Unique Efficiency of Methionine Metabolism in Premenopausal Women May Protect against Vascular Disease in the Reproductive Years

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ABSTRACT Premenopausal women develop occlusive artery disease less frequently than postmenopausal women. In coronary heart disease, higher blood levels of homocysteine-cysteine mixed disulphide have been reported. Therefore, in healthy subjects, we studied the role of menopausal status in the transsulphuration of methionine in 10 premenopausal and 10 postmenopausal women. To exclude the role of aging, we compared these results with those in 10 younger and 10 older men of comparable age groups. An oral methionine load (0.1 g/kg of body weight) was administered after overnight fasting. Before and during 8 h, thereafter, serum levels of methionine, homocystine, and homocysteine-cysteine mixed disulphide were measured. In the fasting state, serum methionine levels were similar in the premenopausal women and both groups of men. Postmenopausal women had significantly lower fasting levels. Peak levels and clearances of methionine after loading did not differ between the groups. In the fasting state, homocystine was never detectable; yet, after methionine loading, slight homocystinemia was present in 12 out of 20 men, and was more pronounced in all postmenopausal women. However, homocystinemia did not occur in any of the premenopausal women after loading. Fasting serum homocysteine-cysteine mixed disulphide levels did not differ between both groups of men and postmenopausal women. In premenopausal women, both fasting and postloading disulphide levels were significantly lower than in any other group. We conclude that premenopausal women have a unique efficiency of methionine

handling, and thereby are preserved against the accumulation of homocysteine after methionine loading. We speculate that this phenomenon might account for the lower incidence of vascular disease in women in the reproductive life cycle.

INTRODUCTION

In man, the essential sulphur amino acid, methionine, is metabolized in the transsulphuration pathway (1). Successively, it is converted to S-adenosyl-methionine, S-adenosyl-homocysteine, and homocysteine. Homocysteine lies at a branch point from which sulphur metabolism can be controlled: either it can be remethylated to methionine or converted to cysteine via cystathionine.

In homocystinuria, an inborn error of methionine metabolism that results from a decreased activity of the enzyme cystathionine β -synthase (EC 4.2.1.22) which normally converts homocysteine into cystathionine, homocysteine accumulates in the blood and tissues and is excreted in the urine. The presence of homocystine and of increased levels of homocysteine-cysteine mixed disulphide in these patients reflects accumulation of homocysteine since the latter is readily oxidized and measured as homocystine (homocysteine-homocysteine) and as the mixed disulphide homocysteine-cysteine by amino acid analysis (2).

In the plasma of normal fasting man, the presence of homocysteine-cysteine mixed disulphide only recently has been demonstrated by Gupta and Wilcken (3); the women show significantly lower plasma levels than men (4). We were able to confirm these findings (5). In patients with homocystinuria, precocious arteriosclerosis and serious thromboembolic events are the most life-threatening complications. Moreover, in animal experiments, chronic homocystinemia has been

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Received for publication 9 February 1983 and in revised form 20 July 1983.

shown to produce endothelial damage and arteriosclerosis (6, 7, 8) whereas, in cultures of human endothelial cells, addition of homocysteine and of homocystine to the medium causes cell injury that is considered to play a significant role in the genesis of arteriosclerosis (9, 10). These data led Gupta and Wilcken to suggest that the difference in plasma concentrations of the mixed disulphide between men and women, albeit small, could contribute over the years to the so far unexplained sex difference in the prevalence of occlusive vascular disease.

To further explore sex differences in methionine handling between men and women in different age classes, we studied the capacity to transsulphurate methionine in pre- and postmenopausal women and in groups of younger and older men of comparable age. Therefore, we loaded the subjects orally with methionine and measured during 8 h thereafter the serum profiles of methionine, homocystine, and the homocysteine-cysteine mixed disulphide.

