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Evaluation of Methionine and Related Metabolites in Hyperhomocysteinemia

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Abstract

Aim: This study was conducted to identify plasma methionine, S-adenosyl methionine (SAM), S-adenosyl homocysteine (SAH), folate, vitamin B12, cysteine levels on people whose diets were standardized and divided into 3 groups (low, middle and high) in terms of homocysteine level and aimed to comment on the fact that methionine was essential. Methods: Participitants were divided into three groups in terms of the homocsyteine level. Participitants with homocysteine levels between 5-13 μmol/L were identified as low-level group (I. group), homocysteine levels between 13-30 μmol/ L as middle-level group (II. group), female) and homocysteine level above 30 μmol/L as high-level group (III. group). After seperating sera homocysteine, methionine, cysteine, SAM and SAH were analyzed on HPLC system, folat and vitamin B12 were analyzed on hormon analyzer by electrochemiluminescence method. Results: The differences in methionine, cysteine, SAM, SAH, folate were statistically significant in three groups. Strong positive correlation was found between homocysteine and methionine. Conclusion: It was shown that methionine is syntesized in the case hyperhomocysteinemia and it was concluded that methionine might be non-essential in the case hyperhomocysteinemia. This result should be supported by studies with different protein contaning diets including more participants.

Keywords: Homocysteine, methionine, essential, non-essential

1. Introduction

Homocysteine is an amino acid produced as a product during the metabolism of methionine, which does not bind with any of the known protein structures in the human body. Moreover, homocysteine does not have any other source of synthesis than methionine (Suliman et al., 2000). Reference value for homocysteine in human plasma is estimated to be at about 5-15 μmol/L in the human body (Gallagher et al.,1996). If homocysteine concentration in the plasma exceeds 5-15 μmol/L the condition is called hyperhomocysteinemia. On the basis of epidemiological studies, hyperhomocysteinemia is known to be an independent risk factor for developing carotid, coronary and peripheral vas occlusive conditions (Boushey et al.,1995; Fallest-Strobl et al., 1997).

Homocysteine can be metabolized through either transsulfuration or re-methylation (5). In the case of a deficiency in methionine levels in the body, re-methylation will, as one of two possible separate pathways, convert and metabolize homocysteine into methionine. In a minor re-methylation pathway, independently from folate and vitamin B_{12}, the betaine homocysteine methyl transferase (BHMT) enzyme, which is a methyl transmitter of the betaine methyl group, will convert itself to dimethylglycine while, simultaneously, transforming into homocysteine and thus producing methionine. 5-methylenetetrahydrofolate is a methyl transmitter in the long pathway. 5-10 methylenetetrahydrofolate will convert into 5-methylenetetrahydrofolate with the methylenetetrahydrofolate reductase (MTHFR) enzyme's mediation.

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While a methyl group of the 5-methylenetetrahydrofolate converts into homocysteine on one hand, mediated by the vitamin B_{12} dependent methionine synthase enzyme, and producing methionine on the other hand, it will also emanate tetrahydrofolate. This tetrahydrofolate will in turn transform into 5-10 methylenetetrahydrofolate. The folate in the re-methylation pathway functions as both co-enzyme and co-factor – and this process repeats itself in a circular pattern (Finkelstein, 1998; Nelen et al.,1998; Schwartz et al.,1997). Homocysteine will, in the case of an excess of methionine or when a cysteine syntheses is required, follow the transsulfuration pathway. The transsulfuration pathway is regulated by the cystathionine β synthesis enzyme (CBS), which is a B_6 vitamin dependent enzyme. Homocysteine is metabolized into cystathionine through the catalysis of CBS. Cystathionine, however, is hydrolyzed into cysteine. Cysteine in turn is hydrolyzed into sulphate, which is disposed of through the urine (Ueland et al.,1993).

