Nutrition 45 (2018) 41–48

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Effects of betaine supplementation on nitric oxide metabolism, atherosclerotic parameters, and fatty liver in guinea pigs fed a high cholesterol plus methionine diet

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ARTICLE INFO

Article history:
Received 27 June 2016
Accepted 11 July 2017

Keywords:
Betaine
Hypercholesterolemia
Hyperhomocysteinemia
NO bioavailability
Oxidative stress
Atherosclerosis
Liver

ABSTRACT

Objective: The aim of this study was to investigate the effect of high cholesterol (CHOL) and CHOL + methionine (MET) diets on atherogenic and oxidative index parameters and on the factors that influence nitric oxide (NO) bioavailability. Also, attempts were made to determine whether dietary betaine (BET) resulted in any improvement in the changes that occurred after CHOL + MET administration.

Methods: Guinea pigs were fed chow containing 1.5% CHOL with or without 2% MET for 10 wk. A third group received the CHOL + MET + BET diet. Control groups were given standard chow or standard chow + BET. Arginine, NO, nitrotyrosine (NT), and asymmetric dimethylarginine (ADMA) levels; lipid profile; and dimethylarginine dimethylaminohydrolase (DDAH) activity were measured. The liver and aorta were subjected to histopathologic analysis.

Results: The CHOL + MET diet caused higher serum CHOL and homocysteine levels, but no further increases were seen in aortic CHOL and diene conjugate (DC) levels and histopathologic lesions as compared with the CHOL group. Hepatic lipids and DC levels were also higher, and histopathologic lesions were more severe. CHOL + MET feeding increased ADMA and NT levels as compared with those of the CHOL-fed group. When BET (1 g/kg body weight/d) was added to the CHOL + MET diet, homocysteine and lipid levels decreased and histopathologic changes were reversed. BET diet decreased serum ADMA and hepatic and aortic DC levels and partly restored DDAH activity.

Conclusions: BET supplementation may be effective in preventing hyperlipidemia, disturbed NO availability, oxidative stress, and the development of fatty liver and atherosclerotic lesions that might result from excess amounts of cholesterol and methionine in the diet.

Introduction

Nitric oxide (NO) is a vasoactive substance that participates in the integrity of blood vessel walls. Reduced endothelium-derived NO synthesis gives rise to endothelial dysfunction reflected by the impairment of endothelium-dependent vasodilation [1]. NO is synthesized from the precursor arginine by endothelial nitric oxide synthase (eNOS) in endothelial cells. Asymmetric dimethylarginine (ADMA), an endogenously produced amino acid, is known to inhibit NOS [1,2]. ADMA is extensively produced by the modification of arginine side chains in proteins. The proteolytic cleavage following methylation liberates both ADMA and symmetric dimethylarginine (SDMA). ADMA inhibits all isoforms of nitric oxide synthase (NOS) by competing with arginine, and an imbalance in the levels of these molecules causes endothelial dysfunction. Several reports indicate that high levels of ADMA in the circulation represent an independent risk for atherosclerosis

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http://dx.doi.org/10.1016/j.nut.2017.07.005
0899-9007/

[1,2]. A positive correlation between plasma ADMA levels and obesity and insulin resistance has also been observed [3,4]. ADMA is metabolized in the liver by the activity of dimethylarginine dimethylaminohydrolase (DDAH), which is a highly oxidation-sensitive enzyme [1,5]. DDAH activity is accepted as a major determinant of endogenous ADMA concentration [5].

Endothelial cell damage is the basic mechanism for initiation and progression of atherosclerosis. There is strong evidence that hypercholesterolemia, a prominent risk factor for atherogenesis, increases the production of reactive oxygen species (ROS) and leads to vascular dysfunction [1,2]. Hypercholesterolemia-induced vascular dysfunction is reported to be associated with endothelial injury mainly via NO-dependent processes [2]. Hyperhomocysteinemia (hHcy) is also considered an independent risk factor for atherosclerotic vascular disease [6].

In a normal diet, proteins are commonly ingested with cholesterol (CHOL) through daily nutrients such as eggs, meat, and milk. In studies that have dealt with atherosclerosis, interest has been focused on methionine (MET) intake because it is the precursor of Hcy [7]. Some investigators have reported that the addition of MET to a high-CHOL diet results in higher levels of Hcy and lipids in the circulation and thereby more profound oxidative stress, endothelial dysfunction, and atherosclerotic changes in comparison to the changes observed in a solely CHOL-enriched diet [7,8]. Because some researchers claim that dietary cholesterol alone is not a risk factor for cardiovascular disease in humans [9], it would be interesting to determine whether MET and CHOL together might be a risk factor.

Betaine (trimethylglycine; BET) is a choline metabolite formed in liver by the action of choline dehydrogenase and betaine aldehyde dehydrogenase. Vegetables are alimentary sources of BET [10]. BET has antioxidant and antiinflammatory properties [10,11] and hepatoprotective potency [11–13]. It has been reported that BET per se does not have a direct antioxidant activity, and that inhibition of oxidative stress by betaine is most probably related to its effect on the metabolism of sulfur-containing substances in the transsulfuration pathway in the liver [13,14]. BET treatment has been reported to increase *S*-adenosylmethionine and glutathione (GSH) levels [12,13] and decrease Hcy levels [11,15]. BET supplementation has been reported to decrease atherogenic risk factor profiles in hyperhomocysteinemic mice [16]. Also, BET diet attenuates atherosclerotic lesions in apolipoprotein E-deficient mice [17]. On the basis of these effects, BET has been introduced as a useful compound in some pathologic conditions, including liver disease and atherosclerosis [11–17].

