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Effects of Dietary Methionine and Folate Supplementation in Ethanol-Fed Rats*

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Chronic alcohol consumption is associated with perturbation of hepatic metabolism of sulphur-containing amino acid. The goal of present study was to evaluate the influence of dietary supplementation of methionine or folate to chronically ethanol-fed rats on the metabolism of sulfur-containing amino acids and one-carbon metabolism. Sprague-Dawley male rats were fed Lieber-Decarli liquid diet with 0% ethanol (control), 36% ethanol (E), 36% ethanol combined with methionine supplement (EM) or folate supplement (EF) for 8 weeks. Hepatic S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), plasma folate and homocysteine (Hcy), urinary excretion of folate and formiminoglutamate were investigated after feeding experimental diets. Growth was retarded by 36% ethanol consupmtion (E, EM and EF) ($p<0.01$). Liver total fat ($p<0.05$) and plasma ALT ($p<0.01$) were increased by methionine supplementation (EM), implicating fatty liver and liver injury. Liver folate was increased slightly by folate supplementation (EF) ($p=0.077$). Urinary folate loss was increased 2.3 fold by ethanol consumption (E) and 17.2 fold by folate supplementation (EF), while decreased by methionine supplementation (EM) ($p<0.0001$). Plasma Hcy was increased 1.9 fold by methionine supplementation (EM) in ethanol-fed rats ($p<0.05$), which was related with decreased methionine synthase activity ($p<0.05$). Hepatic SAM/SAH ratio was depressed by methionine supplementation in ethanol-fed rats (EM) ($p<0.05$). Urinary formininoglutamate (Figlu) excretion after histidine loading was increased by ethanol ingestion and reduced by methionine supplementation ($p<0.001$). Based on these data, methionine supplementation appears to accelerate histidine oxidation. In conclusion, dietary supplementation of methionine to ethanol-fed rats exacerbates alcoholic liver injury possibly by complicating sulphur-containing amino acid metabolism, as while it may have beneficial effects on folate and histidine metabolism.

Key words: Ethanol, Methionine supplementation, Homocysteine, Alcoholic liver disease, Folate

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INTRODUCTION

Methionine is an essential amino acid for the normal growth and development of mammals. Previous studies showed that chronic ethanol consumption alters methionine metabolism in the liver.[1-3] Since the liver is responsible for ethanol metabolism, it is vulnerable to alcohol-induced injury. Ethanol-induced perturbation of methionine metabolism is associated with the inhibition of methionine synthase activity, which causes a decrease in the hepatic levels of the methylating agent, S-adenosylmethionine (SAM), and increased production of potentially toxic agent, homocysteine (Hcy), which is released from the liver.[4,5] Because Hcy is removed inefficiently due to the impairment of methionine synthase activity by ethanol consumption, hepatic S-adenosylhomocysteine (SAH) is accumulated in ethanol-fed rats.[5-7] SAH is the metabolic precursor of Hcy and can be hydrolyzed to Hcy and adenosine when only the products are removed.[2] Elevated Hcy has been reported to play a role in the fatty liver and elevated SAH levels are also associated with liver injury.[6-9] Chronic ethanol feeding depressed hepatic SAM in rodent models[1,10,11] and in micropigs,[5] although changes in SAM level by ethanol consumption have not been consistant.

Effects of methionine supplementation on experimental animals have been diverse.[11-13] Steatosis is the most common alcohol-induced liver disorder. Deficiency of methionine produced hepatic steatosis similar to that seen with ethanol and supplementation with methionine can prevent ethanol-induced fatty liver.[11] This result suggested that these effects occur through increased methionine catabolism and lipotrope methyl-group wastage. Meanwhile, excess dietary methionine disturbed arterial wall morphology in rats[12] and atherosclerotic changes in rabbits[13] although

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the mechanism of methionine-induced disturbances is not clear. Recent study showed that SAM and betaine are effective in alleviating ethanol-induced hepatic steatosis, whereas only betaine can effectively methylate Hcy and prevent the increased release of Hcy by the liver.[7] In the present study it was postulated that dietary methionine supplementation to ethanol-fed rats might accelerate the development of alcoholic liver injury by abnormal methionine and folate metabolism. This postulate is based on the effect of ethanol on the alteration of methionine synthase activity and SAM/SAH metabolism, and the effect of excess methionine on the accumulation of lipid. The goal of the present study was to determine the effects of methionine supplementation in high dose on one-carbon metabolism and liver injury in ethanol-fed rats.

