

Elevated levels of plasma homocysteine in postmenopausal women in Burkina Faso

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Abstract

Background: Low levels of plasma homocysteine have been found in children and adult populations living in Burkina Faso in association with a low prevalence of coronary heart disease. **Methods:** Based on this finding, the levels of plasma homocysteine and other thiols (cysteine, cysteinylglycine, glutathione) in postmenopausal women living in Burkina Faso were evaluated with the aim of investigating whether age and life conditions influence plasma homocysteine and other thiol levels. **Results:** It was found that in older postmenopausal women the mean level of homocysteine was higher ($16.4 \pm 6.6 \mu\text{mol/L}$) than in fertile women ($6.8 \pm 1.2 \mu\text{mol/L}$) and that this increase was correlated with cysteine levels ($166.6 \pm 44.6 \mu\text{mol/L}$). While the glutathione level in postmenopausal women was lower ($3.6 \pm 2.3 \mu\text{mol/L}$) compared with fertile women ($7.0 \pm 1.7 \mu\text{mol/L}$), cysteinylglycine levels were within the normal range ($29.9 \pm 9.3 \mu\text{mol/L}$). No correlation was found between homocysteine levels and serum folate, vitamin B₁₂, vitamin B₆, cystatin C and serum creatinine levels. The older the woman was, the higher was her plasma homocysteine level: levels up to $20.2 \pm 9.1 \mu\text{mol/L}$ were found in those >70 years old. **Conclusions:** The elevated levels of homocysteine in the postmenopausal women of Burkina Faso must be viewed as a characteristic of older age and its metabolic consequences.

Keywords: Burkina Faso; homocysteine; postmenopausal women.

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Introduction

Plasma levels of total homocysteine (Hcy) are the result of interplay between genetic and environmental factors (1). Hcy is the demethylated derivative of methionine, which is derived from diet, a recycling pathway via 5'-deoxy-5'-(methylthio)adenosine and remethylation of Hcy. Approximately 50% of intracellular Hcy is remethylated to methionine and the remainder is converted to cystathionine through a reaction catalysed by the vitamin B₆-dependent cystathionine- β -synthase. In turn, cystathionine is converted to cysteine, which is required for the synthesis of many compounds, including the important thiol glutathione (Figure 1).

Several earlier studies associated moderate hyperhomocysteinaemia (HHcy) with a higher risk of coronary and other vascular diseases (2–5). Increases in plasma Hcy levels were attributed to interruption of the coordinated regulation of S-adenosylmethionine, Hcy trans-sulfuration and remethylation. Since this last step requires a methyl group derived from a cofactor (N⁵-methyltetrahydrofolate, cobalamin, betaine or choline), deficiency may result in an abnormal increase in plasma Hcy (1). The supply of vitamin B₆, vitamin B₁₂ and folic acid in the diet of HHcy subjects reduced plasma levels of Hcy, but it did not seem to modify thrombotic risk (6–8). Previous studies have shown that age, gender and race are the main factors influencing Hcy levels in humans (2, 9, 10). In addition, plasma Hcy levels are inversely correlated with renal function, which is influenced by the ageing process. In Europe, several studies have demonstrated that plasma Hcy levels are higher in both the elderly (11) and postmenopausal woman (12) when compared with other adults with or without cardiovascular disease (3). In fact, healthy centenarians show the highest Hcy levels (13). Since the mechanism that gives rise to HHcy in the elderly has not been clearly elucidated, we decided to measure plasma levels of Hcy, thiols, vitamin B₆, vitamin B₁₂, serum folate and cystatin C, and serum levels of creatinine in postmenopausal women living in Burkina Faso, where low levels of Hcy in children and adults were previously observed.

Material and methods

Inclusion and exclusion criteria

A total of 75 of the 360 postmenopausal subjects at Centre Delwende of Tanghin (Ouagadougou, Burkina Faso) were

