Original article

MOLECULAR METABOLISM

Methionine and choline regulate the metabolic phenotype of a ketogenic diet

Pavlos Pissios *, Shangyu Hong, Adam Richard Kennedy, Deepthi Prasad, Fen-Fen Liu, Eleftheria Maratos-Flier

ABSTRACT

Low-carbohydrate ketogenic diets are commonly used as weight loss alternatives to low-fat diets, however the physiological and molecular adaptations to these diets are not completely understood. It is assumed that the metabolic phenotype of the ketogenic diet (KD) is caused by the absence of carbohydrate and high fat content, however in rodents the protein content of KD affects weight gain and ketosis. In this study we examined the role of methionine and choline in mediating the metabolic effects of KD. We have found that choline was more effective than methionine in decreasing the liver steatosis of KD-fed mice. On the other hand, methionine supplementation was more effective than choline in restoring weight gain and normalizing the expression of several fatty acid and inflammatory genes in the liver of KD-fed mice. Our results indicate that choline and methionine restriction rather than carbohydrate restriction underlies many of the metabolic effects of KD.

Keywords Ketogenic diet; Weight loss; Fatty acid oxidation; Methionine metabolism; Methylation

1. INTRODUCTION

Ketogenic diets are an accepted adjunct to pharmacologic therapy for the treatment of intractable epilepsy in children and are being investigated as means for the treatment of other cognitive disorders [1–3]. In addition to central nervous system effects, low-carbohydrate ketogenic diets are also considered as a potential alternative to low-fat, high-carbohydrate dieting in preventing obesity and its metabolic complications [4]. Human trials have shown that the weight loss achieved on low carbohydrate diets is equivalent to caloric restriction on a high carbohydrate diet and may also confer cardiovascular benefits [5–9].

Ketogenic diet (KD) induces a unique metabolic state in mice including weight loss, low circulating glucose and triglycerides, high serum ketones and increased energy expenditure [10]. In the genetically obese ob/ob mouse model, KD improves glucose tolerance independently of weight loss [11]. Metabolic adaptations to KD include decreased expression of gluconeogenic and lipogenic genes, while expression of genes regulating fatty acid oxidation and ketogenesis is increased, reflecting the use of fatty acids and ketones as a primary source of energy [10]. The fibroblast growth factor 21 (FGF21), originally identified as highly inducible by KD and PPARα agonists, mediates some of the metabolic effects of KD [12–14]. Undesirable side effects of KD in rodent models have also been reported. These include loss of lean mass, liver steatosis, inflammation and bone loss [10,15–17].

The specific nutrients in KD responsible for the metabolic phenotype are largely unknown. Recent studies demonstrate that weight gain in mice and rats depends on the protein content of KD [18,19]. Consistent with that, current formulation of KD is restricted to only about 10%, by weight, of casein, the sole protein source. Because of the low protein content and high caloric density, the methionine restriction in KD is approximately 75% compared to chow, which is close to 80% methionine restriction used in other studies [20]. In addition, KD contains no additional choline beyond what is present in the ingredients. For these reasons we investigated the effect of the relative methionine and choline restriction on the metabolic phenotype of KD.

Our results show that methionine supplementation of KD reverses the weight loss of mice, normalizes multiple metabolic parameters and regulates the expression of genes in fatty acid oxidation in the liver. Choline on the other hand is effective in reducing the fatty liver of mice fed KD. Thus, the relative methionine and choline restriction of KD underlies several of the metabolic effects of this diet.

2. MATERIAL AND METHODS

2.1. Animal husbandry

Wild type male C57BL6/J mice were purchased from Jackson labs and placed on varying diets at 8 weeks of age. Typically, 8 mice were assigned to each experimental group. KD was administered in glass vials on the bottom of the cages. Mice were either singly housed or group housed in cages with cotton fiber bedding liners (Alpha pad) and had ad libitum access to food and water. Measurements of food intake and energy expenditure required single housing of mice. Otherwise studies were performed in group-housed mice. Food intake was measured twice a week for 21 days starting at week 2 on KD. Several

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cohorts were run with similar results. Energy expenditure was measured after 4 weeks on the diets after acclimatization in the Comprehensive Laboratory Monitoring System (CLAMS) (Columbus, Ohio). Body composition was measured 24 h before acclimatization for CLAMS with EchoMRI from EchoMRI LLC (Houston, TX). All procedures were approved by the BIDMC IACUC.

2.2. Diets

Chow diet (#5008) was purchased from Lab Diets (St. Louis, MO). Ketogenic diet (KD) (# F3666) was purchased from Bioserv (Frenchtown, NJ). KD was modified by the addition of the following components. Methionine was mixed with KD to a final concentration of 0.4% w/w (KDM). Choline (as choline bitartrate) was mixed with KD to a final concentration of 0.2% w/w (KDC). Table 1 contains the macronutrient composition of the four diets and their relative content of methionine and choline by weight.