METHODS

Amino acid measurements before and after L-methionine loading were made in four groups of healthy subjects: (a) 10 older men aged 45–61 yr (53 ± 6 , mean $\pm$ SD), (b) 10 younger men aged 22–35 yr (30 ± 3 , mean $\pm$ SD), (c) 10 women aged 45–59 yr (54 ± 3 , mean $\pm$ SD) who all were postmenopausal for at least 0.5–10 yr (median 5). Their gonadotropins (luteinizing hormone 20–100 IU/liter, follicle-stimulating hormone, 30–120 IU/liter) and estradiol levels (< 0.1 nmol/liter) were without exception in the postmenopausal ranges. (d) 10 young women aged 14–42 yr (25 ± 9 , mean $\pm$ SD) who all had regular menses and did not take oral contraceptives. Neither between the groups of postmenopausal women and older men nor between the groups of young women and young men was the age difference significant ($P > 0.10$). None of the subjects took any drug or vitamin supplements. They all had normal renal function (serum urea, 3.0–7.0 mmol/liter; creatinin, 60–100 μ mol/liter), normal liver function (serum glutamic pyruvic transaminase and serum glutamic oxalacetic transaminase < 25 U/liter), and normal plasma levels of vitamin B6 (44–96 nmol/liter) and folic acid (8–30 nmol/liter).

After an overnight fast, an oral L-methionine load was administered at 9.00 a.m. in a dose of 0.1 g (0.7 mmol)/kg body weight. From the height and the weight of the individual subjects, body surface was derived by using the nomogram based on the formula of Du Bois and Du Bois (11). The mean, methionine loads in the respective groups were 4.17 ± 0.09 SEM, 3.89 ± 0.07 , 3.78 ± 0.10 , and 3.71 ± 0.15 g/m² body surface. During the test, they stayed in the metabolic ward and used a methionine-free breakfast and luncheon with a total content of 14 mg methionine in 2 g of proteins (12), 95 g of carbohydrates, and 31 g of fats (670 cal, 2,814 J). Informed consent was obtained from all volunteering subjects after approval of the protocol by the hospital's Ethical Committee.

Methionine, homocystine, and the homocysteine-cysteine mixed disulphide in the serum were measured by ion-exchange chromatography (LC 2000 amino acid analyser, Biotronik Wissenschaftliche Gerät, Munich) in venous blood samples taken at 9.00 a.m. and immediately before and 1, 2, 3, 4, 6, and 8 h after methionine loading. Metabolic clearances were calculated from the formula by which doses me-

thionine administered (μ mol/kg body weight) were divided by the area under the disappearance curves (μ mol/liter $\times$ hour) after fitting the serum methionine data to a bi-exponential equation by using the PHARMFIT computer program (13). The serum for amino acid analysis was deproteinized within 10 min after sampling by addition of equal volumes of 12.5% sulphosalicylic acid. Gamma-amino-butyric acid was used as an external standard. The deproteinized serum was stored at -20°C until analysis. All samples from one individual were measured in the same run within 4 wk to avoid systematic decreases in levels of sulphur-containing amino acids, which are observed after storage periods longer than 2 mo. Trace amounts of homocystine and homocysteine-cysteine mixed disulphide < 0.1 μ mol/liter are denoted as not detectable (ND) and quantitated as 0 in calculations of means.

Statistical analysis was performed by using Wilcoxon's two sample test (P) and Wilcoxon's paired rank test (P^*). Mean values ± 1 SEM are given.

RESULTS

Serum amino acid levels in the fasting state and maximal concentrations after methionine loading (Figs. 1 and 2, Table I) were studied.

