Biomedicine & Pharmacotherapy 91 (2017) 408–414

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Effect of arginine:lysine and glycine:methionine intake ratios on dyslipidemia and selected biomarkers implicated in cardiovascular disease: A study with hypercholesterolemic rats

Ravula Venkatesh[a], Krishnapura Srinivasan[b], Sridevi Annapurna Singh[a,*]

[a] Department of Protein Chemistry and Technology, CSIR – Central Food Technological Research Institute, Mysuru, 570020, India
[b] Department of Biochemistry, CSIR – Central Food Technological Research Institute, Mysuru, 570020, India

ARTICLE INFO

Article history:
Received 16 January 2017
Received in revised form 6 April 2017
Accepted 17 April 2017

Keywords:
Amino acid ratios
Hypercholesterolemia
ADMA
SDMA
Homocysteine
Nitric oxide

ABSTRACT

The effect of intake ratios of arginine (Arg): lysine (Lys) and glycine (Gly): methionine (Met) on lipid profile and selected cardiovascular disease markers, was studied, in rats maintained on a hypercholesterolemic diet. The rise in blood cholesterol was countered by 32%, 24%, and 49%, respectively, through increased oral supplementation of Arg, Gly, and Arg + Gly; a corresponding increase in plasma phospholipids at the end of the 8-week study was observed. The elevated plasma cholesterol to phospholipids ratio was countered by 27, 40, and 57%, respectively, through oral supplementation of Arg, Gly, and Arg + Gly. The elevation in hepatic cholesterol was lowered by 18, 29, and 51%, respectively, while phospholipids concentration was concomitantly increased by these amino acids. The elevated cholesterol to phospholipids ratio was, thus, significantly countered in the hypercholesterolemic situation by orally supplemented Arg, Gly, and Arg + Gly. Increased plasma asymmetric dimethylarginine (ADMA) levels, under hypercholesterolemic conditions, were lowered by 12, 15 and 34%, respectively, while plasma symmetric dimethylarginine (SDMA) levels were lowered by 14, 10 and 17%, respectively, with orally supplemented Arg, Gly and Arg + Gly. Only Gly and Arg + Gly decreased plasma homocysteine levels. Total nitric oxide (NO) concentration was considerably increased by Gly supplementation in hypercholesterolemic rats. Thus, altered ratios of Arg:Lys or Gly:Met offered beneficial influence on the lipid profile and plasma levels of ADMA, SDMA and homocysteine in hypercholesterolemic rats. Optimal beneficial effects, among ratios tested, was observed when Arg:Lys and Gly:Met ratios were maintained in ratios of 1:1 and 2:1, respectively.

1. Introduction

Cardiovascular diseases account for nearly 17 million deaths annually [1]. Some of the known risk factors for cardiovascular disease include high levels of blood cholesterol, asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), and homocysteine [2–4]. Quality of dietary protein may be linked to the progress of hypercholesterolemia and atherosclerosis. Earlier studies suggested that vegetable proteins are healthier compared to animal proteins in inducing hypercholesterolemia and both essential and nonessential amino acids could play a significant role in maintenance of health [5,6].

Animal and human clinical studies reveal that feeding or intravenous infusion of L-arginine inhibits platelet aggregation, improves endothelium-dependent dilatation, thereby reducing the risk of atherosclerosis [7–10]. Lysine enhances arginine catabolism by activating kidney arginase, which in turns depletes arginine levels [11]. Thus, lysine is an antagonist to the health benefits of arginine. Arginine:lysine ratio has been reported to be a significant regulatory factor for serum cholesterol levels in rats fed on different food proteins. Soy protein, containing arginine:lysine ratio of nearly 1, reduced cholesterol and triglycerides, although results were inconsistent when the arginine:lysine ratio was above 2 [12].

L-arginine is the substrate for production of nitric oxide (NO) through catalysis by nitric oxide synthase (NOS). Nitric oxide is of great importance in maintaining endothelial homeostasis.

Abbreviations: CVD, cardivascular diseases; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; CON, control; HCD, high cholesterol diet; Arg, arginine; Gly, glycine; Met, methionine; Lys, lysine; NO, nitric oxide; NOS, nitric oxide synthase; DDAH, dimethylarginine dimethylaminohydrolase.

* Corresponding author.
E-mail address: sridevi@cftri.res.in (S.A. Singh).

http://dx.doi.org/10.1016/j.biopha.2017.04.072
0753-3322/

Table 1
Grouping of experimental animals.

S. No.	Animal group	Diet	Amino acid ratio in the study
1	CON*	Control diet	–
2	CON-A	Control diet	Arg:Lys, 1:1
3	CON-G	Control diet	Gly:Met, 1:1
4	CON-AG	Control diet	Arg:Lys, 1:1; Gly:Met, 2:1
5	HCD*	Hypercholesterolemic diet	–
6	HCD-A	Hypercholesterolemic diet	Arg:Lys, 1:1
7	HCD-G	Hypercholesterolemic diet	Gly:Met, 1:1
8	HCD-AG	Hypercholesterolemic diet	Arg:Lys, 1:1; Gly:Met, 2:1

n = 6 per group.
* Ratios of Arg:Lys and Gly:Met in milk casein were 1:2.3 and 1:1.4, respectively.

