Hyperhomocysteinemia and Oxidative Stress in Ischemic Stroke

To the Editor:
We read with interest the data recently published by El Kossi and Zakhary.1 In this study, the authors found significantly higher levels of plasma homocyst(e)ine, lipid peroxides, and oxidation products of nitric oxide, as well as significantly lower plasma vitamin C levels, in 30 patients with thrombotic cerebrovascular stroke within 2 hours from the onset of symptoms compared with healthy control subjects.2 Furthermore, the authors observed a strong positive correlation between homocyst(e)ine and lipid peroxides and an inverse relationship both between plasma vitamin C and homocyst(e)ine levels and between plasma vitamin C and lipid peroxide levels. As the authors noted, oxidative stress may play an important role in the pathogenesis of ischemic brain injury,2 and homocyst(e)ine might act as pro-oxidant in stroke.3 With their work, they concluded that indeed the association between hyperhomocyst(e)inemia and indexes of oxidative stress might reflect an ischemia-related free radical hyperproduction, and lowering plasma homocysteine levels might help in prevention of oxidative damage to the brain in stroke patients.1

We studied 42 patients (24 men and 18 women, aged 85.6±2.3 years) 5.1±1.4 days after the onset of ischemic stroke of thromboembolic origin. Patients with transient ischemic attack, showing that high doses of vitamin C are ineffective on plasma homocyst(e)ine levels observed in our study is supported by recent trials and antioxidant effects of vitamin C.7 Although the differences between our results and those of El Kossi and Zakhary could result from the differences of age between samples of subjects studied (our patients are roughly 30 years older) and to the time of the evaluation (our patients were studied roughly 3 days later), there is a great need for further studies in this field, in such a way that the reciprocal relationships between ischemic stroke, age, pro-oxidant effects of hyperhomocyst(e)inemia, and antioxidant vitamins are sorted by the age-dependent, relatively low levels of these vitamins also in controls.4 Thirty percent of our stroke patients were shown to be hyperhomocyst(e)inemic, which confirmed previous research, but plasma homocyst(e)ine levels were not related to vitamin B12 or folic acid serum levels. A study of 19 stroke patients5 showed increased plasma homocyst(e)ine levels both before and after methionine load compared with those found in controls, but stroke patients showed higher mean levels of serum vitamin B12 and folic acid than controls.

Our data might be explained by the biological complexity of our sample of subjects (ie, oldest-old subjects), which has not yet been described in great detail. It is conceivable that low blood levels of vitamin C, vitamin B12, and folic acid considered pathological in younger individuals may be considered ‘normal’ in very old subjects, in relation to a lower homeostatic level of specific metabolic pathways. This could be true for both old healthy subjects and old stroke patients. Nevertheless, the lack of a correlation between plasma vitamin C levels and homocyst(e)ine levels observed in our study is supported by recent trials showing that high doses of vitamin C are ineffective on plasma homocyst(e)ine levels in patients with coronary artery disease6 and that supplementation with B-group vitamins reduces mildly elevated homocyst(e)ine levels whether or not this supplementation contains an antioxidant mixture incorporating vitamin C.7

Although the differences between our results and those of El Kossi and Zakhary could result from the differences of age between samples of subjects studied (our patients are roughly 30 years older) and to the time of the evaluation (our patients were studied roughly 3 days later), there is a great need for further studies in this field, in such a way that the reciprocal relationships between ischemic stroke, age, pro-oxidant effects of hyperhomocyst(e)inemia, and antioxidant vitamins are sorted out. The elucidation of these aspects of the pathogenesis of stroke could have important nutritional as well therapeutic implications.8

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Letters to the Editor

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To the Editor:
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**Stress-Related Intracerebral Hemorrhage and the Water-Hammer Effect**

*To the Editor:*

Lammie et al.\(^1\) described thalamic hemorrhage following emotional upset in an elderly man with old lacunar infarcts in other parts of the brain. The case supported Caplan’s hypothesis that acute rises in blood pressure or cerebral blood flow may cause rupture of the small perforating arteries,\(^1\) which branch at almost right angles from the middle and posterior cerebral arteries to supply, among others, the thalamus and basal ganglia.

At autopsy of cases of not only infarct but also hemorrhage, I paid attention to the frequent occurrence of potential sources of small arterial emboli in the heart or at carotid artery atheromatous plaques. That emboli might be related to hemorrhage made no sense until, as a retiree, I began to poke into the physiology and physics\(^3,4\) of flow.

A water-hammer phenomenon was studied in the late 19th century.\(^3\) When flow of fluid in a pipe is stopped by sudden closure of a valve, the kinetic energy of the upstream fluid is reduced to zero very rapidly, creating a high pressure at the valve and causing a pressure wave to move upstream from it. Downstream, momentum reduces pressure. The primary waves are followed by secondary (“bouncing”) ones, until the fluid comes to rest.\(^4\) The theory\(^3,4\) is complicated, but the brief upstream rise of pressure (\(\Delta p\)) at rapid closure of valves may be calculated (G.A. Öhman, personal communication, 2000) from the rather simple equation

\[
\Delta p = \frac{\rho v^2}{2E} \left[\frac{D}{d} + \frac{d}{D}\right] + \frac{\rho v^2}{2K} \left[\frac{D}{d}\right]
\]

I use it to calculate a theoretical rise in pressure in the middle cerebral artery at embolic occlusion at its first major lateral bifurcation, located downstream from the orifices of its perforating arteries.