Older and younger men. The individual fasting methionine levels in the older men ranged from 14 to 41 μ mol/liter and in the younger men from 16 to 47 μ mol/liter. The mean fasting methionine levels (27 ± 2 vs. 30 ± 3 μ mol/liter) did not differ between both groups, neither did the mean maximal concentration after loading. The individual peak levels after loading ranged from 871 to 1,801 μ mol/liter in the older group and from 623 to 1,487 μ mol/liter in the younger group. From the individual postload curves, we calculated a mean clearance of 0.10 ± 0.03 liter/h per kilogram for the older men and 0.07 ± 0.02 liter/h per kilogram for the younger men ($P > 0.10$). Homocystine was not present in measurable amounts in any of the 20 men in the fasting state whereas, after methionine loading, homocystine levels rose by detectable concentrations in 12 out of these 20 men to a maximum of 0.4–1.9 μ mol/liter. The mean homocysteine-cysteine mixed disulphide levels in the fasting state (3.1 ± 0.3 vs. 3.5 ± 0.5 μ mol/liter) were similar in both groups; they ranged from 1.9 to 4.4 μ mol/liter in the older men and from 1.1 to 5.6 μ mol/liter in the younger men. After loading, the disulphide levels rose significantly in all of them ($P^* < 0.01$) to maximal concentrations that ranged from 8.4 to 16.0 μ mol/liter in the older men and from 7.8 to 16.2 μ mol/liter in the younger men. The mean levels in both groups increased by 8.5 ± 0.8 μ mol/liter ($321 \pm 46\%$) in the older group and by 9.0 ± 0.7 μ mol/liter ($326 \pm 63\%$) in the younger group ($P > 0.10$).

Postmenopausal women. The fasting methionine levels ranged from 16 to 27 μ mol/liter, whereas the mean concentration in postmenopausal women (21 ± 1 μ mol/liter) was slightly lower than in both groups of men ($P < 0.05$). The peak levels after loading ranged from 774 to as high as 1,406 μ mol/liter. In contrast to

FIGURE 1 Individual and mean (heavy bars) serum amino acid levels in the fasting state and peak concentrations after methionine loading (given in micromoles/liter) in 10 normal older men (●—●) and 10 normal young men (○—○). Concentrations $<0.1 \mu\text{mol/liter}$ are denoted as nondetectable.

FIGURE 2 Individual and mean (heavy bars) serum amino acid levels in the fasting state and peak concentrations after methionine loading (given in micromoles/liter) in 10 normal postmenopausal women (●—●) and 10 normal premenopausal women (○—○). Concentrations $<0.1 \mu\text{mol/liter}$ are denoted as nondetectable.

TABLE I
Means ($\pm$ SEM) and Ranges of Serum Amino Acid Levels in the Fasting State and the Maximal Concentrations after Methionine Loading in Normal Men and in Normal Post- and Premenopausal Women

	Age	Methionine		Homocystine		Homocysteine-cysteine Mixed Disulphide	
		Fasting	After load*	Fasting	After load	Fasting	After load
	yr				$\mu\text{mol/liter}$		
Men							
Older men	53 $\pm$ 2	27 $\pm$ 2	1132 $\pm$ 80	ND†	0.6 $\pm$ 0.2	3.1 $\pm$ 0.3	11.6 $\pm$ 0.7
(n = 10)	45–61	14–41	871–1801		N.D.(5)§–1.9	1.9–4.4	0.4–16.0
Young men	30 $\pm$ 1	30 $\pm$ 3	993 $\pm$ 92	ND	0.8 $\pm$ 0.2	3.5 $\pm$ 0.5	12.5 $\pm$ 0.8
(n = 10)	22–35	16–47	623–1487		N.D.(3)–1.7	1.1–5.6	7.8–16.2
Postmenopausal women	54 $\pm$ 1	21 $\pm$ 1	1107 $\pm$ 53	ND	2.9 $\pm$ 0.3	2.6 $\pm$ 0.4	18.4 $\pm$ 1.5
(n = 10)	45–59	16–27	774–1406		1.3–4.6	1.3–4.9	10.3–25.7
Premenopausal women	25 $\pm$ 3	26 $\pm$ 2	1033 $\pm$ 60	ND	ND	0.9 $\pm$ 0.3	6.8 $\pm$ 0.4
(n = 10)	14–42	16–38	760–1376			0.2–2.6	5.0–9.1

* Maximal levels achieved 1–8 h after methionine loading (0.1 g/kg).

