F) and males (H) (F; *$P$=0·004; **$P$<0·0001; $n$=8–11).

total cholesterol levels in females ($F$=11·7; $P$=0·003). Whereas levels in WTs and BDNF mutants fed SC were comparable, HFD administration elicited higher increases in cholesterol levels in WTs than in mutant females (Fig. 5A). HFD induced a 69% increase in cholesterol levels in WTs relative to SC diet ($P$<0·0001), compared with a 31% increase in mutant females relative to mutants fed SC ($P$=0·0002). There were no significant differences in the levels of serum cholesterol among any of the male experimental groups (Fig. 5B).

No differences were observed in levels of circulating triglycerides under SC conditions (Fig. 5C). However, whereas HFD intake significantly increased triglycerides levels in WT females relative to SC ($P$=0·007), it did not impact triglyceride content in BDNF mutant females, which exhibited normal levels of this lipid (Fig. 5C). There were no significant differences in the levels of serum triglycerides among any of the male experimental groups (Fig. 5D). These data indicate that diet-induced dyslipidemia is diminished by depletion of BDNF in the liver.

### *Pparα and Fgf21 expression is enhanced in the liver of BDNF mutants fed HFD*

We sought to elucidate the molecular mechanisms underlying the protective effects against HFD challenge facilitated by hepatic BDNF depletion. For this, we measured hepatic expression levels of genes associated with the PPARα signaling pathway, including *Pparα*, *Sirt1*, and *Fgf21*. *Pparα* is a ligand-activated transcription factor that regulates fatty acid β-oxidation and catabolism, reduces hepatic fat storage, improves insulin sensitivity, and is positively regulated by *Sirt1*, a $NAD^+$-dependent protein deacetylase (Stienstra *et al.* 2007, Purushotham *et al.* 2009). FGF21, for its part, is a

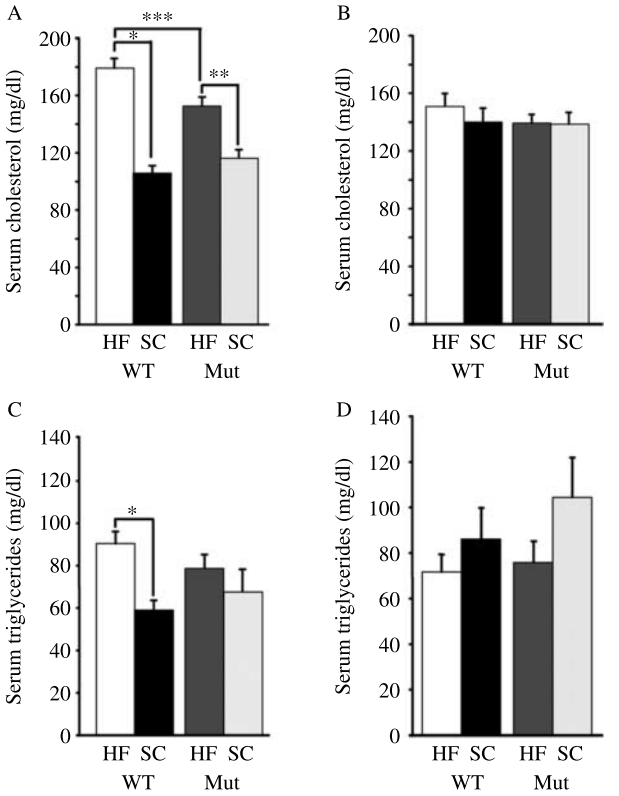


**Figure 5** Circulating levels of lipids in BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice. (A) Serum cholesterol levels in female wild-types (WT) and BDNF$^{2L/2LAlb\text{-}cre}$ mutants (Mut) fed standard chow (SC) or a high-fat (HF) diet. *$P$<0·0001; **$P$=0·0002; ***$P$=0·003; $n$=5). (B) Serum levels of cholesterol in wild-type and BDNF mutant males ($n$=5). (C) Serum levels of triglycerides in female wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice fed HFD or SC. *$P$=0·007; $n$=5. (D) Circulating levels of triglycerides in wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant male mice ($n$=5).