A blood flow velocity (\(v\)) of 0.36 m/s in the middle cerebral artery during anesthesia\(^3\) is low. The density (\(\rho\)) of the blood is \(\approx 1050 \text{ kg/m}^3\). The compressibility (\(K\)) of blood may be close to that of water, 4.8 \(10^{-10} \text{ Pa}^{-1}\). At autopsy the internal diameter (\(d\)) of one undistended middle cerebral artery seemed to be \(\approx 2.2 \text{ mm}\) and its wall thickness (\(\delta\)) \(\approx 0.25 \text{ mm}\), both possibly underestimated. The elasticity modulus (\(E\)) of the artery may be unknown, and I use that of a rubber specimen, 5.5 \(10^5 \text{ Pa}\).\(^4\) If embolic occlusion is sudden, these figures result in a pressure increase (\(\Delta p\)) of 69 mm Hg transmitted upstream in the middle cerebral artery past the orifices of its perforating arteries.

To be sudden, the time of valve closure must not exceed twice the length of the upstream pipe divided by the velocity of the pressure waves of sound in the fluid in the pipe studied, which can be calculated from the data given.\(^7\) If a middle cerebral artery \(\approx 20 \text{ mm long}\) is held as the upstream pipe, the occlusion, to be sudden, must occur in 1.6 ms. If 80 mm of the carotid artery is included, 8 ms is sufficient. At high blood and embolus flow velocity, occlusion of the middle cerebral artery might be sudden in a physical sense.

During brain activity and emotional upset, brain blood flow velocity is higher than during anesthesia, and \(\Delta p\) is directly proportional to \(v\). The elasticity of the rubber may differ from that of the middle cerebral artery, the wall stiffness of which increases with age. In a model of the artery made of steel with a high \(E (2.1 \times 10^{11} \text{ Pa})\), the other figures result in a \(\Delta p\) of 3788 mm Hg. Fibrinoid changes of the small perforating arteries\(^1\) may increase their fragility.

The above supports the hypothesis\(^1\) that acute rises in brain blood flow velocity may trigger intracerebral hemorrhage: If combined with embolic occlusion of middle and posterior cerebral arteries downstream from perforating artery orifices, a high velocity ought to result in a local blood pressure exceeding that elsewhere in the circulation. Retrospectively, I regret that I, in cases of hemorrhagic stroke, never looked for downstream emboli. All factors in the equation can be quantified, and biophysicists might be able to test this hypothesis in models of the carotid-vertebral and cerebral arteries. The high frequency of primary hemorrhage in the brain compared with other sites might be related to the thicker and more resistant media of extracranial arteries of perforating artery diameter, but this quality of extracranial arteries of cerebral artery size may increase \(\Delta p\) (equation). This water-hammer mechanism is not dealt with in my textbooks of physiology. Medline gave 21 hits on “water hammer” (pulse, etc), but none dealt with primary intracerebral hemorrhage.

Göran A. Öhman, PhD (Laboratory of Heat Engineering, Åbo Akademi University), gave generous help but declined authorship, citing lack of insight in blood flow in humans.

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**Serial Measurement of Vascular Endothelial Growth Factor and Transforming Growth Factor-β1 in Serum of Patients With Acute Ischemic Stroke**

*To the Editor:*

Slevin et al.\(^1\) reported on elevated serum levels of vascular endothelial growth factor (VEGF) in patients with acute ischemic stroke. They claim to demonstrate a relationship between stroke volume and circulating VEGF (tissue damage, hypoxia, and VEGF expression) and concluded from their data that VEGF may play an important role in the pathogenesis of ischemic stroke and could be of value in future treatment strategies. A moderate correlation between peripheral blood leukocyte counts and serum levels of VEGF was noted.

