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Original Article

Homocysteine and hypertension in persons with spinal cord injury

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Study design: Cross-sectional analysis of a convenience sample of locally recruited participants, including both patients and volunteers.
Objectives: To determine whether there is an association between plasma homocysteine and hypertension in persons with spinal cord injury (SCI).
Setting: Spinal Cord Injury Service of the Veterans Affairs Palo Alto Medical Center (California, United States of America).
Methods: The incidence of hypertension, dyslipidemia, insulin resistance, and the presence of metabolic syndrome were determined in 168 individuals with SCI (mean age 50.2 ± 12.8 years). Fasting lipids, insulin, glucose, plasma homocysteine, and anthropometric data was gathered for each subject.
Results: Blood pressure values ($P<0.001$) and mean arterial pressure ($P<0.05$) increased with higher plasma homocysteine levels. Homocysteine values were also significantly greater among individuals with hypertension compared with those who were normotensive or prehypertensive ($P<0.0001$). There was an inverse relationship between plasma homocysteine levels and glomerular filtration rate and effective renal plasma flow ($P<0.05$).
Conclusions: Plasma homocysteine levels are elevated in persons with SCI who have hypertension and inversely related to renal function, which suggests that renal dysfunction may be a link between homocysteine and hypertension in persons with SCI.
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Keywords: homocysteine; hypertension; renal function; spinal cord injury

Introduction

Hypertension contributes to the mortality and morbidity from cardiovascular disease, insulin resistance, and obesity.[1] The seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) established guidelines that outline the detection, prevention, and treatment of hypertension[2] and are based on its association with obesity and type I/II diabetes.[3] According to the JNC7, approximately 60% of American adults have either prehypertension or hypertension with overweight groups being disproportionately affected.[4] Prehypertension is defined as blood pressure (BP) ranging from 120/80 to 139/89 mmHg, and identifies those individuals in whom early intervention, for example the adoption of a healthy lifestyle, could reduce BP, decrease the rate of progression of BP to hypertensive levels, or prevent hypertension entirely.[4]

Beginning in the 1960s, persons with spinal cord injury (SCI), especially those with paraplegia, were noted to have a higher prevalence of hypertension than their able-bodied counterparts.[5] Hypertension was noted to differ from the temporal paroxysmal elevation BP common among individuals with SCI and caused by autonomic hyperreflexia.[6] The increased prevalence of visceral obesity, metabolic syndrome, diabetes, and lower physical activity levels in persons with SCI compared to ambulatory individuals raises the overall risk for hypertension in this population. Renal failure remains a primary cause of death in persons with SCI in which hypertension is an important contributing factor.[7] Improvements in overall care, particularly of the nephropathic bladder, have led to a decline in renal failure for persons with SCI.[8] In its place, cardiovascular

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disease has emerged as a major comorbidity, and hypertension is a major risk factor cardiovascular disease.[9–11]

Plasma homocysteine, a thiol-containing amino acid formed by the demethylation of methionine, has been shown to be an indicator of cardiovascular risk.[12–14] A 5 μmol/l plasma homocysteine increment elevates the relative risk of coronary artery disease risk by a factor (40%) similar to that for an increase in serum cholesterol of 20 mg/dl.[15] The European Concerted Action Project showed that an increased plasma homocysteine level confers a risk of cardiovascular disease similar to that for smoking or hyperlipidemia. Moderately elevated plasma homocysteine levels are observed in the presence of hypertension in patients with cardiovascular disease.[16] The SCI population is similar to the able bodied population in that plasma homocysteine tends to increase with age and is higher in men than women; however, plasma homocysteine is generally higher among persons with SCI compared with the able-bodied.[17] The purpose of this study was to determine if there is an equivalent association between increased plasma homocysteine and hypertension in persons with SCI.

Methods

Subject characteristics

A sample of 168 persons with SCI was recruited through the Spinal Cord Injury Center, Veterans Affairs Palo Alto Health Care System and local community for this study, and all gave written informed consent. Of the participants, 73 (43%) had paraplegia and 95 (56%) had tetraplegia; 150 were male; and 104 were white, 11 black, 11 Hispanic, six Asian, and 36 of unknown ethnicity. Tetraplegia was defined as a neurologic injury T1 (thoracic 1) level or above, and paraplegia was any injury level below T1. All participants had stable SCI with a mean duration of injury of 19.1 ± 13 years. Participants were evaluated at the Palo Alto VA for hypertension. Medical information was gathered from questionnaires as well as medical records. Information on renal function, including clinical diagnosis of renal dysfunction, was obtained from medical records within 6 months of the evaluation date. The participants' treatment drug regimens were noted, including those that affect BP and homocysteine, such as lipid-lowering agents, antihypertensive drugs, and folic acid. The presence of arrhythmias was determined from clinically documented history or chronic use of antiarrhythmic medications.

