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New testing strategy detects population-wide vitamin and mineral deficiencies

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Johns Hopkins researchers have demonstrated that levels of certain proteins in the bloodstream may be used to estimate levels of essential vitamins and minerals without directly testing for each nutritional factor. The team's use of a new strategy allowed them to indirectly measure amounts of multiple nutrients in multiple people at the same time, an advance that should make it possible in the future to rapidly detect nutritional deficiencies of an entire population, apply remediation efforts and test their worth within months instead of years.

A summary of the study, which analyzed the levels of five vitamins and minerals in 500 undernourished Nepalese children, was published in the October issue of *The Journal of Nutrition*.

"Currently, levels of each vitamin or mineral are measured by different tests which are often performed in different labs, so the whole process can take three or four years to detect widespread deficiencies," says Keith West, Dr.P.H., M.P.H., the George G. Graham Professor of Infant and Child Nutrition. "That's too long to wait when the proper growth and cognitive development of children are on the line."

According to West, over 30 vitamins and minerals are essential to human health, and conventional methods for measuring their levels rely on running multiple different tests for each person. The time and cost involved are high enough to be entirely prohibitive at the population level (several hundreds of thousands of dollars), especially in developing countries, he says.

To overcome this barrier, the team focused on what all vitamins and minerals have in common: that each does its job by interacting with proteins throughout the body. Because methods already exist for simultaneously identifying the relative amounts of hundreds of proteins in a single sample of blood, the team wondered if some of those protein levels could be correlated with the levels of their associated nutrients, and thus act as "proxies" for the nutrients.

Using blood samples taken from 500 6- to 8-year-old Nepalese children, the researchers first analyzed the levels of vitamins and minerals according to conventional methods, and then they used a method called mass spectrometry to identify and quantify proteins levels in the same samples. They focused on five nutrients (vitamins A, D and E, and copper and selenium) and five proteins that were already known to be closely related to them. However, instead of performing a separate test for each nutrient's protein, they analyzed all five proteins, plus many others in the samples, in a single experiment.

"Mass spectrometry allows us to measure the quantities of 500 to 1,000 proteins in the blood at one time," says Robert Cole, Ph.D., director of the Mass Spectrometry and Proteomics Facility. "Not only that, we can mark all of the proteins from a single sample with a chemical tag that identifies them in the resulting data," he adds, and "because there are eight different tags available, we could tag eight different samples and then mix them together and analyze the eight samples at the same time, directly comparing the samples and saving a lot of time."

At the heart of their experiment, says West, lies the assumption that there are proteins in the bloodstream whose quantities reliably change with the levels of certain nutrients. For example, retinol binding protein (RBP) binds to vitamin A and carries it through the bloodstream to every part of the body, so the researchers theorized that levels of RBP in the children's blood would be a good proxy for their vitamin A levels. To

test this assumption, they compared their mass spectrometry results with those of conventional methods for measuring nutrient levels, and found that, for each nutrient, there were often not just one but several proteins whose levels were significantly correlated with the nutrient levels obtained by conventional means.

According to West, there is reason to believe that other vitamins and minerals will also have good proxy proteins. Their goal is to create a simple, portable test kit that would measure many proxy proteins from a single sample in a single test for under $100 per sample. "That would allow us to determine the level of nutrient deficiencies in a whole population within a few months," says West. "Then we could implement a remedy, like fortifying foods with particular nutrients—something tailored to the needs and habits of the particular population—and then follow up with more tests later to make sure the remedy is working."

The lure of easily and cheaply monitoring many nutrient-related proteins at once also opens the possibility that the new method could be used to monitor nutritional changes in a population over time. The team expects this technique could also be used to measure natural changes, like hormone levels, in healthy subjects and to track changes in protein levels that occur due to the progression of difficult-to-define diseases like Alzheimer's.

More information: dx.doi.org/10.3945/jn.113.175018

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