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# Antihypertensive Treatment and Homocysteine Concentrations

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**Thiazides and angiotensin-converting enzyme (ACE) inhibitors are first-choice drugs for lowering elevated blood pressure and hence risk of cardiovascular disease. Homocysteine (tHcy) is another and independent cardiovascular risk factor and has been reported to be elevated in patients on antihypertensive therapy. As these studies reported only associations, a preliminary, randomized, prospective treatment study was performed in 40 hypertensive patients. We investigated the major determinants of tHcy concentrations after treatment with hydrochlorothiazide (HCT) or captopril: vitamins B6, B12, folic acid, and creatinine and cystatin C as parameters of renal function. A total of 21 Patients were treated with HCT and 19 with captopril, for, respectively, 31 and 29 days. HCT, but not captopril, raised tHcy by 16% ($P = .003$) and also creatinine and cystatin C ($P = .025$ and $P = .004$, respectively). This tHcy increase may offset the desired cardioprotection conferred by lowering the blood pressure.**


ELEVATED BLOOD PRESSURE is an acknowledged risk factor for cardiovascular disease. Various treatment schemes are used worldwide to lower blood pressure and, consequently, cardiovascular risk.[1] Plasma homocysteine (tHcy) is regarded as another independent cardiovascular risk factor and is presently attracting much attention. Two recent studies have reported an association of treatment with antihypertensive drugs of the class of diuretics on the one hand with tHcy elevations on the other hand.[2,3] Such treatment-associated increase in tHcy may be a cause for concern because it might counteract the desired cardioprotection due to the lowering of blood pressure. The 2 aforementioned studies were observational studies, however, and the described patients treated with diuretics had certain features known to influence tHcy, such as advanced age, and alteration in kidney function[2] and vitamin concentrations. Moreover, as these studies reported only associations, which do not allow causal conclusions, we performed a randomized, prospective treatment study aimed at investigating the plasma concentrations of specific vitamins ($B_6$, $B_{12}$, and folic acid) and renal function before and after treatment with hydrochlorothiazide (HCT). Renal function was assessed not only by creatinine, but also by means of cystatin C, which is a low molecular mass plasma protein that has been proposed as a new and very sensitive serum marker of changes in glomerular filtration rate (GFR).[4] A second group of patients, receiving another frequently used antihypertensive drug, the angiotensin-converting enzyme (ACE) inhibitor captopril, was studied for comparison.

## SUBJECTS AND METHODS

### *Study Subjects and Treatment*

A total of 40 Patients (19 men and 21 women) were recruited and included consecutively in this prospective intervention study in a cardiological outpatient unit. The patients had been informed that they would be randomized and allocated to either treatment group according to a randomization list produced before the start of the study. The patients were assigned to receive either 25 mg HCT (n = 21) or an ACE inhibitor (n = 19, 50 mg captopril, in 2 daily doses of 25 mg), the assignment being made by the cardiologist in charge who was unaware of any result for tHcy. The inclusion criteria allowed both sexes, age between 30 and 85, and mild and uncomplicated essential hypertension. We excluded patients whose tHcy levels might have been influenced by multivitamin therapy, vegetarian diet, folate-depleting or folate-inhibiting drugs (phenytoin, carbamazepine, omeprazolein, estrogens, methotrexate, lansoprazole, azaribine, penicillamine, theophylline), more than 2 alcoholic drinks per week, renal failure (serum creatinine $> 120$ $\mu$mol/L), recent stomach or small bowel resection, and/or a history of pernicious anaemia, malignancy, or homocystinuria. Assessments of vitamin or fortified food intake were made in both groups at baseline and after treatment by means of self-administered questionnaires. The study had been approved by the Ethics Committee of the University

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Hospital, and written informed consent had been obtained from each patient.

### *Laboratory Methods*

Before the start of the antihypertensive medication, a baseline fasting blood sample was collected, cooled immediately to 4°C, transferred within 30 minutes to the Institute of Clinical Chemistry, and there centrifuged. Routine clinicochemical parameters were immediately measured, and were stored at −20°C for determinations of tHcy and vitamins. A second fasting blood sample was taken after 4 to 6 weeks of treatment (depending on the patient's availability) and processed in the same way. Renal parameters, folate, and cobalamine were determined in serum. Pyridoxal 5-phosphate and tHcy were measured in EDTA plasma. tHcy was determined as total tHcy by high-performance liquid chromatography (HPLC) with fluorescence detection,[5] both samples from the same patient being measured in 1 run. The assay has a between-run coefficient of variation of 5%. Cobalamin (vitamin $B_{12}$) and folate were determined using commercial test kits (Abbott, Wiesbaden, Germany), creatinine by a commercial enzymatic method (Roche Diagnostics, Mannheim, Germany), cystatin C by latex-enhanced immunonephelometry (Dade-Behring, Marburg, Germany), and vitamin $B_6$ with the aid of a commercial HPLC test with fluorescence detection. Blood pressure was measured to the nearest 2 mm Hg using a manual sphygmomanometer and according to the guidelines of the German Hypertension League.

### *Statistical Analysis*

All results are expressed as means with their standard deviations. The values obtained before and after the assigned antihypertensive treatment were tested for significant differences by the Wilcoxon signed-rank test. In all calculations $P < .05$ was taken as the significance threshold. Comparability of the 2 treatment groups was tested at baseline for the major outcome variables by the Mann-Whitney *U* test. No differences were detected (all $P > .05$).

## RESULTS

Treatment both with HCT and with the ACE inhibitor lowered the systolic and diastolic blood pressure in each group (all $P < .003$) confirming compliance with the therapy. HCT raised tHcy by 16% ($P = .003$) with concomitant increases in creatinine and cystatin C ($P = .025$ and $P = .004$, respectively). The vitamins known to influence tHcy were unaffected (Table 1). The ACE inhibitor did not increase or decrease any of these parameters except cystatin C, which showed an increasing trend ($P = .053$).

