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Influence of Aromatase Inhibitors on Plasma Total Homocysteine in Postmenopausal Breast Cancer Patients

Gun B. Anker,[1] Helga Refsum,[2] Per M. Ueland,[2] Dag C. Johannessen,[1] Ernst A. Lien,[2] and Per E. Lønning[1*]

In this study, we evaluated the effect of estrogen suppression with three aromatase inhibitors, aminoglutethimide (n = 30), formestane (n = 12), and exemestane (n = 10), and the progestin megestrol acetate (n = 21) on plasma total homocysteine (tHcy) in patients suffering from advanced breast cancer. Treatment with 1 g/day aminoglutethimide for 2 and 3–5 months increased plasma tHcy by a mean value of 24.5% [95% confidence interval, 10.5–40.4%] at 2 months and 35.8% (95% confidence interval, 18.2–55.9%) at 3–5 months, corresponding to increases in the mean plasma tHcy of 1.90 and 3.67 μmol/L, respectively. In contrast, none of the other treatment options influenced plasma tHcy concentrations. The finding that aminoglutethimide, but none of the other aromatase inhibitors or megestrol acetate, influenced plasma tHcy suggests that this effect is achieved by mechanisms not related to suppression of plasma estrogens or to the glucocorticoids administered in concert.

Aromatase inhibitors have a well-defined role in the treatment of advanced breast cancer. The first generation drug, aminoglutethimide, has been used for two decades *(1)*, and currently several new, more potent and specific drugs have been developed *(2)*.

Numerous studies have shown that the antiestrogen agent tamoxifen not only induces remission in advanced breast cancer but also prolongs relapse-free as well as overall survival rates when administered to breast cancer patients in the adjuvant setting *(3)*. However, long-term treatment with tamoxifen may cause undesirable side effects, such as an increased risk of endometrial carcinoma *(4)*. This, combined with the favorable effects achieved with novel aromatase inhibitors in advanced breast cancer *(5, 6)*, has led to increased interest in the use of aromatase inhibitors in the adjuvant setting.

A major concern related to the long-term use of aromatase inhibitors is the possible detrimental influence of estrogen suppression on cardiovascular risk factors. There is evidence that tamoxifen reduces the risk of cardiovascular morbidity when administered to patients with early breast cancer *(7, 8)*. We found *(9)* that tamoxifen treatment, similar to estrogen substitution therapy *(10)*, reduces plasma total homocysteine (tHcy)[3]concentrations. Substantial evidence links increased plasma tHcy to an increased risk of cardiovascular disease *(11, 12)* and mortality *(13)*.

Only one small study has compared the long-term effects of aminoglutethimide to placebo treatment in the adjuvant setting *(14)*. This study suggested an increased incidence of cardiovascular events related to aminoglutethimide treatment.

In the present study, we explored possible effects of aminoglutethimide treatment on plasma tHcy in patients suffering from advanced breast cancer. To examine whether changes in plasma tHcy could be the result of plasma estrogen suppression, other biochemical effects of aminoglutethimide, or an effect of glucocorticoids administered in concert, we also evaluated possible alterations in plasma tHcy in patients treated with novel, selective aromatase inhibitors (formestane and exemestane) and in patients receiving progestins (megestrol acetate) in pharmacological doses. Megestrol acetate suppresses plasma estrogen but has also glucocorticoid agonistic effects *(15)*.

Departments of [1] Oncology and [2] Pharmacology, Haukeland University Hospital, N-5021 Bergen, Norway.
*Author for correspondence. Fax 47-55-972046; e-mail Per.Lonning@onko.haukeland.no.
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[3] Nonstandard abbreviations: tHcy, total homocysteine; RBC, red blood cell; and tCys, total cysteine.