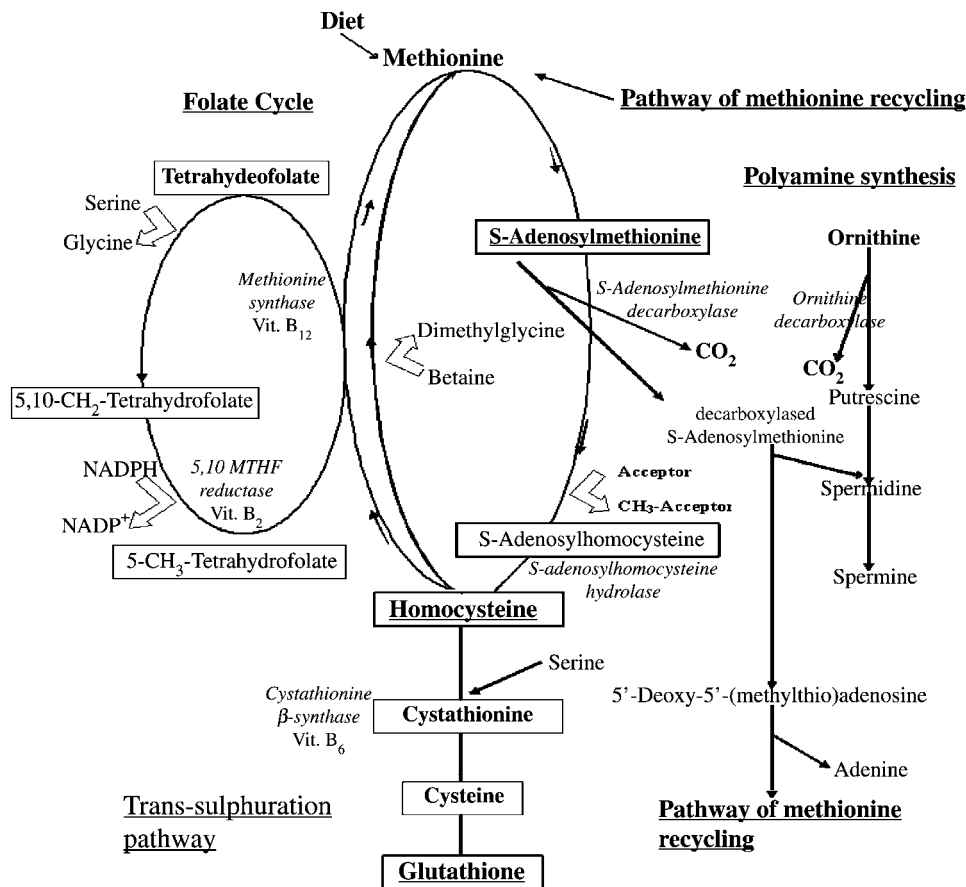


Figure 1 Methionine cycle (from Chillemi et al. 2004) (28).

included in this study. The centre receives older women neglected by their families, who are thus destined to a cruel end, the result of a popular tradition in Burkina Faso whereby older women are banished from the home when they are felt to be useless. All subjects enrolled were from Ouagadougou or nearby villages. Their diet remained the same: millet flour with vegetable sauce, cereals, pork (rarely or no more than once a week), and local seasonal fruits. The diet of all subjects was hypocaloric, averaging 1200–1400 kcal/day. No vitamin supplementation was given. The women carried out simple household chores inside Centre Delwende of Tanghin.

All the subjects were selected according to the Eurage Senieur Protocol criteria (14). A group of 25 healthy fertile women (30–45 years) was enrolled from the staff of Centre Delwende of Tanghin and served as controls. We checked weight, height and blood pressure in a seated position using a standard protocol. Other measurements included heart rate (radial artery) and ventilation rate. The body mass index (BMI) was calculated for all subjects using the formula $\text{weight}/\text{height}^2$. All subjects involved in this study gave a full social and medical history and underwent a physical examination. They gave their informed consent for the study orally. Exclusion criteria included: weight over the 97th percentile for age, systolic pressure greater than mean + 2SD for age, clinical evidence of atherosclerotic cardiovascular disease, diabetes mellitus, renal or hepatic disease, thyroid disease, cardiomyopathy, anticonvulsant medication and Alzheimer's disease, which are all known to be associated with high plasma Hcy levels. The study was approved by the Ethical Committee of Centre Medical St. Camille (CMSC) of Ouagadougou.

Collection, processing and storage of blood samples

Blood samples (10 mL of peripheral blood: 5 mL in a plain tube and 5 mL in EDTA) were collected in the morning after an overnight fast. On the preceding day, the diet of subjects was regular and no restriction was prescribed (i.e., meat). Tubes containing blood in EDTA were immediately centrifuged (within 2 min) at $1500 \times g$ for 10 min at 4°C. Plasma was collected and stored at -80°C in 250- μL aliquots. Tubes containing blood without additive were left to stand at room temperature for 30 min. Serum was then separated for centrifugation and stored at -80°C (in 250- μL aliquots).

