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Reduction of homocysteine in elderly with heart failure improved vascular function and blood pressure control but did not affect inflammatory activity.

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Short title: Homocysteine and chronic heart failure

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Abstract

Introduction: We have previously shown that hyperhomocysteinemia is common in elderly heart failure patients, and is in these associated with endothelial dysfunction, impaired vasodilatory capacity and a low-grade inflammation. In the present study we examined if supplementation with B₆, B₁₂ and folate could normalize the hyperhomocysteinemia and if this, in turn, would improve the associated parameters.

Methods: Open study without placebo-control on heart failure patients with plasma homocysteine > 15 µM. Measurements of cutaneous vascular reactivity, blood pressure, inflammatory activity and endothelial function were performed before and after intervention with intra-individual comparisons.

Results: The treatment reduced homocysteine to near normal values and enhanced the hyperaemic response to acetylcholine related to the response to heat. The mean arterial blood pressure and pulse rate was reduced. There was no effect on inflammatory activity, plasma levels of von Willebrand factor, subjective health quality or the hyperaemic responses to sodium nitroprusside or local warming.

Interpretation: Hyperhomocysteinemia in heart failure patients is multifactorial in origin. Folate deficiency, inflammatory activity and reduced renal function could be contributing. It is suggested that supplementation with B-vitamins can improve the vasodilatory capacity and reduce the blood pressure but additional studies are required to confirm this.

Key Words: endothelium, heart failure, homocysteine, inflammation, von Willebrand factor, vasodilation.

Introduction

There are several reports that heart failure is associated with a reduced vasodilatory capacity. This is suggested to be of importance for the progression of the heart failure syndrome and for the development of the patient's subjective symptoms. In a previous study on elderly heart failure patients we observed that the reactivity in cutaneous microvessels to both endothelium-dependent and – independent stimuli was attenuated (Andersson *et al.* 2003). This dysfunction seemed to be associated with a low grade inflammatory response. We also found that more than a third of the patients had clinical hyperhomocysteinemia, defined as plasma Homocysteine (Hcy) > 12 μ M (De Vriese *et al.* 2002). In accordance, the mean plasma Hcy concentration was elevated in the heart failure group when compared to age- and sex matched controls. The Hcy level correlated positively with the severity of the heart failure, several inflammatory markers and to von Willebrand factor (vWf), a marker of pro-thrombotic endothelial dysfunction. It is not clear which mechanism that induces the hyperhomocysteinemia. It is possible that Hcy is elevated as a part of an acute phase response. Such increases are described in the first phase both after stroke (Hovard *et al.* 2002) and myocardial infarction (Senaratne *et al.* 2000). It has been suggested that immune activation and the subsequent inflammatory response can induce a rise in Hcy (Schroecksnadel *et al.* 2003). In agreement, Hcy is increased in patients with inflammatory disease such as rheumatoid arthritis, and is then related to markers of inflammation (Yxfeldt *et al.* 2003). One possible mechanism is that inflammation stimulates the degradation of vitamin B₆, which leads to an increase

in plasma Hcy (McCarty 2000), but also effects on folate and cobalamines are suggested as well as direct production of Hcy in immune cells (Schroecksnadel *et al.* 2003).

There is an abundance of reports suggesting a causal relationship between elevated Hcy and cardiovascular and thromboembolic disease (*Wald et al.* 2002). Several mechanistic pathways are proposed to be involved. For example, hyperhomocysteinemia is reported to induce endothelial dysfunction and to impair vasodilatory capacity (see Splaver *et al.* 2004). Hcy enhances vascular inflammation in animal models and augments the production of the pro-thrombotic tissue factor (Hofmann *et al.* 2001). This is possibly due to direct proinflammatory effects: Hcy increases production of hydroxyl radicals (see Splaver *et al.* 2004), activates the transcription factor NF- κ B and induces production of proinflammatory cytokines in monocytes (van Aken *et al.* 2000); Wang *et al.* 2001). Hcy could thus be induced as a part of an inflammatory response, but at the same time contributes to disease progression when exerting pro-oxidative effects by itself and amplifying oxidative stress (Schroecksnadel *et al.* 2003).