Patients and Methods

PATIENTS

Blood samples for plasma tHcy measurements were collected from a total of 73 postmenopausal women with advanced breast cancer before and at various time intervals during treatment with different endocrine treatment modalities. The drugs were administered as second- or third-line treatment regimens. All patients gave their verbal informed consent to participate in the study. The median age was 69 years (range, 31–90 years). Four different groups of patients were enrolled.

Patients treated with aminoglutethimide. Thirty patients were treated with aminoglutethimide 250 mg four times per day together with cortisone acetate 50 mg twice per day for the first 2 weeks, followed by aminoglutethimide 250 mg four times per day with cortisone acetate 25 mg twice per day. *(16)*. One patient subsequently had her dose of aminoglutethimide reduced to 250 mg twice per day because of toxic side effects.

Patients treated with exemestane or formestane. Ten patients were treated with exemestane, a novel steroidal aromatase inhibitor *(17)*. The drug was administered by the oral route in a dose of 25 mg once daily. Twelve patients were treated with formestane *(18)*, another steroidal aromatase inhibitor. This drug must be given parenterally, and was administered as intramuscular injections of 250 mg every second week.

Patients treated with megestrol acetate. Twenty-one patients received the synthetic progestin megestrol acetate in a single daily dose of 160 mg *(19)*.

BLOOD-SAMPLING PROTOCOL

Previous endocrine therapy was terminated ≥4 weeks before enrollment in the protocol. Blood samples were collected before and at different intervals (1–12 months) during treatment when the patients attended the clinic for follow-up visits. Fasting blood samples were collected into 10-mL EDTA-containing evacuated tubes, which were centrifuged within 30 min at 0–2 °C. The plasma fractions were stored at −20 °C until analysis. Red blood cell (RBC) folate was determined in the pellet. In addition, blood was collected in vials without additives to obtain serum for cobalamin and folate determinations.

DETERMINATION OF tHcy, CYSTEINE, COBALAMIN, AND FOLATE IN BLOOD

Plasma tHcy and total cysteine (tCys) were determined by a modification of an automated procedure based on derivation with monobromobimane and HPLC separation *(20)*. The CV of the method with respect to tHcy as well as tCys previously had been found to be ~3% *(20)*, and had been confirmed by later measurements (unpublished observations). On the basis of the Hordaland Homocysteine study *(21)* with unpublished follow-up results, the upper 95% limit of plasma Hcy for 70-year-old women is ~18 μmol/L, which is considered as the upper health-related reference limit for plasma Hcy in our population.

Serum cobalamin was determined with a microparticle enzyme intrinsic factor assay on an IMx system from Abbott Laboratories. Serum and RBC folate were assayed using the Quantaphase folate RIA produced by Bio-Rad. Serum creatinine was determined using the alkaline picrate method in the CHEM 1 system (Technicon).

STATISTICAL METHODS

Previous studies by our group *(9, 13)* revealed that plasma tHcy is best fitted to a log-normal distribution. Thus, pretreatment concentrations are described by their geometrical mean values with 95% confidence intervals, and values during treatment are expressed as percentages of the pretreatment concentrations for the same patients from whom we had obtained samples at the different time intervals together with 95% confidence intervals of the mean. In addition to confidence intervals, plasma tHcy concentrations obtained before and during treatment were compared using the Wilcoxon matched-pairs sign-rank test. The *P* values were corrected for multiple comparison according to the Bonferroni rule. Correlations were tested by the Pearson least-square method. All *P* values are expressed as two-tailed.