Chemistry determination

Participants were asked to fast for 12 h prior to blood sampling. Medications were not discontinued. Blood levels were determined for plasma homocysteine and serum creatinine.

Plasma homocysteine levels were analyzed using the IMx Homocysteine assay and analyzer (Abbott Laboratories, Abbott Park, IL, USA). Hyperhomocysteinemia was defined as plasma homocysteine levels $\geqslant 15$ μmol/l.[12] Tertile ranges were obtained from the population distribution for plasma homocysteine levels as: <8.5, 8.5–10.7, and $\geqslant 10.8$ μmol/l.

BP was classified using the JNC7 guidelines for hypertension (Table 1).[18]

Table 1 Seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7) guidelines for blood pressure classification

JNC7 classification	*Blood pressure range (mmHg)*
Normal	$<120/80$
Prehypertensive	120/80–139/89
Stage 1 hypertension	140/90–159/99
Stage 2 hypertension	$\geqslant 160/100$

Mean arterial pressure (MAP) was also used to categorize BP values using the following formula:

$$\text{Mean Arterial Pressure} = (\text{SBP} + 2\text{DBP})/3$$

where SBP = systolic BP and DBP = diastolic BP. Pulse pressure was defined as SBP−DBP.

Renal function

Data on renal function were not complete for all participants. Renal function was evaluated by several methods including serum creatinine, estimated creatinine clearance, estimated glomerular filtration rate (GFR), effective renal plasma flow, and renal ultrasound. Creatinine levels were measured using SYNCHRON Creatinine Reagent and LX System (Beckman Coulter, Fullerton, CA, USA). We used the Cockcroft–Gault formula to estimate creatinine clearance:[19] Serum creatinine values were available for 146 participants.

$$\text{Creatinine clearance (ml/min)} = (140 - \text{age}) \times \text{Weight (kg)}/72 \times \text{Serum creatinine (mg/dl)}$$

For female subjects, the result is multiplied by 0.85. Although this formula tends to underestimate the true GFR,[20] it is widely accepted for clinical use, including those with SCI.[21,22] We also estimated GFR using the Modification of Diet in Renal Disease (MDRD) Study formula in a sub-population of the study sample.[23]

$$\text{MDRD} = 170 \times \text{Serum creatinine}^{-0.999} \times \text{age}^{-0.176}\ \text{Serum urea nitrogen}^{-0.17} \times \text{Albumin}^{0.318}$$

For female subjects, the result is multiplied by 0.762 and for individuals of black ethnicity, the result is multiplied by 1.18.[24] Renal scan data to assess total effective renal plasma flow (ERPF) were available in 81 participants and renal ultrasound were available in 64 participants. The year-to-year variation found in ERPF measurements among individuals with SCI has been shown to be unaffected by age, gender, level and degree of lesion, and time since injury.[25,26] Chronic kidney

disease was defined as GFR <60 ml/min per 1.73 m^2 or by the clinical assessment of disease. Ultrasound renal abnormalities were considered to be the presence of significant cysts, calcification, cholelithiasis, hepatomegaly, wall thickening, or hydronephrosis.

Statistical analysis

All statistical analyses were performed using NCSS 2001 (NCSS, Kaysville, UT, USA). ANOVA were used to assess statistical differences in BP, mean arterial pressure, pulse pressure, plasma homocysteine, serum creatinine, Cockcroft–Gault creatinine clearance, GFR, and ERPF for the presence or absence of hypertension or renal dysfunction between different categories for homocysteine and hypertension. Differences in proportions were assessed by χ^2 analysis. Linear regression was used to study associations between homocysteine and ERPF.

Results

Hypertension was present in 76 (45%) individuals. Of these, 51 (67%) had paraplegia and 25 (33%) had tetraplegia. Persons with paraplegia had significantly higher systolic and diastolic BPs, mean arterial pressure, and pulse pressure than those with tetraplegia. Mean plasma homocysteine was not significantly different between those with paraplegia (9.76 ± 3.2) and tetraplegia (10.2 ± 3.9). Hyperhomocysteinemia was present in 11 participants. There was no significant difference in GFR or in total ERPF between individuals with tetraplegia and paraplegia. There were no significant differences in MDRD GFR values between groups. Plasma homocysteine levels for participants taking folic acid supplements were significantly higher than those who were not taking folic acid supplements ($P<0.05$).

