Psychoneuroendocrinology (2013) 38, 1381—1387

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Plasma homocysteine levels increase following stress in older but not younger men

Ulrike Kuebler [a], Michael Linnebank [b], Alexander Semmler [b], Birgit Stoffel-Wagner [c], Roberto La Marca [a], Ulrike Ehlert [a], Petra H. Wirtz [d,*]

[a] Department of Clinical Psychology and Psychotherapy, University of Zurich, Zurich, Switzerland
[b] Department of Neurology, University Hospital Zurich, Zurich, Switzerland
[c] Institute of Clinical Chemistry and Clinical Pharmacology, University Bonn, University Hospital of Bonn, Bonn, Germany
[d] Biological and Health Psychology, University of Bern, Bern, Switzerland

Received 22 September 2012; received in revised form 4 December 2012; accepted 6 December 2012

KEYWORDS
Homocysteine;
Age;
Stress reactivity;
Acute mental stress;
TSST;
Cardiovascular disease risk

Summary
Background: The incidence and prevalence of cardiovascular disease (CVD) increases with age. Some evidence suggests that mental stress may increase plasma homocysteine (Hcy), an amino acid relating to CVD. However, none of these studies assessed age effects on Hcy stress reactivity, nor did they control for age. The objective of this study was (a) to investigate whether Hcy reactivity to psychosocial stress differs between younger and middle-aged to older men and (b) to study whether psychosocial stress induces Hcy increases independent of age.
Methods: Twenty eight younger (20—30 years) and 22 middle-aged to older (47—65 years) apparently healthy men underwent an acute standardized psychosocial stress task combining public speaking and mental arithmetic in front of an audience. Blood samples for Hcy measurements were obtained immediately before and after, as well as 10 and 20 min after stress. Moreover, salivary cortisol was repeatedly measured to test the effectiveness of the stress task in triggering a neuroendocrine stress response.
Results: Hcy reactivity to stress differed between age groups ($F(1.4, 60.7) = 5.41$, $p = .014$). While the older group displayed an increase in the Hcy response to stress ($F(2.5, 39.8) = 3.86$, $p = .022$), Hcy levels in the younger group did not change ($p = .27$). Psychosocial stress per se did not change Hcy levels independent of age ($p = .53$).
Conclusions: Our findings suggest that psychosocial stress does not evoke an Hcy response per se, but only in interaction with age pointing to a mechanism by which mental stress may increase CVD risk in older individuals.

* Corresponding author at: University of Bern, Department of Psychology, Biological and Health Psychology, Alpeneggstrasse 22, 3012 Bern, Switzerland. Tel.: +41 31 631 5790; fax: +41 31 631 4155.
E-mail address: petra.wirtz@psy.unibe.ch (P.H. Wirtz).

0306-4530/$ — see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.psyneuen.2012.12.003

1. Introduction

The incidence and prevalence of atherosclerotic and thrombotic cardiovascular disease (CVD) increase progressively with age (Herrera et al., 2010). To explain the higher atherothrombotic risk and ultimately cardiovascular mortality with increasing age, several studies have attempted to elucidate changes in atherogenic and thrombogenic factors during aging (Weinsaft and Edelberg, 2001; Torregrossa et al., 2011).

Homocysteine (Hcy) has been firmly established as an independent predictor of atherosclerotic and thrombotic CVD (McCully, 1996; Wald et al., 2002). Moreover, plasma Hcy tends to rise in healthy persons parallel with age (Ellinson et al., 2004). Hcy is a sulfur-containing amino acid derived from the dietary amino acid methionine (Castro et al., 2006). Hcy plasma levels are regulated by a complex metabolic pathway involving genetics, nutrition, and renal function (Refsum et al., 1998; Castro et al., 2006). Notably, the age-related increase in Hcy levels may result from suboptimal intake or absorption of vitamin B6, B12, and folate acting as key cofactors for Hcy metabolism, and decreased renal clearance of Hcy (Selhub et al., 1993; Castro et al., 2006). The mechanisms by which elevated Hcy levels relate to CVD are not fully understood. However, compelling evidence from experimental models indicates that Hcy is responsible for arterial changes and vascular damage by impairing bioavailability of nitric oxide and antioxidant defense, enhancing lipid peroxidation (Papatheodorou and Weiss, 2007), causing protein modification (Jakubowski, 2001, 2004), and thrombosis (Undas et al., 2006; Jakubowski et al., 2008), or activating inflammation (Jakubowski, 2005; Bogdanski et al., 2008). Notably, abundant epidemiological and experimental evidence suggests a potential causal role of mild to moderate hyperhomocysteinemia in the pathogenesis of CVD (McCully, 1996; Wang et al., 2007; Dragani et al., 2012). However, recent intervention trials have raised suspicion about this notion as treatment of mild hyperhomocysteinemia with B vitamins resulted in lower Hcy levels without subsequent reduction of CVD incidence (Clarke et al., 2010). Interestingly, the vitamin B induced Hcy reduction was associated with reduction of stroke risk (Saposnik et al., 2009). In addition, some Mendelian randomization studies found an increased CVD risk in subjects with lifelong mildly to moderately elevated plasma Hcy due to polymorphisms in Hcy-metabolizing enzymes (Wald et al., 2002; Casas et al., 2005; Cronin et al., 2005), whereas others did not detect an association (Clarke et al., 2012). Notably, intervention trials as well as Mendelian randomization studies have several limitations (for review see (Smulders and Blom, 2011)) and results need to be interpreted with caution.