METHODS

1. Reagents

Lactobacillus casei (7469) was obtained from American Type Culture Collection (Manassas, VA, USA). Folic acid depleted casein medium and 7-fluoro-benzo-2-oxa-1, 3-diazole-4-sulfonate (SBDF) were obtained from Difco Laboratories (Detroit, MI, USA) and Wako Chemicals (Osaka, Japan), respectively. L-Homocystine, tri-n-butylphosphine, DL-methionine, folic acid, SAM and SAH were purchased from Sigma (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). 5-[$^{14}CH_3$]-tetrahydrofolate (62 μCi/μmole) was purchased from Amersham (Piscataway, NJ, USA). All chemicals were of the highest purity commercially available.

2. Animals and Diet

Animal experiments followed protocols approved by the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications NO. 80~23, revised 1996). Male Sprague-Dawley rats weighing 170~180 g were housed in wire-mesh cages with a daily light cycle from 0600 to 2000 hr and controlled temperature (20±2 ℃) and humidity (50±5%). They were quarantined for 1 wk, during which time they were fed a non-purified diet and water *ad libitum*.

After a one-week adjustment, 28 rats were assigned to four groups by a randomised block design, with nine rats in each group. After quarantine, the rats were acclimated for 3 days to the control liquid diet that is essentially the same as the diet described by Lieber and DeCarli[14] with the exception of reducing total lipid content from 39.6 g/L to 23 g/L and supplementing dextrin-maltose for the energy deficit. Ethanol was gradually introduced over 7 days into the diets of those rats that were fed the ethanol-containing diets, then the rats were fed Lieber-Decarli liquid diet with 0% ethanol (control), 36% ethanol (E), 36% ethanol supplemented with methionine (EM) or folate (EF) for the next eight weeks. The compositions of the experimental diets are listed in Table 1. As daily dosage of methionine supplemented to EM rats was calculated from food consumption (EM diet contained 2 g/L of methionine), each EM rat received 170 mg methionine per day, Rats of E, EM and EF group were fed ad libitum and control rats were pair-fed to ethanol-fed group (E). Food consumption was measured every day and rats were weighed once a week.

Table 1. Dietary composition of the applied liquid diet

Groups[1)]	Control	E	EM	EF
Ethanol (% of Calories)	0	36	36	36
Methionine (g/L diet)	0.3	0.3	2	0.3
Folate (mg/L diet)	0.5	0.5	0.5	2

[1)] Control; 0% ethanol diet, E; 36% ethanol diet, EM; 36% ethanol diet supplemented with methionine, EF; 36% ethanol diet supplemented with folate

3. Sample Collection

Twenty-four hr urine samples were collected during seventh week. Twenty-four hr urine samples for formimino-glutamate (Figlu) measurement were collected in tubes containing 1 M HCl (1 mL) after histidine loading. L-Histidine (250 mg/100 g body wt.) was loaded by feeding 40 mL of liquid diet containing L-histidine.

Following 8 wks of feeding experimental diets, rats were anesthetized and blood was collected by heart puncture using a heparinized syringe. Blood was immediately centrifuged for 15 min at 3,000 rpm to collect plasma. Livers were removed, weighed and rapidly frozen. Samples were stored at -70 ℃ until use.

4. Biochemical Analyses

Folate was analysed by a microtiter plate assay using L. casei (ATCC 7469) according to Tamura.[15] Liver was treated with pig kidney conjugase and then assayed for folate. Hepatic ALT and AST activity was measured by using commercial kit (Bayer, USA). Total lipid in liver tissues were extracted by the procedure of Folch *et al.*[16] and quantified after evaporation. Plasma Hcy was analysed using modification[17] of HPLC method described by Araki and Sako.[18] Hepatic methionine synthase activity was measured as described by Chen *et al.*[19] Hepatic and brain SAM and SAH levels were measured by the method of Fell *et al.*[20] Urinary Figlu was determined by enzymatic method of Tabor and Wyngarden.[21]

5. Statistical Analysis

Results were expressed as mean±SE. Significant differences among the groups were determined by one-way ANOVA using SPSS 12.0 for window (SPSS, Inc., Chicago, IL). If the effects of ethanol feeding and the supplementation of methionine or folate were significant, subgroup analyses were performed by one-way ANOVA with Duncan's multiple range tests for post hoc comparisons. Statistical significance was accepted at the $p<0.05$.