The trans methylation pathway found in the homocysteine chain cycle is the process in which methionine is metabolized into homocysteine. Just as methionine adjoins the structures of newly synthesized proteins, S-adenosyl can enzymatically transform into methionine (SAM) with the help of ATP. The SAM's methyl group may, with the aid of DNA methyltransferase, break off and transform into S-adenosyl homocysteine (SAH). With the SAH's adenosyl section's hydrolytic fragmentation, homocysteine is produced (Finkelstein, 1998).

The metabolism of homocysteine has been schematized in Figure 1.

Despite methionine being an essential amino acid, it's thanks to the methionine chain cycle that the human body is able to synthesis it from homocysteine. Many previous studies have found a link between homocysteine and methionine. Except methionine levels have not previously been determined in any studies where hyperhomocysteinemia has been detected. There is no way of knowing if homocysteine levels rise or remain level in cases when methionine levels are at stable normal levels. In the metabolism chain cycle of homocysteine, this study has determined and analyzed the levels of not only methionine, but also other metabolites such as SAM and SAH and other cofactors such as folate and vitamin B_{12}. Thus, this study has aimed to determine whether or not homocysteine levels increase independently from methionine and whether or not methionine concentrations are sufficient in persons suffering from hyperhomocysteinemia.

2. Methods

Healthy volunteers whose homocysteine levels have been determined have been divided into the three following groups based on their homocysteine levels: those with low homocysteine levels around 5-13 µmol/L (I. Group), those with medium homocysteine levels around 13-30 µmol/L (II. Group) and those with high homocysteine levels above 30 µmol/L (III. Group). There were 75 individual subjects in each group at the onset of the study. The subjects' diets were questioned on the basis of their food consumption records. 35 subjects from each group were picked out for the study group. These subjects were people who were not on diets targeting weight gain or weight loss or diets meant to deal with specific illnesses. The subjects were followed up by a dietitian for at least 3 months and their diets were monitored and their energy levels measured using the Schofield Equation. By way of the homocysteine HPLC system, a commercial kit was used following the instructions attached to the kit. The cysteine levels of the subjects were analyzed using the homocysteine chromatograph.

To analyze the methionine, a commercial kit containing a set of 5 amino acids was utilized. Plasma SAM and SAH analysis were analyzed based on the method described by Melnyk et al. as well as the method prescribed in the instructions of commercial kit (Melynk et al.,2000). Per 200 µL of plasma, 40 µL of acid from a 40% trichloroacetic acid was added. It was incubated in room temperature 30 minutes after being vortexed. The plasma was later centrifuged at +4 ºC at a speed of 10000 g. The HPLC device was calibrated so as to automatically take a 20 µL sample. and the results were presented in nmol/L. The experiment then proceeded with the use of the Roche Elecsys' E170 (Roche Diagnostics GmbH, Mannheim - Germany) hormone analyzer, with separate Cobas kits produced to measure folate and vitamin B_{12} levels – which were then applied using the electroluminescence method. The statistical analysis were performed by using SPSS for Windows 19.0 software. Kolmogorov-Smirnov test was used to determine the normality distrubition of groups. In comparing the parameters between the groups, the One-Way ANOVA (one way variance analysis) and Kruskall Walis tests were performed, while the Tukey test was applied to compare the subgroups. Pearson's correlation analysis was used to determine of the correlations between parameters. p values less than 0.05 was considered statistically significant at 95% confidence interval.

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

3. Results

The three groups divided according to their levels of homocysteine are presented with their age and gender distributions to homocysteine, methionine, SAM, SAH, folate, vitamin B_{12} and cysteine distributions (Table 1). There were statistically significant differences in the distribution of all numeric data, except for vitamin B_{12} levels, in the groups which consisted of subjects divided in terms of homocysteine levels. The correlations between all the subjects' data are presented regardless of groups (Table 2). The correlation between homocysteine and methionine is shown in Figure 2.