**Table 1. Total tHcy and Related Parameters Before and After Therapy With HCT and an ACE Inhibitor**

| | HCT (n = 21) | ACE Inhibitor (n = 19) |
|---|---|---|
| Ratio (male:female) | 13:8 | 8:11 |
| Age (yr) | 63 ± 5 | 67 ± 6 |
| Duration of treatment (d) | 31 ± 6 | 29 ± 6 |
| tHcy baseline (μmol/L) | 12.5 ± 3.5 | 13.3 ± 4.8 |
| tHcy after treatment (μmol/L) | 14.5 ± 4.9 | 14.1 ± 5.0 |
| *P** | .003 | .157 |
| Folate baseline (ng/mL) | 9.4 ± 3.8 | 8.2 ± 3.5 |
| Folate after treatment (ng/mL) | 8.3 ± 3.0 | 8.7 ± 3.9 |
| *P* | .332 | .382 |
| Cobalamin baseline (pg/mL) | 396 ± 133 | 430 ± 213 |
| Cobalamin after treatment (pg/mL) | 401 ± 148 | 418 ± 189 |
| *P* | .345 | .507 |
| PLP baseline (ng/mL) | 12.2 ± 7.8 | 19.2 ± 14.6 |
| PLP after treatment (ng/mL) | 14.0 ± 10.7 | 18.3 ± 14.2 |
| *P* | .145 | .163 |
| Creatinine baseline (μmol/L) | 81 ± 20 | 90 ± 27 |
| Creatinine after treatment (μmol/L) | 86 ± 19 | 92 ± 24 |
| *P* | 0.025 | 0.327 |
| Cystatin C baseline (mg/L) | 0.86 ± 0.21 | 0.97 ± 0.37 |
| Cystatin C after treatment (mg/L) | 0.90 ± 0.24 | 1.03 ± 0.36 |
| *P* | .004 | .053 |

NOTE. All values are means with standard deviations.
*According to the Wilcoxon signed-rank test.

## DISCUSSION

The major finding of this study, an increase in tHcy-under treatment with HCT, confirms the report of the 2 earlier studies[2,3] and extends our understanding by suggesting the mechanism responsible for the effect. This underlying mechanism is most probably a concomitant deterioration of renal function. The importance of renal function for tHcy concentrations is well documented[6] and explains the increases seen in the elderly[7] and in patients with kidney diseases, and the very high levels in renal end-stage disease.[8] In our study, 2 parameters of renal function deteriorated after HCT: serum creatinine and cystatin C, which serves as a specific measure of GFR.[9] The other antihypertensive drug used in the present study, captopril, did not have any statistically significant effects on tHcy or creatinine and increased cystatin C only with borderline significance.

Morrow et al[10] proposed as an alternative mechanism a decrease of the folate concentration in red blood cells as a consequence of a depletion of water-soluble vitamins under diuretics. Because such an effect can be observed only after long-term treatment (due to the erythrocyte half-life of 120 days), we could not observe a folate decrease in serum after 6 weeks. If such a folate depletion occurs in addition to the reported renal impairment, the final increase in tHcy might actually exceed the 16% observed by us and reach the 70% described by Morrow et al.[10]

The finding that a drug that increases creatinine and cystatin C also increases tHcy corresponds to our earlier observations using fibrates for lipid lowering. Both fenofibrate and bezafibrate increase creatinine and cystatin C and also tHcy.[9] Gemfibrozil, which is another fibrate, but does not affect the parameters of renal function, accordingly did not increase tHcy.[10] If drug-induced deteriorations of renal function do generally increase tHcy, hyperhomocysteinaemia may not be infrequent. Other drugs that increase creatinine, and which therefore might also increase tHcy, are phenytoin,[11] lithium, and the nonsteroidal antiinflammatories.[12] This possibility should be investigated further, because some of these drugs are used for longer periods and may therefore enhance the cardiovascular risk.

The tHcy increase after HCT may be clinically important, because this increase may counteract the desired cardiovascular protection conferred by lowering of the blood pressure. In this context, it may be mentioned that in some studies, the use of HCT (MRC,[13] MRFIT[14]) to lower the risk of coronary heart

disease proved to be less successful than expected. tHcy increases may now be added to the unfavorable side effects profile of HCT, such as increases in low-density lipoprotein (LDL)-cholesterol and glucose or hypokalemia.[15]

Although this study is preliminary, it may be advisable to monitor tHcy in patients treated with HCT. If tHcy increases substantially and HCT therapy has to be continued, normalization can be tried by supplementing vitamins, which lower tHcy (folic acid, vitamins $B_6$, and $B_{12}$).

The finding that hyperhomocysteinaemia can be provoked by HCT and by some fibrates is also interesting for another reason. The association between elevated tHcy and cardiovascular disease was found predominantly in retrospective studies in which cardiovascular patients (cases) were compared with matched individuals free from cardiovascular disease (controls). Because cardiovascular patients are frequently treated with HCT or fibrates or both, it is conceivable that their higher tHcy concentrations might have been caused in the first place by their drug treatment, which was most probably less administered in the control group. This warrants a reassessment of these case-control studies, with evaluation of only those cases and controls who received neither HCT nor fibrates known to increase tHcy.

In conclusion, it has been shown that hydrochlorothiazide treatment increases tHcy, probably via an alteration of renal function. This side effect may give reason for (1) closer monitoring of tHcy in the patients concerned, (2) an investigation of this side effect with other drugs affecting renal function, and (3) reassessment of retrospective studies investigating patients and controls treated with tHcy-raising drugs.

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