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Nutrition Discussion Forum

How reliable and robust are current biomarkers for copper status? – reply by Danzeisen *et al.*

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The response by Brewer & Althaus to our recent review on biomarkers for Cu[1] bears testimony that the subject is topical and of public, scientific and commercial interest. It is valuable that a private company like Pipex Pharmaceuticals, Inc. is now interested in an area that was long thought to be peripheral to human health.

We acknowledge that there is disagreement regarding the prevalence of Cu deficiency and the use of ceruloplasmin (Cp) as a marker for marginal Cu status. This currently cannot be resolved.

Brewer & Althaus note that many symptoms of Cu deficiency cited by us are observed in animal models, and not in humans. This is a weakness inherent to fundamental scientific research, and the authors (Brewer & Althaus) must be aware that they base their own arguments regarding the role of Cu in Alzheimer's disease (AD) on no fewer than eleven animal studies. In addition, all cited studies showing a negative effect of Cu on the progression of AD are carried out in rabbits fed extremely high cholesterol levels in the diet, or in animals that are spontaneously hypercholesterolaemic. This rather artificial animal model is cited with surprising confidence by Brewer & Althaus in three instances.

There is no evidence to support the statement that 'copper in drinking water and copper in supplements [...] bypasses the liver for a time and is available to directly penetrate the blood brain barrier.' In contrast, Cu is thought to be taken up into the brain by ATP7A or Ctr1 in a controlled manner (see, for example, Nishihara *et al.* [2] and Kuo *et al.* [3]).

We strongly feel that the term 'free Cu' is inappropriate, as it has long ago been demonstrated that 'free Cu' is extremely unlikely to exist in biological systems. It is too reactive, and will be bound to proteins or amino acids immediately. We refer to a classic paper by O'Halloran *et al.*, which appeared in 1999 in the journal *Science* [4]. Brewer and colleagues use the term 'free Cu' to describe 'non-Cp-bound Cu', and we suggest that the more accurate term be used.

Ultimately, however, there may be less disagreement between us and Brewer & Althaus, as we refer to a recent publication by Brewer[5]: in this paper, Cp is mentioned as an acute-phase reactant (especially in atherosclerosis) and as a marker of inflammation, which in our view limits its usefulness as a biomarker for Cu. Brewer also correctly discusses that there is significant controversy over the role of Cu in the pathogenesis of AD. He mentions animal studies in which an increase in brain Cu due to a mutant ATP7B Cu transporter resulted in a reduction of Aβ in the brain[6], supplementation with Cu in an AD mouse model which lowered Aβ production and increased longevity[7], and human AD studies in which cognitive decline correlated positively with low plasma levels of Cu[8]. We agree with Brewer's fair assessment of this controversial issue.

Brewer further underlines the importance of Cu on page 327 of the same paper[5]: 'Deficiency leads to anemia and bone marrow suppression, followed by a neurologic syndrome called a myelopathy... the most bioavailable source of copper is in meat. [...] vegetarian diets are much more borderline in providing adequate copper...' And on page 328: 'It is clear, of course, that copper levels must not be lowered into the range where the activities of copper-dependent enzymes are affected, because that adversely affects the vasculature.' Again, we are in agreement with Brewer's comments.

Brewer & Althaus heavily rely on the recently published data by the Italian group including Squitti and Rossini as ultimate 'proof of concept' of elevated 'free Cu' in AD. Again, we do not disagree with those studies, and instead commend the careful discussion and caution employed by Squitti *et al.* in interpretation of their own data. This may reflect the fact that some of their studies have used highly sophisticated statistics to show what was not conclusive from the experiments. 'Non-ceruloplasmin bound copper ('free') seems slightly elevated in AD patients.' 'The reliability of copper as a marker of AD has yet to be proven and the debate on the toxic or protective role of this metal in AD is still ongoing.' 'Evidence of 'free' copper in AD is still scanty,...' 'The data collected at this stage of the research are surely not sufficient to draw conclusions about the effective implication of copper in AD.' These are all direct citations from Squitti *et al.* [9].

It is curious that Brewer & Althaus use the data described above for extrapolation not only to the development of a device called FreeBound to measure 'free Cu', but also to the use of a chelator (tetrathiomolybdate) binding 'free Cu'. The latter product is called COPREXA, and several press releases by Pipex in late 2007 have announced the testing of this product in pre-clinical and clinical AD studies.

The concept of Cu chelation in AD is not new, and has been thoroughly explored by Prana Biotechnology, a company based in Australia. After the first compound Clioquinol, a Cu chelator, failed in clinical trials, Prana's scientific co-founder is now publishing statements such as 'Based upon our current findings, to normalize brain metal levels in AD, a drug would need to increase copper levels and decrease zinc levels while preventing pooling of these metal ions in the amyloid mass.'[10]

We want to stress that the role of Cu in AD and many other diseases remains controversial, and that there is not enough data to justify the use of Cu chelation in AD. A Cu chelator cannot be expected to solve problems as diverse as AD, macular degeneration and fibrotic disease, as advertised by Pipex. While Cu can be toxic when present in excess, it is an essential nutrient. The 'removal' of any essential nutrient, including Cu, must be viewed with caution.

Conflict of interest

R. D. is an employee of the International Copper Association. The other authors are affiliated with independent universities or research institutes and have received research grants from the International Copper Association.

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