2.3. Metabolite measurements

Mice were fasted for 4 h, anesthetized by ketamine/xylazine cocktail and blood was collected by transcardial puncture. Glucose was measured by tail bleed with One-Touch Ultra glucometer (LifeScan). Serum triglycerides, cholesterol and beta-hydroxybutyrate were measured with the kits from Stanbio (Boerne, TX). Insulin was measured by ELISA from Crystal Chem (Downers Grove, IL). Non-esterified fatty acids (NEFA) were measured with the NEFA-HR (2) assay from Wako (Richmond, VA). Choline and lactate were measured by enzymatic assays from Biovision (Milpitas, CA). Liver triglycerides and cholesterol were extracted by the Folch method and measured with the enzymatic assays from Stanbio (Boerne, TX).

2.4. mRNA extraction and QPCR

Mice were fasted for 4 h, anesthetized by a ketamine/xylazine cocktail and tissues were rapidly dissected and snapped frozen in liquid nitrogen. Liver RNA was extracted with the RNeasy kit from Qiagen (Valencia, CA). cDNA was made with the Quantitect cDNA synthesis kit from Qiagen (Valencia, CA). Quantitative PCR was performed with the SybrGreen Master Mix from Life Technologies (Carlsbad, CA) on a 7800HT PCR (Applied Biosystems). Primer sequences are available upon request. Details of the microarray analysis were reported in Kennedy et al. [10] and the complete results of the microarray experiment are available on the NCBI Geo web site (GDS2738).

2.5. Histology

Liver samples were fixed overnight in 10% buffered formalin. They were paraffin embedded, sliced and stained with hematoxylin and eosin by the BIDMC histology core.

2.6. HPLC measurements

HPLC method for SAM/SAH was adopted from She et al. [21] with minor modifications. Briefly, liver samples were homogenized in 5 vol of ice-cold 0.4 N perchloric acid, centrifuged at 12,000*g* and analyzed on the Breeze HPLC system from Waters (Milford, MA). Samples (10 μl) were injected onto the Atlantis T3 column (Waters) (50 mm, 4.6 mm i.d, 3 μm particle size) protected by a 20 mm guard (Atlantis dC18) and eluted isocratically in 30 mM $NH_4H_2PO_4$ (pH 3), 8 mM sodium heptane sulfonate, 20% methanol at 2 ml/min with a 10 min total run time. Peaks were identified by co-elution with standards at 254 nm (Waters UV/VIS detector 2487) and the area under the curve determined with the Breeze software v3.2.

	Chow	KD	KDC	KDM
Protein (%)	23.5	8.6	8.6	8.6
Fat (%)	6.5	75.1	75.1	75.1
Carbohydrate (%)	49.4	3.2	3.2	3.2
Methionine (gm/kg)	4.3	2.2	2.2	6.2
Choline (gm/kg)	2.0	0.274	2.274	0.274

Table 1: Macronutrient composition of the experimental diets.

2.7. Statistics

Statistical differences were evaluated by one-way ANOVA followed by a posthoc test (Tukey's HSD) in JMP10 Pro from SAS (Cary, NC). Significance was set at alpha < 0.05 by one-way ANOVA followed by posthoc Tukey's HSD. The following scheme is used throughout the manuscript to denote statistical significance: [a]relative to chow, [b]relative to KD, [c]relative to KDC, [d]relative to KDM.

All data are expressed as means ($n=6$–8) $\pm$ standard error (SE). To satisfy the assumption of equal variance, some data were log transformed before ANOVA to normalize the variance between the cohorts.

3. RESULTS

3.1. Methionine but not choline supplementation reverses the weight loss of mice on KD

We speculated that the relative restriction of methionine and choline in KD might be responsible for some of the metabolic phenotypes. To test this hypothesis, we fed cohorts of C57BL6/J mice for 7 weeks with standard chow, KD, KD + 0.4% methionine (KDM) or KD + 0.2% choline (KDC) to achieve standard percentages of methionine and choline [22,23]. Mice need several days to adjust to diets of different texture and when switched to the ketogenic diets this resulted in the initial weight loss as we have previously reported [10]. From the second week on, mice consuming the methionine supplemented KD started to gain weight and at the end of the study weighted significantly more than the KD cohort (26.4 g vs 21.0 g respectively), while the weight of the KDC cohort was not different from the KD cohort (Figure 1A).

Liver weight was significantly decreased in mice on KD and KDC compared with chow but not in mice fed KDM (Figure 1B). Lean mass was also significantly increased in KDM-fed mice compared to KD and KDC-fed mice (Figure 1C). Total fat content by MRI was not significantly different between the cohorts although a trend towards decreased fat was seen in KD-fed mice and normalization in KDC and KDM-fed mice (Figure 1D).

Blood glucose, triglycerides and NEFA were not significantly changed by the KD diets (Table 2). B-hydroxybutyrate (BHB) was significantly increased by KD (8.8-fold) compared with chow. KDC had no effect on β-hydroxybutyrate but KDM tended to lower the ketone levels. Plasma choline and cholesterol were significantly increased by choline supplementation of KD. Finally, serum insulin of the KD and KDC cohorts was below the detection limit of the assay and therefore insulin could not be reliable determined. In the KDM-fed mice, insulin was measurable but significantly decreased compare with chow (Table 2).