RESULTS

1. Physiological Variables after Methionine Loading

Body weights of ethanol-fed rats (E, EM, EF) were lower than controls although control group was pair-fed to the ethanol group (E) ($p<0.001$) (Table 2). Liver weights were not significantly changed by ethanol ingestion and by either supplementation of methionine or folate (Table 2).

Liver total lipid was examined to determine hepatic steatosis (Table 2). Hepatic total lipid was increased by dietary methionine supplementation (EM), but not changed by dietary folate supplementation in ethanol-fed rats. Plasma ALT and AST activities of the rats were examined to determine liver injury (Table 2). Plasma ALT was increased by ethanol ingestion slightly ($p< 0.05$) and significantly by supplementation of methionine combined with ethanol ingestion in rats.

Table 2. Effect of dietary methionine and folate supplementation on food intake and physiological variables of ethanol-fed rats

Groups[1)]	Control	E	EM	EF
Body Weight	525.0± 9.2[2)a3)]	473± 8.7[b]	456.1± 9.1[b]	483.6± 9.9[b]
F.E.R[4)]	0.041± 0.002[a]	0.035± 0.001[c]	0.038± 0.001[b]	0.034± 0.001[c]
Liver Weight (g)	13.55± 0.50[NS]	14.35± 0.66	13.08± 0.65	15.45± 0.74
Liver Weight (g/100g B.W.)	3.15± 0.48[NS]	3.03± 0.12	2.86± 0.12	3.19± 0.12
Liver Total Fat (mg/g tissue)	83.7±12.7[b]	64.8± 4.9[b]	119.5±13.3[a]	80.3± 5.0[b]
AST (units/L)	114.8±15.0[NS]	115.8±10.8	157.8±15.8	120.6±17.0
ALT (units/L)	45.6± 1.5[b]	61.7± 5.2[ab]	79.3±10.1[a]	61.8± 1.3[ab]

[1)] Control; 0% ethanol diet, E; 36% ethanol diet, EM; 36% ethanol diet supplemented with methionine, EF; 36% ethanol diet supplemented with folate
[2)] Values are mean±S.E. for 7 rats.
[3)] Values with different superscript letter within a column are significantly different at $p<0.05$ by Duncan's multiple-range test.
[4)] Food Efficiency Ratio=Body weight gain (g)/Food intake (L)
NS; not significant

2. Plasma and Liver Folate Levels and Uninary Folate Excretion

Chronic ethanol ingestion has been associated with folate deficiency. Table 3 showed that supplementation of methionine (EM) did not affect plasma and liver folate levels in ethanol-fed rats. Plasma ($p=0.120$) and liver folate ($p=0.077$) tended to be increased slightly by folate supplementation. Excretion of urinary folate was increased 2.3 fold by ethanol feeding (E) and 17.2 fold by folate supplementation combined with ethanol feeding ($p<0.0001$) (Table 3).

Table 3. Effect of dietary methionine and folate supplementation on plasma and liver folate and urinary folate excretion in ethanol-fed rats

Groups[1)]	Control	E	EM	EF
Plasma Folate (ng/mL)	93.70±5.85[2)ab3)]	87.99±3.67[ab]	81.88±4.04[b]	98.06±6.90[a]
Liver Folate (μg/g Liver)	7.16±0.10[ab]	6.91±0.37[b]	7.32±0.36[ab]	8.57±0.63[a]
Urinary Foate (μg/24 hr)	2.83±0.49[a]	6.52±1.76[a]	2.97±0.65[a]	48.85±3.11[b]

[1)] Control; 0% ethanol diet, E; 36% ethanol diet, EM; 36% ethanol diet supplemented with methionine, EF; 36% ethanol diet supplemented with folate
[2)] Values are mean±S.E. for 7 rats.
[3)] Values with different superscript letter within a column are significantly different at $p<0.05$ by Duncan's multiple-range test.
NS; not significant