Routine haematological study

Clinical chemistry tests were performed by the central laboratory of Centre Medical St. Camille of Ouagadougou using standard methods. We considered the upper reference limit for serum creatinine to be 123.16 $\mu\text{mol}/\text{L}$ and used 2SD from the normal value as the limit for other laboratory parameters.

Plasma homocysteine

The determination of circulating plasma Hcy and other thiols was carried out by HPLC (15) at the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy. Daily quality control was carried out using a control sample prepared from a plasma pool processed at the beginning and at the end of the analytical session. The inter-assay coefficient of variation was 3.6%. Plasma Hcy values $>15 \mu\text{mol}/\text{L}$ were considered elevated.

Table 1 Clinical and laboratory parameters in African fertile and postmenopausal women living in Burkina Faso.

| Parameter | Fertile women (n=25) | Postmenopausal women (n=75) |
|--|-------------------------|------------------------------------|
| Age, years | 35.9, 30–45 36.4±5.5 | 67, 50–90 66.5±8.9 ^d |
| BMI, kg/m ² | 25.2±1.4 | 24.2±2.6 ^b |
| Haemoglobin, g/L | 142±24 | 131±24 ^c |
| Blood glucose, mmol/L | 4.66±0.66 | 4.71±0.66 |
| Blood nitrogen, mmol/L | 20.0±4.5 | 22.3±3.4 ^b |
| Serum cholesterol, mmol/L | 4.56±0.89 | 4.51±0.82 |
| Serum triglycerides, mmol/L | 1.08±0.11 | 0.97±0.10 ^d |
| Serum creatinine, µmol/L | 63.65±9.7 | 69.83±14.14 ^c |
| Cystatin C, mg/L | 0.69±0.08 | 0.86±0.24 ^d |
| Serum iron, µmol/L | 14.86±4.00 | 13.25±3.29 ^c |
| Serum ASP transaminase, U/L | 20.0±2.4 | 23.7±2.6 ^d |
| Serum ALT transaminase, U/L | 22.0±10.1 | 23.7±8.6 |
| Serum folate, nmol/L | 13.37±4.98 | 13.37±5.1 |
| Serum vitamin B ₆ , nmol/L | 26.7±11.7 | 24.7±12.9 |
| Serum vitamin B ₁₂ , pmol/L | 614.7±251.0 | 527.7±277.8 |
| Systolic blood pressure, mm Hg | 132.0±4.2 | 139.9±22.5 ^a |
| Diastolic blood pressure, mm Hg | 82.0±3.4 | 79.5±13.6 |

ALT, alanine aminotransferase. Student t-test: ^a p=0.084; ^b p=0.070; ^c p<0.05; ^d p<0.0001.

Other biochemical studies

Serum folate, vitamin B₆ and vitamin B₁₂ levels were measured at the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy.

Serum vitamin B₁₂ was measured by microparticle enzyme immunoassay and serum folate by ion capture assay on an AxSYM Analyser (Abbott Diagnostics, Abbott Park, USA). The inter-assay coefficient of variation was 1.7% for both vitamin B₁₂ and folate. A folate level < 11.33 nmol/L was considered hypofolataemia, and a serum vitamin B₁₂ level < 115.86 pmol/L was considered low. This method measures all folate in serum, including N⁵,N¹⁰-methylenetetrahydrofolate and N⁵-methyltetrahydrofolate.

Vitamin B₆ was measured by HPLC using a commercially available kit (Immunodiagnostik, Bensheim, DE). We considered vitamin B₆ values < 20.23 nmol/L to be low.

Cystatin C was also measured at the Clinical Biochemistry Laboratory of Catholic University, Rome, Italy, using a C PET kit (Dako, Italy). Values ranging from 0.69 to 2.30 mg/L were considered normal.

Statistical methods

Data are presented as mean±SD. Statistical comparisons were performed using the Student t-test when appropriate, with p<0.05 considered to be statistically significant. To analyse the relationship between Hcy and the variables age, serum folate, vitamin B₆, vitamin B₁₂, cystatin C, creatinine, blood systolic and diastolic pressure, we used multiple linear correlation. This method gives the measure and significance of co-variations among variables using linear analysis. The

correlation indexes (r) and multiple linear correlation analysis were calculated with the SPSS-10 program for Windows (SPSS Inc, Chicago, IL, USA).

