High-fat diet consumption reduces hepatic folate transporter expression via nuclear respiratory factor-1

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Received: 12 January 2018 / Accepted: 17 August 2018

Abstract

Folate is an essential micronutrient for biological function. The liver, a primary organ for folate metabolism and storage, plays an important role in folate homeostasis. Proton-coupled folate transporter (PCFT) and reduced folate carrier (RFC) are the major folate transporters responsible for folate uptake at basolateral membrane of hepatocytes. Low serum folate levels are frequently associated with obesity. We investigated the mechanism that regulated folate status in a mouse model with diet-induced obesity. Mice (C57BL/6J) were fed a high-fat diet (60% kcal fat) for 8 weeks. Mice displayed increased hepatic lipid accumulation and decreased folate levels in the liver and serum compared to mice fed a normal chow diet (10% kcal fat). High-fat diet-fed mice had low expression of PCFT and RFC and decreased nuclear respiratory factor-1 (NRF-1)/DNA-binding activity. Treatment with NRF-1 siRNA or palmitic acid reduced folate transporter expression in hepatocytes. Inhibition of NRF-1 mediated folate transporter expression significantly reduced intracellular folate levels. These results suggest that chronic consumption of high-fat diets impairs folate transporter expression via NRF-1-dependent mechanism, leading to reduced hepatic folate storage. Understanding the regulation of folate homeostasis in obesity may have an important implication in current guideline of folate intake.

Key messages

- Serum and liver folate levels are decreased in diet-induced obese mice.
- Chronic high-fat diet consumption impairs expression of hepatic PCFT and RFC.
- NRF-1 regulates hepatic folate transporters expression and folate levels.

Keywords

Folate
High-fat diet
Proton-coupled folate transporter
Reduced folate carrier
Nuclear respiratory factor-1

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00109-018-1688-8) contains supplementary material, which is available to authorized users.

Introduction

Folate is a water-soluble B vitamin obtained from diets. This micronutrient is essential for biological functions including sulfur-containing amino acid metabolism, nucleotide synthesis, and methylation reactions [1, 2]. Due to the implementation of mandatory folic acid fortification policy, dietary deficiency of folate is believed to be uncommon in generally healthy populations in Western countries [3, 4]. However, despite adequate intake of folate from diets and supplements, obese individuals and patients with non-alcoholic fatty liver disease (NAFLD) are reported to have low folate levels in the circulation [5, 6, 7, 8]. In addition, low folate status is also observed in patients with malabsorption, impaired kidney function, or liver disease, i.e., in vulnerable senior population [9, 10, 11]. Owing to the increased epidemic of obesity and prevalence of NAFLD in the recent decades [12], folate deficiency is an emerging issue, especially for patients with obesity [7, 13].

Obesity is one of the most common risk factors for various chronic diseases [14]. It has been reported that low levels of serum folate are associated with enhanced NAFLD severity in obese patients [8, 15]. However, the mechanism that leads to low folate status in obese patients is not fully understood. Chronic consumption of high-fat diets is a major

contributor to NAFLD and obesity [16, 17]. The high-fat diet fed rodents develop hepatic histopathological features such as lipid accumulation, oxidative stress, and inflammation, which resemble NAFLD [18, 19]. In previous studies, we observed that folic acid supplementation reduced oxidative stress as well as improved hepatic lipid and glucose metabolism in mice fed a high-fat diet [20, 21]. However, it is not clear whether chronic consumption of high-fat diets affects folate homeostasis. The liver is a primary organ responsible for folate metabolism and storage as well as folate distribution to the circulation [22, 23, 24]. Mammals lack the enzymatic capacity for folate biosynthesis, and therefore, folate must be acquired from dietary sources to meet physiological requirement [2]. Following intestinal absorption, folate enters the hepatic portal circulation and is taken up by the liver via folate transporters [24, 25]. The proton-coupled folate transporter (PCFT, encoded by SLC46A1 gene) and the reduced folate carrier (RFC, encoded by SLC19A1 gene) are transmembrane proteins that mediate folate uptake in hepatocytes. These two folate transporters are abundantly expressed at the basolateral membrane of hepatocytes [2, 25]. Regulation of folate transport is important to ensure adequate folate storage in the liver and to maintain folate levels in the systemic and enterohepatic circulation [22, 26]. The nuclear respiratory factor-1 (NRF-1) is recently identified as a key transcriptional factor that controls the expression of PCFT gene expression in the intestine [27]. However, the regulation of hepatic folate transporters in the context of obesity is unclear.

In the present study, we evaluated the impact of chronic consumption of a high-fat diet on hepatic folate transporter expression and folate status in mice, and the mechanisms involved. Mice fed a high-fat diet for 8 weeks displayed hepatic lipid accumulation and a significant decrease in folate transporter (PCFT, RFC) expression in the liver. We investigated the mechanisms by which high-fat diet reduced hepatic folate transporter expression. We demonstrated that downregulation of NRF-1 led to reduced expression of folate transporters in the liver of mice fed a high-fat diet.

Materials and methods

Animals

Male C57Bl/6J mice aged 6 weeks were purchased from Central Animal Care Services (University of Manitoba, Winnipeg, MB, Canada) and were isolated for 1 week quarantine before the beginning of the experimental period. Mice were fed a normal chow diet (D12450B) or a high-fat diet (D12492) for 8 weeks. The normal chow diet consisted of 10% kcal fat, 20% kcal protein, and 70% kcal carbohydrate with 2 mg of folic acid/kg of diet, while the high-fat diet consisted of 60% kcal fat, 20% kcal protein, and 20% kcal carbohydrate with 2.6 mg of folic acid/kg of diet (Supplementary Table 1). All the purified diets were formulated and prepared by Research Diets Inc. (New Brunswick, NJ, USA). Mice were kept on a 12-h light/12-h dark cycle with access to food and water ad libitum. Mice were sacrificed after 8 weeks and blood was collected for serum preparation. Animal body weights were recorded prior to feeding and at the end of the experimental feeding period. Following euthanasia, tissues were collected and immediately placed in liquid nitrogen, then kept frozen at − 80 °C until further analysis. Lipids in the liver tissue were extracted according to the Folch method [28]. The total lipid levels in the liver were measured using the sulfo-phospho-vallin colorimetric reaction, according to manufacturer's instructions (BQ Kits, San Diego, CA, USA). All procedures were performed in accordance with the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and approved by the University of Manitoba Protocol Management and Review Committee.

Cell culture

HepG2 cells (human hepatoblastoma cell line HB8065; American Type Culture Collection, Manassas, VA, USA) were cultured in DMEM (VWR, Mississauga, ON, Canada) supplemented with 10% FBS, in a humidified atmosphere at 37 °C with 5% CO_2. Cells were plated at a density of 5×10^5 and treated with or without palmitic acid (0.3 mM). It was previously reported that plasma concentrations of palmitic acid in patients ranged from 0.1 to 0.3 mM [29]. Other studies showed that treatment of HepG2 cells with 0.3 mM palmitic acid caused significant changes in lipid

metabolism and cytokine expression [19, 21, 30]. In the present study, palmitic acid (PA; Sigma-Aldrich, Oakville, ON, Canada) was dissolved in 10% BSA (essentially fatty acid free; Sigma-Aldrich, Oakville, ON, Canada) and 5% ethanol with light shaking overnight at 37 °C [19, 21]. Cell viability was examined after incubation with palmitic acid (0.1 –0.3 mM) for 12 h by MTT assay using 3-(4,5-dimethylthiazol-2-Yl) -2,5-diphenyltetrazolium bromide. Treatment with various doses of palmitic acid did not significantly affect cell viability (Supplementary Fig. 1). Cells incubated with the 10% BSA were used as control.