Based on this, and our previous results we hypothesised that in heart failure high Hcy levels contribute to the endothelial dysfunction, reduced vasodilatory capacity, inflammatory activity and, in turn, low health quality. The present study was conducted to investigate if vitamin supplementation, with doses used in ordinary health care, has the ability to normalise plasma Hcy in

elderly heart failure patients. Furthermore, if this affected the parameters mentioned above.

Materials and methods

Ethics.

The study was conducted in accordance with the Declaration of Helsinki. The Ethics Committee of Lund University approved of the protocol (LU 516-02). Informed consent was obtained from all subjects.

Subjects.

Patients diagnosed with heart failure based on symptoms, clinical signs and an elevated plasma level of N-terminal pro-brain natriuretic peptide (NT-proBNP) and with plasma Hcy $>15 \mu\text{M}$ were included. Exclusion criteria were presence of tremor or cognitive impairment which could jeopardize the measurements. Fourteen patients were included, most of them outpatients in primary care. Two were female. Mean age was 81 ± 1 years and body mass index 26 ± 1 . After inclusion the participants were interviewed by a research nurse and asked to answer questions regarding their subjective health, tobacco and caffeine use, physical capacity and medication. They were also given a briefing on their pharmacotherapy.

One subject was active smoker and one used oral tobacco. Seven were former smokers but most of them had quit more than twenty years ago. The severity of the heart failure was quantified in several ways: the mean

estimated New York Heart Association functional class was 2.8 ± 0.2 and mean estimated maximal walking distance 400 meters with a range from 10 to 1000 m. The plasma NT-proBNP level is given below. The patients were on average treated with nine different drugs (range one to sixteen). Cardiovascular pharmacotherapy is summarized in table 1. It was not changed during the treatment period except for a self-reported improvement in compliance (below).

Measurements.

On the first visit plasma samples were taken for measurement of: interleukin-6 (IL-6), soluble interleukin2-receptor (sIL2r), C-reactive protein, (CRP; high sensitive method), cobalamines, folate, von Willebrand factor (antigen; vWf), HbA1c, creatinine and uric acid. Plasma samples were analysed at Department of Chemistry, Lund University Hospital. Creatinine clearance was calculated according to the Cockcroft-Gault formula: $(140 - \text{age}) \times \text{body weight (kg)} \times \text{K} / \text{serum creatinine } (\mu\text{M})$. K (constant) was 1.25 for men and 1.03 for women).

The cutaneous blood flow response to the endothelium-dependent vasodilator acetylcholine (ACh), the endothelium-independent dilator sodium nitroprusside (SNP) administered by iontophoresis, and to local warming was determined as in our former study. ACh (2%) was administered five times using anodal current, and SNP four times using cathodal current. After this the vasodilatory response to local warming (+44 C for 10 min) was determined. For details see; (Andersson *et al.* 2003).

Treatment was then given for six weeks with a daily dose of 3 mg pyridoxine (vitamin B₆), 0.8 mg folate and 0.5 mg cyanocobalamine (vitamin B₁₂) (TrioBe[®], Recip, Sweden). At the end of the treatment period all measurements were repeated.

Data, statistical analysis.

Data are given as mean \pm standard error of the mean. Statistical analysis was performed by the Students two-tailed t-test for paired data. For several of the measurements we did not obtain data from the initial stimulations, due to technical difficulties. We do therefore not present the area under the stimuli-response curve. Instead the maximal value (= the value after the last stimulation) is given. Since the baseline blood flow differed between the measurements (see below) the relative changes are presented. Calculations were performed using StatView 5.0, Berkeley, CA.

Table 1

Pharmacological treatment	Number of subjects
Loop diuretics	9
Aldosterone inhibitor	5
Digitalis	4
β- adrenergic antagonists	4
Calcium channel blockers	3
Angiotensin-converting-enzyme inhibitors	4
Angiotensin receptor blockers	2
Anticoagulantia/thrombocyte inhibitor	13
Long acting nitroglycerin	2
Statins	4
Insulin	3

Results

Effect on homocysteine and vitamin concentrations.