In addition to age, Hcy, and their interaction, mental stress is a further factor that has been associated with CVD risk. Accumulating evidence indicates a strong impact of mental stress on the pathogenesis of atherosclerotic and thrombotic CVD (Brotman et al., 2007). It has been suggested that repeated episodes of acute or chronic stress may initiate or promote the atherosclerotic process via effects on Hcy (Black and Garbutt, 2002). Indeed, a study assessing basal Hcy levels in non-smoking war veterans with and without Post Traumatic Stress Disorder (PTSD) found highest Hcy levels in war veterans with PTSD (Jendricko et al., 2009). As PTSD is likely to add to chronic stress load, the study's findings point to a potential association between stress and higher Hcy plasma levels. In terms of acute stress reactivity, studies in rodents show increases in plasma Hcy immediately after restrained stress (de Oliveira et al., 2004; de Souza et al., 2006). However, human studies investigating Hcy reactivity to acute stress are rare and results are contradictory. While two studies reported increases in plasma Hcy levels in response to acute mental stress in middle-aged to older women (Stoney, 1999) and in young men (Sawai et al., 2008), another study including pre- und postmenopausal women found no Hcy stress reactivity (Farag et al., 2003). Moreover, none of these studies assessed age effects on Hcy stress reactivity, nor did they control for age. To date, it has not yet been investigated whether a potential Hcy change following acute mental stress might be age-dependent, which could shed light onto the complex mechanisms in the interface between age, Hcy, stress reactivity, and their interactions.

Therefore, we aimed to elucidate whether a potential Hcy stress reactivity differs between younger and middle-aged to older men. We hypothesized that older men show higher Hcy responsiveness to acute stress than younger men. Moreover, we investigated whether Hcy levels do change following mental stress independent of age.

2. Methods

2.1. Participants

This study is part of a larger project assessing psychobiological stress reactivity in healthy men (Wirtz et al., 2008). The Ethics Committee of the State of Zurich, Switzerland, formally approved the research protocol. For the purpose of this part of the study we intentionally selected from an existing study sample of $N = 63$ apparently healthy men aged between 20 and 65 years a group of younger men without age-related CVD risk (age range 20—30 years) and a group of middle-aged to older men with age-related CVD risk (age range 45—65 years) for analysis of Hcy levels from frozen plasma samples. Notably, the threshold of 45 years of age for increased age-related CVD risk was based on previous literature (Roger et al., 2011). Applying these age criteria for group inclusion the final sample of this study comprised 28 subjects aged 20 to 30 years (''younger men'') and 22 subjects aged from 47 to 65 years (''older men'') rendering a total sample size of $N = 50$. All subjects provided written informed consent.

The study was conducted between April 2004 and August 2005. In the main study, we intentionally recruited nonsmoking men aged between 20 and 65 years who were in good physical and mental health as confirmed by a telephone interview using an extensive health questionnaire. Specific exclusion criteria were obtained from the subjects' self-report and included clinical psychosomatic and psychiatric diseases, regular strenuous exercise, alcohol and illicit drug abuse, any heart disease, varicosis or thrombotic diseases, elevated blood sugar and diabetes, elevated cholesterol, liver, and renal diseases, chronic obstructive pulmonary diseases, allergies and atopic diathesis, rheumatic diseases, and current infectious diseases. In addition, participants were