3. Liver Methionine Synthase and Plasma Homocysteine

Several studies[1,4-7)] have found that prolonged ingestion of large quantities of ethanol causes inhibition of methionine synthase. Methionine synthase activity is required for biological methylation via the production of methionine and SAM, and also for DNA synthesis via the one-carbon metabolism of the folate cycle. Hepatic methionine synthase activity was depressed by the supplementation of methionine combined with ethanol ingestion (EM) for 8 wks ($p<0.05$) (Table 4). This result appeared to explain the increases in plasma homocysteine levels markedly by methionine supplementation to ethanol-fed rats (Table 4).

Table 4. Effect of dietary methionine and folate supplementation on hepatic methionine synthase activity and plasma homocysteine in ethanol-fed rats

Groups[1)]	Control	E	EM	EF
Homocysteine (nmole/mL)	19.73±2.81[2)b3)]	19.95±0.89[b]	36.96±5.39[a]	25.80±5.42[ab]
MS Activity (nmole/hr/mg)	3.25±0.29[a]	2.88±0.24[ab]	2.20±0.44[b]	2.52±0.32[ab]

[1)] Control; 0% ethanol diet, E; 36% ethanol diet, EM; 36% ethanol diet supplemented with methionine, EF; 36% ethanol diet supplemented with folate
[2)] Values are mean ± S.E. (n=7)
[3)] Values with different superscript letter within a column are significantly different at $p<0.05$ by Duncan's multiple-range test.

4. Liver and Brain SAM and SAH

Hepatic and brain SAM and SAH levels were determined

Table 5. Effect of dietary methionine and folate supplementation on hepatic and brain SAM and SAH in ethanol-fed rats

Groups[1)]	Control	E	EM	EF
Liver SAM (nmole/g)	55.73±2.74[NS]	53.55±2.16	52.25±4.36	50.68±3.05
Liver SAH (nmole/g)	18.21±1.15[2)ab3)]	17.38±1.43[ab]	19.99±0.81[a]	16.01±0.64[b]
Liver SAM/SAH	3.10±0.17[ab]	3.08±0.20[ab]	2.63±0.30[b]	3.22±0.27[a]
Brain SAM (nmole/g)	14.09±0.47[NS]	13.35±0.35	13.64±0.43	13.77±0.29
Brain SAH (nmole/g)	1.53±0.10[NS]	1.64±0.13	1.80±0.16	1.86±0.13
Brain SAM/SAH	9.43±0.66[NS]	8.39±0.58	7.85±0.55	7.65±0.60

[1)] Control; 0% ethanol diet, E; 36% ethanol diet, EM; 36% ethanol diet supplemented with methionine, EF; 36% ethanol diet supplemented with folate
[2)] Values are mean±S.E. for 7 rats.
[3)] Values with different superscript letter within a column are significantly different at $p<0.05$ by Duncan's multiple-range test.
NS; not significant

(Table 5). Mean hepatic SAH levels tended to be increased by supplementing methionine (EM) while decreased by supplemention of folate in ethanol-fed rats (EF). Contrary to this, liver SAM levels was not affected by either supplementation of methionine (EM) or folate (EF). In this context, liver SAM/SAH ratio was decreased by methionine supplementation. However, brain SAM and SAH were not altered either by supplementation of methionine (EM) or folate (EF).

5. Urinary Fomiminoglutamate Excretion

Excretion of urinary Figlu was analyzed to determine the effect of methionine supplementation on histidine metabolism. Uninary Figlu excretion was increased by ethanol feeding (E) while decreased to the level lower than control by methionine supplementation in ethanol-fed rats (Fig. 1). Twenty-four hour urinay Figlu excretion of folate-supplemented rats (EF) was similar to that of ethanol-fed group (E).

