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Letters

Comment

—to: Looker HC, Fagot-Campagna A, Gunter EW et al. (2003) Homocysteine as a risk factor for nephropathy and retinopathy in Type 2 diabetes. Diabetologia 46:766–772

To the Editor: We read with interest the recent paper by Looker et al. [1] who presented a study on homocysteine as a risk factor for nephropathy and retinopathy in Type 2 diabetes. We would like to add some comments and observations relating to possible underlying mechanisms that may be of interest.

Pathophysiologically, both diabetes mellitus and hyperhomocysteinaemia are associated with oxidant stress, in particular the formation of superoxide [2]. We found that in aortae of diabetic rabbits, homocysteine augments the impairment of acetylcholine-stimulated relaxation and cGMP formation, in vitro, but had no effect in age-matched control rabbits [2]. These effects were reversed with superoxide dismutase (SOD), indicating that homocysteine augments superoxide formation in aortae from diabetic animals. Since superoxide reacts with nitric oxide (NO) to form peroxynitrite (ONOO), effectively reducing the bioavailability of NO [2], this readily explains the observed effects in our studies. Since diminished NO formation is now firmly associated with vasculopathy [2], these data consolidate that homocysteine and diabetes mellitus interact to augment diabetic angiopathy, in part, through diminished NO formation.

These observations could also explain why increased concentrations of homocysteine are not always associated with diabetic angiopathy [3]. We showed that 10 µmol/l homocysteine (considered within the normal range in humans) is sufficient to inhibit NO formation in aortae from diabetic animals, whereas in aortae from non-diabetic animals, concentrations of homocysteine as high as 1 mmol/l are required to elicit similar effects [2]. Thus, plasma concentrations of homocysteine might not necessarily be at the "risk factor level" for homocysteine to have an angiopathic effect in diabetic patients.

We also established that the potency of homocysteine on the inhibition of NO-mediated relaxation was potentiated by copper in the normal rat aorta [4]. It was therefore suggested that it may not be the absolute concentrations of homocysteine alone but the relative concentrations of copper that determines the vaculopathic effect of homocysteine through an augmentation of the auto-oxidation of the amino acid [4]. It is of interest, therefore, that copper concentrations are increased in the plasma of patients with both hyperhomocysteinaemia and diabetes mellitus and that increased concentrations of copper are themselves a risk factor for cardiovascular disease [5]. It has also been reported that plasma copper concentrations are correlated with increased homocysteine concentrations in patients with peripheral arterial disease [6]. To consolidate this proposal, we found that copper augmented the impairment of acetylcholine-stimulated relaxation and cGMP formation in aortae from diabetic but not the control rabbits matched for age [7]. We are currently investigating homocysteine-copper interactions in the same animal model. All studies cited using animal models were given humane care in compliance with the National Institute of Health Guidelines for animal research and were approved by the United Kingdom Home Office.

Finally, since diabetes mellitus and hyperhomocysteinaemia are both associated with the overproduction of O_2^- and a reduction of NO formation in arterial tissue [5], it is reasonable to speculate that diabetes mellitus and homocysteine and copper exert an interactive negative effect on NO formation through a common denominator system(s). There are several candidate mechanisms: (i) diabetes mellitus augments the intra-arterial (auto)oxidation of homocysteine and therefore the generation of superoxide; (ii) homocysteine and copper further impair the already reduced activity of SOD and other antioxidant systems; (iii) homocysteine and copper increase the activity of enzymes that generate superoxide [e.g. nicotinamide adenine dinucleotide phosphate (NADPH) oxidase].

Further studies on the interactions between diabetes mellitus, homocysteine and copper on these and other systems are required to determine whether they play a role in diabetic angiopathy. These lines of evidence also point to the potential use of antioxidants, in particular SOD mimetics, to treat diabetic angiopathy.

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Abbreviations: SOD, Superoxide dismutase; NO, nitric oxide.

Comment

—to: Krebs M, Brehm A, Krssak M et al. (2003) Direct and indirect effects of amino acids on hepatic glucose metabolism in humans. Diabetologia 46:917–925

To the Editor: The paper by Krebs et al. [1] illustrates a potential difficulty in the interpretation of measurements of gluconeogenesis in man, which applies to the large number of published studies using similar techniques. In these papers, total gluconeogenesis is estimated by subtracting net glycogenolysis (measured by tracking hepatic glycogen content using nuclear magnetic resonance) from endogenous glucose production (EGP) (measured isotopically using [6,6-$^2H_2$]glucose). We have pointed out [2] that this calculation ignores the possible effect of hepatic intralobular functional heterogeneity.

The estimation of EGP depends on the rate of dilution of glucose-specific activity by new unlabelled glucose, the latter principally derived in the fasting state from hepatic periportal gluconeogenesis and hepatic glycogenolysis. As blood passes down the hepatic sinusoid, the specific activity of its glucose content is diluted by unlabelled glucose. During the subsequent passage through the perivenous zone, uptake of glucose [2, 3] reduces the amount of this decreased specific-activity glucose that eventually reaches the assumed single compartment volume of glucose distribution. Thus this compartment receives a lower amount of diminished specific-activity glucose than it would in the absence of perivenous glucose uptake (PVGU), and EGP is underestimated to varying degrees, depending on the conditions. It follows that when the rate of glycogenolysis is subtracted from EGP, the remaining new glucose production is underestimated.

Biological considerations suggest that the interpretation offered by Krebs et al. could be correct in the particular circumstances of their study. But the fact that technology permissible in man does not allow estimation of PVGU should not cause investigators to lose sight of the possibility that results obtained with techniques like those used in [1] could have a very different explanation from those commonly offered. The alternative explanation is that increases in EGP could be due to failure of PVGU, rather than increased gluconeogenesis. An example of this mechanism is seen in adult rats fetally programmed by maternal protein restriction to develop glucose intolerance; their perfused livers overproduce glucose, largely because of failure of perivenous glucose uptake as a result of localised perivenous depletion of glucokinase [2, 3]. In these studies measurements of hepatic glucose uptake, gluconeogenesis and glycogen were made directly, by methods not involving isotopic dilution. The point is of practical importance, as knowledge of the precise biochemical pathology could influence the development of therapeutic strategies in Type 2 diabetes.

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Abbreviations: EGP, endogenous glucose production; PVGU, perivenous glucose uptake.

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