4. Discussion

Methionine and homocysteine are precursors to each other. The construction of one means the destruction of the other as a feature, and this cycle makes up the chain cycle of methionine. Despite methionine being an essential amino acid, it's thanks to the methionine chain cycle that the human body can synthesize homocysteine (Finkelstein, 1998; Nelen et al.,1998; Schwartz et al.,1997). Homocysteine is neither obtained through diet (Verhoef et al., 2005) nor has another source of synthesis than through methionine (Suliman et al., 2000). Protein catabolism is not possible without the entrance of homocysteine into the protein.

Through the HPLC method, in a study on patients with rheumatoid arthritis who had their plasma homocysteine levels measured, the levels of homocysteine, cysteine and methionine were found to be statistically and significantly high in the group of ill subjects versus those of the control group. The team of researchers, when examining the standard diets of the subjects, inferred that the significant difference could be explained by a mistake in the regulation of either the transsulfuration pathway or the homocysteine cycle (Hernanz et al., 1999).

Moreover, in the studies which were able to determine the level of homocysteine and methionine levels in the plasma, it was found that the patients with high levels of homocysteine also had statistically significant high methionine levels – and thus supports a positive correlation between homocysteine and methionine levels (Boston et al.,1995; Hong et al.,1998).

With the determination of the levels of methionine of the groups in our study, we found that the increase in homocysteine and methionine levels are parallel and that the increase in methionine between the groups was significant. Furthermore, a clear significant positive correlation between homocysteine and methionine was established. These results are in line with already existing studies in literature. With the hallmark of this study being the detail to the methionine-homocysteine cycle's SAM, SAH, folate, vitamin B_{12} and cysteine concentrations in all test subjects as the source of the heightened levels of methionine deriving from heightened levels of homocysteine.

In the case when homocysteine levels in the body increase or the levels of methionine decrease, the homocysteine is known to re-methylate (Fallest-Strobl et al., 1997). In the light of this knowledge and the findings of our study, for any reason that homocysteine concentration levels increase; if homocysteine has any other source than methionine, which we do not know, and if there are sufficient cofactors and coenzymes available without defects in the cycle, then we can conclude that a specific level of methionine will be synthesized.

While homocysteine, through methylation pathway, is re-methylated into methionine, it is catabolized to cysteine through the transsulfuration pathway via cystathionine. People with high plasma homocysteine levels, have statistically and significantly low cysteine levels (Melynk et al.,2000). In the same study, a negative correlation was found between the levels of plasma homocysteine and plasma SAM/SAH ratio with plasma pyridoxyl 5-phosphate concentrations. Researchers suggest that the decrease in plasma pyridoxyl 5-phosphate concentrations arise from dietary deficiency in vitamin B_6 and the decrease in cysteine concentrations support this explanation (Melynk et al.,2000). When we examined the cysteine results of the groups in our study, we found that cysteine levels are the lowest in the group that has the highest levels of homocysteine, that in all three groups there it was observed that increases in the homocysteine lead to decreases in cysteine levels. The differences between the groups are statistically significant. Likewise, there is a negative correlation between cysteine and SAM, SAH and methionine levels.

Concluding our study; regardless of cause, through the aid of methionine synthesis, the methionine synthesizes and the methionine levels are thus found to be high in people with a high level of homocysteine while they have normal levels of folate, B_1, B_6 and B_{12} vitamins and no enzyme defects. In this case when the aspect of the cycle that consists of a working methionine synthase reaction and since the methionine concentration is high, we can establish that there must be an unknown problem in the transformation pathway from homocysteine to cysteine and that the cysteine synthesis is decreased and weakened. To consolidate the cause of the decrease in cysteine concentrations, one must measure the levels of the cofactor; vitamin B_6 concentrations as one of the reactions in the homocysteine to cysteine transformation. Also, it might be productive to research and study the activities of the CBS and γ- cystathionase ezymes, which are reactional enzymes in this cycle.

