



Assessing High-Dose B-Vitamin Therapy in Diabetes

Unexpected Result Indicates Worsened Outcomes

By Genna Rollins

Studies have linked high homocysteine levels to the risk of diabetic nephropathy, retinopathy, and vascular disease. Research also has shown that B-vitamin therapy can lower plasma concentrations of homocysteine and improve endothelial function, suggesting a role for this therapy in preventing vascular events and slowing the progression of diabetic nephropathy. This issue of Strategies reports on new research that explored the impact of B-vitamin therapy in diabetics.

About 40% of diabetics in the U.S. eventually develop diabetic nephropathy, a significant cause of chronic kidney disease that has a tremendous societal burden and enormous health consequences for individual patients. High levels of plasma total homocysteine have been associated with the risk of developing diabetic nephropathy, as well as retinopathy and vascular diseases such as stroke and myocardial infarction (MI). Studies have shown that B-vitamin therapy can lower homocysteine levels and improve endothelial function. Canadian researchers recently tested the hypothesis that high-dose B-vitamin therapy would slow progression of diabetic nephropathy and prevent vascular events (JAMA 2009;303:1603-9).

"There's very strong evidence that homocysteine is a risk factor for vascular disease, not only for heart attack and stroke, but also probably for diabetic small vessel disease. There's also evidence that diabetics have on average higher homocysteine levels and that those with higher levels have more small vessel disease," explained senior author J. David Spence MD, MBA, professor of neurology and clinical pharmacology at the University of Western Ontario and director of the Stroke Prevention and Atherosclerosis Research Centre at Robarts Research Institute in London, Ontario. "We thought that treating the homocysteine, which has been shown to impair endothelial function, might slow down the decline in renal function."

Spence and his colleagues tested their theory in the Diabetic Intervention with Vitamins to Improve Nephropathy (DIVINe) trial, a five-center study of 238 participants with type 1 or 2 diabetes and a clinical diagnosis of diabetic nephropathy. The subjects were randomized 1:1 to receive either a single B-vitamin tablet with 2.5 mg/d of folic acid, 25 mg/d of vitamin B₆, and 1 mg/d of vitamin B₁₂, or placebo. The participants were predominantly white men with type 2 diabetes and had a mean baseline glomerular

filtration rate (GFR) of 54.7 mL/min/1.73m², meaning that nearly two-thirds had at least stage 3 chronic kidney disease.

After enrollment, participants had clinic visits every 6 months for up to 3 years, with annual measurement of plasma total homocysteine, serum folate, and serum B₁₂. In addition, radionuclide GFR was measured at baseline, 18 months, and 36 months, and GFR also was estimated every 6 months using a 3-hour timed creatinine clearance started 1 hour after a single dose of cimetidine, which was used to prevent tubular secretion of creatinine. The researchers also estimated GFR using the Modification of Diet in Renal Disease formula. The primary outcome was progression of nephropathy as assessed by change in GFR. Secondary outcomes included start of dialysis, occurrence of vascular events and all-cause mortality, cognitive decline, and amputation.

The researchers found that participants receiving B-vitamin therapy experienced a mean decrease in total homocysteine of 2.2 μmol/L versus a mean 2.6 μmol/L increase in the placebo group, a mean difference of -4.8 μmol/L in favor of the B-vitamin therapy arm. This finding corresponded with significant increases in mean serum folate and vitamin B₁₂ at all time points in the treatment group.

While those results may have been expected, to their surprise, researchers found that patients in the treatment arm experienced greater declines in GFR, and a significantly greater number of cardiovascular events than those in the placebo group. In fact, the 36-month hazard ratio for a composite outcome of MI, stroke, revascularization and all-cause mortality was double in the vitamin therapy versus placebo group. "We found, to our astonishment, that the high-dose vitamins were associated with a worsening decline of renal function and a higher risk of the combined outcome of vascular events," said Spence. "We were completely flabbergasted. When I first saw the results, I thought we had the randomization code backwards. But then we also observed that these patients had significantly lower levels of homocysteine, so the homocysteine was being lowered even as the vitamins were being toxic."

While other studies have suggested potential harm or at least a neutral benefit from B vitamin therapy, the DIVINE trial is the first, to the researchers' knowledge, to show significant detrimental effects from pharmacological therapy with B-vitamins. The authors proposed at least three mechanisms for this outcome, including that folic acid may promote cell proliferation through its role in thymidine synthesis, that folic acid and vitamin B₁₂ may alter the methylation potential in vascular cells, and that B-vitamin therapy might increase methylation of L-arginine to the nitric oxide synthase inhibitor, asymmetric dimethylarginine.

Since publication of their study, the investigators have also received commentary that the problem may have been the form of vitamin B₁₂, cyanocobalamin, which they used in the study. "There was evidence published in Japan that there's release of cyanide from cyanocobalamin, which doesn't accumulate in healthy people because it's renally excreted, but it does accumulate in renal failure," said Spence. "The mechanism by which cyanide might adversely affect vascular function is that it could interfere with hydrogen sulfide, which is one of the newly recognized gasotransmitters, some of which are good for arteries, and some of which are not."

The results suggest that while lowering homocysteine levels remains an important therapeutic goal, the best means of doing so might not be through high-dose vitamin therapy, according to Elizabeth L. Frank, PhD, DABCC, associate professor of pathology at the University of Utah Health Sciences Center and medical director of analytic biochemistry and calculi at ARUP Laboratories in Salt Lake City. "It's well established that there's an optimum concentration of homocysteine—and it's low—but how you achieve that value, from this study, is not accomplished by taking a vitamin pill," she said. Frank also pointed out that although patients in the treatment arm of DIVINE experienced lower homocysteine levels from baseline, they did not reach what is generally considered the optimal concentration of $<11 \mu\text{mol/L}$. She was not involved in the study.

The study also underscores the challenges for laboratorians in working with clinicians to get a true picture of vitamin status, according to Frank. "One of the disconnects in vitamins and nutrition is that nutritionists think in terms of intake—recommended daily allowances or dietary reference intake. But in the laboratory, we measure concentration in plasma or whole blood and hope that it reflects an adequate amount of the active compound at the site of action of the vitamin, which is in the cell," she explained. "We can't necessarily measure the right form of the vitamin in the right specimen and we don't have a lot of information that links what we measure to consumption and intake."

Spence agreed that labs need to look closely at how they are measuring vitamins. "Maybe they need to think more clearly about how they're reporting the adequacy of vitamin B₁₂ levels," he suggested. "There's not enough understanding that a serum B₁₂ is not an adequate way to diagnose sufficiency or adequacy of B₁₂, because what's being measured is total B₁₂, not active B₁₂."

Spence and his colleagues are energetically pursuing the unexpected negative outcome associated with high-dose B-vitamin therapy. Already, his team is looking at the use of mesna, a thiol, as a homocysteine-lowering agent in patients with renal impairment. "The biggest advances come from surprises. When that happens, you have to say, 'wait a minute, what's going on?'" he said. "I suspect we'll be doing a lot of that in the future."

Dr Spence has received consulting fees from Pan American Laboratories and Medice Arzneimittel Pütter GmbH & Co. He also has, with others, a patent pending on the use of mesna, a thiol, to reduce homocysteine levels in patients receiving dialysis.