Mean plasma homocysteine was $17.9 \pm 0.6 \mu\text{M}$ at inclusion. Vitamin supplementation significantly reduced this to 13.8 ± 1.1 ($p < 0.01$). The level decreased in all subjects except for one with end stage renal disease (calculated creatinine clearance = 13 ml/min), in whom an increase was observed despite elevated plasma vitamin levels. It should be noted that only four patients reached the currently accepted upper limit for Hcy of $12 \mu\text{M}$ (De Vriese *et al.* 2002). In all

participants there was a marked elevation of plasma folate indicating a good compliance to the study drug. The mean concentration increased from 15.8 ± 2.7 nM at inclusion (range 6.4 – 37.0) to 43.6 ± 0.7 ($p < 0.001$). Four of the subjects had subnormal folate levels (< 10.0) before treatment. The plasma concentration of cobalamines was 537 ± 91 pM on first visit (range 183-1475). None of the subjects had subnormal values. After treatment the level had increased to 636 ± 85 ($p < 0.05$). Our laboratory has no ability to analyse vitamin B₆.

Plasma samples.

Before treatment the calculated creatinine clearance was 49 ± 5 ml/min corresponding to a moderate decrease in renal function. It was not affected by the treatment. Neither was the plasma levels of uric acid, HbA1c, soluble interleukin2 receptor (sIL2r), IL-6 or vWf or the severity of heart failure, measured as plasma NT-proBNP, affected by the vitamin supplementation (Table 2).

Table 2

	<u>Before treatment</u>	<u>After treatment</u>
NT-proBNP (ng/l)	2607 ± 637	2474 ± 469
HbA1c (%)	5.7 ± 0.4	5.3 ± 0.2
Uric acid (µM)	422 ± 27	423 ± 37
sCRP (mg/l)	13.2 ± 6.8	13.6 ± 7.1
IL-6 (ng/l)	6.1 ± 1.5	4.9 ± 0.8
sIL-2r (kU/l)	856 ± 163	728 ± 73
vWf (ag) (IU/ml)	2.04 ± 0.18	1.95 ± 0.20
Creatinine (mg/l)	140 ± 34	143 ± 37

General circulatory parameters.

The mean arterial blood pressure and heart frequency was lower at the second measurement (90.2 ± 3 vs. 95.8 ± 3 and 70 ± 3 vs. 75 ± 3 respectively; both $p < 0.05$). The pulse pressure did not differ between measurements.

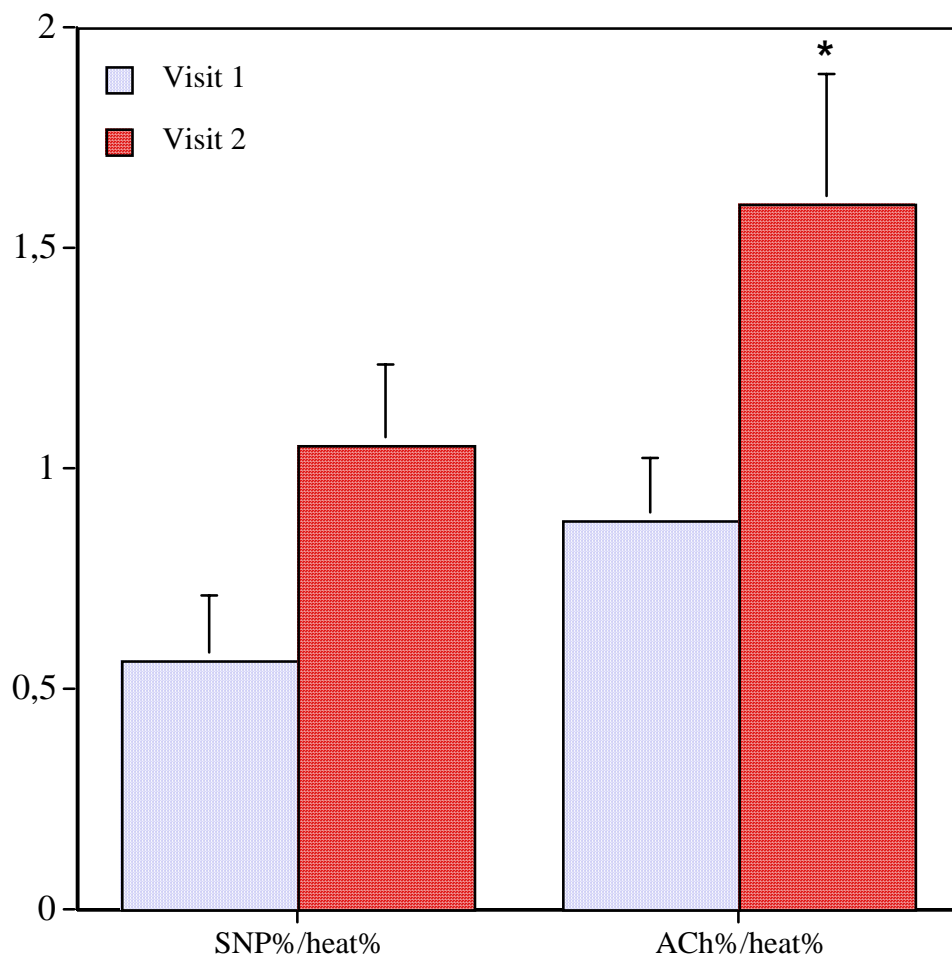
Health quality assessment and pharmacological treatment.