Results

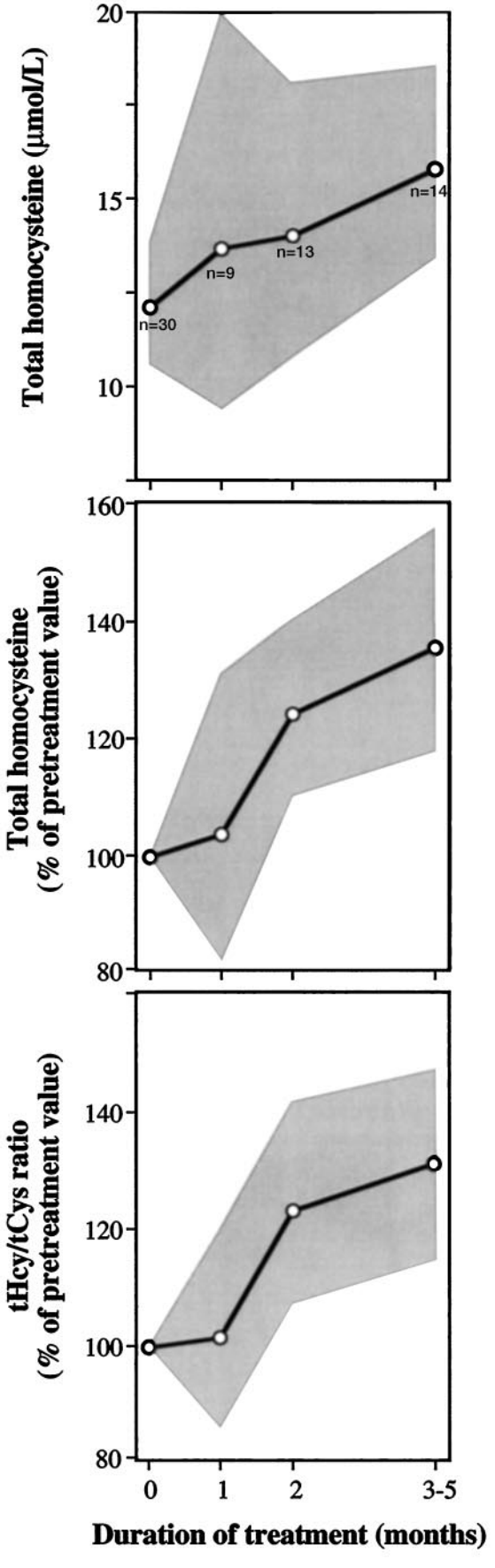
PRETREATMENT VALUES

Pretreatment values for plasma tHcy in the group treated with aminoglutethimide are shown in Fig. 1. For the total group of patients, the mean pretreatment tHcy was 12.6 μmol/L.

Three patients in the group treated with formestane, two patients in the aminoglutethimide group, one patient in the exemestane group, and three patients in the megestrol acetate group had tHcy above the value defined as the upper health-related limit for postmenopausal women in our laboratory (18 μmol/L; see *Patients and Methods*). All patients had serum and RBC folate as well as serum cobalamin concentrations above the lower health-related limit. Two patients had slightly increased serum creatinine. Although there was a trend for a negative correlation between plasma tHcy and cobalamin ($r = -0.36$) and plasma tHcy and serum folate ($r = -0.30$) in the pretreatment samples from the aminoglutethimide-treated group, these correlations were not statistically significant ($P > 0.05$). In the total population, a weak, nonsignificant correlation between RBC folate and plasma tHcy ($r = -0.36$; $P > 0.05$) was seen, but not between plasma tHcy and any of the other indicators.

INFLUENCE OF ENDOCRINE TREATMENT ON PLASMA tHcy AND THE tHcy/tCys RATIO

Treatment with aminoglutethimide was associated with a significant change in plasma tHcy (Fig. 1). Although short-term (1 month) treatment with aminoglutethimide



had no effect on tHcy, treatment for >2 months was associated with a significant increase (mean increases of 24.5% and 35.8%; $P < 0.025$ and $P < 0.01$ after 2 months and 3–5 months, respectively). The mean plasma tHcy increased from 12.2 to 15.8 μmol/L after 3–5 months on treatment.

The selectivity of the increase in plasma tHcy during aminoglutethimide treatment was further documented by an increase in the tHcy/tCys ratio with no increase in tCys. Although treatment with aminoglutethimide for 1 month had no significant influence on the tHcy/tCys ratio, treatment for 2 months and 3–5 months caused a significant increase (mean increases of 23.4% and 30.2%, respectively; Fig. 1).