Results

The clinical and biochemical data for the women studied, who were divided into two groups according to the presence or absence of menstruation and their age, are reported in Table 1. The BMI of all women was normal for local standards. The laboratory parameters did not differ substantially between the two groups, with the exception of serum creatinine, cystatin C and aspartate aminotransferase (ASP) transaminase, which were all significantly higher in postmenopausal women, while haemoglobin, serum triglycerides and serum iron were significantly higher in the younger women. However, all these parameters were within the normal ranges for age. The results for Hcy and thiol determinations are reported in Table 2. We found that in all postmenopausal women the plasma levels of Hcy were significantly higher (mean value 16.4±6.6 µmol/L) than in fertile women (6.8±1.2 µmol/L), as well as plasma cysteine levels (mean value 166.6±44.6 vs. 106.3±13.0 µmol/L, respectively). Cysteinylglycine levels were 29.9±9.3 µmol/L in postmenopausal women, while in fertile women the mean level was 36.1±11.0 µmol/L. Glu-

Table 2 Values for homocysteine and other thiols in African fertile and postmenopausal women living in Burkina Faso.

| Subjects | Hcy, µmol/L | Cys, µmol/L | CysGly, µmol/L | GSH, µmol/L | Hcy/Cys ratio | Hcy/CysGly ratio | Hcy/GSH ratio |
|--|-----------------------|-------------------------|-----------------------|----------------------|--------------------------|--------------------------|-------------------------|
| Fertile women (30–45 years) | 6.8±1.2 | 106.3±13.0 | 36.1±11.0 | 7.0±1.7 | 0.064±0.081 | 0.188±0.092 | 0.971±0.045 |
| Postmenopausal women (50–90 years) | 16.4±6.6 ^c | 166.6±44.6 ^c | 29.9±9.3 ^a | 3.6±2.3 ^c | 0.097±0.035 ^b | 0.586±0.266 ^c | 5.297±3.38 ^c |

Hcy, homocysteine; Cys, cysteine; CysGly, cysteinylglycine; GSH, glutathione. Student t-test: ^a p=0.007; ^b p=0.005; ^c p=0.0001.

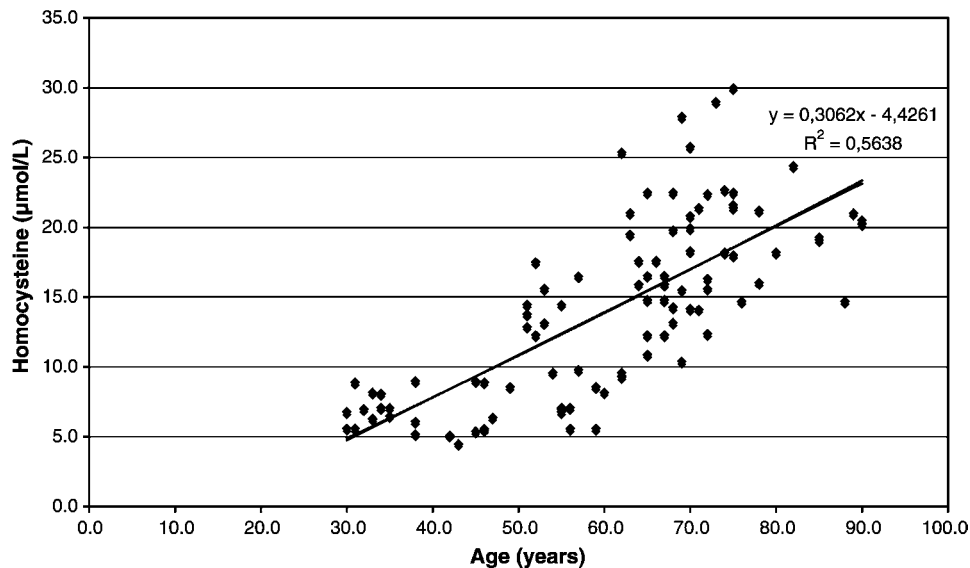


Figure 2 Correlation between Hcy and age in fertile and post-menopausal women living in Burkina Faso.

