

Betaine alleviates hepatic lipid accumulation via enhancing hepatic lipid export and fatty acid oxidation in rats fed with a high-fat diet

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(Submitted 15 October 2014 – Final revision received 9 February 2015 – Accepted 11 March 2015 – First published online 29 April 2015)

Abstract

High-fat diet (HFD) induces hepatic lipid accumulation, which is associated with increased hepatic lipid export and fatty acid oxidation. Betaine, a natural methyl group donor, may alleviate hepatic lipid accumulation. In this study, we investigated the effect of betaine on hepatic lipid export and fatty acid oxidation in rats fed with a HFD. Rats were fed with a HFD or a HFD supplemented with betaine (HFD + betaine) for 8 weeks. Hepatic lipid accumulation was significantly reduced in the HFD + betaine group compared with the HFD group. Hepatic lipid export, measured by the expression of hepatic broblast growth factor 21 (BFGF21), was significantly increased in the HFD + betaine group compared with the HFD group. Hepatic fatty acid oxidation, measured by the expression of hepatic carnitine palmitoyltransferase I (CPT1), was significantly increased in the HFD + betaine group compared with the HFD group. Expressions of hepatic broblast growth factor 21 (BFGF21) were increased (P < 0.05) and hepatic carnitine palmitoyltransferase I (CPT1) were increased (P < 0.05) in the HFD + betaine group compared with the HFD group.

Key words:

Betaine; High-fat diet; Hepatic lipid accumulation; Hepatic lipid export; Fatty acid oxidation.

(N,N,N,N-tetra-methyl-β-alanine) (betaine) is a naturally occurring trimethylated homocysteine derivative. It has been reported that betaine plays a role in numerous biological processes, including cell growth, cell differentiation, and cell survival (1). Betaine is also known to be a natural methyl group donor, which is essential for the synthesis of many biologically important molecules, including DNA, RNA, and proteins (2). In recent years, betaine has been shown to have beneficial effects on various metabolic disorders, including obesity, insulin resistance, and non-alcoholic fatty liver disease (3). In fact, betaine supplementation has been shown to improve hepatic lipid export and fatty acid oxidation in rats fed with a high-fat diet (4). This suggests that betaine may be a potential therapeutic agent for the treatment of hepatic lipid accumulation.

The present study was designed to investigate the effect of betaine on hepatic lipid export and fatty acid oxidation in rats fed with a high-fat diet. We found that betaine supplementation significantly reduced hepatic lipid accumulation and increased hepatic lipid export and fatty acid oxidation. These findings suggest that betaine may be a potential therapeutic agent for the treatment of hepatic lipid accumulation.

Materials and methods

Animal experimental procedure

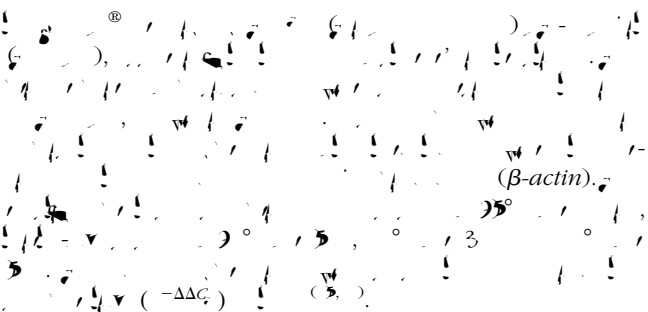
Eighteen male Wistar rats were divided into three groups: control (C), high-fat diet (HFD), and high-fat diet plus betaine (HFD + betaine). The rats were fed with their respective diets for 8 weeks. At the end of the study, the rats were sacrificed and the livers were removed for analysis.