The vitamin supplementation did not change the patients' subjective health quality or walking distance. At inclusion 13 of the subjects felt that they were well informed on the rationale for their pharmacological treatment. Five subjects did regularly refrain from taking one or several of the prescribed drugs. This number was reduced to two subjects at the follow-up visit. At the first visit nine patients complained of problems related to the pharmacological treatment. The number

was reduced to five at the follow-up. More than half of the group had technical help for correct dosing.

Vasodilatory responses.

The basal blood flow (before stimulation) differed between measurements. For ACh the value was 20.0 ± 6.7 flow units at the first visit and 7.2 ± 1.4 at the second ($p < 0.01$). Corresponding values were 7.25 ± 1.4 and 19.8 ± 6.8 (n s) for SNP and 17.4 ± 3.5 and 11.6 ± 1.6 ($p < 0.05$) for heat stimulation, respectively. The differences were obviously due to random variation. Stimulation with ACh induced an increase in blood flow of $578 \pm 110\%$ at first visit and 782 ± 105 at the second (n s; $p = 0.07$). For SNP the corresponding values were $394 \pm 99\%$ and 524 ± 92 (n s). Local warming induced a blood flow increase of $721 \pm 114\%$ before treatment and 564 ± 76 after (n s). There was a significant enhancement of the response to ACh when it was expressed as relative (per cent) to the response to heat (Fig. 1). The response to SNP, calculated the same way, did not reach statistical significance ($p = 0.056$). These responses did not correlate to HCy levels, however.

Fig. 1

Discussion

The present study was conducted in order to further investigate previous findings related to Hcy (Andersson *et al.* 2003). The two study populations were similar with regard to NYHA functional class, BMI, MAP, Hcy concentration and medication heart failure. The present group was older, however, and had a more reduced renal function. No side effects of the vitamin treatment were reported from the participants.

Our results show that vitamin supplementation can reduce the elevated Hcy levels in heart failure patients. The treatment did not induce a complete normalisation of Hcy, however, and it can be speculated that the doses used were suboptimal. We can only speculate to which extent each of the three vitamins contributed to the result. A number of the subjects in the present study had subnormal plasma folate concentrations although the mean plasma level was within the normal range. Folate deficiency could thus be one explanation for the elevated Hcy. A similar conclusion was drawn from an earlier study with a larger group of psychogeriatric patients. Those patients were comparable in age to our group but had even higher Hcy levels (Nilsson *et al.* 1999). The two groups also differed in that none of the subjects in our study had subnormal levels of cobalamines before treatment whereas that was common in the psychogeriatric group. Our group had thus a higher mean plasma level of cobalamines. It might thus be that the mechanisms leading to hyperhomocysteinemia differ between conditions and that cobalamine deficiency is of less importance in heart failure. We could not measure vitamin B₆ but the present data together with the correlation between inflammation and Hcy seen previously fit in with the hypothesis mentioned earlier of a reduced availability of pyridoxine phosphate (McCarty 2000).

The study participants had in general, mild to moderate renal failure. This is a well-known determinant of the Hcy level (De Vriese *et al.* 2002 and references therein). The reduction in Hcy did not affect the creatinine values. The one subject with end stage renal disease exhibited an increased Hcy level

despite elevated vitamin concentrations. This is in accordance with previous findings and is probably explained by the fact that the hyperhomocysteinemia in patients with renal failure to a large extent is due to a marked reduction of Hcy clearance from plasma. Folate does not affect the plasma elimination of Hcy but enhances the remethylation in the tissues, which leads to a lower influx into the plasma compartment. In our group of patients this effect might be sufficient to override the defect in elimination and obtain new lower steady state levels. This is not possible in end stage renal disease, however (De Vriese *et al.* 2002). Taken together it could be that several factors contribute to the hyperhomocysteinemia; renal dysfunction, inflammatory activity and vitamin deficiency being among these.