Western immunoblotting

The protein levels of PCFT and RFC were determined by western immunoblotting analysis. In brief, liver proteins (10–60 μg) were separated by electrophoresis in an 8 or 10% SDS polyacrylamide gel. Proteins were transferred from the gel to a nitrocellulose membrane followed by incubation with primary antibodies that were diluted in 5% milk in TBST (1:1000) overnight at 4 °C. Primary antibodies used for mouse liver samples were as follows: rabbit anti-HCP1 (PCFT) polyclonal antibody (ab25134, Abcam, Cambridge, MA, USA) and mouse anti-RFC-1 monoclonal antibody (sc-271276, Santa Cruz Biotechnology, Dallas, TX, USA). Primary antibodies used for human HepG2 cell lysates were as follows: rabbit anti-HCP1 (PCFT) polyclonal antibody (NBP1-06603, Novus Biologicals, Littleton, CO, USA), rabbit anti-SLC19A1 (RFC) polyclonal antibody (ab62302, Abcam, Cambridge, MA, USA), and rabbit anti-NRF-1 polyclonal antibody (#12381, Cell Signaling, Danvers, MA, USA). Secondary antibodies were diluted in 5% milk in TBST (1:1000). Membranes were subsequently probed with secondary antibodies including HRP-conjugated anti-mouse IgG antibody (#7076, Cell Signaling, Danvers, MA, USA) or anti-rabbit IgG antibody (#7074, Cell Signaling, Danvers, MA, USA) for 1 h at room temperature, and visualized using the Luminata Crescendo chemiluminescent HRP detection reagent (Millipore (Canada) Ltd., Etobicoke, ON, Canada). To ensure equal protein loading, the same membranes were reprobed with rabbit anti-β-actin monoclonal antibody (1:2000; #4967, Cell Signaling, Danvers, MA, USA).

Real-time PCR analysis

Total RNA was prepared from the liver tissue and cells using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. In brief, 2 μg of RNA was converted to cDNA by reverse transcription. The mRNA levels of various genes were assessed using the iQ5 real-time PCR detection system (Bio-Rad, Mississauga, ON, Canada). The real-time PCR reaction mixture consisted of 2 μl of cDNA product, 0.4 μM of 5′ and 3′ primers, and iQ-SYBR green supermix reagent (Bio-Rad, Mississauga, ON, Canada). The cycle threshold (CT) values of the target genes were normalized against β-actin CT values. Relative mRNA levels were quantified according to the ΔΔCt method. The primer sequences (Thermo Fisher Scientific, Waltham, MA, USA) are listed (Supplementary Table 2).

Electrophoretic mobility shift assay

Nuclear proteins were prepared from the liver tissue and HepG2 cells [31, 32]. DNA oligonucleotides were biotin-labeled according to the Biotin 3′ End DNA labelling kit (Thermo Fisher Scientific, Waltham, MA, USA). The sense strand sequences containing the NRF-1 binding site used in the study were as follows: 5′*GCGCAGGCGCAGA*CAGCACAGACTGGT3′ (mouse; GenBankTM accession number NM_026740) and 5′GACGCCG*GGCGCAGGCGCA*GACAGCG3′ (human; GenBankTM accession number EU185738.1). The core consensus sequence of NRF-1 is italicized [27]. Electrophoretic mobility shift assay (EMSA) was performed using the LightShift Chemiluminescent EMSA Kit (Thermo Fisher Scientific, Waltham, MA, USA). The labeled oligonucleotides were added to a reaction mixture (20 μl) that contained 2 μg of nuclear extract according to manufacturer's instructions, with some minor modifications. Poly [d (A-T)] (50 ng per reaction) was used as a nonspecific DNA competitor. The NRF-1 binding site is a GC-rich sequence and use of poly [d (I-C)] can compete out the NRF-1 binding site. Reaction mixtures were incubated at room temperature for 20 min. The cold competition assay was performed by incubating nuclear extracts with 1000-fold excess of unlabeled oligonucleotides before addition of labeled probe. Following the

incubation, reaction mixtures were loaded in a 6% nondenaturing polyacrylamide gel to allow for separation of DNA-protein complexes and transferred to a nylon membrane for detection using the Chemiluminescent Nucleic acid Detection Module Kit (Thermo Fisher Scientific, Waltham, MA, USA). The supershift assay was performed by incubating liver nuclear proteins (2 μg) with 2 μl and 4 μl of anti-NRF-1 antibody (#12381, Cell Signaling, Danvers, MA, USA) for 20 min followed by nondenaturing polyacrylamide gel (4%) electrophoresis.

Cell transfection

HepG2 cells were transfected with NRF-1 siRNA duplex oligoribonucleotides (Stealth RNAiTM, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For a negative control, cells were transfected with StealthTM RNAi negative control (Invitrogen, Carlsbad, CA, USA) consisting of a scrambled sequence that was unable to inhibit gene expression. At 48 h after transfection, the cells were collected, and mRNA levels of NRF-1, PCFT, and RFC were measured. Cells were also collected after 72-h transfection, and protein and folate levels were measured.

Folate measurement

Total folate concentrations in the liver and serum, as well as in cultured hepatocytes, were determined using the *L. rhamnosus* microplate assay, as followed by manufacturer's instructions provided in the Folic acid Vitamin B9 Microbiological Test Kit (Alpco, Salem, NH, USA).

Histological staining

A portion of the liver tissue was soaked in 10% neutral-buffered formalin overnight and embedded in paraffin. Paraffin-embedded cross sections were prepared on glass slides. Liver sections were deparaffinized and stained with hematoxylin and eosin (H&E) staining to examine histological changes of the liver [31]. Images of H&E sections were captured using an Olympus BX43 light microscope equipped with a Q-Color3 camera and were analyzed at × 100 and × 200 magnification.

Statistical analysis

The results were analyzed by a two-tailed Student's *t* test, followed by post hoc analysis using the Newman-Keuls test. The data was presented as the means ± S.E. A *p* value less than 0.05 were considered statistically significant.