All patients stopped previous endocrine treatment ≥4 weeks before entering this study. However, we previously had demonstrated that tamoxifen may be detected in human tissues for several months after treatment is terminated *(22)*. Thus, data for patients treated with aminoglutethimide were re-analyzed after exclusion of the five patients who had received tamoxifen as their last treatment modality before aminoglutethimide. Also in this subgroup (n = 25), aminoglutethimide was found to cause a significant increase (mean, 27.4–28.7%) in plasma tHcy (data not shown).

Exemestane and formestane belong to the same subgroup of selective, steroidal aromatase inhibitors and are thought to act by the same biochemical mechanisms. The two drugs were found to have no influence on plasma tHcy, and data from these patients were pooled for statistical analysis. Treatment with these aromatase inhibitors or treatment with the progestin megestrol acetate did not influence plasma tHcy concentrations (data not shown).

VITAMINS AND CREATININE VALUES

To evaluate whether the increase in plasma tHcy caused by treatment with aminoglutethimide could be the result of reduced cobalamin or folate, which are cofactors in Hcy metabolism, we determined serum cobalamin and folate and RBC folate before and during aminoglutethimide therapy. Treatment with aminoglutethimide for 1–5 months caused a nonsignificant increase in all the three indicators (Table 1). No significant alterations in plasma creatinine values were observed during aminoglutethimide treatment.

Discussion

Several studies have addressed the influence of menopause as well as hormone replacement therapy on cardiovascular risk factors in postmenopausal women *(23)*;

Fig. 1. Influence of treatment with aminoglutethimide on plasma tHcy expressed as absolute concentration (*top*), percentage of the pretreatment value (*middle*), and the tHcy/tCys ratio (*bottom*) as a percentage of the pretreatment value.

Data are given as geometric means, and the *shaded areas* indicate 95% confidence intervals of the mean.

Table 1. Serum folate and cobalamin and RBC folate before and during treatment with aminoglutethimide.

Time, months	n	Serum folate, % of pretreatment[a]	n	Serum cobalamin, % of pretreatment[a]	n	RBC folate, % of pretreatment[a]
0	30	100.0	30	100.0	16	100.0
1	7	106.6 (79.3–143.3)	7	125.4 (87.7–179.1)	7	98.2 (83.9–115.0)
2	12	110.7 (80.6–162.2)	12	128.7 (103.1–160.6)	8	127.4 (85.1–190.6)
3–5	14	109.4 (87.6–136.6)	14	111.6 (97.5–127.8)	3	124.6 (104.9–148.0)

[a] Geometric means with 95% confidence intervals of the mean. $P > 0.2$ in all situations.

however, there is only limited information about the physiological effect of low plasma estrogens in postmenopausal women *(24)*. The incidence of cardiovascular morbidity and mortality increases substantially in women after menopause *(25)*, and even a moderate increase in the hazard ratio for cardiovascular death may outweigh an improvement in relapse-free survival in patients with early breast cancer who have estrogen deprivation as adjuvant therapy.

Previous findings suggested that an increased risk of cardiovascular diseases *(14)* associated with aminoglutethimide adjuvant treatment may be partly the result of an increase in plasma cholesterol and triglycerides *(26)*. This increase in plasma cholesterol of ~15% *(26)* corresponds to an increase in the risk of myocardial infarction of ~35–50% *(27)*; the increase in cardiovascular deaths in the adjuvant trial was higher, however, with eight patients in the aminoglutethimide treatment arm compared with only three placebo-treated patients *(14)*. Although these results should be interpreted carefully because the number of events was small, they raise the possibility that factors other than an increase in plasma cholesterol may increase the risk of cardiovascular diseases in patients treated with aminoglutethimide.

