Methods in Micronutrient Metabolism

Editor's Note: This article is part of a series developed by the Nutrition and Dietetic Educators and Preceptors group on emerging topics of interest in dietetics education.

As nutrition students graduate and move forward in their careers, being able to decipher the details of nutrition research will be of the utmost importance if they are to stay current in the field. Many studies that provide the foundation for dietetics practice have their foundation in both animal and human studies. One area that can present a struggle for nutrition students and recent graduates, however, is understanding the methods used in the research. While we do not expect students and recent graduates to walk into a minerals or vitamins metabolism laboratory and be able to conduct research, having some insight into the relevant methods will facilitate their comprehension of research literature and allow them to critically evaluate the applicability of such studies to their dietetics practice. Surprisingly, references for students and nutrition educators to gain an understanding of the methods appear to be lacking.

This article will highlight the most common methods used at the laboratory bench, and give examples wherein each of these methods is used in micronutrients research.

LABORATORY METHODS USED IN MICRONUTRIENTS RESEARCH

As a nutritionist searching for a description of micronutrient laboratory methods, what one will find is a plethora of resources written in the form of laboratory methods in chemistry, biochemistry, molecular biology, and immunology. While it is true that these resources provide the basic tools for the micronutrients laboratory, a publication aimed at a nutrition audience is lacking.

Studies of micronutrients are generally performed either in vivo (in whole animals) or in vitro (outside the whole animal, as in a test tube), with the purpose of examining the effects of nutritional excesses and/or deficiencies, mechanisms, and interactions. The types of analytical tools that are employed in the laboratory allow for the isolation and quantification of metabolites and often utilize tools that have their foundation in biochemistry, molecular biology, and immunology.

Centrifugation

Centrifugation provides a means of separating materials that vary in density, such as molecules and cellular components (eg, nuclei, mitochondria, lysosomes, endoplasmic reticulum), by spinning a solution that contains these items at many times the force of gravity. To separate cellular components, often ultracentrifuges capable of spinning at up to 2,000,000 times the force of gravity are utilized and the samples (placed in test tubes) are spun through a solution of increasing density (such as sucrose or glycerol) in a method called density gradient ultracentrifugation. With this method, components matching the density of the solution will partition into that layer. One can then collect the various fractions for additional analysis or treatment.

Chromatography

Chromatography is a technique for separating a mixture of compounds into individual compounds based on properties such as size, charge, or polarity. In the context of polarity or charge, the separation depends on the relative affinity of the components for a stationary phase relative to a mobile phase. Size separation takes advantage of the property of small molecules to move faster through a stationary matrix than large molecules. The stationary phase most often takes the form of a polymeric matrix that is packed into a column (column chromatography), whereas the carrier, mobile phase is most often liquid (liquid chromatography) or gas (gas chromatography). Various forms of liquid chromatography include high-performance liquid chromatography and fast protein liquid chromatography; while both use more advanced equipment, they work on the same basic principles. By changing the composition of the mobile phase, one is able to selectively release each compound from the stationary phase. By collecting samples from the end of the column at selected intervals, one can then confirm the composition of the samples with any number of methods. Chromatography is often used to isolate and purify compounds such as peptides or proteins. For more on chromatography, see Karp[1] and Heftman.[2]

Confocal Fluorescence Imaging

Confocal fluorescence imaging is one of many immunohistochemical methods that capture information about the cellular location of antigens based on the specificity of the antigen–antibody interaction. Confocal fluorescence imaging, in particular, provides a method of producing three-dimensional images of a specimen. Fluorescence from an illuminated specimen is captured through a pinhole to reduce out-of-focus fluorescence and thus enhance the resolution of the image.[1] Fluorescence imaging requires that the specimen or molecule of interest be labeled with a fluorophore, a molecule that, when struck with one wavelength of light, will emit light at a different wavelength. By selectively labeling molecules inside or on the surface of cells with different fluorophores, one can, for example, determine the relative distribution of molecules within the cell or their distribution on the cell membrane. To facilitate the aforementioned process, the fluorophore is most often

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conjugated to an antibody. Then cells are exposed to the fluorophore-conjugated antibody. The antibody will bind to its antigen, allowing localization of the antigen upon microscopic imaging. For further reading, visit the Optical Microscopy Primer.[3]

Electrophoresis

Agarose gel electrophoresis is a technique used to separate and determine the length of DNA and RNA strands. Samples containing the molecules are added to wells created in the agarose gel, and are separated by applying an electrical field that moves the negatively charged DNA and RNA molecules through the agarose gel matrix toward the positively charged anode. Shorter sequences of nucleotides travel more readily through the pores of the gel, while longer sequences will be retarded in their movement, thus creating a separation. Detection is often facilitated with a dye, such as ethidium bromide, that binds within the bases of the DNA or RNA. For an animation of the process, visit the DNA Learning Center at the Cold Spring Harbor Laboratory.[4]

Polyacrylamide gel electrophoresis is a technique that can be used to separate proteins in an electric field in a manner similar to agarose gel electrophoresis based on their size or charge. For size separation, proteins are coated with sodium dodecyl sulfate to give the proteins a negative charge. Detection of the separated proteins can be facilitated by a dye such as Coomassie blue. Detection and quantification of specific proteins can be enhanced with a method called Western blotting, which involves transferring the proteins from the gel to a nitrocellulose membrane, which is then exposed to antibodies for specific proteins of interest. Quantification of the amount of protein is facilitated by a secondary antibody–enzyme–substrate complex in which the addition of substrate results in production of a colored product, the intensity of which is proportional to the amount of protein present. Western blotting is thoroughly described by Mahmood and Yang.[5]

Enzyme Assays

Enzyme assays can be used to assess the status of nutriture of micronutrients of patients given the role of vitamins and minerals as cofactors in metabolic enzymes. In an enzyme assay, one measures the activity of the enzyme based on the appearance of product or disappearance of substrate, whose presence is often detected spectrophotometrically (see section on Spectrophotometry). In the laboratory, using samples obtained from a patient, these assays can be performed before and after the addition of a required cofactor, in which case a large increase in activity of the enzyme suggests that the patient may be deficient in that cofactor. For example, superoxide dismutase in red blood cells is a copper-dependent enzyme used to assess copper status. For more on enzyme assays, visit the UC Davis Chem Wiki.[6]

ELISA

Enzyme-linked immunosorbent assay (ELISA) is a method designed to quantify the amount of a specific protein or hormone (ie, antigens) present in a solution and takes advantage of the selective binding properties of an antibody to a single antigen. Briefly, ELISAs are often

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