† Not detectable.

§ Number of patients indicated in parentheses.

the fasting levels, the mean peak levels did not differ from those in men. A clearance of methionine of 0.08 ± 0.02 liter/h per kilogram was calculated for this group. As in men, fasting homocystine levels were not detectable in these postmenopausal women. After loading with methionine, homocystinemia occurred in each of the women with maximal levels ranging from 1.3 to 4.6 $\mu\text{mol/liter}$. The mean homocystine levels in these women (2.9 ± 0.3 $\mu\text{mol/liter}$) was significantly higher than in both groups of men ($P < 0.01$). The fasting homocysteine-cysteine mixed disulphide concentrations in the postmenopausal women ranged from 1.3 to as high as 4.9 $\mu\text{mol/liter}$, and their mean (2.6 ± 0.4 $\mu\text{mol/liter}$) did not differ significantly from that in both groups of men. The peak levels achieved after methionine loading ranged from 10.3 to 25.7 $\mu\text{mol/liter}$ and the mean maximal level (18.4 ± 1.5 $\mu\text{mol/liter}$) was significantly higher than in both groups of men ($P < 0.01$).

Premenopausal women. The mean fasting methionine level (26 ± 2 $\mu\text{mol/liter}$) in the premenopausal women was slightly higher than in the postmenopausal group ($P < 0.05$). The mean peak level after loading (range from 760 to 1,376 $\mu\text{mol/liter}$) did not differ from that in postmenopausal women. When compared with both groups of men, the mean fasting and the postloading methionine levels in the premenopausal women were virtually identical. The clearance of methionine, 0.08 ± 0.02 liter/h per kilogram, was not significantly different from the mean clearance in the other three groups ($P > 0.10$). Homocystine was not detected in any of the premenopausal women in the fasting state as was assessed in the postmenopausal women and in both groups of men. In contrast to the homocystine

profiles after methionine loading in the postmenopausal women and in both groups of men, homocystinemia did not occur in any of these premenopausal women. Furthermore, the mean fasting disulphide level (0.9 ± 0.3 $\mu\text{mol/liter}$) in the premenopausal group was at least three times lower than the mean value in any of the other groups ($P < 0.02$ – 0.01). After methionine loading, individual peak disulphide increments in the premenopausal women (range 4.2–7.4 $\mu\text{mol/liter}$, mean 5.9 ± 0.8 $\mu\text{mol/liter}$) were without exception lower than in the postmenopausal women (range 7.9–22.9 $\mu\text{mol/liter}$, mean 15.8 ± 4.9 $\mu\text{mol/liter}$, $P < 0.00025$). The mean peak disulphide increment in the premenopausal women was also significantly lower than that in both groups of men ($P < 0.02$ vs. the older men and $P < 0.005$ vs. the younger men).

DISCUSSION

The present study shows that in men fasting methionine levels are higher than in women as reported earlier (5, 14, 15). However, our data demonstrate that this difference only holds for women in the postmenopausal state. No such difference was found between men and premenopausal women. Our data contradict the earlier statement that fasting methionine levels do not differ between pre- and postmenopausal women (16). Indeed, in premenopausal women, these levels appeared to be significantly higher than in postmenopausal women. After methionine loading, the maximal methionine levels and the clearances in the premenopausal women were comparable with those in the other three groups. These results indicate a similar load to the transsulphuration pathway in the four groups. The present

data illustrate that methionine loading uncovers a so far unreported difference in the occurrence of homocystinemia between pre- and postmenopausal women. Furthermore, the data in both groups of men strongly suggest that the unique absence of homocystinemia after loading in premenopausal women is not due to their young age. As first suggested by Mudd and Poole (17) and afterwards demonstrated by others (4, 5), the mean fasting homocysteine-cysteine mixed disulphide levels in men are higher than in women. Our study clearly illustrates that this observation only holds true for premenopausal women, whereas these levels in postmenopausal women were not discernible from those in young and older men. It is intriguing that, in the fasting state, mean levels of the disulphide in the premenopausal women were on the average three times lower than in postmenopausal women whereas no homocystine was found in both groups. This unique characteristic of methionine handling in premenopausal women is further illustrated by the significantly lower increment of the disulphide despite a similar methionine load (also, after correction of the dose per kilogram for body surface) at equally high serum levels of this amino acid thereafter. Comparing the increments of the disulphide levels in the men and women of both age groups, the conclusion seems inevitable that age per se cannot be the factor responsible for the observed difference between pre- and postmenopausal women.