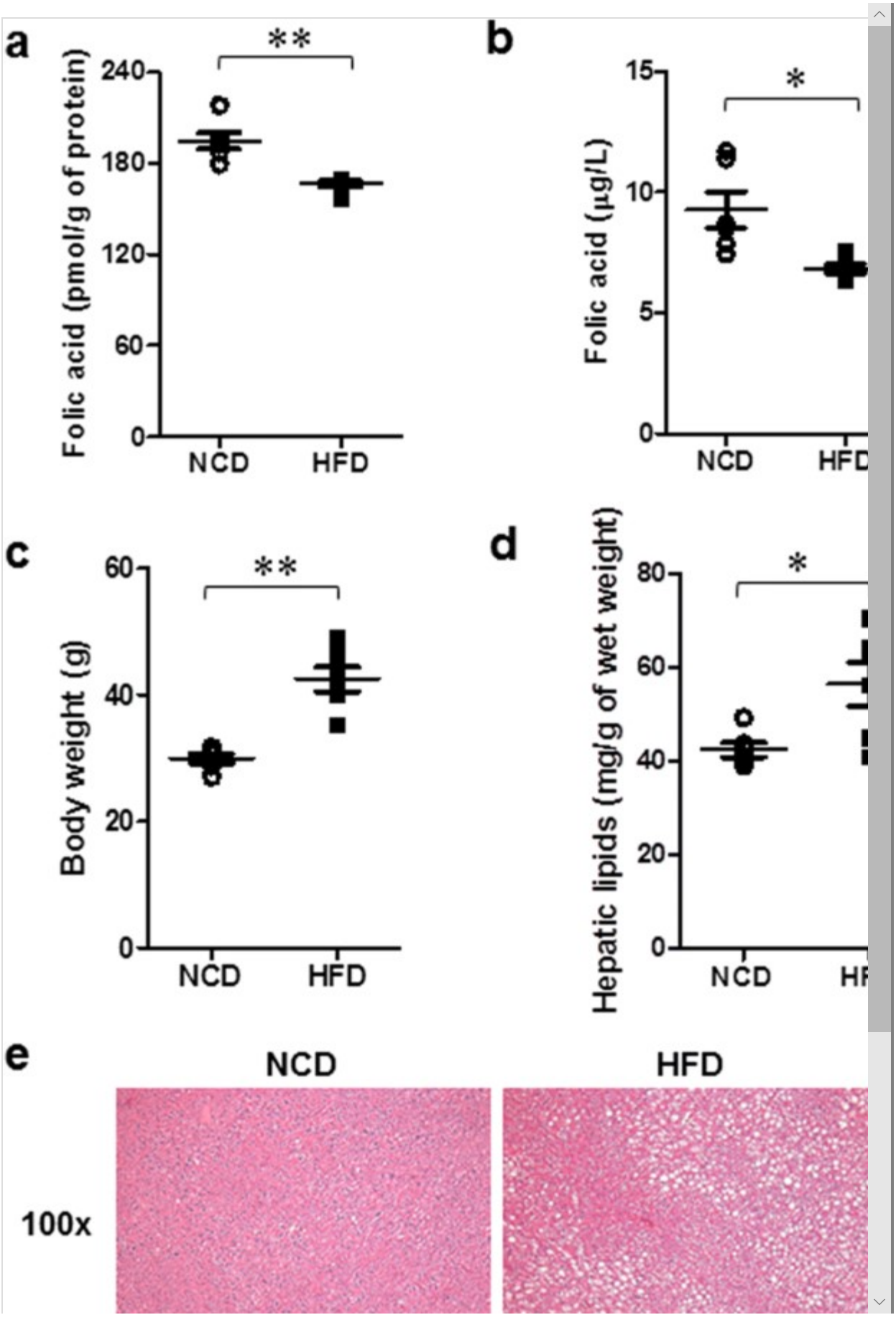
Results

High-fat diet feeding reduced folate levels in the liver and serum

High-fat diet feeding for 8 weeks caused a significant decrease in folate levels in the liver (Fig. 1a, 194.52 ± 5.33 pmol/g protein versus 166.31 ± 1.96 pmol/g protein, 15% decrease) and in the serum (Fig. 1b, 9.27 ± 0.74 μg/L versus 6.83 ± 0.19 μg/L, 25% decrease). Mice fed a high-fat diet exhibited a significant increase in body weight compared to those maintained on a control diet (Fig. 1c). There was a significant elevation of total lipid levels in the liver (Fig. 1d, 42.46 ± 1.52 mg/g tissue versus 56.51 ± 4.71 mg/g tissue, 23% increase). Hepatic lipid accumulation was further examined by H&E staining. Mice fed a high-fat diet had an increased accumulation of lipid vacuoles in the liver compared to the control mice (Fig. 1e).

Fig. 1

Body weight, liver lipids, and folate levels. Mice were fed a normal chow diet (NCD) or a high-fat diet (HFD) for 8 weeks. **a** Liver folate and **b** serum folate levels were measured. **c** Body weight and **d** hepatic lipids were measured. **e** Representative H&E staining images of liver sections are shown. The bar on the images represents 100 μm. Results are expressed as means ± S.E. ($n = 6$). *$p < 0.05$ and **$p < 0.01$ when compared with the value obtained from the control group (NCD)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**
240
180
120
60
0
Folic acid (pmol/g of protein)
NCD
HFD
b
*
15
10
5
0
Folic acid (μg/L)
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60
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Body weight (g)
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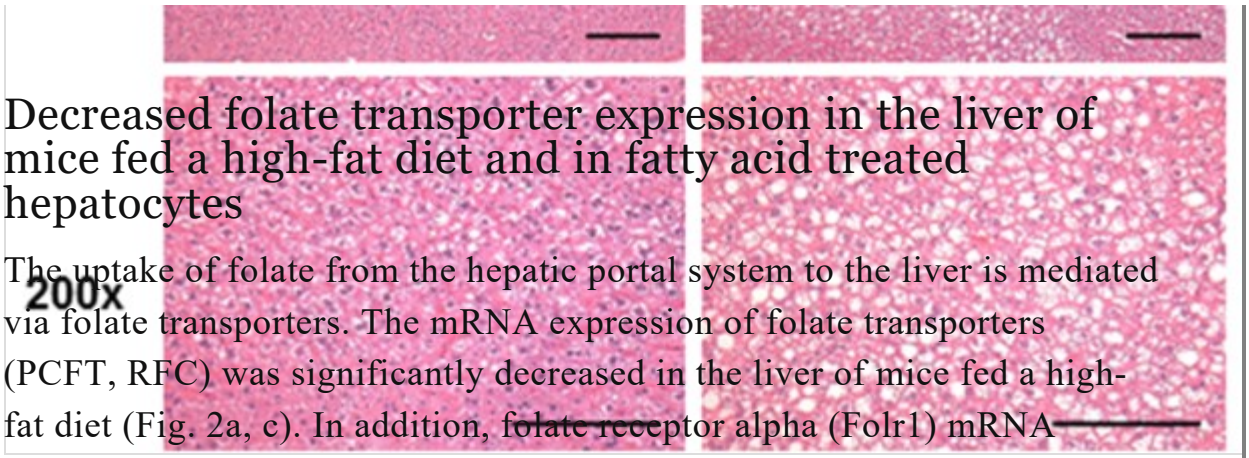