These findings are interesting. However, in our opinion, several points need to be considered in order to interpret these results more accurately. Under physiological conditions virtually no soluble VEGF is present in the blood. The main transporter of VEGF in the blood stream are thrombocytes,\(^2\) and unfortunately no data on platelet counts are reported in the article by Slevin and colleagues. Platelets contain large amounts of VEGF (approxi-
mately 1.74 pg/10^6 platelets), and there is a tight correlation between blood platelet counts and serum VEGF, slightly influenced by the mean platelet volume. Thus, in serum samples, VEGF predominantly released from platelets during the in vitro clotting process is measured. When citrated plasma samples are analyzed instead (where platelets remain intact), only very low VEGF levels can be found. In patients with a massive platelet activation and destruction in vivo (eg, those suffering from idiopathic thrombocytopenic purpura and thrombotic thrombocytopenic purpura), we found elevated VEGF levels in both serum samples and citrated plasma (71 pg/10^6 platelets and 93 pg/10^6 platelets, respectively). Also, patients with acute myocardial infarction have high levels of circulating VEGF, most likely as a result of platelet activation in vivo (authors' own unpublished data). Thus, soluble VEGF measured in serum or plasma samples from patients with chronic vascular disease might reflect platelet activation and destruction. Activation and destruction of platelets occurs frequently in patients with cerebrovascular events, and therefore it seems likely that the elevated VEGF levels in stroke patients described by Slevin et al merely reflect this phenomenon, notwithstanding the fact that patients with stroke may have elevated platelet counts. Also, their finding that patients with coronary heart disease have the highest VEGF levels fits well into this assumption. Therefore, for a more accurate interpretation of their data on VEGF blood levels in patients with stroke, the appropriate control samples should be taken from patients with atherosclerosis instead of age-matched healthy individuals.

Finally, we would favor another interesting point, that is, the use of elevated VEGF blood levels as a screening parameter for fatal events in patients with chronic vascular disease. This issue is currently being evaluated.

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Response

Gunsilius et al suggest that patients suffering from acute ischemic stroke may have increased expression of blood platelets which could account for the increase in vascular endothelial growth factor (VEGF) concentration observed in serum, following in vitro clotting. Although we did not measure this parameter, blood platelet concentration has been measured in several studies in patients suffering from stroke. These studies demonstrated either a reduction in platelet expression or showed no statistical difference between control, healthy volunteers and stroke patients. A similar study showed a reduction in platelet concentration in patients suffering from large infarct (Li) compared with small infarct (Si), suggesting that greater tissue damage and stroke volume might in fact result in a reduction in circulating platelets. Furthermore, the authors went on to show that serum expression of TGF-β, also known to be synthesized in platelets, was not related to stroke or its severity. It is not likely, therefore, that increased expression of VEGF described in stroke patients in our study is associated with changes in blood platelet concentration.

Gunsilius et al also claim that the VEGF concentration in human platelets is approximately 1.74 pg/10^6, however, a figure of 0.56 pg/10^6 was reported recently, suggesting difficulty in accurate measurement of this parameter.

It is possible that increased activation and destruction of platelets in and around the infarcted area may contribute to the overall increase in serum VEGF, as Gunsilius et al suggest; however, there are many other well-documented sources. For example, VEGF expression is upregulated in neurons, endothelial cells, and astrocytes in the penumbra region after stroke. Similarly, activated macrophages and neutrophils associated with the acute inflammatory response also express and release significant quantities of VEGF. We believe that increased VEGF expression occurs as a result of a combination of these factors, and furthermore, the same argument may be applied to patients suffering from chronic vascular diseases such as atherosclerosis, where in the latter stages the inflammatory response coupled with platelet aggregation could result in generation of larger-than-normal quantities of VEGF. The pathological and biochemical changes that occur during atherosclerotic plaque formation are currently under investigation in our laboratories.

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Acute Ischemic Stroke Trials

To the Editor:

We congratulate Duncan and colleagues on their illuminating report regarding outcome measures in acute stroke trials. However, we wish to point out that their claim to have analyzed all “phase II and phase III randomized trials of pharmacological interventions in the acute phase of stroke, published in English in 1980 or later” may be overstated. They review only 51 trials. Our systematic review of all randomized acute ischemic stroke trials reported in English has identified a larger number. In our ongoing study of trends in acute
ischemic stroke clinical trial design, after review of MEDLINE, the Cochrane Stroke Review Group Database, the Ottawa Stroke Trials Registry, and other sources, we have identified 131 randomized, pharmacologic, acute ischemic stroke trials with full-length reports from 1900 to 1999, including 108 trials reported in the years 1980 to 1999.2,3 Similarly, Bath and colleagues4 identified a considerably larger number of trials in the years 1980 to 1996 than are contained in the report of Duncan and colleagues. The possibility that their identification of extant acute stroke trials was incomplete does not substantially alter the important insights offered by the study of Duncan and colleagues. However, we do feel it is important that stroke researchers generally appreciate the true scope of acute ischemic stroke clinical trials in the literature. We are happy to provide our unified list of acute trials on request.

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Response

Dr Saver and his colleagues were kind enough to provide me with their list of acute trials. I have carefully compared the list of trials generated by Dr Saver with those included in our study. The major explanations for the differences are that we did not include studies that were limited to safety and tolerability; 2 major trials1,2 were published in December 1999, after we had completed our review; and our search strategies were slightly different and produced some incongruities in the lists. For example, we included 4 studies in our review that are not listed in Dr Saver's database.

As Dr Saver and his colleagues noted, identification of other acute stroke trials does not alter the conclusions of the review. Neither the Atlantis trial nor the Citicoline trial used new outcome measures or selected unique cutoffs to define recovery.

Thank you for your interest in our study and your respectful challenges.

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Stroke. 2001;32:275-278
doi: 10.1161/01.STR.32.1.275

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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