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SYSTEMS BIOLOGY APPROACHES TO NUTRITION

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Summary

Systems biology is an integrative approach to the study of biology. It integrates information gathered from reductionist experiments and various high-density profiling tools to understand how the parts of the system interact with each other and with other external factors such as diet. The science of nutrition is well suited to a systems biology approach. The tools of systems biology can be applied to settings relevant to nutrition with the goal of better understanding the breadth and depth of the impact that changing nutrient status has on physiology and chronic disease risk. However, there are many challenges to appropriately applying the systems biology approach to nutritional science. Among the challenges are those related to cost, study design, statistical analysis, data visualization, data integration, and model building.

Introduction

Reductionism versus Systems Biology: A Changing Paradigm

Nutrition requires an understanding of disciplines such as physiology, cell biology, chemistry, biochemistry, and molecular biology among others. In contrast to this broad view, we apply reductionist experimental approaches to advance our understanding of specific nutrient functions. However, while these approaches have been useful, significant issues limit their utility. For example, it can be difficult to translate mechanism-focused research in cells into the complex physiology of a whole organism. As a result, biological models developed from reductionist experiments often fail to explain why gene knockout mice studies do not have the expected phenotype (e.g. the facilitated diffusion model used to describe intestinal calcium absorption is being challenged by the results from calbindin D_{9k} and TRPV6 knockout mice (Benn *et al.*, 2008; Kutuzova *et al.*, 2008)). Even after extensive examination of a problem with reductionist approaches, we often find that gaps exist in our understanding. It is clear that re-applying the approaches we have traditionally used to investigate nutritional questions is unlikely to yield a different outcome. Because of this we need new approaches that complement traditional reductionist approaches but which give us a new, broader perspective of how nutrients are influencing human biology. Systems biology is such an approach.

Systems biology has been described as an approach to biological research that combines reductionist techniques with an "integrationist" approach to identify and characterize the components of a system, and then to evaluate how each of the components interacts with one another and with their environment. The goal of the systems

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TABLE 1.1 Definitions related to systems biology

Term	Description
Genomics	The study of the genomes of organisms including influences of DNA sequence variation on biology and the impact of modifying DNA and histones on DNA function (i.e. epigenomics)
Transcriptomics	The study of transcripts from the genome including messenger RNA and non-coding RNA such as micro RNA
Proteomics	The study of proteins in a biological system including their level, location, physical properties, post-translational modifications, structures, and functions
Metabolomics	The study of the unique chemicals (metabolites) that are produced as a result of cellular processes, e.g. small molecules such as lipids, metabolites of intermediary metabolites
Ionomics	The study of the mineral nutrient and trace element composition of an organism
Next-generation sequencing	High-throughput DNA sequencing technologies that parallelize the sequencing process thereby producing millions of sequences at once
Cluster	A graphical representation of relationships between data based on similarities in their concentrations or changes in concentrations
Pathway	A graphical representation of biological data organized on the basis of accepted relationships (e.g. glycolysis; signaling through the insulin receptor; lipoprotein transport)
Network	A complex graphical representation of biological data that is developed from the experimental data. This will include known relationships (pathways) and new relationships linking pathways

biology approach is to integrate many types of information so that you get a more complete view of a system (Kohl *et al.*, 2010). This definition has a flexibility that is very attractive to nutrition. The notion of a "system" can be applied narrowly to a cell, where the parts are individual biochemical and signaling pathways and the "environment" is the growth factors and hormones that regulate these pathways. However, it can be applied more broadly to a person, where the integration relates to the physiologic systems and the "environment" is lifestyle variables such as diet. For example, we know that calcium influences bone metabolism *but* we know that this relies upon the efficiency of intestinal calcium absorption and renal calcium excretion as well as on hormones produced at various sites (e.g. PTH in the parathyroid gland, 1,25-dihydroxyvitamin D in the kidney). Thus, our understanding of how dietary calcium intake influences bone is enhanced by looking at the interactions between multiple tissues rather than just focusing only on bone.

Systems Biology as Discovery Tool

Systems biology is an approach but within this approach are also three classes of novel tools necessary for a successful systems biology analysis. First, there are the high-density phenotyping platforms that allow simultaneous measurement of whole classes of biological compounds, i.e. omics methods such as genomics, transcriptomics, proteomics, metabolomics, and ionomics (Table 1.1). Next, the information from these platforms must be analyzed to identify the important changes resulting from a treatment. This requires the application of sophisticated statistics. Third, the information must be annotated and integrated with prior knowledge: this is the field of bioinformatics.