Lowered concentrations of NO may cause impaired endothelial function thereby leading to atherosclerosis. L-arginine can also be methylated through the action of methyltransferase to produce ADMA and SDMA. Both ADMA and SDMA are elevated in chronic renal insufficiency, high blood pressure, diabetes mellitus, hyperhomocysteineamia and dyslipidemia [13–16]. ADMA has been recognized as the competitive endogenous inhibitor of NO Synthase (NOS) [17]. Under hypercholesterolemic conditions, ADMA and its symmetrical stereoisomer SDMA concentrations are raised in the blood [18]. SDMA, although not an inhibitor of NOS, competes with ADMA and arginine for cell entry. Therefore, it may indirectly influence NO production [13]. A recent meta-analysis by Schlesinger et al., reveals that both ADMA and SDMA are independent risk markers for cardiovascular disease events [19].

Homocysteine has been recognized as a risk factor for cardiovascular disease, although controversial, and is derived from methionine, a sulfur containing essential amino acid [20,21]. Homocysteine also inhibits NO synthesis by post-translational inhibition of dimethylarginine dimethylaminohydrolase (DDAH) enzyme activity, causing accumulation of ADMA [22]. Glycine prevents the ill-effects of methionine induced hypercholesterolemia by lowering homocysteine levels [23]. Other studies also confirm down-regulation of plasma cholesterol when lower methionine to glycine ratio is ingested. Thus, glycine has a cardio protective effect [24].

The aim of the present study is to examine the effective concentration and combination of amino acids that are believed to be influential in cardiovascular health. We hypothesize that the effects of arginine and glycine would be reinforced when given in combination. Arginine to lysine and glycine to methionine ratios were maintained as specific levels in the diet to see their effect on the plasma lipid profile, ADMA, SDMA, and homocysteine.

2. Materials and methods

2.1. Materials

Arginine, glycine, cholesterol, bile salts and nitrite/nitrate assay kit were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cellulose and vitamins were obtained from Himedia Laboratories (Mumbai, India). Choline chloride, DL-methionine and all mineral salts for preparation of AIN-93 diet were purchased from SISCO Research Laboratory (Mumbai, India). Casein was procured from Nimesh Corporation (Mumbai, India). ADMA ELISA kit and SDMA ELISA kit were purchased from Immundiagnostik (Bensheim, Germany). Homocysteine ELISA kit was procured from Creative Diagnostics (Shirley, NY, USA). All solvents for the study were of analytical grade and distilled before use.

2.2. Animal study

Experimental protocols were approved by the institutional animal ethics committee. Male Wistar rats [OUTB-Wistar, IND-cft (2c)] weighing 110 ± 5 g were grouped (n = 6 per group) by random distribution and housed in polycarbonate cages, in an approved animal house facility under a 12 h light / dark cycle at CSIR-Central Food Technological Research Institute, Mysuru, India. Rats were divided into eight groups as listed in Table 1. Rats had free access to fresh diet and water. The gain in body weight was monitored at regular intervals. The animals were maintained for a total duration of 8 weeks on the semisynthetic AIN-93 diet [25]. Hypercholesterolemia was induced by including 1% cholesterol and 0.25% bile salts in the diet. Arginine and glycine, nonessential amino acids, were administered through oral gavage from the fourth week of the study. After 8 weeks, rats were fasted overnight (12 h) and sacrificed under anesthesia using diethyl ether. Blood was drawn by cardiac puncture. The plasma was separated by centrifuging the

Table 2
Influence of orally supplemented arginine and glycine on body weight and organ weights in hypercholesterolemic rats.

Animal group	Final Body weight	Liver	Kidney	Heart	Lungs	Spleen
CON	284.7 ± 17.5	7.85 ± 0.77	1.54 ± 0.23	0.78 ± 0.08	1.52 ± 0.13	0.68 ± 0.14
CON-A	273.8 ± 24.9	6.97 ± 0.89	1.54 ± 0.15	0.78 ± 0.10	1.38 ± 0.13	0.60 ± 0.09
CON-G	275.0 ± 20.6	7.56 ± 0.75	1.57 ± 0.22	0.79 ± 0.07	1.18 ± 0.52	0.71 ± 0.18
CON-AG	276.7 ± 18.2	7.24 ± 0.52	1.52 ± 0.13	0.77 ± 0.08	1.27 ± 0.16	0.60 ± 0.08
HCD	295.5 ± 5.8	10.55 ± 0.63*	1.65 ± 0.11	0.79 ± 0.02	1.40 ± 0.10	0.68 ± 0.12
HCD-A	287.7 ± 16.4	9.43 ± 1.14**	1.57 ± 0.13	0.78 ± 0.05	1.48 ± 0.31	0.63 ± 0.04
HCD-G	288.2 ± 28.8	10.04 ± 1.21	1.63 ± 0.29	0.75 ± 0.09	1.30 ± 0.15	0.65 ± 0.07
HCD-AG	277.0 ± 13.5	9.08 ± 0.95**	1.47 ± 0.12	0.73 ± 0.08	1.25 ± 0.22	0.62 ± 0.04

Values (g) are Mean ± SD of six rats per group.
* Significant compared to CON ($P < 0.01$).
** Significant compared to HCD ($P < 0.05$).