metabolic regulator under the transcriptional control of *Pparα* that regulates lipid metabolism and to which anti-diabetic and lipid-lowering effects have been ascribed (Kharitonenkov *et al.* 2005, 2007, Badman *et al.* 2007, Lundasen *et al.* 2007, Dostalova *et al.* 2009). Levels of *Pparγ* mRNA were also measured. Livers of WTs and BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed SC contained similar levels of *Pparα*, *Sirt1*, *Pparγ*, and *Fgf21* mRNA (Fig. 6). In contrast, BDNF mutants fed a HFD displayed a 2·3-fold increase in *Pparα* mRNA compared with WTs fed a similar diet ($P$=0·002; Fig. 6). Furthermore, whereas WTs responded to HFD challenge with a 110% increase in *Fgf21* mRNA expression in the liver compared with their SC-fed counterparts ($P$=0·03), BDNF mutants responded to the diet challenge with a more pronounced and dramatic 377% elevation in levels of *Fgf21* mRNA ($P$=0·001; data not shown). Indeed, BDNF mutants fed a HFD displayed a 4·3-fold ($P$=0·007) increase in hepatic expression of FGF21 compared with WTs fed a similar diet (Fig. 6). Levels of PPARγ were similar in both groups of animals fed HFD (Fig. 6).

Because *Pparα* and *Fgf21* mRNA content was elevated in the livers of BDNF mutants fed HFD, we examined whether expression of genes linked to this pathway previously and involved in lipid handling was also altered in the mutants (Badman *et al.* 2007). Consistent with their elevated levels of FGF21, BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed HFD had 3·3-fold ($P$=0·0004) and 1·9-fold ($P$=0·01) increases in the levels of expression of ACADM and ACADL respectively (Fig. 6). We also examined the expression of CD36, a fatty acid transporter that mediates triglyceride clearance. There was a trend toward a threefold increase in transcript levels of *Cd36* in the livers of BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed HFD compared with WTs; however, this did not reach statistical significance (Fig. 6).

Pertinent to the improved glucose homeostasis exhibited by BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed HFD, we examined liver levels of the gluconeogenesis-related transcript G6Pase. Additionally, we measured hepatic levels of Glut1. Levels of *G6Pase*