3.2. Effect of choline and methionine supplementation of KD on food intake and energy expenditure

To understand the mechanism underlying the changes in body weight, we measured food intake and energy expenditure by indirect calorimetry. Respiratory exchange ratio (RER) of KD was close to 0.7 and not

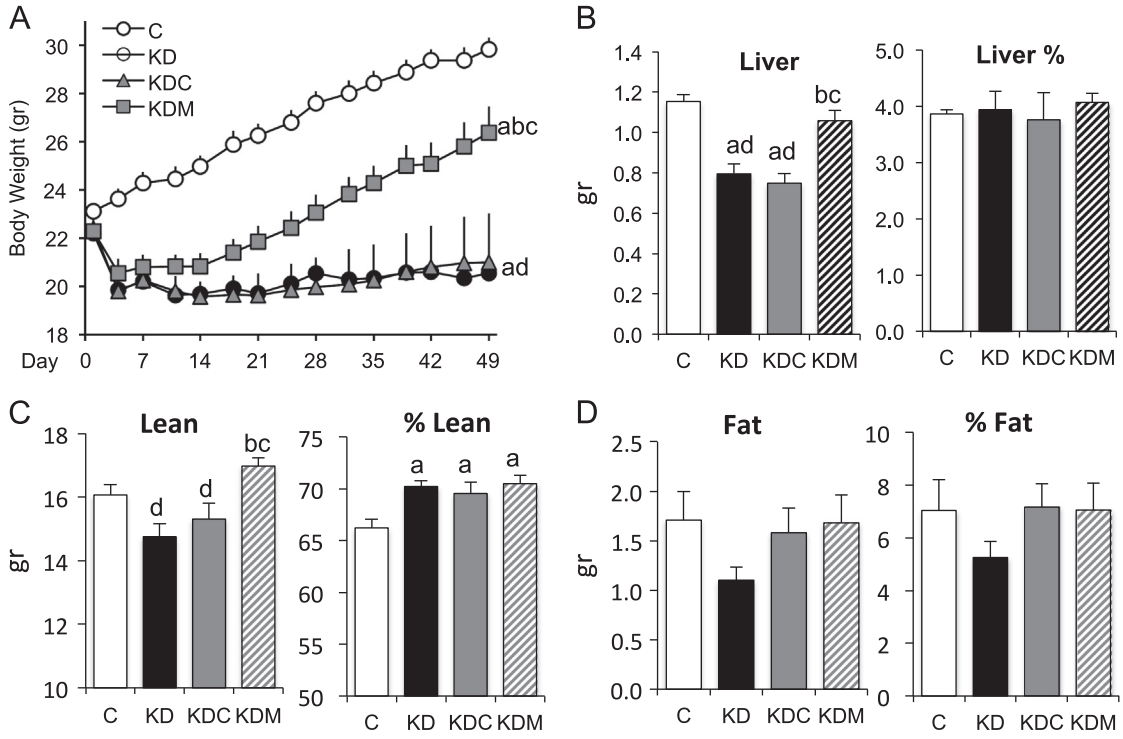


Figure 1: Methionine but not choline supplementation reverses the weight loss of mice consuming KD. (A) Body weight of mice consuming chow, ketogenic diet (KD), KD supplemented with 0.4% methionine (KDM) or KD supplemented with 0.2% choline (KDC). (B) Liver weight (absolute and % of body weight) of the four dietary cohorts. (C) Lean mass (absolute and % of body weight) by MRI. (D) Fat mass (absolute and % of body weight) by MRI. $N=6–8$, relative to chow, [b]relative to KD, [c]relative to KDC, [d]relative to KDM at alpha < 0.05 by one-way ANOVA followed by posthoc Tukey's HSD.

	Chow	KD	KDC	KDM
BHB (mM)	0.19 (0.02)	1.67 (0.31)[a]	1.57 (0.48)[a]	1.1 (0.21)a
NEFA (mEq)	0.22 (0.02)	0.29 (0.04)	0.25 (0.06)	0.21 (0.03)
TG (mg/dl)	54.6 (4.1)	42.6 (3)	51.6 (5)	49.6 (4.3)
Cholesterol (mg/dl)	75.3 (5)	100 (9.9)	109.4 (8.6)[a]	103.6 (12.6)
Choline (mM)	0.11 (0.01)	0.09 (0.01)	0.14 (0.01)[ab]	0.11 (0.01)
Glucose (mg/dl)	130.8 (8.9)	96 (16.6)	107.9 (22.2)	138.3 (6.7)
Insulin (ng/ml)	0.71 (0.1)	nd	nd	0.24 (0.06)a
Lactate (mM)	3.68 (0.3)	4.07 (0.39)	3.47 (0.34)	3.59 (0.36)

