Host: This is the podcast from *Clinical Chemistry*. I am Bob Barrett.

In 1994, *Clinical Chemistry* reported that healthy volunteer's total plasma homocysteine concentrations were higher in serum than EDTA plasma. Since its publication, the paper by Dr. Donald W. Jacobsen and his colleagues has been cited more than 275 times.

The September issue of *Clinical Chemistry* included a Citation Classic article by Dr. Jacobsen that follows the mediator and marker controversy for homocysteine that's continued for more than ten years.

Dr. Jacobsen is currently working for the Department of Cell Biology at the Cleveland Clinic, and he is our guest in this podcast today.

Tell us Dr. Jacobsen, just what is homocysteine and plasma total homocysteine?

Dr. Donald W. Jacobsen: Homocysteine is a sulfur-containing amino acid and an intermediary metabolite produced in the methionine cycle which is found in all cells in the body. Homocysteine is a bio-marker for cardiovascular diseases, including coronary artery disease, cerebral vascular disease, and peripheral vascular occlusive disease.

It is also a bio-marker for dementia and Alzheimer's disease, complications of pregnancy, which would include pre-eclampsia and neural tube defects and osteoporosis and hip fracture.

The clinical diagnostic assays for homocysteine actually measure what is called plasma total homocysteine, and plasma total homocysteine is the sum of all circulating forms of homocysteine in blood. These include both reduced and oxidized species.

Reduced homocysteine with its sulfhydryl group is chemically very reactive in circulation and it participates in thiol-disulfide exchange reactions leading to the formation of disulfide that is oxidized species including homocysteine, homocysteine-cysteine mixed disulfide and predominantly protein-bound homocysteine. In normal plasma, approximately 80% of total homocysteine is found as protein-bound homocysteine.

Host: So then what is hyperhomocysteinemia?

Dr. Donald W. Jacobsen: Hyperhomocysteinemia, or elevated blood homocysteine, is a pathological condition in which blood levels of plasma total homocysteine rise. The normal range for plasma total homocysteine in adults is 5 to 12 micromoles per liter.

Unfortunately, this normal range does not consider the age factor. As we grow older, plasma total homocysteine increases from around five micromole per liter at birth to up to 25 micromoler per liter in extreme old age.

I strongly believe that ages just at normal ranges have to be used in clinical diagnosis, but this is rarely done. Premenopausal females have lower total homocysteine levels than their age match to male counterparts. This suggests that either muscle mass or hormones play a role in determining homocysteine levels.

I use three working ranges to categorize hyperhomocysteinemia. Mild would be 20 to 30 micromole per liter, moderate hyperhomocysteinemia 30 to 100 micromole per liter, and severe hyperhomocysteinemia is greater than 100 micromoles per liter.

In clinical studies that I participated in here at the Cleveland Clinic during the 1990s, we found that up to 50% of patients with coronary artery disease had mild hyperhomocysteinemia. Approximately 60% of hard transplant recipients developed mild hyperhomocysteinemia within two to six months, post-transplant. Nearly all patients with end-stage renal disease had mild-to-moderate hyperhomocysteinemia.

Host: So what causes hyperhomocysteinemia?

Dr. Donald W. Jacobsen: Hyperhomocysteinemia is caused by both genetic and acquired factors. Homocystinuria, a disease characterized by the excretion of large amounts of homocysteine in the urine, is an autosomal recessive disorder in which there are severe deficiencies of the enzyme, methylenetetrahydrofolate reductase, MTHFR for short, B12-dependent methionine synthase or cystathionine beta-synthase. These rare inborn errors of homocysteine metabolism occur in 1 to 100,000 to 1 in 50,000 live births.

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Patients with homocystinuria have severe hyperhomocysteinemia with levels ranging from 100 to 500 micromoler. Keep in mind that a normal level would be less than 12 micromoler. If left untreated, homocystinurics experience mortality and morbidity due to cardiovascular disease.

Now, there are less severe mutations in the genes for the enzyme MTHFR and MS, methionine synthase, and these result in polymorphisms that cause slightly elevated levels of total plasma homocysteine. Keep in mind that homocysteine metabolism is driven by five B-Complex vitamins.

Deficiencies of folate, Vitamin B12, and Vitamin B6 cause hyperhomocysteinemia. Individuals who are either folate or B12-deficient often have severe hyperhomocysteinemia. Lifestyle can also affect plasma levels of homocysteine.

Hyperhomocysteinemia is associated with chronic alcoholism, strict vegans usually have lower plasma homocysteine levels than individuals who consume large amounts of animal protein.

Host: Can hyperhomocysteinemia be treated?

Dr. Donald W. Jacobsen: Yes. The major cause of homocystinuria, the severe disease, is an enzyme deficiency of cystathionine beta-synthase. Now this enzyme is dependent upon Pyridoxine, or Vitamin B6, which participates in the transsulfuration pathway. It converts homocysteine to cysteine, that is the pathway does.