Although there was no direct correlation between plasma homocysteine levels and BP, there was a modest correlation between log-transformed homocysteine values and SBP ($r=0.30$, $P<0.05$). Plasma homocysteine levels were mildly correlated with SBP ($r=0.30$, $P<0.05$) and DBP ($r=0.37$, $P<0.001$) in participants who were not taking hypertensive medications and/or folic acid supplements ($n=84$). In addition, for increasing tertiles of plasma homocysteine, systolic BP rose significantly (Figure 1). Subjects with a history of hypertension had significantly higher mean homocysteine levels than those with no history of hypertension, although the values were within the normal range for both groups (Figure 2). Further, participants with hypertension and on hypertensive medications still had significantly higher plasma homocysteine levels ($P<0.0001$). Using the JNC7 classification of hypertension, mean homocysteine levels rose significantly with increasing BP (Figure 3 and Table 2). When JNC7 classifications were analyzed for systolic or diastolic BP values alone, the association of plasma homocysteine values with rising BP remained significant ($P<0.050$ for systolic and diastolic BP, respectively). This relationship

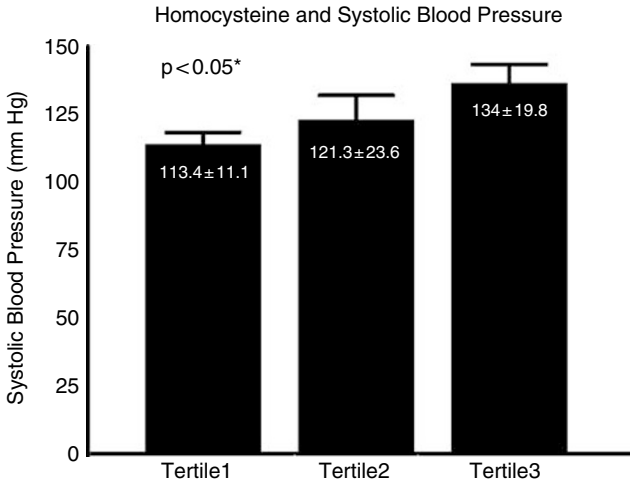

Figure 1 Mean and standard deviation (SD) of systolic blood pressure (mmgHg) for tertiles of increasing plasma homocysteine levels, where tertile 1 is homocysteine 1–4.9 μmol/l, tertile 2 is homocysteine 5–15 μmol/l, and tertile 3 is homocysteine >15 μmol/l

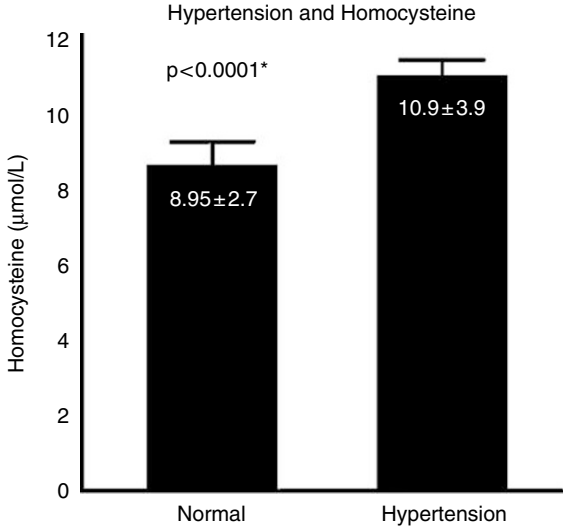

Figure 2 Mean and SD of plasma homocysteine (μmol/l) levels for subjects with normal blood pressure and those with hypertension. Both groups included persons taking antihypertensive medications

remained significant when taking into account antihypertensive medications and folic acid supplements. Plasma homocysteine levels were also significantly elevated for increasing levels of pulse pressure ($P<0.050$). Plasma homocysteine levels were mildly associated with mean arterial pressure ($r=0.29$, $P<0.05$).

Plasma homocysteine levels were inversely related to GFR (Figure 4). In the cohort analyzed by renal scan, homocysteine was significantly and inversely correlated with total ERPF (Figure 5). The inverse relationship

between renal dysfunction and homocysteine was further supported by the significantly higher homocysteine levels observed in participants with renal abnormalities as determined by renal ultrasound ($P<0.05$). Creatinine clearance for participants with normal kidney function was more than double that of those with chronic kidney disease (Figure 6a). Conversely, plasma homocysteine level for participants with chronic kidney disease was nearly double of those with normal kidney function (Figure 6b).