The possibility that alterations in tHcy during treatment with aminoglutethimide could be secondary to alterations in blood lipids may be considered. However, most data thus far suggest only a weak correlation between plasma tHcy and plasma cholesterol *(11–13, 28)*, and we consider these indicators in general to be independent risk factors for cardiovascular disease *(29)*.

We found that treatment with aminoglutethimide caused a significant and substantial increase in plasma tHcy and the ratio of tHcy to tCys. Treatment with aminoglutethimide for 3–5 months was found to increase plasma tHcy by a mean of 3.7 μmol/L. Notably, a recent prospective analysis indicated that an increase in plasma tHcy of 4 μmol/L may increase the risk of coronary heart disease by ~40% *(11)*.

It is difficult from our data to identify the mechanism by which aminoglutethimide increases plasma tHcy. The finding of a nonsignificant increase in cobalamin and folate refutes a hypothesis that aminoglutethimide may exert this effect by reducing the vitamin concentrations. In contrast to treatment with aminoglutethimide, we found that treatment with the steroidal aromatase inhibitors formestane and exemestane as well as treatment with progestins in pharmacological doses had fewer and nonsignificant effects on plasma tHcy. This may suggest that aminoglutethimide does not exert its effect on plasma tHcy by suppressing plasma estrogens. Megestrol acetate administered at a dose of 160 mg in a single daily dose has substantial glucocorticoid agonistic effects *(15)*. Thus, our data suggest that the increase in plasma tHcy seen during administration of aminoglutethimide is not caused by cortisone acetate administered in concert. However, aminoglutethimide has additional biochemical effects, including induction of liver mixed function oxidases *(30, 31)*. This should be considered as a mechanism whereby drugs may increase the plasma concentrations of tHcy and thereby increase cardiovascular risk. Notably, the observation that enzyme-inducing antiepileptic drugs also increase tHcy *(32)* support this notion.

In summary, our data show that aminoglutethimide treatment increases plasma tHcy in breast cancer patients. This effect may be of little importance in advanced breast cancer, but it may have significant implications in the adjuvant setting. The effect seems to be drug-specific and not related to estrogen suppression in general because similar alterations were not observed in patients treated with the steroidal aromatase inhibitors formestane and exemestane or the progestin megestrol acetate. Our findings emphasize the importance of a thorough evaluation of the pharmacodynamic profiles, including side effects, of drugs used for long-term treatment of early cancers.

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References

1. Santen RJ, Worgul TJ, Lipton A, Harvey H, Boucher A. Aminoglutethimide as treatment of postmenopausal women with advanced breast carcinoma. Ann Intern Med 1982;96:94–101.
2. Lønning PE. Pharmacology of new aromatase inhibitors. Breast 1996;5:202–8.
3. Clarke M, Collins R, Davies C, Godwin J, Gray R, Peto R. Tamoxifen for early breast cancer: an overview of the randomized trials. Lancet 1998;351:1451–67.
4. Wolf DM, Jordan VC. Gynecologic complications associated with long-term adjuvant tamoxifen therapy for breast cancer. Gynecol Oncol 1992;45:118–28.
5. Dombernowsky P, Smith I, Falkson G, Leonard R, Panasci L,

Bellmunt J, et al. Letrozole, a new oral aromatase inhibitor for advanced breast cancer: double-blind randomized trial showing a dose effect and improved efficacy and tolerability compared with megestrol acetate. J Clin Oncol 1998;16:453–61.