NS British Journal of Nutrition

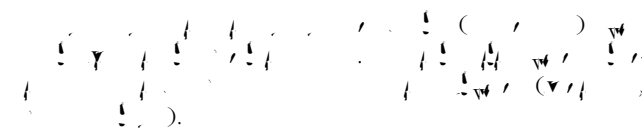
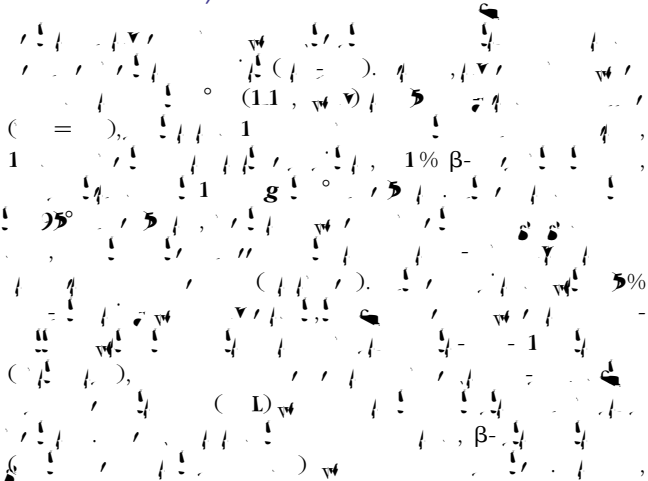
Table 2. Primer-pairs of target genes used for real-time PCR

| Gene | Forward primer (from 5' to 3') Reverse primer (from 5' to 3') | PCR product size (bp) | GenBank accession number |
|----------------|--|-----------------------|--------------------------|
| <i>β-Actin</i> | GGA AAT CGT GCG TGA CAT TA AGG AAG GAA GGC TGG AAG GAG | 183 | NM_031144 |
| <i>BHMT</i> | GGGCAGAAGGTCAATGAAGCT ACCAATGCATCCCCTTCGT | 108 | NM_030850 |
| <i>PPARα</i> | TGCGGACTACCAGTACTTAG CGACACTCGATGTTTCAGTGC | 167 | M88592 |
| <i>FGF21</i> | CGACAGAGGTATCTCTACACAGATGACG GATCCATAGAGAGTTCATCTGGTTGT | 206 | NM_130752 |
| <i>AMPK</i> | TGTGACAAGCACATTTTCCAA CCGATCTCTGTGGAGTAGCAG | 156 | NM_019142-2 |
| <i>CPT1</i> | GCTCGCACATTACAAGGACAT TGGACACCACATAGAGGCAG | 250 | AF020776 |

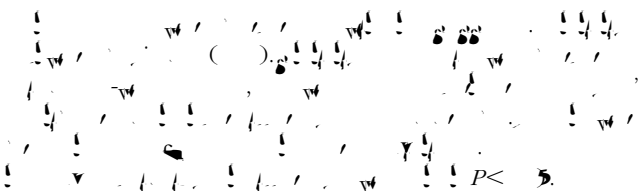
BHMT, betaine-homocysteine methyltransferase; *FGF21*, fibroblast growth factor 21; *AMPK*, AMP-activated protein kinase; *CPT1*, carnitine palmitoyltransferase 1.



Western blot analysis

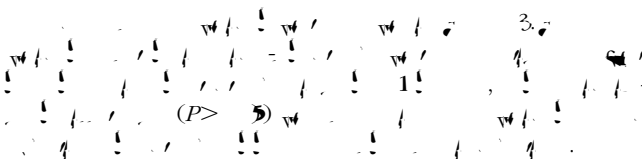


Statistical analysis



Results

Assessment of body weight



Effects of betaine on serum lipid metabolites

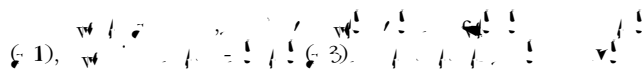


Table 3. Changes of body weight during 4 weeks (g)
(Mean values and standard deviations, *n* 7)

| | T1 | | T2 | | T3 | | T4 | |
|------|--------|-------|--------|-------|--------|-------|--------|-------|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 0 d | 100.02 | 0.15 | 100.15 | 0.28 | 99.91 | 0.13 | 99.95 | 0.36 |
| 7 d | 150.00 | 2.58 | 148.75 | 3.14 | 152.12 | 3.46 | 149.12 | 3.03 |
| 14 d | 202.00 | 5.42 | 205.5 | 6.75 | 207.75 | 7.45 | 206.17 | 7.60 |
| 21 d | 228.74 | 16.83 | 244.13 | 17.55 | 227.61 | 26.12 | 253.83 | 31.47 |
| 28 d | 290.58 | 9.86 | 296.03 | 14.26 | 287.26 | 15.80 | 305.75 | 25.67 |

T1, basal diet; T2, basal diet with betaine administration; T3, high-fat diet; T4, high-fat diet with betaine administration.