Despite the reduction in plasma Hcy the measured markers of inflammatory activity remained constant. The data does thus not support the hypothesis that high Hcy augments the inflammatory response in heart failure. The co-variation between inflammatory parameters and Hcy seen in our former study is then rather explained by the opposite relationship; that inflammation raises Hcy. Neither did the treatment reduce the levels of vWf. This was a somewhat unexpected finding; it has previously been reported that an acute hyperhomocysteinemia induced by methionine loading increases vWf levels significantly (Tam *et al.* 2003). Our results rather suggest that, in heart failure, the relationship between this marker of endothelial dysfunction and Hcy are influenced by a common upstream event but do not directly affect each other. In

accordance, it has previously been reported that vWf is increased in humans with high Hcy but the association is, at most, weak (Becker *et al.* 2000).

The participants could not detect any marked improvement in their subjective health quality. It should be kept in mind that the subjects were old and most of them had multiple chronic diseases. It thus seems likely that minor or moderate improvements in physical abilities could pass unnoticed and that this parameter thus has a low sensitivity. Interestingly the opportunity to discuss the pharmacotherapy markedly improved the participants concordance and reduced the discomforts they felt were related to medication. The blood pressure was significantly lower at the second visit. Since this study was open and without placebo control it is not possible to conclude if this was due to the increased adherence to the prescribed pharmacological treatment or to an unspecific effect. There are, however, previous reports that treatment with pyridoxine plus folic acid had a reducing effect on the blood pressure (van Dijk *et al.* 2001) and in a recent study it was shown that higher folate intake is associated with reduced risk of incident hypertension (Forman *et al.* 2005). It might thus be hypothesised that vitamin supplementation has a direct blood pressure reducing effect in heart failure patients with hyperhomocysteinemia, possibly mediated by an improvement of vascular function.

Vitamin supplementation has in several previous studies been shown to improve vasodilator capacity concomitant with a reduction in Hcy (references in Splaver *et al.* 2004). Some controversy remains if this improvement is directly linked to the Hcy reduction or if it is a parallel

phenomenon (De Vriese *et al.* 2002; Stanger *et al.* 2002). The reports are unequivocal; vitamin supplementation seems not to have any beneficial effect on vasorelaxation when Hcy is elevated secondary to renal failure (De Vriese *et al.* 2002). Similarly, it has recently been reported that supplementation with B-vitamins and folate does not improve flow-mediated vasodilation in older adults with mild hyperhomocysteinemia (Carlsson *et al.* 2004). In our study the mean vasodilatory response to ACh and SNP was higher at the second visit but these changes did not reach statistical significance. The ACh induced hyperaemia was significantly elevated, however, when related to the heat response. This suggests that the high Hcy concentration contributes to the impaired vasodilatory capacity in heart failure patients but also the improved compliance to prescribed medication could have influenced the results. Additional studies are thus required to establish the link between homocysteine and vascular function in this condition and perhaps for a longer time period.

In conclusion, the present data suggests that the hyperhomocysteinemia seen in heart failure is multifactorial in origin. Folate deficiency, inflammatory activity and reduced renal function are likely to contribute to the elevation, among others. Vitamin supplementation reduces plasma Hcy in this condition and it is suggested that this treatment could improve the endothelium-dependent vasodilatory capacity and reduce blood pressure. We found no evidence of an improvement of the pro-thrombotic endothelial function.

Study limitations

The small number of participants and the open-label design limits the study. Furthermore, the variations in the base-level flow could have influenced the vasodilatory results. These data could also have been affected by the increased compliance to the prescribed drugs.

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Figure legends

Fig. 1. The cutaneous vasodilatory responses to SNP and ACh (maximal response expressed as per cent of basal blood flow) expresses as per cent of the response to local warming; before and after vitamin supplementation. *= p<0.05.

Table legends

Table 1. Cardiovascular pharmacotherapy taken by the study subjects.

Table 2. Plasma concentrations of parameters related to heart failure and inflammation before and after vitamin treatment.