Moreover, in the case when there are sufficient substrates, cofactors and enzymes that make up the methionine chain cycle, we have established that homocysteine to methionine synthesis takes place and the increment in homocysteine increases the methionine concentration levels. In fact, the question of which one replenishes the other of the two arises; "does the increment in homocysteine increase the methionine concentrations or vice versa?". Hence, the daily intakes of food and beverages were recorded when the subjects were grouped and selected according to a set diet adjusted to a certain energy level; thus, we've tried to standardize the intake of methionine among the subjects.

Furthermore, due to methionine being synthesized in adequate quantities in patients suffering from hyperhomocystenemia, we can state that "methionine was non-essential in patients suffering from hyperhomocystenemia". Bhagavan N.V. declares in his biochemistry book, that "as long as sufficient homocysteine and a methyl group source can't be found, methionine is essential, though it is not essential because cysteine can be synthesized from methionine"(Bhagavan,2001). Our findings are complementary and parallel to Bhagavan's conclusion.

Finally, hyperhomocystenemia increases methionine concentration levels. This is the first study which establishes that methionine is non-essential to people with hyperhomocystenemia. Future studies including larger series and variable protein intakes are required to support our conclusion.

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Table 1. Distrubition of data and test statistics p values among groups

	Group 1 n=35	Group 2 n=35	Group 3 n=35	Among Groups (p)	Group 1-2 (p)	Group 2-3 (p)	Group 1-3 (p)
Age (years)	31,4±6,1	39,6±11,9	46,5±13,2	<0,01	0,06	0,25	<0,01
Hcy (µmol/L)	9,2±1,9	20,8±4,0	39,8±6,9	<0,01	<0,01	<0,01	<0,01
Met (µmol/L)	7,7±3,5	15,8±7,3	40,1±14,3	<0,01	0,02	<0,01	<0,01
SAM (nmol/L)	49,6±12,8	89,1±26,8	102,9±34,6	<0,01	<0,01	0,08	<0,01
SAH (nmol/L)	131,3±26,0	168,4±70,1	241,2±75,9	<0,01	0,03	<0,01	<0,01
Folat (ng/ml)	7,9±4,5	7,1±2,9	5,3±2,2	0,05	0,56	007	0,05
Vit B_{12} (pg/ml)	294,7±118,3	265,0±111,0	252,1±96,3	0,25	0,51	0,88	0,24
Cys (µmol/L)	185,4±36,5	131,5±40,3	86,9±39,1	<0,01	<0,01	<0,01	<0,01

Results are expressed as mean±sd, Hcy: Homocysteine, Met: Methionin, SAM: S-adenosyl methionin, SAH: S-adenosyl homocysteine, Vit B_{12}: Vitamin B_{12}, Cys: Cysteine, p: test statistics p value.

Table 2. Correlation coefficients (r) among data

	Hcy	Vit B_{12}	Folat	SAM	SAH	Met	Cys	Age
Hcy (r)	1							
Vit B_{12} (r)	-0,172	1						
Folat (r)	-0,315**	0,055	1					
SAM (r)	0,534**	-0,221*	-0,156	1				
SAH (r)	0,589**	-0,082	-0,081	0,350**	1			
Met (r)	0,789**	-0,148	-0,255**	0,565**	0,470**	1		
Cys (r)	-0,709**	0,157	0,168	-0,484**	-0,390**	-0,634**	1	
Age (r)	0,520**	0,088	-0,069	0,228*	0,230*	0,372**	-0,331	1

Hcy: Homocysteine, Vit B_{12}: Vitamin B_{12}, Met: Methionin, SAM: S-adenosyl methionin, SAH: S-adenosyl homocysteine, Cys: Cysteine *:$p<0.05$, **:$p<0.01$