Table 2: Blood metabolites of the chow, KD, KDC and KDM cohorts.
nd: Values below the sensitivity of the assay; values are means ± SE. N=6–8, a relative to chow, b relative to KD, c relative to KDC, d relative to KDM at alpha < 0.05 by one-way ANOVA followed by posthoc Tukey's HSD.

changed by either choline or methionine supplementation reflecting continued fatty acid utilization as their main source of energy (Figure 2A). Caloric intake per mouse of the various versions of KD was not different from chow (Figure 2B) but the choline-supplemented cohort tended to eat more than when caloric intake was normalized to lean mass (Figure 2C). Mice fed the KD-modified diets increased their energy expenditure compared to chow on a whole animal basis, even though they were significantly smaller than the chow-fed mice and these differences became even more pronounced when normalized to lean mass (Figure 2D–F). Energy expenditure of the methionine-supplemented group trended lower compared to KD when normalized to lean mass (Figure 2E and F).

3.3. Effect of choline and methionine on liver steatosis in KD-fed mice

One of the consequences of KD in mice is liver steatosis [10,17,24]. Liver histology of KD-fed mice shows a combination macro- and microvesicular steatosis with an apparent improvement in the KDC cohort (Figure 3A). Consistent with the histology, triglyceride content of the liver was increased almost 7-fold by KD compared with chow and was significantly decreased (2-fold) by choline supplementation of KD but not by methionine (Figure 3B).

3.4. Methionine regulates multiple metabolic pathways in KD-fed mice

We and others have previously reported that KD suppresses lipogenic gene expression and increases the expression of genes in fatty acid oxidation, ketogenesis and inflammation in the liver [10,17,24]. We therefore determined the effect of methionine and choline supplementation on the above pathways. KD suppressed the expression of lipogenic genes, such as fatty acid synthase (FAS) and stearoyl Co-A desaturase 1 (SCD1) and to a smaller degree expression apolipoprotein B (apoB) and microsomal triglyceride rich protein (MTTP), involved in VLDL secretion. Methionine and choline supplementation of KD did not reverse the suppression of the above genes (Figure 4A). KD significantly increased (2–25 fold) the expression of several fatty acid oxidation genes, such as acyl-coA dehydrogenase long chain (Acadl), fatty acid translocase (Cd36), cytochrome p450 A10 (cyp4A10), cytochrome p450 4A14 (cyp4A14), 3-hydroxybyturate dehydrogenase (Bdh) and 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2) (Figure 4B). Methionine supplementation reversed the increase in the expression of the above genes by KD. Notably, Fgf21, which is an essential mediator of the metabolic effects of the ketogenic diet, was also significantly decreased by methionine supplementation of KD. Choline had no effect on the expression of fatty acid oxidation genes. Uncoupling protein (Ucp2), previously shown to be upregulated by KD in the liver was also significantly suppressed by methionine supplementation (Figure 4B). Next, we analyzed the expression of inflammatory genes in the livers of