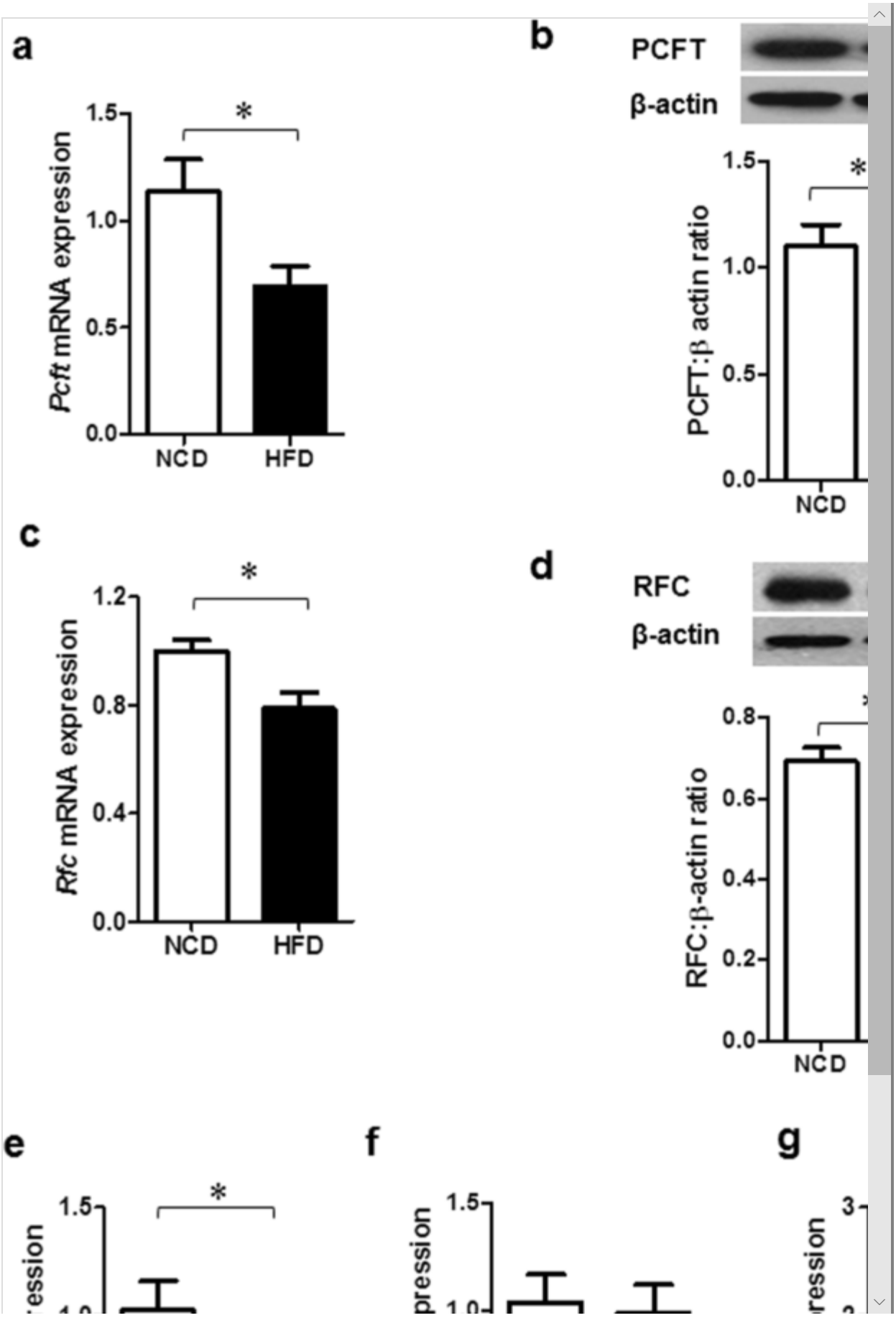
Decreased folate transporter expression in the liver of mice fed a high-fat diet and in fatty acid treated hepatocytes

The uptake of folate from the hepatic portal system to the liver is mediated via folate transporters. The mRNA expression of folate transporters (PCFT, RFC) was significantly decreased in the liver of mice fed a high-fat diet (Fig. 2a, c). In addition, folate receptor alpha (Folr1) mRNA expression was also significantly reduced in response to high-fat diet feeding (Fig. 2e). In accordance with these results, there was a marked reduction of PCFT and RFC protein in the liver of mice fed a high-fat diet (Fig. 2b, d). The ATP-binding cassette subfamily G member 2 (ABCG2) is the predominant ABC transporter that exports folate from the liver [33]. High-fat diet feeding did not affect mRNA expression of ABCG2 (Fig. 2f). The multidrug resistance-associated protein 3 (MRP3) is another ABC transporter that exports folate from the liver [25]. High-fat diet feeding significantly increased mRNA expression of MRP3 in the liver (Fig. 2g). Such upregulation of MRP3 expression might be an adaptive response to low folate levels in the circulation [34]. Palmitic acid is a major saturated fatty acid in the high-fat diet. Incubation of HepG2 cells with palmitic acid resulted in a significant decrease in PCFT and RFC mRNA expression (Fig. 3a, c), as well as protein levels (Fig. 3b, d). Furthermore, incubation of cells with palmitic acid resulted in a significant reduction of intracellular folate concentrations (Fig. 3e). These results indicated that high-fat diet feeding or fatty acid treatment impaired hepatic folate transporter expression.

Fig. 2

Folate transporter mRNA and protein expression in the liver. Mice were fed a normal chow diet (NCD) or a high-fat diet (HFD) for 8 weeks. **a** PCFT mRNA expression and **b** protein expression were measured. **c** RFC mRNA expression and **d** protein expression in liver tissue were measured. **e** Folr1, **f** ABCG2, and **g** MRP3 mRNA expression in liver tissue was measured. The mRNA levels were determined by real-time PCR analysis and the protein

levels were determined by western immunoblotting analysis. Results are expressed as means ± S.E. ($n = 6$). *$p < 0.05$ and **$p < 0.01$ when compared with the value obtained from the control group (NCD)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Pcft mRNA expression
1.5
1.0
0.5
0.0
*
NCD
HFD
b
PCFT
β-actin
PCFT:β actin ratio
1.5
1.0
0.5
0.0
*
NCD
c
Rfc mRNA expression
1.2
0.8
0.4
0.0
*
NCD
HFD
d
RFC
β-actin
RFC:β-actin ratio
0.8
0.6
0.4
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NCD
e
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g
3