6. Buzdar A, Jonat W, Howell A, Jones SE, Blomqvist C, Vogel CL, et al. Anastrozole, a potent and selective aromatase inhibitor, versus megestrol acetate in postmenopausal women with advanced breast cancer: results of overview analysis of two phase III trials. J Clin Oncol 1996;14:2000–11.
7. Rutquist LE, Mattson A. Cardiac and thromboembolic morbidity among postmenopausal women with early-stage breast cancer in a randomized trial of adjuvant tamoxifen. J Natl Cancer Inst 1995;85:1398–406.
8. McDonald CC, Alexander FE, Whyte BW, Forrest AP, Stewart HJ. Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomized trial. Br Med J 1995;311:977–80.
9. Anker G, Lønning PE, Ueland PM, Refsum H, Lien EA. Plasma levels of the atherogenic amino acid homocysteine in postmenopausal women with breast cancer treated with tamoxifen. Int J Cancer 1995;60:365–8.
10. Mooren MJ, Wouters MGAJ, Blom HJ, Schellekens LA, Eskes TKAB, Rolland R. Hormone replacement therapy may reduce high serum homocysteine in postmenopausal women. Eur J Clin Investig 1994;24:733–6.
11. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. Int J Epidemiol 1995;24:704–9.
12. Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995;346: 1395–8.
13. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337:230–6.
14. Jones AL, Powles TJ, Law M, Tidy A, Easton E, Coombes RC, et al. Adjuvant aminoglutethimide for postmenopausal patients with primary breast cancer: analysis at 8 years. J Clin Oncol 1992;10: 1547–52.
15. Lundgren S, Helle SI, Lønning PE. Profound suppression of plasma estrogens by megestrol acetate in postmenopausal breast cancer patients. Clin Cancer Res 1996;2:1515–21.
16. Kvinnsland S, Lønning PE, Dahl O. Treatment of breast carcinoma with aminoglutethimide. Acta Radiol Oncol 1984;23:421–4.
17. Johannessen DC, Engan T, Di Salle E, Zurlo MG, Paolini J, Ornati G, et al. Endocrine and clinical effects of exemstane (PNV 15921), a novel steroidal aromatase inhibitor, in postmenopausal breast cancer patients: a phase I study. Clin Cancer Res 1997;3: 1101–8.
18. Goss PE, Powles TJ, Dowsett M, Hutchison G, Brodie AMH, Gazet J-C, et al. Treatment of advanced breast cancer with an aromatase inhibitor, 4- hydroxyandrostenedione: phase II report. Cancer Res 1986;46:4823–6.
19. Lundgren S. Progestins in breast cancer treatment. Acta Oncol 1992;31:709–22.
20. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: Automated determination and sample stability. Clin Chem 1993;39:263–71.
21. Nygård O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA 1995;274: 1526–33.
22. Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Res 1991;51:4837–44.
23. Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med 1992;117:1016–37.
24. Cauley JA, Gutai JP, Glynn NW, Paternostro-Bayles M, Cottington E, Kuller LH. Serum estrone concentrations and coronary artery disease in postmenopausal women. Arterioscler Thromb 1994; 14:14–8.
25. Dallongeville J, Marecaux N, Isorez D, Zylberberg G, Fruchart J-C, Amouyel P. Multiple coronary heart disease risk factors are associated with menopause and influenced by substitutive hormonal therapy in a cohort of French women. Atherosclerosis 1995;118:123–33.
26. Bonneterre J, Ghalim N, Nguyen M, Puchois P, Demaille A, Demanquilly C, et al. Variations in lipoproteins during aminoglutethimide therapy. Breast Cancer Res Treat 1987;10:197–200.
27. Holme I. An analysis of randomized trials evaluating the effect of cholesterol reduction on total mortality and coronary heart incidence. Circulation 1990;82:1916–24.
28. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrøm LE, Ueland PM, et al. Plasma homocysteine as a risk factor for vascular disease: the European concerted action project. JAMA 1997;277:1775–81.
29. Refsum H, Ueland PM, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. 1998;49:31–62.
30. Murray M, Cantrill E, Farrell GC. Induction of cytochrome P450 2B1 in rat liver by the aromatase inhibitor aminoglutethimide. J Pharmacol Exp Ther 1993;265:477–81.
31. Lønning PE. Aminoglutethimide enzyme induction: pharmacological and endocrinological implications. Cancer Chemother Pharmacol 1990;26:241–4.
32. Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease, and drug therapy. J Lab Clin Med 1989;114:473–501.