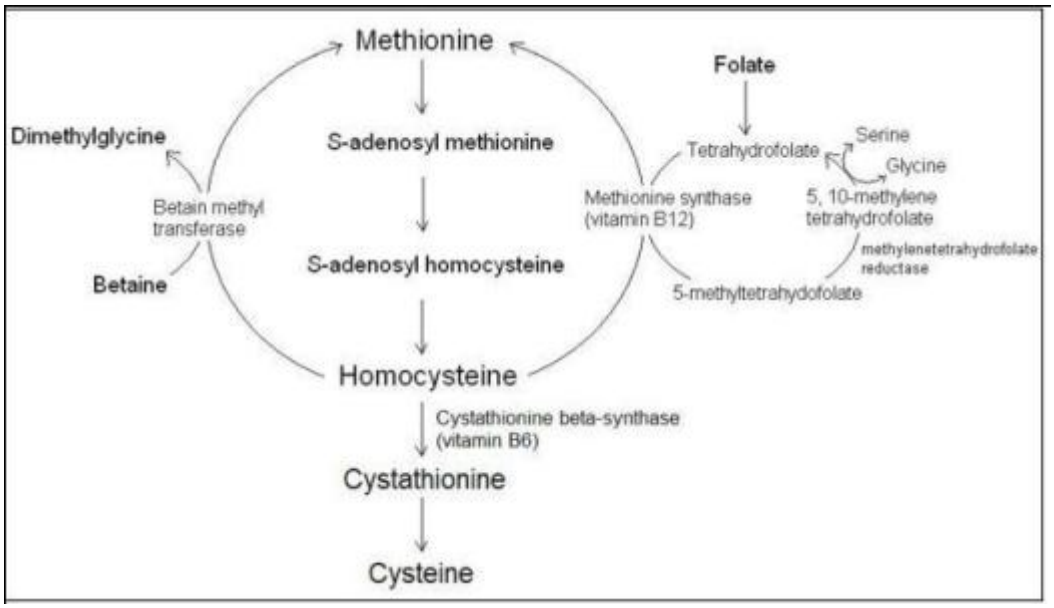

Figure 1. Homocysteine metabolism

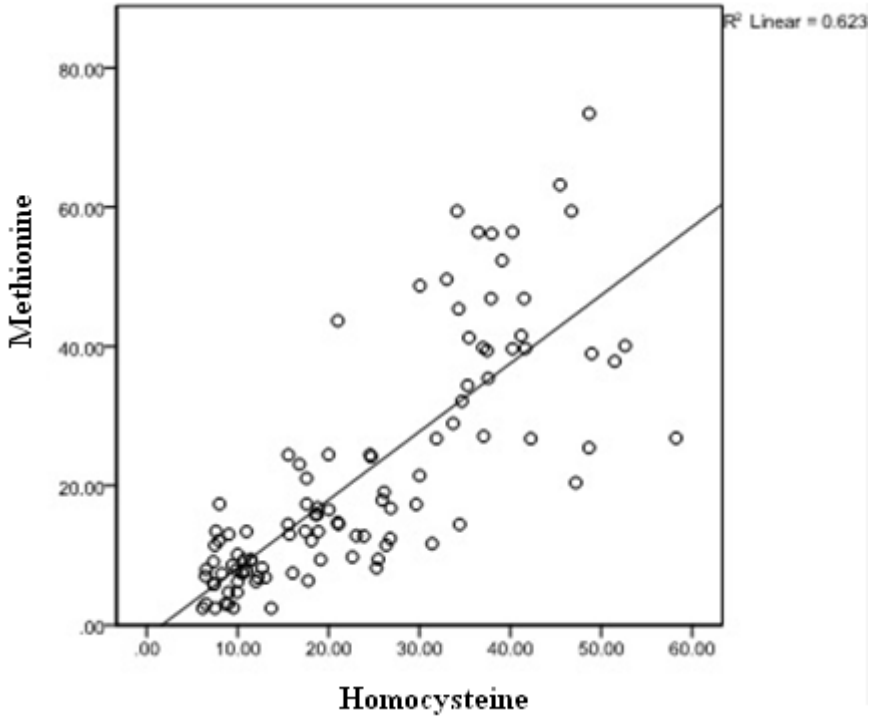

Figure 2. Correlation graph between homocysteine and methionine