Plasma homocysteine appeared to be associated with arrhythmias. The mean plasma homocysteine level for participants with arrhythmias was 50% greater than for those with normal heart rhythm ($P<0.001$). This relationship was supported by the significantly higher homocysteine levels among participants who were taking antiarrhythmic medications ($16.5\pm7.8\,\mu$mol/l) compared with those who were not ($9.64\pm3.0\,\mu$mol/l).

Discussion

Evidence has suggested an association between hypertension and plasma homocysteine levels in the general population.[27,28] A recent review of these studies showed that for each 5 μmol/l increase in plasma homocysteine there was an increase in SBP and DBP of 0.7 and 0.5 mmHg in men and 1.2 and 0.7 mmHg in women,

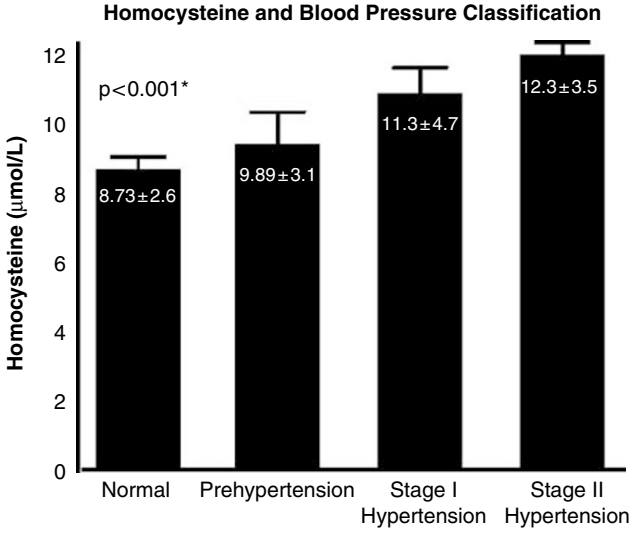

Figure 3 Mean and SD of plasma homocysteine (μmol/l) levels for subjects characterized with normal blood pressure (BP $<120/80$ mmHg), prehypertensive (BP 120/80–139/89) stage I hypertension (BP 140/90–159/99) and stage II hypertension (BP $\geqslant 160/100$), by JNC7 criteria

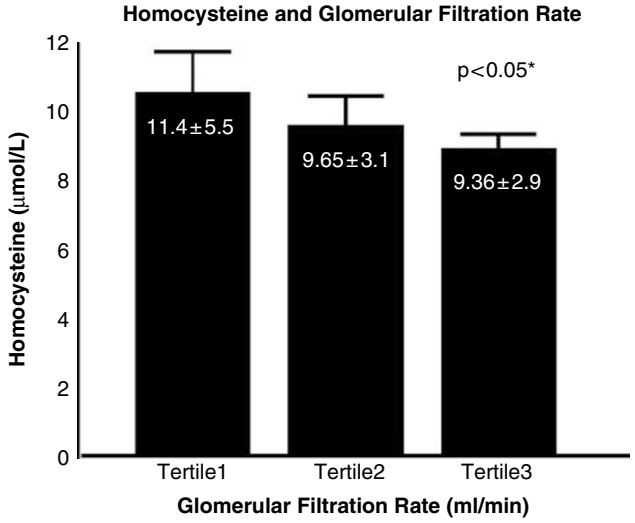

Figure 4 Mean and SD of plasma homocysteine levels for tertiles of increasing glomerular filtration rate (GFR), where tertile 1 is GFR <99 ml/min, tertile 2 is GFR 99–132 ml/min, and tertile 3 is GFR $\geqslant 132$ ml/min

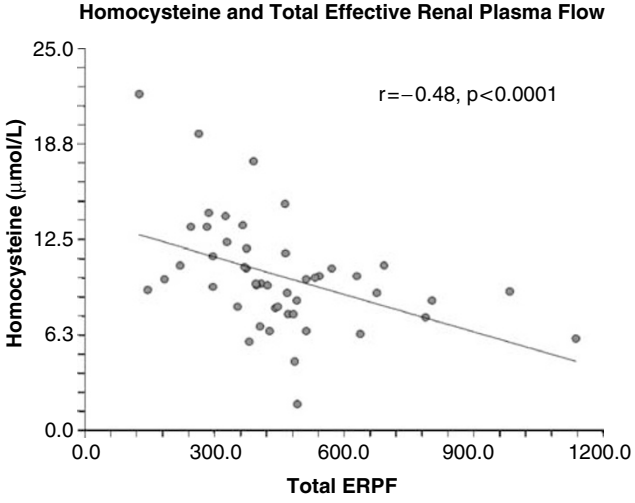

Figure 5 Linear regression of plasma homocysteine (μmol/l) levels with total effective renal plasma flow