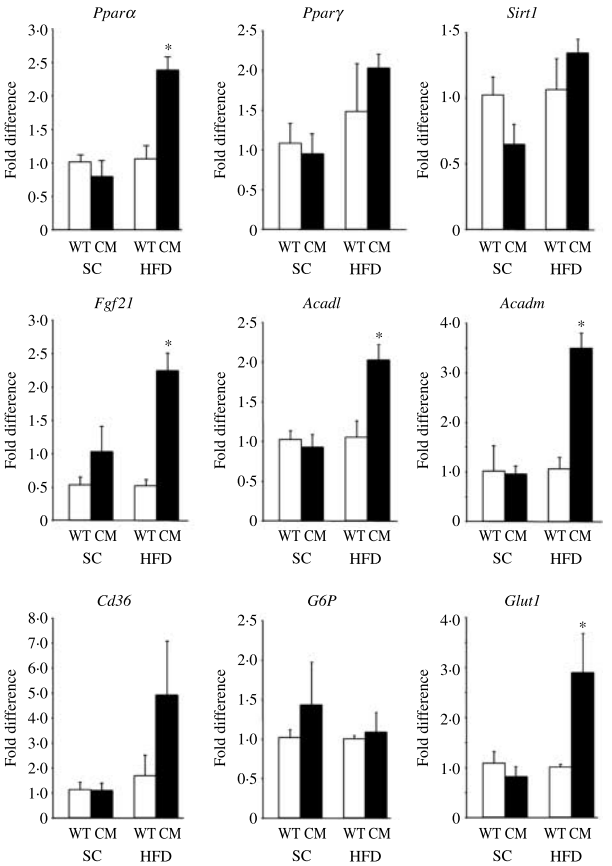


**Figure 6** Liver content of *Pparα*, *Sirt1*, *Fgf21*, *Acadl*, *Acadm*, *Cd36*, *G6Pase*, and *Glut1* mRNA in wild-type and BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice. Quantitative RT-PCR results obtained using mRNA obtained from livers of wild-types (WT) and BDNF$^{2L/2LAlb\text{-}cre}$ conditional mutants (CM) fed standard chow (SC) or a high-fat diet (HFD) for 3 months. Data are expressed as fold differences determined using the comparative $C_t$ method. *$P$<0·05.

mRNA were comparable in WTs and BDNF$^{2L/2LAlb\text{-}cre}$ mutants fed similar diets (Fig. 6). However, when we compared genotype-matched mice, we found that WTs fed a HFD experienced a 48% increase ($P=0{\cdot}005$) in *G6Pase* mRNA expression in the liver relative to WTs fed SC. In contrast, BDNF$^{2L/2LAlb\text{-}cre}$ mice did not exhibit significant increases in G6Pase expression following the HFD challenge (data not shown). Moreover, transcript content of Glut1 was significantly elevated in the liver of mutants fed HFD compared with WTs challenged with the same diet (Fig. 6; $P=0{\cdot}05$). In summary, the data show that hepatic depletion of BDNF results in increased content of PPARα, FGF21 and their target genes involved with glucose and lipid homeostasis under HFD conditions.

## Discussion

We sought to ascertain the physiological role of BDNF secreted by hepatic tissue. Here, we have shown that in contrast to its effects in the brain, where it acts to suppress appetite and maintain energy balance, BDNF expression in the liver is not required for the regulation of food intake or body weight. Importantly, we demonstrated that depletion of hepatic BDNF reduced the hyperglycemic, hyperinsulinemic, and hyperlipidemic effects of increased dietary fat intake. Furthermore, diet-induced increases in ALT and AST were diminished in liver-specific BDNF mutants. The protective effects of hepatic BDNF depletion were associated with enhanced PPARα and FGF21 content in the liver. The work described here suggests a previously unrecognized role of hepatic BDNF in facilitating some of the detrimental effects of dietary stress by suppressing the effects of PPARα and FGF21. These are significant findings as they bring us closer to understanding the pathological mechanisms leading to metabolic disorders and liver disease in individuals afflicted with diet-induced obesity.

### *Hepatic BDNF and the emergence of metabolic disturbances*

We interrogated the role of hepatic BDNF in energy, glucose, and lipid homeostasis under basal conditions and following increased dietary fat intake. BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice exhibited normal eating behavior and body weights when fed either SC or an HFD, indicating that hepatic BDNF expression is not required to maintain energy balance. Furthermore, both WT and BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice adapted to the higher caloric content of the HFD by eating less, thus maintaining normal body weights, probably due to their hybrid C57BL/6 and 129 background. It was previously demonstrated that WT 129 mice gained significantly less weight in response to prolonged HFD consumption compared with WTs in a C57BL/6 background (Kokkotou *et al.* 2005). These investigators also found that melanin-concentrating hormone (MCH) null mice in a 129 background fed an HFD had reduced daily caloric intake compared with MCH$^{-/-}$ mutants fed an SC diet. This caloric adaptation did not occur in MCH$^{-/-}$ mice in a C57BL/6 background (Kokkotou *et al.* 2005).