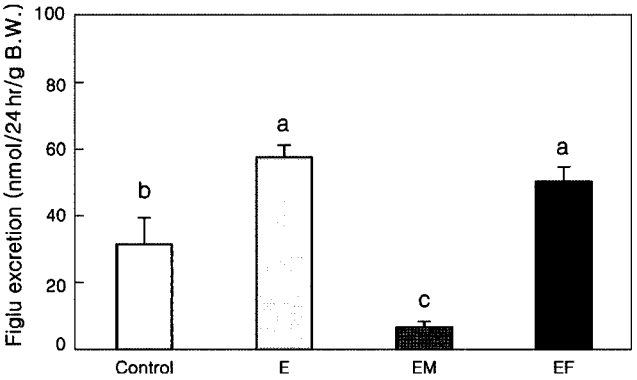

Fig. 1 Effect of dietary methionine and folate supplementation on urinary excretion of formininoglutamate in ethanol-fed rats. Values are mean±S.E. for 7 rats.
Bars with different superscript letter are significantly different at $p<0.05$ by Duncan's multiple-range test.
Control; 0% ethanol diet, E; 36% ethanol diet, EM; 36% ethanol diet supplemented with methionine, EF; 36% ethanol diet supplemented with folate

DISCUSSION

Ethanol ingestion and its effects on one-carbon metabolism have long been studied in the past. However, reports about the influence of methionine supplementation in ethanol-fed animals and its possible effects on ethanol-induced toxicity are limited. One-carbon metabolism and methionine metablolism are important processes in the liver, and alteration of these pathways may contribute various diseases, including coronary, cerebral, hepatic, and vascular diseases.[22] It has been revealed that ethanol feeding impairs methionine metabolism by inhibiting the enzyme methionine synthase. Inhibition of methionine synthase leads to impaired remethylation of Hcy to produce methionine and subsequently to decreased production of SAM.[1,23,24]

The present study showed that the activity of methionine synthase was inhibited by ethanol feeding and methionine supplementation ($p<0.05$) (Table 4). Consequence of methionine synthase inhibition was the accumulation of Hcy in the circulation. Plasma Hcy level was inversely well correlated with hepatic methionine synthase activity. From this result, supplementation of dietary methionine in high dose appears to impair methionine synthase activity further, which, in turn, produces homocysteinemia by methionine supplementation in ethanol-fed rats. Barak *et al.*[4] showed that chronic ethanol consumption increases Hcy accumulation in the isolated hepatocytes from ethanol-fed rat liver. However, contrary to this observation, Hcy level in the liver tissue was far below the detection level in control and all experimental groups of this study (data not shown). Therefore, Hcy appears to be released efficiently by active transport from the hepatocytes in control and all experimental groups.

Hcy is known to induce aortic alterations in rats.[25-27] Matthia *et al.*[28] showed that oral administration of methionine to normotensive and spontaneously hypertensive rats elevated serum Hcy and accompanied aortic change and, in spontaneously hypertensive rats, serum showed higher Hcy and cystathionine level. The methionine-related aortic changes were more pronounced and developed earlier with the dose and the length of time of dietary methioinine application. Hirche *et al.*[29] demonstrated a positive correlation between circulating plasma Hcy and plasma cholesterol in adult rats fed the high-methionine diet (6.82 g/kg diet) and suggested that dietary methionine induced elevation of plasma Hcy is associated with an increase of plasma cholesterol. Thus, they concluded that disturbed protein metabolism due to methionine supplementation in ethanol-fed rats is of importance as a risk factor for cardiovascular disease.

Clearly, ethanol feeding elevates urinary folate excretion

and thus appears to contribute to the depletion of plasma and liver folate.[17] Plasma and liver folate tended to be depleted slightly by ethanol feeding in these animals during the 8 weeks of this experiment (Table 3). Folate supplementation compensated plasma and liver folate completely. Table 3 demonstrated marked increase in urinary folate excretion by folate supplementation. This observation showed that large quantity of supplemented folate is lost through urinary excretion. Interestingly, methionine supplementation to ethanol-fed rats reduced urinary folate excretion ($p<0.0001$), which suggested the protective effect of methionine on urinary folate excretion and folate depletion from liver.