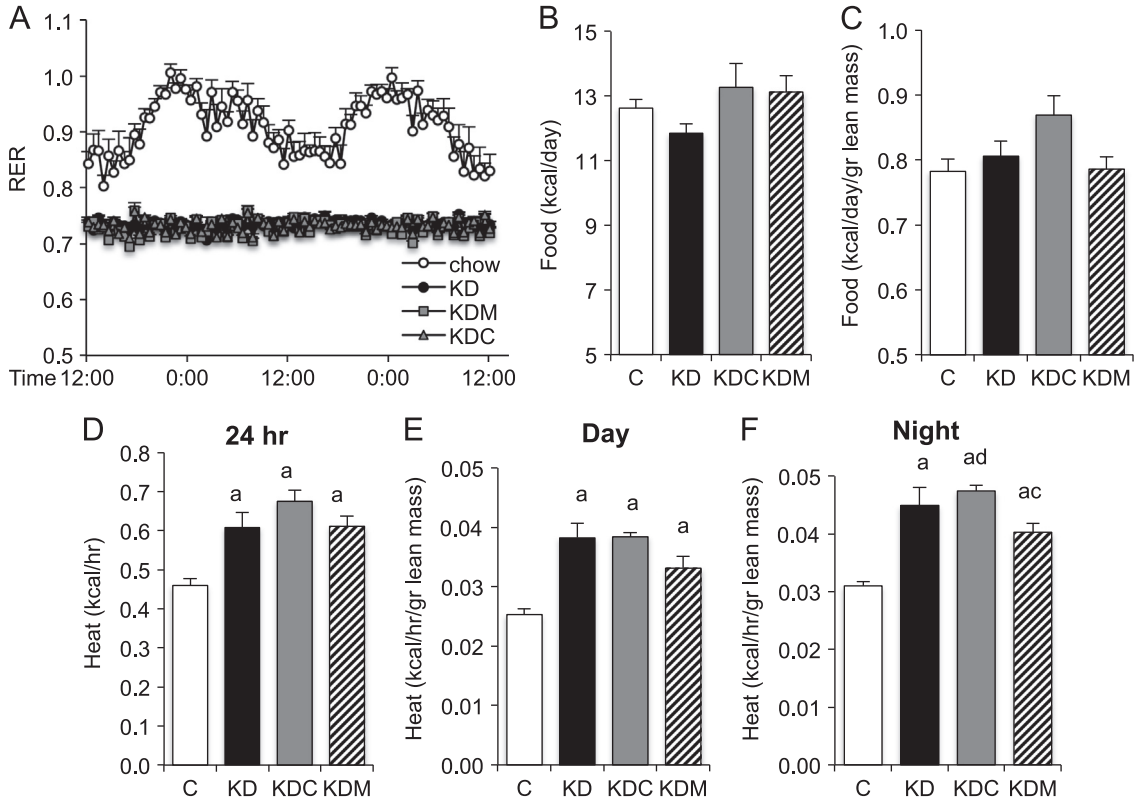


Figure 2: Effect of methionine or choline on food intake and energy expenditure in mice consuming KD. (A) Respiratory Exchange Ratio (RER) over 48 h of chow, KD, KDC and KDM mice. (B) Average daily food intake per animal basis. (C) Average daily food intake normalized to lean mass. (D) 24-hr energy expenditure per animal basis. (E) Light phase energy expenditure normalized to lean mass. (F) Dark phase energy expenditure normalized to lean mass. $N = 6–8$, [a]relative to chow, [b]relative to KD, [c]relative to KDC, [d]relative to KDM at alpha < 0.05 by one-way ANOVA followed by posthoc Tukey's HSD.

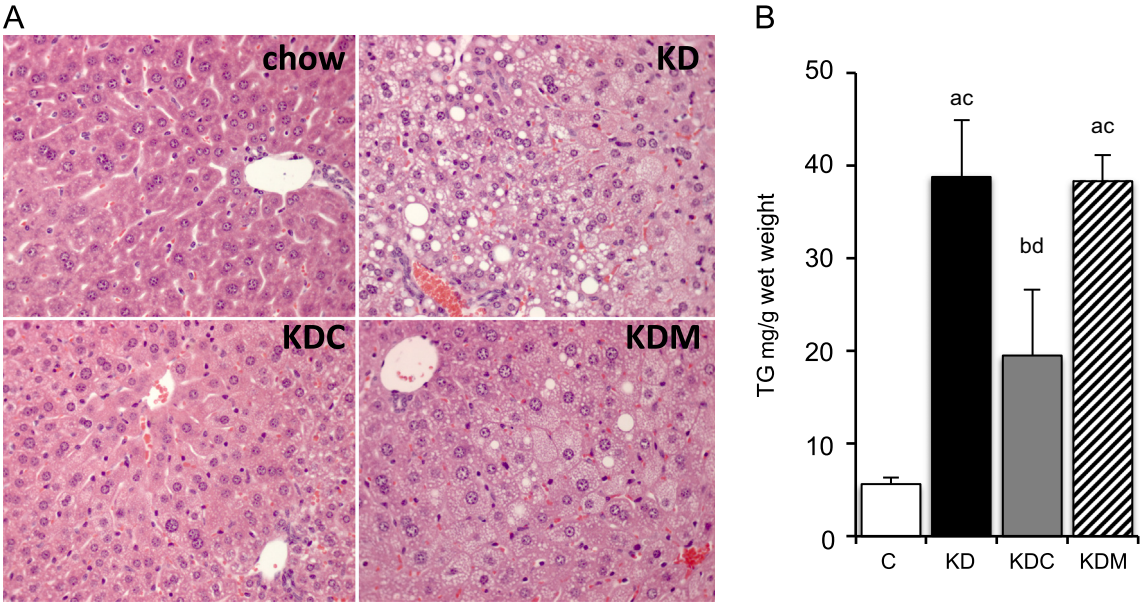


Figure 3: Choline but not methionine reduces liver steatosis of KD-fed mice. (A) Liver histology of mice consuming the 4 different diets. (B) Liver triglycerides (TG) of mice consuming the 4 different diets. $N = 6–8$, [a]relative to chow, [b]relative to KD, [c]relative to KDC, [d]relative to KDM at alpha < 0.05 by one-way ANOVA followed by Tukey's HSD.

KD mice. Expression of several proinflammatory and fibrogenic cytokines, tumor necrosis factor alpha (Tnfα) and transforming growth factor beta (TGFβ), as well as adhesion markers, intercellular cell adhesion molecule (Icam) and vascular cell adhesion molecule (Vcam) were increased by KD, and methionine supplementation abolished or attenuated this increase (Figure 4C).