Approximately 50% of individuals who are deficient in CBS respond to pharmacological doses of Vitamin B6. Non-responders are treated by dietary methionine restriction, increased folic acid, Vitamin B12, and betaine.

Total homocysteine levels can be dramatically reduced by this treatment, but it is rarely normalized in individuals with CBS deficiency. Nevertheless, treated homocystinurics remain largely free of cardiovascular events.

Now what about treatment of mild hyperhomocysteinemia?

The answer is, definitely, yes. A cocktail of folic acid containing 1 to 5 milligrams, Vitamin B12, a half to one milligram and Vitamin B6, 50 to 100 milligrams will lower plasma total homocysteine in most

individuals by 20 to 25%. This homocysteine-lowering therapy as it's called, is the basis for numerous secondary intervention trials that have been conducted since the year 2000.

Host: With that in mind, is there any evidence that elevated total homocysteine plays a causal role in cardiovascular disease. Does it mediate disease progression, or is it merely a bio-marker of the disease process?

Dr. Donald W. Jacobsen: The answer is controversial. We know very little about mechanisms of homocysteine pathology. It is often suggested that elevated homocysteine limits the viability of nitric oxide or can cause oxidative stress and endoplasmic reticulum stress. And it can also decrease the so-called methylation potential, defined as the ratio of S-adenosylmethionine to S-adenosylhomocysteine.

But again, these suggestions lack mechanistic insight. The evidence of elevated homocysteine plays a causal role in the development of cardiovascular disease is strong in the case of homocystinuria where total plasma homocysteine levels range from 100 to 500 micromoler.

Our lab found that homocysteine could induce the production of monocyte chemoattractant protein 1 and interleukin-8, and these are potent chemokines that are involved in early atherosclerosis called “atherogenesis.” This study was done in human aortic endothelial cells.

One should consider also the ApoE knockout mouse, which is a model for spontaneous atherosclerosis.

When hyperhomocysteinemia is induced in these animals by dietary or genetic means, the rate of atherogenesis and disease progression is accelerated dramatically. But the actual mechanism of homocysteine toxicity is likely to involve its reactivity with proteins.

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Homocysteine and homocystine, that's an oxidized form, can attack cysteine residues in proteins and formed mixed disulfide adducts. These so-called S-homocysteine-related protein adducts may suffer loss of function.

Homocysteine, because of its unique structure can also form a five-membered ring called “homocysteine thiolactone.” And this form of homocysteine is also very reactive and can attack protein lysine residues and the products are called N-homocysteine-related protein. Again, there is a possible loss of function of these protein molecules.

Host: Well, if it's so easy to lower total homocysteine in patients with cardiovascular disease, then why have the homocysteine-lowering secondary intervention trials been so disappointing?

Dr. Donald W. Jacobsen: Again, this is another controversial area. Keep in mind the secondary intervention trials recruit subjects with advanced atherosclerotic disease. In advanced disease, homocysteine maybe a marker and not a mediator. I call this the mediator-to-marker hypothesis.

There is evidence that homocysteine mediates the progression of atherogenesis, that is, early disease, but becomes less important in advanced disease setting. But not all results from the secondary intervention trials are negative. In the HOPE-2 trial, the treatment group, that is, those receiving the cocktail of B vitamins to lower homocysteine had fewer strokes compared to those receiving placebo.

And also the decline and stroke mortality in North America increased dramatically after folic acid was introduced into the North American food supply between 1996 and 1998. This is in contrast to no such accelerated decline in European countries where fortification of foods with folic acid does not occur.

Keep in mind that the folate, that is, folic acid is a major determinant of plasma total homocysteine levels. And after fortification, these levels decreased in the population of North America.

Host: In your opinion, who should be tested for hyperhomocysteinemia?

Dr. Donald W. Jacobsen: Individuals that are at risk for developing cardiovascular disease are certainly candidates for homocysteine testing. So, if there is a strong family history of cardiovascular disease then I think these individuals should be looked at very carefully.

And you should look at the younger individuals, because treatment of hyperhomocysteinemia at a young age, which would be primary intervention,

may be beneficial. Keep in mind that homocysteine is a bio-marker of folate and B12 deficiency.

While folate deficiency is rare in North America since the introduction of folic acid to the food supply, Vitamin B12 deficiency is widespread, particularly among our ever-increasing elderly population. Therefore, the individuals suspected of having B12 deficiency should probably be tested, and actually should be tested for elevated total plasma homocysteine.

Because of our increase in the elderly population of this country, the occurrence of dementia and Alzheimer's disease will obviously increase. Homocysteine is actually a risk factor for these cognitive dysfunctions.

So testing for homocysteine in the elderly population should be done as well.

Host: Dr. Donald Jacobsen is at the Department of Cell Biology at the Cleveland Clinic, and he's been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.

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