Liver content of BDNF significantly influenced the effects exerted by HFD administration. Both WT and mutant mice developed hepatic steatosis following increased fat intake. However, BDNF$^{2L/2LAlb\text{-}cre}$ mutant females fed an HFD trended toward reduced liver weights when compared with WTs fed a similar diet. This was the first indication that ablation of BDNF in the liver conferred some protection against dietary stress. A fatty liver is clinically associated with dyslipidemia, hyperglycemia, and insulin resistance, which are all components of the metabolic syndrome. Indeed, HFD-fed WT mice studied here exhibited mild hyperglycemia, elevated levels of insulin, and dyslipidemia. In contrast, these effects were absent or significantly attenuated in BDNF$^{2L/2LAlb\text{-}cre}$ mice fed an HFD. However, they exhibited a comparably deficient response to a glucose challenge. Relevant to this finding, it is important to note that insulin resistance induced by HFD intake entails organs other than the liver, including skeletal muscle and adipose tissue, which are thought to play a more prominent role in the glucose challenge response (Park *et al.* 2005*a*,*b*). This point is nicely illustrated by mice with global overexpression of PPAR-γ coactivator-1α. Albeit having hepatic insulin resistance, transgenic mice exhibited enhanced insulin sensitivity in muscle tissue and a concomitant improved overall response to a glucose challenge compared with the WT animals (Liang *et al.* 2009). Therefore, it is plausible that persistent expression of BDNF in muscle and adipose tissues of BDNF$^{2L/2LAlb\text{-}cre}$ mice contributed to their impaired glucose tolerance. Nonetheless, the data presented here suggest that BDNF signaling in the liver is likely to facilitate local decreases in insulin sensitivity triggered by HFD consumption.

Similar to WTs, BDNF$^{2L/2LAlb\text{-}cre}$ mice developed fatty livers in response to chronic HFD administration. However, unlike WTs, they maintained normal levels of serum glucose and insulin. The disassociation of hepatic steatosis and glucose homeostasis observed in BDNF mutants fed an HFD is puzzling, as lipid accumulation in the liver is clinically associated with hyperglycemia, hyperinsulinemia, and insulin resistance. However, there are other examples in the literature indicating a divergence of pathways linked to lipid and glucose homeostasis. For example, angioprotein-like protein 4 (ANGPTL4) actions in the liver were associated with improved glucose homeostasis, but also with hepatic steatosis in mice (Xu *et al.* 2005). Furthermore, liver-specific depletion of PTEN, which negatively regulates the PI3K/AKT pathway, resulted in enhanced insulin sensitivity in the liver, but also in liver steatosis (Stiles *et al.* 2004). Notably, whereas alterations in ANGPTL4 and PTEN also resulted in increased levels of circulating lipids compared with control mice, the BDNF mutants described here appeared to have improved lipid clearance from the bloodstream under HFD conditions compared with the WTs.

A concomitant effect of lipid excess is liver inflammation and injury. BDNF mutants had decreased levels of serum ALT and AST compared with the WTs, suggesting that hepatocellular damage induced by the HFD challenge was diminished in the absence of BDNF. An interesting observation of our studies was that while lack of hepatic BDNF was protective against insulin resistance in both males and females, protection against defective lipid clearance or increased levels of ALT and AST was evident only in mutant females and males respectively. Sex differences revealed in some of the parameters that we examined are not entirely surprising. Sexual dimorphism in body weight regulation, fat distribution and expression of genes linked to metabolic function was reported previously (Bjorntorp 1996, Legato 1997, Blaak 2001, Regitz-Zagrosek *et al.* 2006, Priego *et al.* 2008). Furthermore, sex-dependent differences in lipid handling under HFD conditions were also reported in previous studies (Priego *et al.* 2008).

### *Mechanisms facilitating resistance to metabolic alterations in BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice*