Table 2 Mean $\pm$ standard deviation for age, body mass index (BMI), homocysteine, and creatinine levels by JNC7 blood pressure classification

JNC7 class	*Age*	*BMI (Kg/m^2)*	*Homocysteine(μmol/l)*	*Creatinine (mg/dl)*
Normal ($n=65$)	51.9 ± 12.9	26.1 ± 5.34	8.73 ± 2.6	0.724 ± 0.18
Prehypertension ($n=55$)	56.1 ± 12	28.5 ± 4.88	9.89 ± 3.07	0.778 ± 0.23
Stage 1 hypertension ($n=27$)	58.3 ± 11.3	28.5 ± 4.16	11.3 ± 4.72	0.789 ± 0.38
Stage 2 hypertension ($n=14$)	56.9 ± 12.4	28.7 ± 6.1	12.3 ± 3.53	0.900 ± 0.27

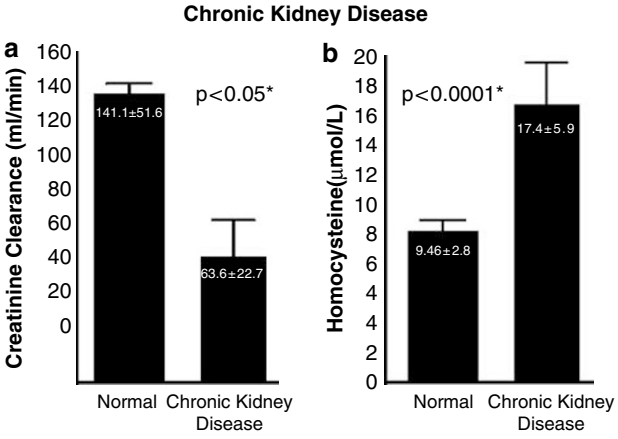

Figure 6 (**a**) Mean and SD of creatinine clearance (ml/min) for individuals with normal kidney function and those with chronic kidney disease, and (**b**) mean and SD of plasma homocysteine (μmol/l) level for individuals with normal kidney function and those with chronic kidney disease

respectively.[29] The findings of the current study corroborates these results in persons with SCI and suggests that a possible link between these two risk factors (elevated plasma homocysteine and hypertension) is renal dysfunction. In agreement with prior studies, we observed that plasma homocysteine levels mildly correlated with BP,[27–29] although the degree of hypertension was strongly related to changes in homocysteine levels. In addition, there was a strong relationship between the classification of hypertension by the JNC7 guidelines and plasma homocysteine levels.

We also found an inverse relationship between plasma homocysteine and renal function, as measured by GFR, in agreement with previous studies.[30] This relationship is unlikely attributable to bladder dysfunction associated with SCI-specific bladder management. Several studies have shown that bladder management techniques do not significantly affect renal function in persons with SCI.[31,32] We also found an inverse correlation between plasma homocysteine and total ERPF. This is similar to the findings of Wollesen *et al*,[33] who demonstrated that homocysteine is inversely related to GFR in patients with diabetes.

Our findings agree with those of other studies in demonstrating a connection between plasma homocysteine and hypertension and suggest the association may be based on impairment of renal function. However, it should be noted that there are several limitations in our study. Although the use of the Cockcroft–Gault formula is an accepted approximation of renal function, it may overestimate glomerular filtration.[19] Impairment of renal function was confirmed by measurement of ERPF in only one-third of the participants. Our study cohort consisted of only 168 individuals of whom the vast majority was male, which may have skewed our results considering the gender differences in hypertension. A larger study with complete renal assessment is necessary to provide confirmation of the proposed link between renal dysfunction, homocysteine, and hypertension in persons with SCI.

In conclusion, our results show that, similar to the general population, elevated plasma homocysteine is associated with hypertension in persons with SCI. This association appears to be linked with impairment of renal function. A better understanding of the association between hypertension and homocysteine and the potential role of renal dysfunction would shed further light on the increased risk of cardiovascular disease in persons with SCI as well as the able-bodied population. Confirmation of our results by larger investigations in the general population as well as by *in vivo* molecular evidence would suggest that the increased cardiovascular disease risk profile implied by elevated homocysteine levels is associated with the elevated presence of hypertension through a renal function-mediated mechanism.

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