In this study, we wanted to investigate the changes in some parameters involved in the generation of oxidative stress and development of liver damage and atherosclerosis in guinea pigs fed a high CHOL + MET diet. Attempts have also been made to evaluate the effectiveness of BET supplementation on these factors together with the parameters that influence NO bioavailability, that is, ADMA, arginine, nitrotyrosine (NT), and DDAH activity.

Materials and methods

Chemicals

CHOL and BET were supplied by Alfa Aesar (Thermo Fischer Scientific, Germany). L-MET and other chemicals were obtained from Sigma-Aldrich (USA).

Animals

Male Dunkin-Hartley guinea pigs weighing 695 ± 38.6 g (aged 4–6 mo) were obtained from the Aziz Sancar Experimental and Medical Research Institute, Istanbul University. They were housed in a light- and temperature-controlled room on a 12/12-h light/dark cycle and kept in wire-bottomed stainless-steel cages (two or three animals per cage). The study protocols were approved by the Animal Care and Use Committee of Istanbul University.

Treatments

Animals were divided into five groups, each containing seven or eight animals. 1) The control group was fed a commercial guinea pig chow containing 88% dry matter, 16.5% crude protein, 3.5% crude fat, 12.0% crude fiber, and 6.9% crude ash, to which a salt and vitamin mixture supplemented with ascorbic acid was added. 2) The BET group ate a commercial feed to which 2% (w/w) BET was added. 3) The CHOL group was fed a diet supplemented with 1.5% (w/w) CHOL. 4) The CHOL + MET group was fed a CHOL (1.5%) + MET (2%, w/w)-supplemented diet. 5) The CHOL + MET + BET group received a diet containing 1.5% CHOL, 2% MET, and 2% BET. Diets were prepared by the Barbaros Denizeri Company (Gebze) and kept at 4°C. The animals were allowed free access to food and water.

At the end of the 10-wk period, animals were anesthetized with sodium thiopental (50 mg/kg, intraperitoneally) after an overnight fast. Blood was obtained by cardiac puncture and collected in dry tubes. Aliquots of serum were stored at −80°C until analyzed. Liver and aorta were rapidly removed, washed in 0.9% NaCl, and stored at −80°C.

Determinations in serum

Total CHOL, low-density lipoprotein (LDL)-CHOL, high-density lipoprotein (HDL)-CHOL, and triacylglycerol (TG) levels and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured with a Cobas Integra 400 instrument on the day of sacrifice. Serum Hcy levels were measured with the chemiluminescence immunoassay using Immulite 2000 XPI (Siemens Medical Solutions Diagnostics, USA). Serum NO levels were estimated as total nitrite + nitrate using a spectrophotometric commercial kit (Oxford Biomedical Research, USA). Serum NT levels were measured with the enzyme-linked immunosorbent assay (Cell Biolabs). Serum arginine and ADMA levels were determined using the high-performance liquid chromatography (HPLC) fluorometric method after samples had been treated with *o*-phthaldialdehyde to convert methylarginines to a fluorescent compound [18].

Determinations in the liver

NO and NT levels

A portion of liver tissue was homogenized in ice-cold 25 mM Hepes-NaOH buffer (pH 7.5) containing 150 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 2% glycerol, and protease-inhibitor cocktail. Homogenates were centrifuged at 16 000*g* for 10 min at 4°C. Supernatants were collected, and NO and NT levels were measured using kits as described earlier.

Protein levels

Protein levels were determined using the bicinchoninic acid method [19].

Cholesterol and triacylglycerol levels

Lipids were extracted with chloroform:methanol (2:1). After evaporation, the crude extract was dissolved in ethanol:ether (3:1) for the determination of liver CHOL and TG levels [20]. These levels were determined using kits provided by Bio-Science Medical (Madrid, Spain).

Table 1
Effect of BET on body and liver weights and liver index* in guinea pigs fed CHOL and CHOL + MET diets

	Control (n = 8)	BET (n = 7)	CHOL (n = 8)	CHOL + MET (n = 8)	CHOL + MET + BET (n = 7)
Body weight (g)	790 ± 58.0[a]	800 ± 57.9[a]	651 ± 88.7[b]	633 ± 57.2[b]	736 ± 56.4[ab]
Liver weight (g)	25.5 ± 2.82[a]	26.3 ± 2.65[a]	54.5 ± 9.82[b]	57.2 ± 4.80[b]	49.8 ± 2.93[b]
Liver index (%)	3.23 ± 0.22[a]	3.29 ± 0.27[a]	8.48 ± 1.83[b]	9.07 ± 0.65[b]	6.78 ± 0.52[c]

Mean values within each row not sharing a common superscript letter are significantly different at $P < 0.05$
* Liver index = liver weight/body weight × 100.