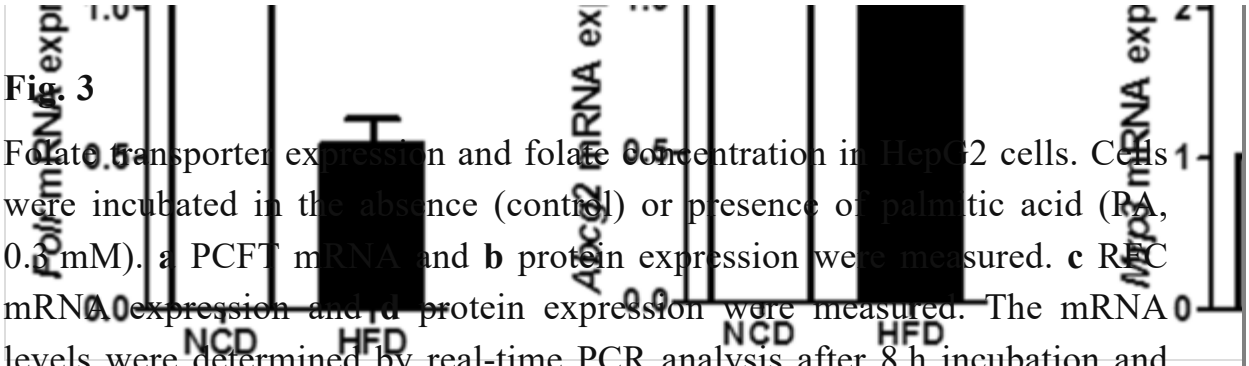
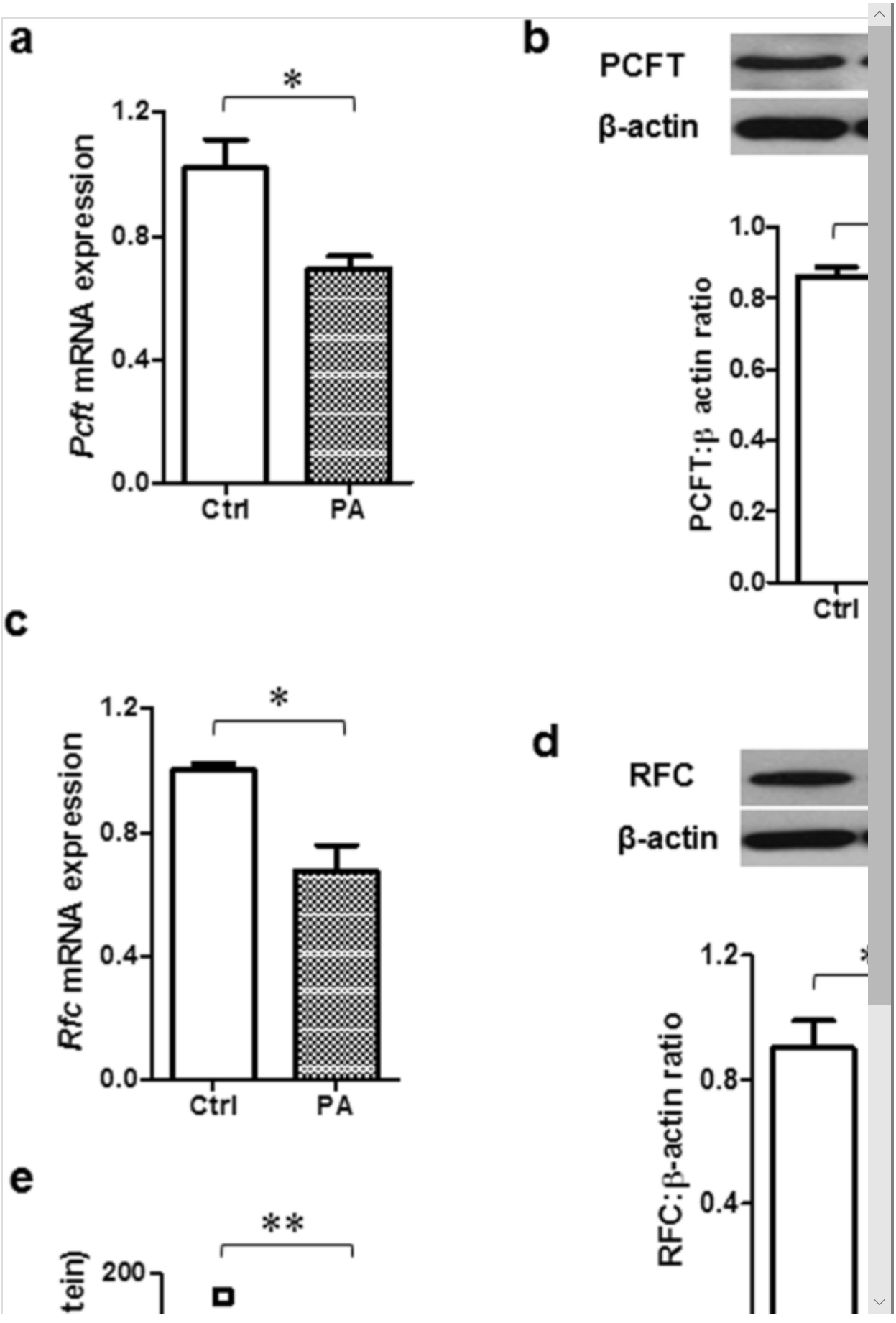


Fig. 3

Folate transporter expression and folate concentration in HepG2 cells. Cells were incubated in the absence (control) or presence of palmitic acid (PA, 0.2 mM). **a** PCFT mRNA and **b** protein expression were measured. **c** RFC mRNA expression and **d** protein expression were measured. The mRNA levels were determined by real-time PCR analysis after 8 h incubation and the protein levels were determined by western immunoblotting analysis after 12 h incubation. **e** Intracellular folate concentration was measured after 48 h incubation. Results are expressed as means ± S.E. ($n = 4–6$). *$p < 0.05$ and **$p < 0.01$ when compared with the value obtained from the control cells


a
*
1.2
0.8
0.4
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Pcft mRNA expression
Ctrl
PA
b
PCFT
β-actin
1.0
0.8
0.6
0.4
0.2
0.0
PCFT:β actin ratio
Ctrl
c
*
1.2
0.8
0.4
0.0
Rfc mRNA expression
Ctrl
PA
d
RFC
β-actin
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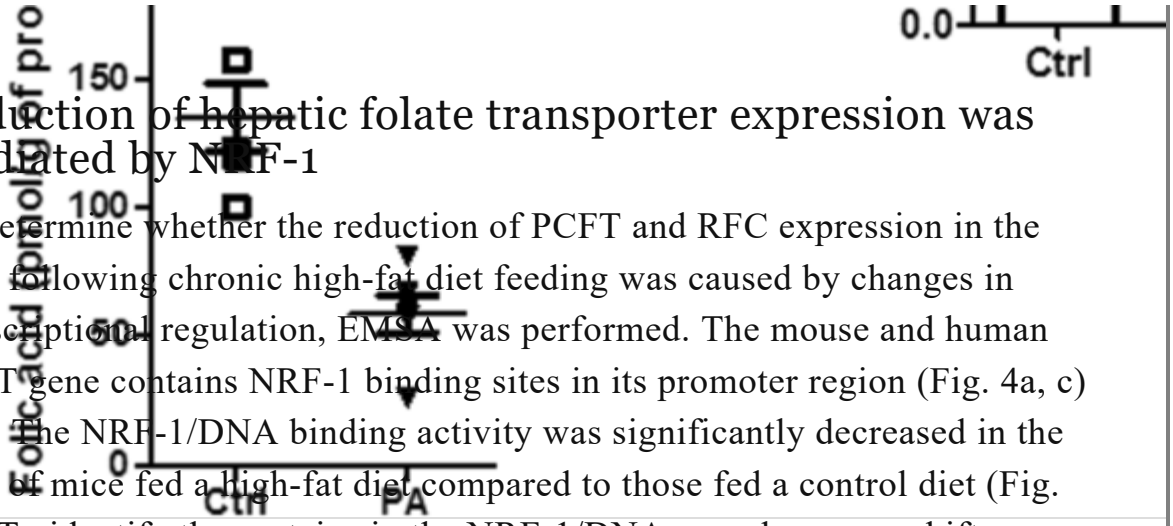