Systems Biology and Omics Tools for Biomarker Discovery

Omics analyses are often used to profile a biological state and then essential elements of the profile are used as a biomarker. Theoretically, the more independent traits one incorporates into a biomarker, the less likely it will be that the biomarker will be influenced by extraneous/confounding factors. To illustrate this point we can look to the field of iron metabolism. Nutritional iron status can be evaluated by measuring serum ferritin (high ferritin = high iron status) but this parameter is confounded by chronic inflammation (high inflammation = high ferritin) that can mask iron deficiency (Wang *et al.*, 2010). The serum levels of other proteins are also affected by the changes in iron status, e.g. hepcidin (high levels = high iron status) and soluble transferrin receptor (low levels = high iron status). Whereas hepcidin is affected negatively by inflammation (Nemeth and Ganz, 2009), transferrin receptor is not (Beguin, 2003). Thus, by simultaneously assessing the serum levels of ferritin, transferrin receptor, and a serum marker of chronic inflammation (e.g. C-reactive protein), one can assess iron status and remove the confounding caused by the inflammation associated with acute or chronic disease. The approach of using omics to identify measurements that can be combined to make an effective biomarker has been applied to the assessment of certain cancers (Sikaroodi *et al.*, 2010) and some argue that this approach may be useful for the assessment of nutrient status or of nutrition-related conditions that have proved resistant to the single marker approach (e.g. micronutrients such as zinc) (Lowe *et al.*, 2009).

Use of Systems Biology to Define New Modes of Regulation by a Nutrient or Metabolic State

A second way to use systems biology is to identify the groups of genes/transcripts/proteins/metabolites coordinately regulated under specific conditions. These groups could be organized within known biological pathways or as random groupings driven by statistical correlation, i.e. networks that expose new relationships not previously recognized from traditional reductionist research.

Understanding the Systems Biology Approach

It is an over-simplification to imply that there is just one way to do systems biology research but this section will attempt to provide a framework for approaching a nutritional research problem from the systems biology perspective (see Figure 1.1 for a summary of the steps in the framework).

Experimental Design

This is the single most important step of a systems biology research project for several reasons (Allison *et al.*, 2006). First, adequate experimental planning is necessary to focus the research and to use resources efficiently. One will likely need multiple time points to collect data on multiple phenotypes. For example, early time points may be more informative for measurement of direct transcriptional regulation (e.g. using transcriptomics or chromatin immunoprecipitation coupled to high-density DNA sequencing

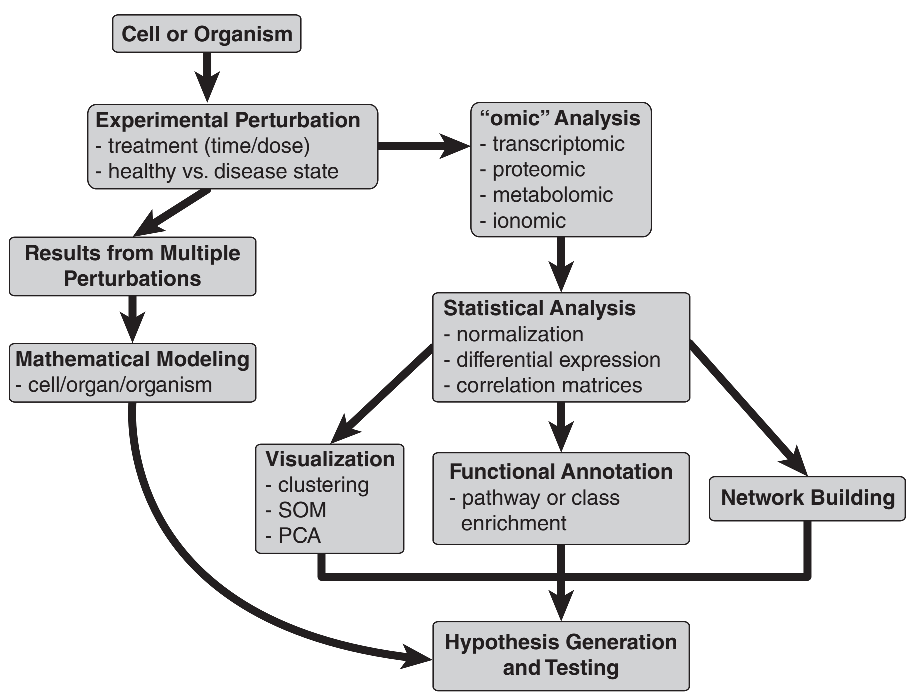

FIG. 1.1 An overview of the steps in a systems biology analysis.