(P < 0.05), 11.1 ± 2.3 vs 3.2 ± 0.8 (P < 0.05),
(P < 0.05). 14.5 ± 3.1 vs 3.5 ± 1.2 (P < 0.05),
 18.2 ± 4.5 vs 2.1 ± 0.6 (P < 0.05).
(P < 0.05). 15.3 ± 3.8 vs 2.5 ± 0.9 (P < 0.05).
L.L. 10.5 ± 2.7 vs 2.8 ± 1.0 (P < 0.05).
 12.1 ± 3.2 vs 3.0 ± 1.1 (P < 0.05).

Oral administration of betaine effectively alleviated the excessive accumulation of fat in the liver

11.5 ± 2.8 vs 2.2 ± 0.7 (P < 0.05),
 13.2 ± 3.5 vs 3.1 ± 1.1 (P < 0.05),
 15.8 ± 4.1 vs 2.5 ± 0.8 (P < 0.05),
 17.4 ± 4.8 vs 2.3 ± 0.6 (P < 0.05).
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(1), (3) ($P < 0.05$), AMPK (1, 3).

(1), (3) ($P < 0.05$), AMPK (1, 3).

Betaine increased the activity, gene and protein expression of fibroblast growth factor 21, and elevated the gene expression of AMP-activated protein kinase in the liver

(1), (3) ($P < 0.05$), FGF21 (1, 3) ($P < 0.05$), AMPK (1, 3) ($P < 0.05$).

Discussion

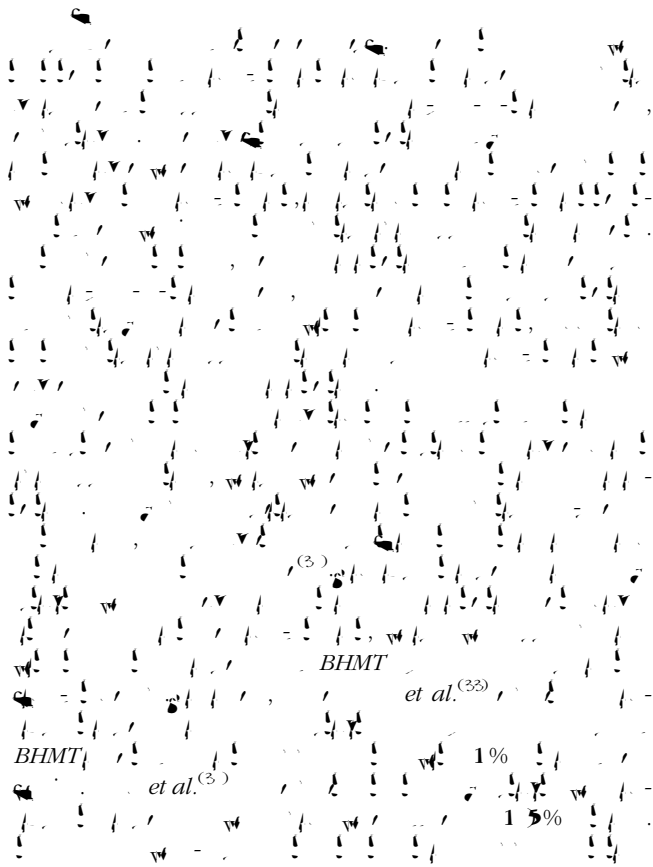
(1), (3) ($P < 0.05$), AMPK (1, 3) ($P < 0.05$).