Urinary Figlu excretion after histidine loading was significantly increased by ethanol consumption and decreased by methionine supplementation markedly (Fig. 1). Based on these data methionine supplementation appears to accelerate histidine oxidation. Research showed that methionine supplement increases SAM, which is allosteric inhibitor of 5, 10-methylene tetrahydrofolate reductase (MTHFR).[30,31] Thus, inhibition of MTHFR resulted in a change in one-carbon moiety forms of folate derivatives, and facilitated the metabolic pathways that require THF, which include histidine oxidation by providing functional folate derivatives. Hidiroglou *et al.*[32] reported that rat liver treated with ethanol had similar total hepatic folate concentrations compared to control rats, but different proportions of folate subspecies: higher methylated tetrahydrofolate; higher unsubstituted folate; and lower formylated tetrahydrofolate compared with control rats. Methionine supplement may have protective effect on the alteration of folate subspecies caused by chronic ethanol consumption.

Ethanol-induced liver injury was exacerbated by methionine supplementation in this study. Chronic dietary supplementation of methionine in ethanol-fed rats produced hepatic steatosis and increased ALT (Table 1). Fatty liver occurs very commonly in alcoholics. Chronic ethanol intake leads to the development of liver dysfunction, which eventually decreases in the release of lipoprotein from the liver.

A number of interactions have been described between ethanol and the metabolic pathways of methionine and its metabolites. Trimble *et al.*[11] demonstrated that, in rats, ethanol feeding results in a loss of methionine and choline by the stimulation of methyl group catabolism. In the similar context, Parlesak *et al.*[33] showed that methionine supplementation up to 240 mg per day neutralized ethanol-induced liver injury in male Wistar rats subjected to a 90% jejunoileal bypass surgery, and thus supplementation with methionine can prevent ethanol-induced fatty liver.[11] In contrary to this observation, excess dietary methionine disturbed arterial wall morphology in rats[12] and caused atherosclerotic changes in rabbits.[13] From this observation, it was speculated that dose of methionine treated may be important criteria for being either beneficial or toxic to liver cells and one-carbon metabolism of animals.

In summary, dietary supplementation of methionine in high dose to ethanol-fed rats exacerbated alcoholic liver injury possibly by complicating sulphur-containing amino acid metabolism, and, in turn, by accumulating lipid in liver. Meanwhile, methionine supplementation appears to have beneficial effects on folate and histidine metabolism.

Literature cited

1) Barak AJ, Beckenhauer HC, Tuma DJ. Ethanol feeding inhibits the activity of hepatic N5-methyltetrahydrofolate: homocysteine methyltransferase in the rat. *IRCS Med Sci* 13:760-761, 1985
2) Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem* 1:228-237, 1990
3) Tsukamoto H, Lu SC. Current concepts in the pathogenesis of alcoholic liver injury. *FASEB J* 15:1335-1349, 2001
4) Barak AJ, Beckenhauer HC, Kharbanda KK, Tuma DJ. Chronic ethanol consumption increases homocysteine accumulation in hepatocytes. *Alcohol* 25:77-81, 2001
5) Halsted CH, Villanueva JA, Devlin AM, Niemela O, Parkkila S, Garrow TA, Wallock LM, Shigenaga MK, Melnyk S, James SJ. Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. *Proc Natl Acad Sci USA* 99:10072-10077, 2002
6) Halsted CH, Villanueva JA, Chandler CJ, Stabler SP, Allen RH, Muskhelishvili L, James SJ, Poirier L. Ethanol feeding of micropigs alters methionine metabolism and increases hepatocellular apoptosis and proliferation. *Hepatology* 23: 497-505 1996
7) Barak AJ, Beckenhauer HC, Mailliard ME, Kharbanda KK, Tuma DJ. Betaine lowers elevated s-adenosylhomocysteine levels in hepatocytes from ethanol-fed rats. *J Nutr* 1332845-1332848, 2003
8) Ji C, Kaplowitz N. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. *Gastroenterology* 124:1488-1499, 2003
9) Song Z, Zhou Z, Uriarte S, Wang L, Kang YJ, Chen T, Barve S, McClain CJ. S-adenosylhomocysteine sensitizes to TNF-alpha hepatotoxicity in mice and liver cells: a possible etiological factor in alcoholic liver disease. *Hepatology* 40:989-997, 2004
10) Finkelstein JD, Cello JP, Kyle WE. Ethanol-induced changes in methionine metabolism in rat liver. *Biochem Biophys Res Commun* 61:525-531, 1974
11) Trimble KC, Molloy AM, Scott JM, Weir DG. The effect of ethanol on one-carbon metabolism: increased methionine catabolism and lipotrope methyl-group wastage. *Hepatology*