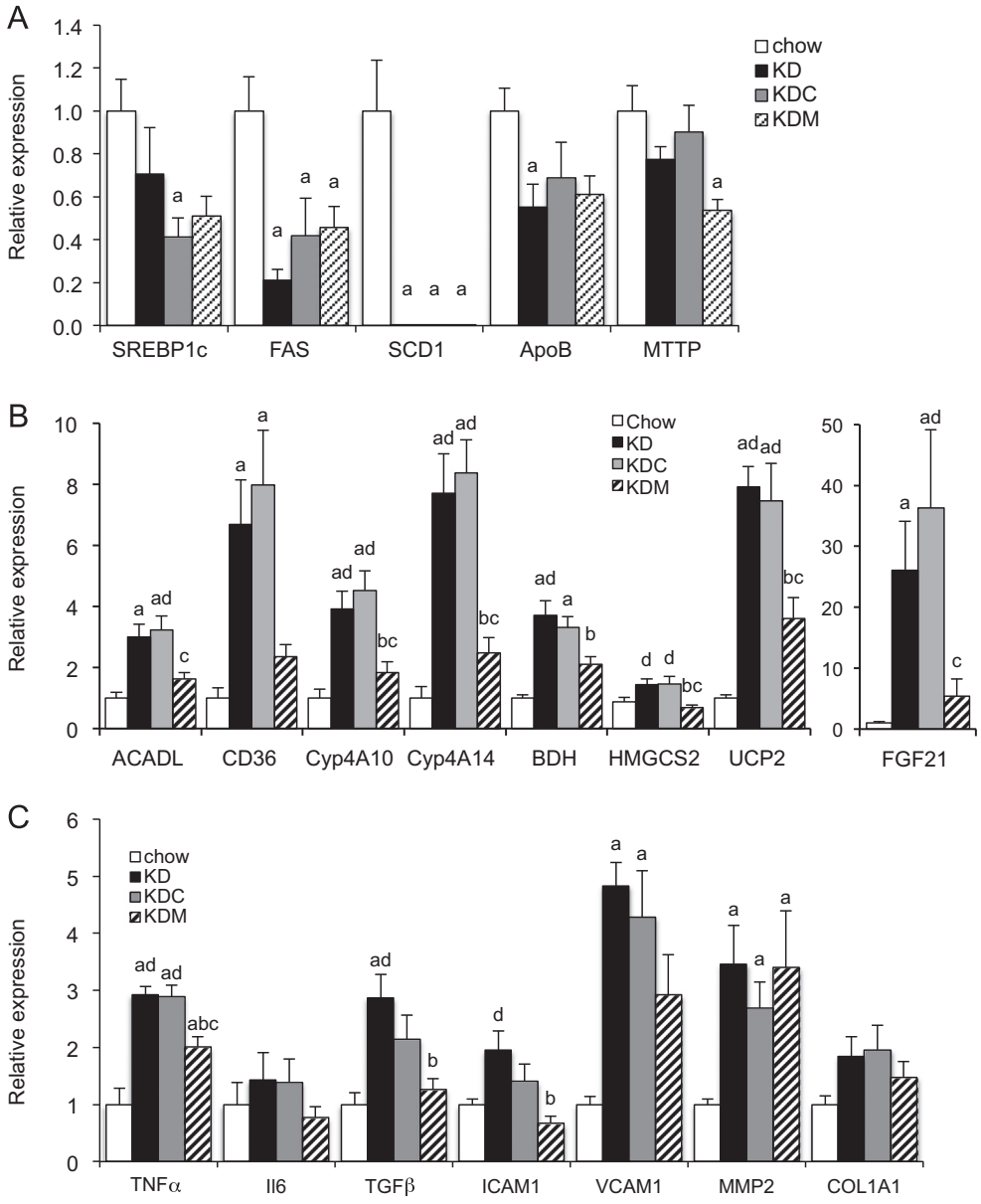


Figure 4: Effect of methionine and choline on expression of genes in fatty acid synthesis, oxidation and inflammation in mice consuming KD. (A) Effect of methionine and choline supplementation of KD on the expression of lipogenic genes Srebp1c, Fas, Scd1, apoB and Mttp. (B) Effect of methionine and choline supplementation of KD on the expression of genes in fatty acid oxidation and ketogenesis, Acadl, Cd36, cyp4A10, cyp4A14, Bdh, Hmgcs2, Fgf21 and UCP2. (C) Effect of methionine and choline supplementation of KD on the expression of inflammatory and profibrotic genes, Tnf, IL6, Tgf, Icam1, Mmp2 and Col1a1. $N=6–8$, [a]relative to chow, [b]relative to KD, [c]relative to KDC, [d]relative to KDM at alpha < 0.05 by one-way ANOVA followed by Tukey's HSD.

3.5. Ketogenic diet regulates multiple genes in methionine metabolism

We have previously performed microarray profiling from mouse livers to describe the metabolic adaptations to KD [10]. Further analysis of the microarray data revealed that a significant subset of genes involved in one carbon metabolism was downregulated by KD (Table 3). These include the betaine-homocysteine methyltransferase (Bhmt) (−3.14-fold), Bhmt2 (−1.88-fold) and S-adenosyl-homocysteine hydrolase (Ahcy) (−1.77-fold), core enzymes in recycling methyl donors. Expression of several major hepatic methyltransferases was also downregulated by KD, such as glycine N-methyltransferase (Gnmt) (−2.95-fold), thioether methyltransferase (Temt) (−2.37-fold), thiopurine methyltransferase (Tpmt) (−1.9-fold) and nicotinamide N-methyltransferase (Nnmt) (−27.87-fold). Expression of other major methyltransferases, such as methionine adenosyl transferase (Mat1α), methionine synthase (Ms), phenylethanolamine methyltransferase (PEMT) and guanidinoacetate methyltransferase (GAMT) was not affected by KD.