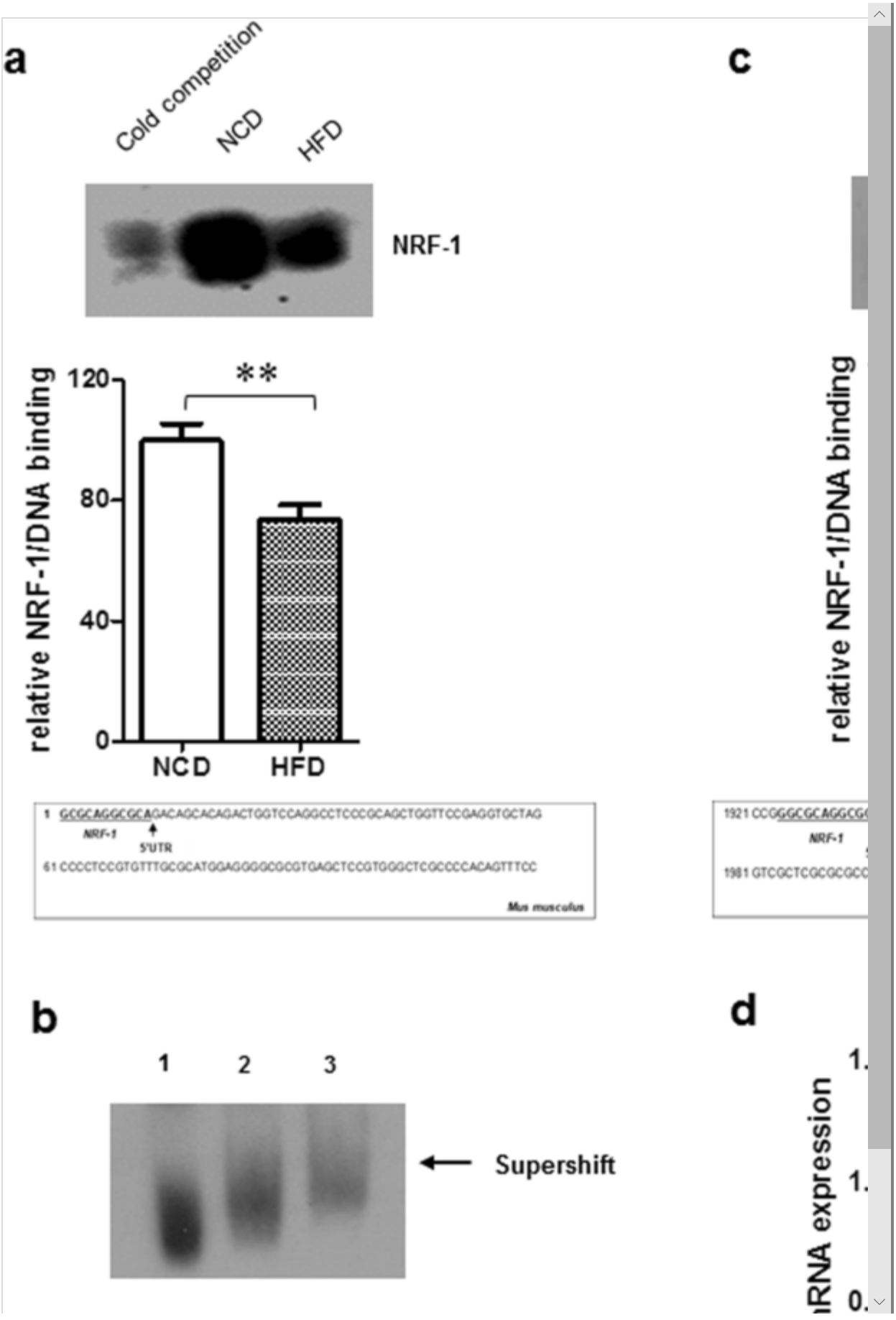
Reduction of hepatic folate transporter expression was mediated by NRF-1

To determine whether the reduction of PCFT and RFC expression in the liver following chronic high-fat diet feeding was caused by changes in transcriptional regulation, EMSA was performed. The mouse and human PCFT gene contains NRF-1 binding sites in its promoter region (Fig. 4a, c) [27]. The NRF-1/DNA binding activity was significantly decreased in the liver of mice fed a high-fat diet compared to those fed a control diet (Fig. 4a). To identify the proteins in the NRF-1/DNA complex, supershift assay was performed using anti-NRF-1 antibodies. Preincubation of liver nuclear proteins with anti-NRF-1 antibodies resulted in a slower migration of the protein-DNA complex (Fig. 4b), indicating that NRF-1 was involved in DNA binding. In addition, palmitic acid treatment also significantly reduced DNA binding activity of NRF-1 in HepG2 cells (Fig. 4c). However, treatment with palmitic acid did not significantly change NRF-1 mRNA expression (Fig. 4d). To further confirm that NRF-1 was involved in the regulation of hepatic PCFT and RFC gene expression, hepatocytes were transiently transfected with either NRF-1 siRNA or scrambled siRNA (negative control). Transfection of cells with NRF-1 siRNA significantly abolished NRF-1 mRNA and protein expression (Fig. 5a, d), as well as inhibited the expression of PCFT and RFC (Fig. 5b, c, e, f). In contrast, scrambled siRNA transfection in hepatocytes had no effect on NRF-1, PCFT, and RFC gene and protein expression (Fig. 5a–f). There was no significant difference in cell viability between NRF-1 siRNA transfection and scrambled siRNA transfection (Supplementary Fig. 2). Palmitic acid treatment did not cause further reduction of PCFT and RFC mRNA expression in siNRF-1 transfected cells (Supplementary Fig. 3). Inhibition of NRF-1 mediated folate transporter expression caused a significant decrease in intracellular folate concentrations (Fig. 5g). These results suggested that NRF-1 might play a role in regulating hepatic folate transporter expression and folate content. In addition to folate transporters, we also measured gene expression of dihydrofolate reductase (DHFR), the rate-limiting enzyme that converts folate to its bioactive form

(tetrahydrofolate). Results showed that following NRF-1 siRNA transfection in HepG2 cells, the mRNA expression of DHFR was significantly elevated (Fig. 5h). It was plausible that in response to low intracellular folate levels caused by decreased folate transporter expression, DHFR might have been upregulated as an adaptive reaction.