18:984-989, 1993
12) Fau D, Peret J, Hadjiisky P. Effects of ingestion of high protein or excess methionine diets by rats for two years. *J Nutr* 118:128-133, 1988
13) McCully KS, Wilson RB. Homocysteine theory of arteriosclerosis. *Atherosclerosis* 22:215-227, 1975
14) Lieber CS, DeCarli LM. Animal models of chronic ethanol toxicity. *Methods Enzymol* 233:585-594, 1994
15) Tamura T. Microbiological assay of folate, in Folic Acid Metabolism in Health and Disease (Picciano MF, Stokstad ELR, Gregory JF eds.). pp.121-137, Wiley-Liss, New York, 1990
16) Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226:497-509, 1957
17) Min H, Im ES, Seo JS, Mun JA, Burri BJ. Effects of chronic ethanol ingestion and folate deficiency on the activity of 10-formyltetrahydrofolate dehydrogenase in rat liver. *Alcohol Clin Exp Res* 29:2188-2193, 2005
18) Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 422:43-52, 1987
19) Chen Z, Crippen K, Gulati S, Banerjee R. Purification and kinetic mechanism of a mammalian methionine synthase from pig liver. *J Biol Chem* 269:27193-27197, 1994
20) Fell D, Steele RD. The effects of vitamin A deficiency on hepatic folate metabolism in rats. *Arch Biochem Biophys* 240: 843-850, 1985
21) Tabor H, Wyngarden L. A method for the determination of formiminoglutamic acid in urine. *J Clin Invest* 37:824-828, 1958
22) Cravo ML, Camilo ME. Hyperhomocysteinemia in chronic alcoholism: relations to folic acid and vitamins B_6 and B_{12} status. *Nutrition* 16:296-302, 2000
23) Barak AJ, Beckenhauer HC, Tuma DJ, Donohue TM Jr. Adaptive increase in betaine: homocysteine methyltransferase activity maintains hepatic S-adenosylmethionine levels in ethanol-treated rats. *IRCS Med Sci* 12:866-867, 1984
24) Barak AJ, Beckenhauer HC. The influence of ethanol on hepatic transmethylation. *Alcohol Alcohol* 23:73-77, 1988
25) Lentz SR. Mechanisms of homocysteine-induced atherothrombosis. *J Thromb Haemost* 3:1646-1654, 2005
26) Stazka J, Luchowski P, Urbanska EM. Homocysteine, a risk factor for atherosclerosis, biphasically changes the endothelial production of kynurenic acid. *Eur J Pharmacol* 517:217-223, 2005
27) Li J, Chai S, Tang C, Du J. Homocysteine potentiates calcification of cultured rat aortic smooth muscle cells. *Life Sci* 74:451-461, 2003
28) Mattias D, Becker CH, Riezler R, Kindling PH. Homocysteine induced arteriosclerosis-like alterations of the aorta in normotensive and hypertensive rats following application of high doses of methionine. *Atherosclerosis* 122:201-216, 1996
29) Hirche F, Schroder A, Knoth B, Stangl GI, Eder K. Methionine-induced elevation of plasma homocysteine concentration is associated with an increase of plasma cholesterol in adult rats. *Ann Nutr Metab* 50:139-146, 2006
30) Krebs HE, Hems R, Tyler B. The regulation of folate and methionine metabolism. *Biochem J* 158:341-353, 1976
31) Stokstad ELR, Chan MM-S, Watson JE, Brody T. Nutritional interaction of vitamin B_{12}, folic acid and thyroxin. *Ann N Y Aca Sci* 355:119-129, 1980
32) Hidiroglou N, Camilo ME, Beckenhauer HC, Tuma DJ, Barak AJ, Nixon PF, Selhub J. Effect of chronic alcohol ingestion on hepatic folate distribution in the rat. *Biochem Pharmacol* 47:1561-1566, 1994
33) Parlesak A, Bode C, Bode JC. Free methionine supplementation limits alcohol-induced liver damage in rats. *Alcohol Clin Exp Res* 22:352-358, 1998