3.6. Effect of methionine and choline on methyltransferase expression and methyl donor balance in the context of KD

We next sought to validate the results from the Affymetrix analysis from the KD-fed mice (Table 3) and also examine the effect of choline and methionine supplementation on methyltransferase expression. QPCR confirmed the significant downregulation of Gnmt (−3-fold), Bhmt (−4-fold) and Nnmt (−8-fold) expression by KD. KD did not affect Mat1α and MS expression, consistent with the microarray data. Cystathionine beta synthase (Cbs) expression was significantly decreased by all ketogenic diets (2-

fold) and Ahcy was trending down (Figure 5A). The only gene normalized by methionine supplementation of KD was Bhmt, while choline did not affect the expression of the genes assayed (Figure 5A).

Since many of the above enzymes regulate the methyl donor balance, we measured by HPLC the methyl donors S-adenosyl-methionine (SAM), S-adenosyl-homocysteine (SAH) and their ratio in the liver of these mice. All three versions of KD did not significantly change the SAM content of the liver compared with chow (Figure 5B). However, KD significantly decreased SAH content (2.5-fold) (Figure 5B). Consequently, SAM/SAH ratio increased significantly from 2.0 in chow-fed mice to 3.7 in KD-fed mice. A further increase in SAM/SAH ratio to 5.1 was observed by choline supplementation of KD while methionine supplementation had no effect on SAM/SAH ratio (Figure 5B).

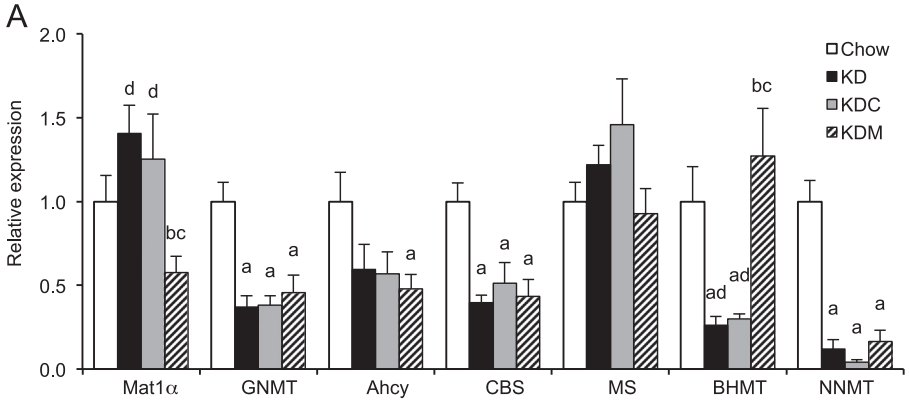
Gene symbol	Probe id	FC	LCB	Gene name
Ahcy	1417125_at	−1.77	−1.44	S-adenosylhomocysteine hydrolase
Gnmt	1417422_at	−2.95	−2.48	Glycine N-methyltransferase
Bhmt	1450624_at	−3.14	−2.55	Betaine-homocysteine methyltransferase
Bhmt2	1418913_at	−1.88	−1.38	Betaine-homocysteine methyltransferase 2
Tpmt	1430889_a_at	−1.90	−1.52	Thiopurine methyltransferase
Temt	1418697_at	−2.37	−1.65	Thioether S-methyltransferase
Nnmt	1432517_a_at	−27.87	−12.38	Nicotinamide N-methyltransferase

Table 3: Gene expression analysis of enzymes in methionine metabolism (Affymetrix).
FC, fold change compared with chow; LCB, lower confidence bound [10].

4. DISCUSSION

Ketogenic diet in mice induces a unique metabolic profile characterized by weight loss, low glucose, high ketones and increased energy expenditure [10]. Although it is assumed that the metabolic profile of KD depends on the absence of carbohydrates and on the high fat content, recent work suggested that weight gain and induction of ketosis depends on the relative fat to protein ratio in the ketogenic diet [18,19].