tathione levels were significantly reduced (mean value $3.6 \pm 2.3 \mu\text{mol/L}$) in all postmenopausal women compared with fertile women ($7.0 \pm 1.7 \mu\text{mol/L}$). The ratios Hcy/cysteine, Hcy/cysteinylglycine and Hcy/glutathione were significantly different between younger and postmenopausal women (see Table 2). In all women studied, plasma Hcy correlated significantly ($r=0.74$, $p<0.0001$) with age (Figure 2). When multiple linear correlation (Table 3) was performed, age correlated significantly with Hcy ($p<0.0001$), while serum folate, vitamin B₆, vitamin B₁₂, cystatin C, creatinine did not. Blood systolic and diastolic pressures also correlated with plasma Hcy, but at lower significance levels ($p<0.005$ and $p<0.05$, respectively).

Discussion

Low plasma Hcy levels have been found in African children and adults living in Burkina Faso (10), which suggests different regulation of Hcy metabolism in these people. This hypothesis was reinforced by the observation that in African subjects the increase in plasma Hcy levels after an oral methionine loading test is lower than in European people (16). The lower levels of plasma Hcy found in African people living in Burkina Faso are associated with a low prevalence of cardiovascular disease (CVD) (10). This correlation was also reported in an African population living in South Africa by Ubbink et al. (17, 18). If the low levels of Hcy in African people living in Burkina Faso explain the phenomenon of "ethnic protection" against CVD, a role for environmental factors in CVD risk in African Americans living in the USA can be postulated, given that a Western lifestyle (19) produces a higher level of Hcy.

In this study we measured plasma Hcy and other thiol levels in both postmenopausal and fertile women in Burkina Faso. Our data show that the levels of Hcy and cysteine were significantly higher in the postmenopausal women than in the fertile women. At the

same time, levels of glutathione were significantly reduced in the postmenopausal women, and cysteinylglycine levels were normal (Table 2). It is significant that the Hcy values of our postmenopausal women were significantly lower than those found by us in European centenarians (100–107 years old; $31.8 \pm 10.4 \mu\text{mol/L}$) (13). It is true that those European subjects were clearly older than the African subjects in the current study. Nevertheless, the analysis of Hcy levels in African women in this study (Figure 2) shows that the Hcy value extrapolated for age >100 years in African women is $\sim 25 \mu\text{mol/L}$, clearly lower than the levels in the European centenarians. This suggests a different genetic background in these two populations.

The different levels of Hcy and other thiols in both fertile and postmenopausal African women could result from the following factors:

1. Nutritional: African people in Burkina Faso eat mainly millet, which contains tannins and reduces the bioavailability of methionine in the diet (16). The postmenopausal women at Centre Delwende of Tanghin consume a traditional hypocaloric diet (1200–1400 kcal/day), with a relatively poor protein intake, but it provides sufficient quantities of vitamins (B₆ and B₁₂) and folate through the presence of fruit and fresh vegetables. It is known that Hcy levels increase with age and that folate, vitamin B₆ and vitamin B₁₂ deficiencies represent a very common risk factor for HHcy in the elderly (20–22). However, this study shows that all postmenopausal women investigated had normal levels of folate, vitamin B₆ and vitamin B₁₂ (Table 1), so it is unlikely that the higher levels of Hcy found in postmenopausal women are a consequence of nutritional deficiency.
2. Genetic: Methylene tetrahydrofolate reductase (MTHFR) influences Hcy metabolism through the folate cycle. The *MTHFR* gene is preserved in sub-Saharan Africa, where the allele frequency of the

Table 3 Linear correlation between homocysteine and the other parameters considered in this study.

| | r | p |
|--------------------------|-------|-------------------|
| Age | 0.748 | <0.0001 |
| Serum folate | 0.026 | NS |
| Vitamin B ₆ | 0.010 | NS |
| Vitamin B ₁₂ | 0.036 | NS |
| Cystatin C | 0.100 | >0.05, <0.10 (NS) |
| Creatinine | 0.105 | >0.05 <0.10 (NS) |
| Blood systolic pressure | 0.213 | >0.0005, <0.005 |
| Blood diastolic pressure | 0.175 | >0.025, <0.05 |

NS, not significant.