Fig. 4

Effect of high-fat diet on NRF-1/DNA binding activity in the liver. Mice were fed a normal chow diet (NCD) or a high-fat diet (HFD) for 8 weeks. **a** The DNA binding activity of NRF-1 in the liver tissue was determined by EMSA. The binding activity in the NCD group was expressed as 100%. The schematic diagram illustrates the promoter region of the PCFT gene in mouse (*Mus musculus*). The NRF-1 binding sequence is shown in bold letters and underlined. The 5′UTR site is indicated by an arrow. **b** The nuclear protein and DNA oligonucleotides were incubated with anti-NRF-1 antibodies (2 μl in lane 2, 4 μl in lane 3) for supershift assay. The shift of the NRF-1/DNA complex is indicated by an arrow. **c** HepG2 cells were incubated with or without palmitic acid (PA, 0.3 mM) for 4 h. The DNA binding activity of NRF-1 was determined by EMSA. The schematic diagram illustrates the promoter region of the PCFT gene in mouse (*Mus musculus*) and human (*Homo sapiens*). The NRF-1 binding sequence is shown in bold letters and underlined. The 5′UTR site is indicated by an arrow. **d** The NRF-1 mRNA in HepG2 cells was determined by real-time PCR analysis after 8-h incubation with or without palmitic acid. Results are expressed as means ± S.E. ($n = 4–6$). $*p < 0.05$ and $**p < 0.01$ when compared with the value obtained from the control group


a
Cold competition
NCD
HFD
NRF-1
relative NRF-1/DNA binding
120
80
40
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**
NCD
HFD
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NRF-1
5'UTR
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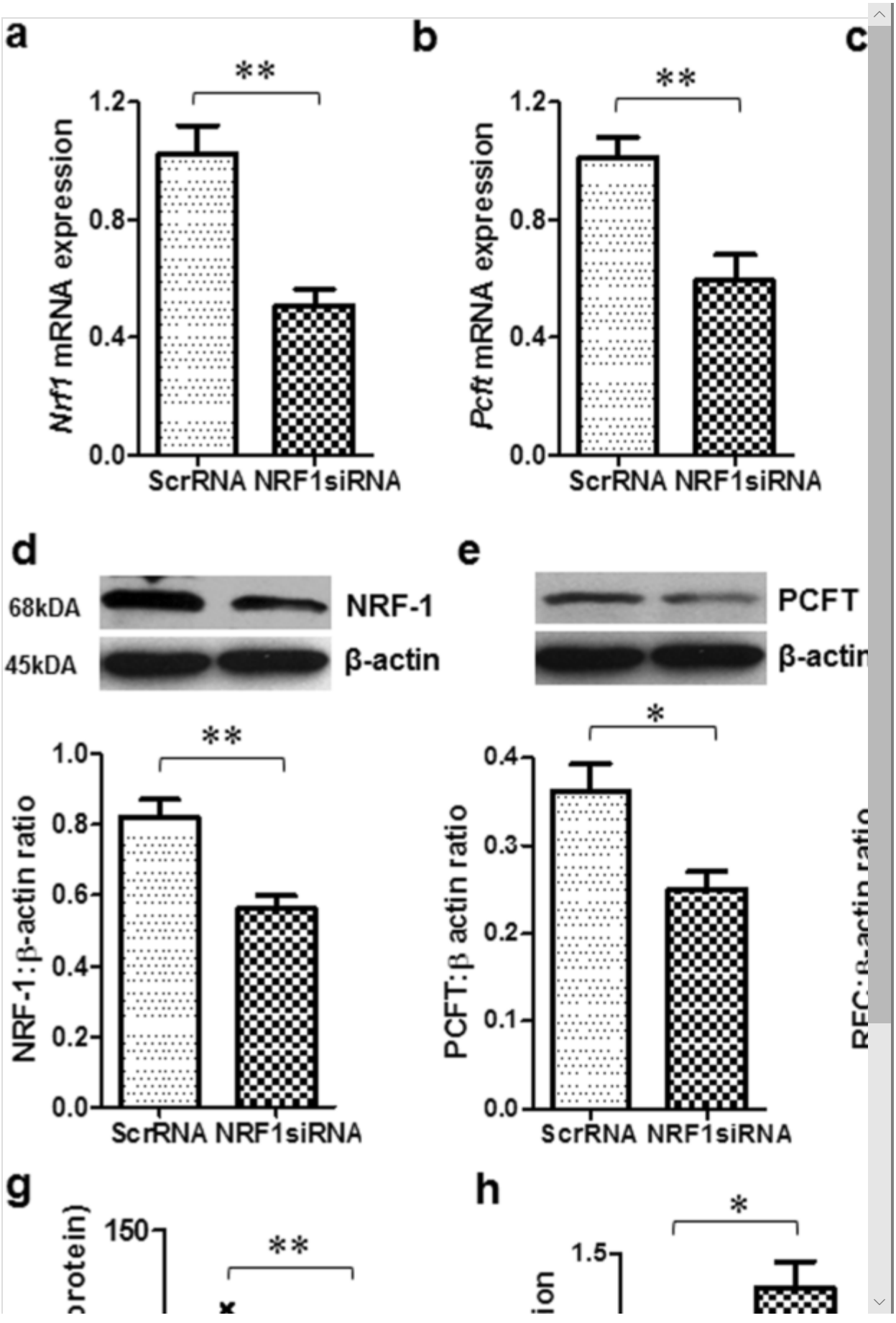
Mus musculus
c
relative NRF-1/DNA binding
1921 CCGGGCGCAGGCGC
NRF-1
1981 GTCGCTCGCGCGCC
b
1
2
3
Supershift
d
1.
1.
0.
RNA expression

Fig. 5

Effect of NRF-1 siRNA transfection on folate transporter expression and folate concentration in HepG2 cells. Cells were transfected with NRF-1 siRNA or scrambled siRNA (negative control). The mRNA levels of **a** NRF-1, **b** PCFT, and **c** RFC were measured. The protein levels of **d** NRF-1, **e** PCFT, and **f** RFC were measured. **g** Intracellular folate concentration was measured. **h** DHFR mRNA was measured. The mRNA and proteins were determined by real-time PCR and western immunoblotting analysis, respectively. Results are expressed as means ± S.E. ($n = 4–6$). *$p < 0.05$ and **$p < 0.01$ when compared with the value obtained from cells transfected with scrambled siRNA