In this study we tested the effect of methionine and choline supplementation of KD, both of which are limiting in the current formulation of rodent ketogenic diet, and provide experimental evidence that methionine restriction underlies several of the key metabolic phenotypes of KD. Thus methionine but not choline significantly increased body weight gain and lean mass of mice on KD. Two pathways potentially contribute to the increased body weight by methionine supplementation of KD. First, methionine is an essential amino acid and integral part of proteins. In this way, methionine might preserve the lean mass in KD fed mice and indirectly affect the metabolic rate. Second, gene expression and indirect calorimetry data suggest that methionine regulates energy expenditure potentially contributing to the increased body weight. Thus methionine but not choline suppressed the expression of uncoupling protein 2 and normalized the expression of several fatty acid oxidation genes increased by KD, such as Fgf21, Acadl, cyp4A10 and cyp4A14, as well as the ketogenic genes Hmgcs2 and Bdh. These scenarios are not mutually exclusive and might operate simultaneously. Our results are in agreement with other studies showing decreased weight gain and increased energy expenditure by methionine restriction despite employing different animal models and diets [20,25,26]. PPARα is the common upstream regulator of the many of the genes assayed suggesting that methionine content of KD regulates PPARα activity. This effect is not specific to KD as protein and methionine restriction have been shown to increase expression of PPARα targets on other diets as well



B

Diet	SAM (pmol/mg)	SAH (pmol/mg)	SAM/SAH
Chow	67.71 (6.73)	33.92 (2.58)	2.01 (0.16)
KD	49.08 (7.37)	13.34 (1.77)[a]	3.68 (0.17)[a]
KDC	62.22 (7.74)	12.33 (1.24)[a]	5.06 (0.37)[abd]
KDM	53.62 (10.91)	13.93 (1.25)[a]	3.72 (0.47)[ac]

Figure 5: Effect of methionine and choline on expression of methyltransferases and methylation potential in mice consuming KD. (A) Expression of methionine cycle and methyltransferase genes in the liver of mice consuming chow, KD, KDC or KDM, (Mat1, Gnmt, Ahcy, Cbs, Ms, Bhmt and Nnmt). (B) Liver content of SAM, SAH and their ratio in mice consuming chow, KD, KDC or KDM. $N=7–8$, [a]relative to chow, [b]relative to KD, [c]relative to KDC, [d]relative to KDM at alpha < 0.05 by one-way ANOVA followed by Tukey's HSD.

[27,28]. The precise mechanism through which methionine regulates the activity of PPARα remains to be elucidated but it could be due to decreased availability of cysteine for which methionine is a precursor, as cysteine supplementation reverses the metabolic changes caused by methionine restriction [29].

Two known adverse consequences of KD are liver steatosis and inflammation [10,17,24]. Liver steatosis was partially improved by choline supplementation of KD, indicating that choline levels are limiting in KD. Choline is a known lipotropic agent and a precursor for the synthesis for phosphatidylcholine, necessary for the assembly and efficient export of liver VLDL triglycerides. While methionine did not affect liver triglyceride content, its restriction led to the induction of a number of proinflammatory and fibrogenic molecules in the liver, such as Tnfα, Tgfβ, ICAM and Vcam, which was reversed by methionine supplementation of KD.

In addition to being structural components of proteins and phospholipids, methionine is a precursor for the universal methyl donor S-Adenosyl-Methionine (SAM) and choline derivative betaine donates its methyl group for the remethylation of homocysteine back to methionine. Early clues that KD might affect methyl donor balance came from our previously published microarray analysis showing a significant decrease in the expression of major hepatic methyltransferases such as Bhmt, Ahcy, Gnmt, Nnmt and others. For this reason we measured SAM, SAH and their ratio to determine whether methyl donor balance could be linked to the phenotypic changes in our experimental cohorts. Somewhat surprisingly KD-fed mice maintained their liver SAM levels compared to chow-fed mice despite limited methionine and choline in this diet. Preservation of liver SAM could be a consequence of the suppressed GNMT expression, which controls SAM levels in hepatocytes [30]. Consistent with the idea of decreased transmethylation, liver SAH levels were reduced on KD. Neither methionine nor choline was able to normalize SAM/SAH ratio and, with the exception of BHMT expression which was restored by methionine, the expression of multiple methyltransferases was not affected, suggesting that other components in KD are responsible for these changes. Our results are consistent with a recent publication reporting that isocaloric protein restriction in the rat decreases SAH content of the liver and increases SAM/SAH ratio, although the mechanism behind these changes might not be identical [27]. Thus some phenotypes of KD such as weight loss, increased energy expenditure and methyl donor balance resemble more the dietary models of protein and methionine restriction which confer a favorable metabolic phenotype [20,25,26], while other aspects of liver physiology such as increased inflammation and steatosis of KD resemble the extreme methionine and choline deficient diets used to induce liver steatosis, inflammation and fibrosis.

In conclusion, we show that some of the metabolic adaptations to KD can be attributed to specific dietary components other than the lack of carbohydrate in KD and its replacement by fat. Limiting methionine content of KD contributes to weight loss, increased expression of fatty acid oxidation and inflammatory genes while the low choline contributes to fatty liver. Thus supplementation of each specific nutrient corrects distinct metabolic consequences of KD. In addition, KD induces significant changes in the liver methyl donor balance and in the expression of several hepatic methyltransferases but these changes are not a consequence of the limiting methyl donors methionine and choline but most likely of other nutrients. Further investigation into the role of specific dietary ingredients on the metabolic phenotype of KD might improve the safety and efficacy of this dietary model and uncover novel regulators of nutrient physiology.

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DISCLOSURES

The authors have nothing to disclose.

CONFLICTS OF INTEREST

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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