To summarize, the unique features of methionine handling in the premenopausal women are: (a) higher fasting methionine levels than in the postmenopausal women, (b) no homocystinemia after loading with methionine in contrast to its invariable presence in the postmenopausal ones, and (c) much lower disulphide levels both before and after methionine loading. Although it would be attractive to hypothesize from these findings that premenopausal women better remethylate homocysteine to methionine, our data do not allow this thesis. Differences in transsulphuration rate or in renal excretion of homocysteine might be alternative explanations.

The methionine handling in premenopausal women is not only unique in comparison with postmenopausal women but also in comparison with young men; the latter showed significantly higher homocysteine-cysteine mixed disulphide levels and, in the majority of them, overt homocystinemia after methionine loading. When considered together, these observations indicate that premenopausal women are better protected against accumulation of homocysteine than young men.

Our data illustrate differences in methionine handling also between the postmenopausal women and the older men. They suggest that homocysteine accumulates much more in older women than in older men. This difference is the more convincing when the slightly higher load of methionine (absolute and per square

meter body surface) in the older men is taken into account. This finding has not been reported so far and again accentuates that pre- and postmenopausal women differ markedly in their handling of methionine, and thus are unlike young and older men who, according to the data in Table I, did not show differences in any of the variables studied.

Our findings finally indicate that a standardized methionine load can lead to overt homocystinemia in normal men and normal postmenopausal women and that an increase in the mixed disulphide levels even up to those found in obligate heterozygotes (18) in normal postmenopausal women could give rise to the erroneous conclusion of the presence of heterozygous classical homocystinuria.

Wilcken and Gupta suggested that the slightly but significantly lower fasting plasma levels of the homocysteine-cysteine mixed disulphide in women in comparison with men might be a contributing factor to the unexplained reduced proneness of women to develop vascular disease (4). Probably most, if not all, women studied by the latter authors were in the premenopausal state as we deduced from the young age of their group. The observation by the same authors of accumulation of homocysteine in patients with chronic renal failure (19, 20, 21) and in young patients with angiographically established coronary artery disease (22) gives further support to the hypothesis that a reduced capacity to metabolize methionine is a risk factor for the development of premature arteriosclerosis.

The data in our study of normal men and women confirm that premenopausal women have lower fasting amounts of homocysteine-cysteine mixed disulphide than men. In addition, our data establish that such a sex difference does not exist between postmenopausal women and older men of similar age. After ingestion of a major methionine load to stress the transsulphuration pathway, premenopausal women do not exhibit homocystinemia and show a significantly lesser rise of homocysteine-cysteine mixed disulphide than postmenopausal women and younger and older men. A number of papers have demonstrated a lower rate of coronary artery disease in premenopausal women as compared with men of the same age, and an increasing incidence of cardiovascular disease in women after menopause is completed naturally or by castration (23-30). Based on our data, we speculate that the unique efficiency in methionine handling in premenopausal women, which leads to a virtual absence of circulating homocystine and much lower levels of the disulphide, might be a natural defense mechanism to protect women in the reproductive life cycle against vascular damage.

ACKNOWLEDGMENTS

We acknowledge the help of Prof. J. M. van Rossum in the computation of metabolic clearances. Details of the PHARM-

FIT computer program can be obtained from the Department of Pharmacology, 6500 HB Nijmegen, The Netherlands, Postbox 9101.

This research was supported by grant (nr. 80. 048) from the Dutch Heart Foundation.

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