C677T mutation is approximately 6–7% in the adult population (23–25). In Europe, the frequency of this mutated allele is approximately 40–45% (26, 27). Results obtained for the postmenopausal women in this study demonstrate that the C677T allele frequency is 3.3% vs. 7.7% found in fertile women (data reported elsewhere). In the presence of the C677T mutation of *MTHFR*, the folate cycle is less active, that is, the amount of Hcy recycled to methionine via the folate cycle is lower and consequently the level of Hcy increases. In sub-Saharan Africa, where the mutated *MTHFR* gene (C677T) seems to be negatively selected by *P. falciparum* (28), Hcy is recycled faster into methionine and the level of Hcy is lower in both children and adults when compared with European Hcy plasma levels.

Nevertheless, the results of this study lead to four conclusions:

- The increased levels of Hcy in postmenopausal women from Burkina Faso are not due to a deficiency of folate, vitamin B₁₂ or vitamin B₆, and no correlation between Hcy, folate, vitamin B₆ and vitamin B₁₂ was found in this study. The healthy alimentation of these women provides adequate vitamin intake.
- The HHcy in our postmenopausal women is not a consequence of renal failure. In fact, cystatin C and serum creatinine did not correlate with Hcy and were within the normal range for older age.
- The ratios Hcy/cysteine, Hcy/cysteinylglycine and Hcy/glutathione were significantly different in young and in postmenopausal women, suggesting progressive imbalance of the trans-sulfuration pathway with ageing as a consequence of reduced recycling of Hcy to methionine.
- The relatively lower levels of Hcy in the people of Burkina Faso compared with Europeans (10) could be due to the higher prevalence of the wild-type allele of *MTHFR*, which, by maintaining an active folate cycle, reduces Hcy levels.

In our postmenopausal women, HHcy is more probably a consequence of a deficiency of NADPH, which determines decreased synthesis of N⁵-methyltetrahydrofolate (Figure 1). However, the levels of folate in fertile and postmenopausal women were comparable, and this seems to conflict with data in the literature

regarding this issue. Ghandour et al. (29) demonstrated that when high oral supplementation of folic acid was given to patients with renal failure to correct HHcy, only 35% of the total folate increase was due to N⁵-methyltetrahydrofolate (the remainder being unmethylated folic acid). When an equimolar amount of folinic acid was supplemented, N⁵-methyltetrahydrofolate accounted for 96% of the total folate increase. In both experiments the total serum folate levels were similar. This means that the levels of serum folate must have been comparable in our fertile and postmenopausal women because of intake of elevated quantities of folate in vegetables. The fraction of N⁵-methyltetrahydrofolate could be lower in postmenopausal women since the method used by us in this study measures all folate species and does not distinguish N⁵-methyltetrahydrofolate from the other species. Thus, Hcy does not correlate with serum folate. The results we obtained in these African postmenopausal women reminded us of patients affected by acute malaria, in whom the presence of *P. falciparum* produces marked consumption of antioxidant substances (30, 31). In acute malaria, the severity of disease correlates positively with Hcy levels and negatively with glutathione levels (23). Old age can thus be paradoxically compared to chronic malaria, as its main metabolic characteristic is an increased requirement for antioxidant substances to counter oxidation (32–34).

It is true that subjects with CVD were not included in this study, so it was not possible to demonstrate if HHcy is involved or not in CVD in Burkina Faso. However, epidemiological data show that CVD is infrequent in this country (in the register of the National Yalagdo Ouedraogo Hospital of Ouagadougou, only 2.67% of admitted patients had cardiovascular pathology; personal communication).

The metabolic consequences of HHcy seem to be linked to age, given that unknown modifying factors are more likely to have a significant role in younger than older people (35, 36). Hcy is essential in the pathogenesis of atherosclerosis, especially when it is associated with smoking (37), reduced physical activity (38) and hypertension (39), conditions which were not present in our subjects.

Since the reaction of S-adenosylhomocysteine hydrolase is reversible (40), HHcy leads to an increase in S-adenosylhomocysteine and consequently reduces the availability of adenosine, essential for cell metabolism. In fact, the cardiovascular effects of HHcy are more evident in younger persons, in whom the processes of growth and development place maximum demand on metabolic functions (41). A decrease in adenosine levels could contribute to the cardiovascular and metabolic damage observed in children affected by a deficit in cystathionine-β-synthase (42), in whom levels of plasma Hcy are extremely elevated.

In conclusion, the elevated levels of plasma Hcy in the postmenopausal women from Burkina Faso must therefore be viewed as a characteristic of older age and of its metabolic consequences.

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