a
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1.2
0.8
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Nrf1 mRNA expression
ScrRNA NRF1siRNA
b
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1.2
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0.0
Pcft mRNA expression
ScrRNA NRF1siRNA
c
d
68kDA
NRF-1
45kDA
β-actin
**
1.0
0.8
0.6
0.4
0.2
0.0
NRF-1:β-actin ratio
ScrRNA NRF1siRNA
e
PCFT
β-actin
*
0.4
0.3
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PCFT:β actin ratio
ScrRNA NRF1siRNA
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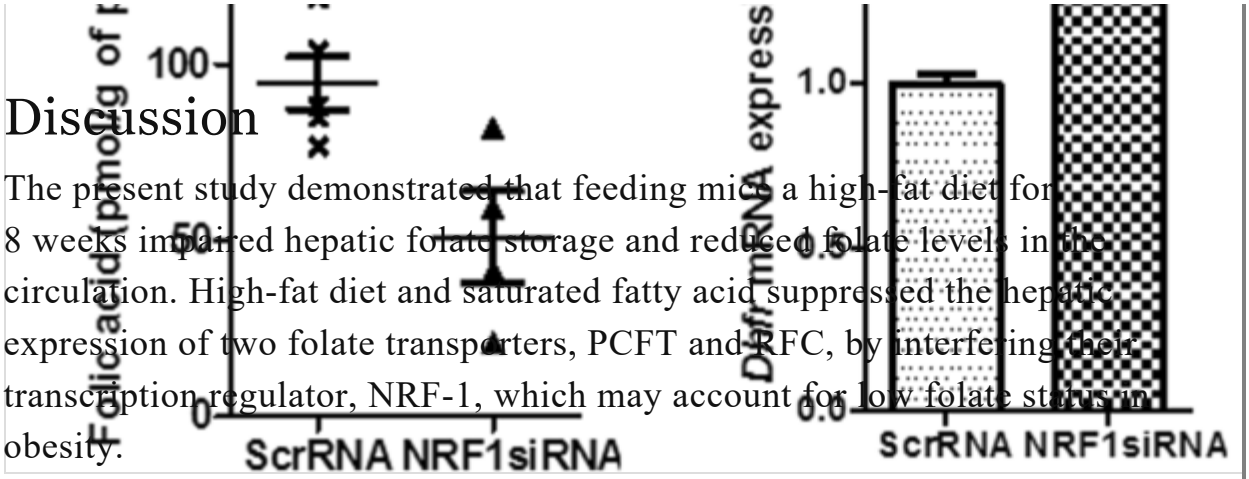
1.5



Discussion

The present study demonstrated that feeding mice a high-fat diet for 8 weeks impaired hepatic folate storage and reduced folate levels in the circulation. High-fat diet and saturated fatty acid suppressed the hepatic expression of two folate transporters, PCFT and RFC, by interfering their transcription regulator, NRF-1, which may account for low folate status in obesity.

The liver is the primary organ for folate storage and is where folate is converted to its active form 5-methyltetrahydrofolate (5-MTHF) that is then delivered to peripheral tissues through the systemic or enterohepatic circulation [13, 23, 24]. Folate transporters (PCFT, RFC) are abundantly expressed at the basolateral membrane of hepatocytes and mediate folate transport from the hepatic portal system to the liver [2]. Changes in hepatic folate transport not only affects folate storage in the liver but also can disrupt systemic folate homeostasis [22]. In the present study, mice fed a high-fat diet for 8 weeks developed fatty liver. Besides inducing hepatic lipid accumulation, high-fat diet feeding significantly reduced the expression of PCFT and RFC in the liver. Such a reduction of folate transporter expression was associated with low folate levels in the liver and serum. Furthermore, incubation of HepG2 cells with palmitic acid, a major saturated fatty acid in the high-fat diet, caused a decrease in folate transporter expression and intracellular folate levels. These results suggested that impaired expression of hepatic folate transporters might be one of the underlying mechanisms leading to low folate status in mice fed a high-fat diet.

The regulation of folate transporter expression in the liver is not well understood. The transcription factor NRF-1 was first identified to regulate gene expression of folate transporters in the intestine [27]. In the present study, we identified several lines of evidence that suggested the involvement of NRF-1 in the regulation of folate transporter expression in the liver. There was a significant reduction of NRF-1/DNA binding

activity in the liver of mice fed a high-fat diet. This finding was consistent with decreased expression of PCFT and RFC in the liver. We then examined the role of NRF-1 in the transcriptional regulation of folate transporter expression in HepG2 cells. Inhibition of NRF-1 by using siRNA transfection significantly reduced folate transporter expression and folate concentrations in HepG2 cells. Although NRF-1 has been implicated as a key transcriptional factor that controls the expression of PCFT, its involvement in RFC expression has not been well established. A recent study has shown that NRF-1 silencing not only reduces PCFT expression but also significantly decreases RFC expression in HeLa cells [27]. However, the molecular mechanism by which NRF-1 is involved in RFC expression remains to be further investigated. The results from the present study suggest that downregulation of NRF-1 may contribute to decreased expression of folate transporters in the liver and reduced folate levels after chronic consumption of high-fat diets.

We previously reported that feeding mice with a high-fat diet induced lipid accumulation and oxidative stress in the liver [19, 20]. Several studies showed that dietary folate deficiency was associated with steatosis due to increased hepatic lipid biosynthesis and reduced lipid export via lipoproteins in mice [35, 36]. A recent study reported that patients with NAFLD had low serum folic acid levels, which was associated with severity of steatosis and hepatocellular ballooning [8]. In line with these clinical findings, the present study identified a low folate status that was associated with fatty liver caused by chronic consumption of a high-fat diet. Currently, effective treatment of patients with NAFLD remains a clinical challenge due to its multifaceted pathogenesis that is still incompletely understood [12, 37]. We previously demonstrated that folic acid supplementation could counteract oxidative stress and improve lipid and glucose metabolism in the liver of mice fed a high-fat diet [20, 21]. These findings suggest that folate can exert hepatic protective effects in the context of diet-induced NAFLD. Obesity is the most common risk factor for NAFLD [38] and obese patients often exhibit low folate levels in the circulation [5, 6]. It was previously reported that folate status was significantly improved in morbidly obese patients after gastric bypass

surgery [13, 39]. Because treatment of obesity was able to restore folate levels in patients, this suggests that obesity may be the underlying cause for the imbalance of endogenous folate levels [13]. Due to increased global prevalence of obesity, folate deficiency is expected to reoccur, which alarms both women in childbearing age and aging population. Further studies are warranted to investigate the relationship between obesity and folate status during pregnancy. Understanding the hepatic regulation of folate transporters and folate levels under pathophysiological conditions is imperative to ensure adequate folate status, which may be beneficial for patients with obesity and NAFLD.

In conclusion, the present study has demonstrated that chronic consumption of high-fat diets reduces the expression of hepatic folate transporters (PCFT, RFC), which, in turn, may account for low folate levels in the liver and circulation. Downregulation of transcription factor NRF-1 may be responsible for reduced expression of hepatic PCFT and RFC. These novel findings suggest that regulation of folate transporter expression in the liver is important in maintaining adequate folate status in the body. As a key nutrient, folate is essential for many biological functions in humans and animals. Although the general populations in Western countries can achieve sufficient dietary folate intakes, findings from our study indicate that prolonged consumption of diets that are high in fats may exert a negative influence on folate homeostasis. A better understanding of the mechanism by which high-fat diets disrupts hepatic folate transporter expression may have a significant implication for management of folate deficiency.

Funding information

This study was supported, in part, by the Natural Sciences and Engineering Research Council of Canada and St. Boniface Hospital Research Centre.

Compliance with ethical standards

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All procedures were performed in accordance with the Guide to the Care and Use of Experimental Animals published by the Canadian Council on

Animal Care and approved by the University of Manitoba Protocol Management and Review Committee.

Conflict of interest The authors declare that they have no conflict of interest.

Electronic supplementary material

ESM 1

(PDF 208 kb)

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