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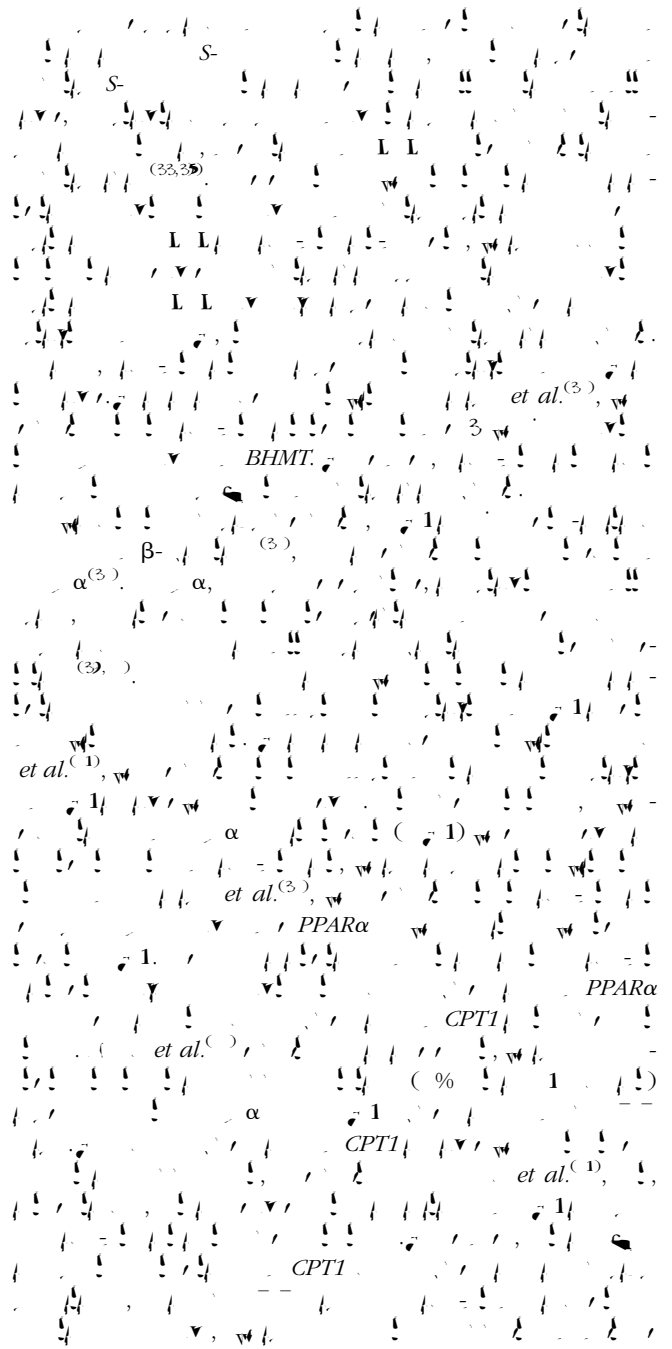
(1), (3) ($P < 0.05$), AMPK (1, 3) ($P < 0.05$).

Table 5. Effects of betaine on hepatic lipid metabolism (Mean values and standard deviations, $n = 7$)

| | T1 | | T2 | | T3 | | T4 | |
|-----------------------------------|---------------------|------|---------------------|-------|---------------------|-------|--------------------|-------|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| TAG (mg/g) | 7.81 ^b | 0.66 | 7.47 ^b | 0.58 | 9.20 ^a | 1.42 | 7.96 ^b | 0.84 |
| NEFA ($\mu\text{mol/g}$ protein) | 32.73 ^b | 9.16 | 39.44 ^b | 11.77 | 57.93 ^a | 12.76 | 67.08 ^a | 12.27 |
| TC (mg/g) | 2.21 ^{b,c} | 0.17 | 2.46 ^{a,c} | 0.47 | 2.58 ^{a,c} | 0.48 | 2.76 ^a | 0.49 |
| Lecithin (ng/g) | 1.00 ^c | 0.05 | 1.10 ^b | 0.05 | 1.04 ^c | 0.05 | 1.17 ^a | 0.02 |



(A) 3.5
3.0
2.5



(B)

(C)

29. ... , et al. ()
 (Sus scrofa domestica). *Comp Biochem Physiol A Mol Integr Physiol* **1** ,
 131-135.
30. ... & ... () ...
Br J Nutr ,
31. ... , et al. (1)
Eur J Lipid Sci Tech **112** , 33-38.
32. ... & ... (5)
Curr Drug Metab , 15.
33. ... (11)
 S- ...
J Biol Chem **28** ,
 33-38.
34. ... , et al. ()
J Hepatol , 31-35.
35. ... , et al. ()
Asian-Austral J Animal Sci **1** ,
 N- ... ()
 1-2.