Consistent with their serum glucose, insulin, and lipid profiles, BDNF$^{2L/2LAlb\text{-}cre}$ mutant mice exhibited elevated content of both PPARα and FGF21 in the liver in response to HFD challenge compared with the WTs. Increased synthesis of FGF21, a metabolic regulator, is a molecular adaptation required to maintain lipid and glucose homeostasis under dietary stress conditions. Expression of FGF21 is most prominent in the liver and positively modulated by PPARα. The chief role of this pathway in metabolic homeostasis is underscored by mice with selective knock down of hepatic FGF21, which exhibited increased circulating levels of triglycerides when fed a HF, ketogenic diet compared with the WTs (Badman *et al.* 2007). Conversely, exogenous administration of FGF21 in diabetic monkeys resulted in reduced levels of total cholesterol, triglycerides, and insulin in the serum (Kharitonenkov *et al.* 2007). Similarly, leptin-deficient *ob/ob* mutant mice had normalized levels of blood glucose and triglycerides following chronic FGF21 treatment (Kharitonenkov *et al.* 2005). Surprisingly, BDNF mutants fed a HFD exhibited levels of hepatic steatosis similar to those of WTs albeit containing elevated levels of FGF21. Moreover, expression levels of the FGF21 target genes, *Acadl* and *Acadm*, which mediate fatty acid oxidation were also increased in the mutant liver. However, it is important to note that there was an indication that hepatic BDNF depletion might also result in the upregulation of the fatty acid transporter CD36, which was shown previously to be downregulated in FGF21 knockdown mice (Badman *et al.* 2007). Increased content of CD36 is associated with enhanced lipid uptake, fat accumulation in the liver, and the onset of hepatic steatosis (Koonen *et al.* 2007). Furthermore, CD36$^{-/-}$ mice exhibited increases in plasma levels of triglycerides (Febbraio *et al.* 1999). Therefore, the effects of increased lipid uptake mediated by the elevated content of CD36 in the livers of BDNF mutants under HFD conditions might diminish the effects of enhanced lipid oxidation, ultimately resulting in hepatic steatosis.

Improved levels of serum glucose and insulin in BDNF$^{2L/2LAlb\text{-}cre}$ mice challenged with an HFD might be related to enhanced suppression of hepatic gluconeogenesis and to increased glucose uptake by GLUT1. Indeed, FGF21 was reported previously to reduce the expression of the gluconeogenic gene *G6Pase*, and to induce the expression of the glucose transporter *Glut1* in adipose tissue (Xu *et al.* 2009). Consistent with these findings and their elevated content of hepatic FGF21, BDNF$^{2L/2LAlb\text{-}cre}$ mice fed HFD did not experience the increase in *G6Pase* expression exhibited by HFD-fed WTs relative to their genetic counterparts fed SC. Moreover, BDNF mutants fed HFD had an elevated content of *Glut1* mRNA in the liver.

In light of the well-ascribed roles of PPARα and FGF21 in preserving glucose and lipid homeostasis under HFD conditions, it is plausible that the improved metabolic adaptations exhibited by BDNF$^{2L/2LAlb\text{-}cre}$ mice are mediated by augmentation of this pathway. The implication of this model is that BDNF contained in the liver acts normally to diminish induction of FGF21 expression under HFD conditions, ultimately facilitating the onset of the metabolic syndrome. A putative mechanism of neurotrophin regulation might entail modulation of hepatic PPARα expression via action on vagal afferents. Afferent fibers of the vagus nerve were shown to mediate increases in PPARα expression in the liver triggered by glucocorticoids, and selective hepatic vagotomy prevented this induction of PPARα (Bernal-Mizrachi *et al.* 2007). BDNF acting via hepatic vagal afferents, which contain TrkB receptors (Ernfors *et al.* 1992, Zhuo *et al.* 1994), might signal the brain to modify efferent autonomic input to the liver, which in turn might influence PPARα expression. Future investigations should aim to determine the effect of excess content of BDNF in the liver on the expression of PPARα and FGF21 under HFD conditions.

In summary, our findings suggest a role of hepatic BDNF signaling in facilitating the emergence of metabolic disorders and liver damage elicited by HFD intake. The contrasting effects of BDNF in the brain versus the liver illustrate the complexity of neurotrophin action, and the necessity of fully dissecting the underlying mechanisms when considering therapies for obesity disorders and associated metabolic syndromes.

## Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1677/JOE-09-0405.

## Declaration of interest

ST and GAC have nothing to declare. MR serves as a consultant for Wyeth.

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