Symposium: Improving Human Nutrition through Genomics, Proteomics and Biotechnologies

Personal Metabolomics as a Next Generation Nutritional Assessment[1,2]

J. Bruce German,*†[3] Matthew-Alan Roberts** and Steven M. Watkins‡

*Nestle Research Center, Lausanne 26, Switzerland; †University of California, Davis, CA 95616; **Nestle Purina Pet Care, St. Louis, MO 63164; and ‡Lipomics Technologies, Inc., West Sacramento, CA

ABSTRACT Nutrition research is in the process of addressing a series of questions related to the future of diet and health. Are all humans the same with respect to their response to diet? If not, humans must be fed differently according to the differences in their genetics and metabolic needs. Are those differences self-evident to the individual or their care-givers? If not, methods must be developed to measure the basis of differences between humans. Are the current sets of diagnostic biomarkers for disease appropriate and sufficient to distinguish the appropriate diets of humans for optimal metabolic health? If not, metabolites must be measured such that the differences in human metabolism are resolvable before they become diseased. Will a small subset of metabolic markers provide an indication of intended and unintended effects of diets that relate to overall metabolism? If not, comprehensive metabolic analyses (metabolomics) must be put in place to ensure that all aspects of health are accurately assessed. Inappropriate dietary choices are accelerating the development of chronic metabolic disease and threatening to overwhelm public health's ability to manage them. Nutrition and food sciences will need to collaborate with other scientific disciplines to develop and implement metabolic assessment technologies and to assemble annotated databases of metabolite profiles in humans, thus building the knowledge needed to link metabolism to diet and health. Biochemical and physiological research must be guided to define the mechanisms by which diet interacts with metabolism in different individuals. Integrating metabolism with the genetic and dietary variables that affect health is the role of nutrition sciences. Integrating personal nutritional value with food's other key values of safety, quality, comfort, delight, convenience and affordability is the role of food science. It is time for these two fields to address a common problem, metabolic health, with coordinated solutions. J. Nutr. 133: 4260–4266, 2003.

KEY WORDS: • *lipomics* • *nutrition* • *lipids* • *genetics* • *metabolite*

The arrival of the human genome has changed the way biological research is conducted and will soon change the way science is applied to nutrition and health. Nutrition research and its public health applications have achieved a major impact on the prevention of diseases caused by deficiencies of essential nutrients. The absolute quantitative levels of essential nutrients that are necessary in the diet to prevent these conditions have been established for the human population at various stages of growth and physiology and are the basis of population-wide surveillance and intervention (1). The diseases that are produced by a deficiency of each of these nutrients have been characterized and point-of-care diagnostics developed to recognize those individuals who are actively experiencing deficiencies (2). Finally, the risks of deficiencies of essential vitamins, minerals, amino acids and fatty acids have been successfully reduced by multinational programs of fortification, enrichment and process modification throughout the food industry, and population-wide dietary guidelines and food education programs have been aimed at the consuming public. Nevertheless, variations in food choices throughout the world continuously lead to the emergence of subpopulations at risk of deficiencies of essential nutrients emphasizing that constant personalized surveillance remains important (3).

Unfortunately, identifying nutrient deficiencies does not necessarily resolve all nutritional problems. New sets of issues associated with diet continue to emerge. Rather than diseases caused by deficiencies of essential nutrients, these new health problems are the result of dietary imbalances and the inability to control metabolism accurately within a range of lifestyles (4). Metabolic disturbances involve more than the essential nutrients; they extend to macronutrient fuels and nonessential nutrients. The rapid increase in the incidence of metabolic disorders of energy regulation, from obesity to diabetes and

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[3] To whom correspondence should be addressed.
E-mail: jbgerman@ucdavis.edu.

atherosclerosis, now includes the majority of citizens of many countries including the most developed countries of the world (5). Such prevalence has caused the public and its oversight health agencies to reevaluate all aspects of environment and lifestyle, from diet to exercise to leisure activities (6). While many common themes are emerging, scientists are agreed in one aspect—no single causal agent is responsible for the problem in all persons affected (7). That is, the same diet practiced by one individual may support their health and quality of life and yet this same diet practiced by another may lead to obesity and subsequent metabolic problems compromising their quality of life. The genetic basis of these differences is being established in the newly emerging fields of genomics, nutrigenetics and nutrigenomics (8). Nevertheless, even before all of the genetic and molecular reasons that can explain the differences between individuals are discovered, it is clear that diversity of the human population is a nutritional reality. Once this diversity is realized, it becomes imperative that the problems of metabolic regulation, and their causes and interventions, will need to be personalized in order to be addressed and finally solved.

Metabolic phenotype

Metabolic phenotype can be described by the simple genetic relationship relating phenotype to the variables that control it.

$$\text{phenotype} = \text{genotype} + \text{environment} + \text{genotype} \times \text{environment},$$

where phenotype is the sum of all functional attributes related to health, *viz.*, "The observable physical or biochemical characteristics of an organism."

Genotype

Genotype is the sequence of all genes and typically considered to constitute all of the polymorphisms within an individual as well. The burgeoning fields of nutrigenetics and nutrigenomics, though still being defined, are already bringing detailed sequence information to the measurement of precisely how individuals vary genetically, and what these differences mean in terms of function and genetic potential (9,10). Nutrigenetics refers to the specific gene sequence differences between humans and how these affect the differences in responses to diet and particular needs for nutrients. Nutrigenomics is the study of the effects of diet on the expression of all genes and their functions.

Environment

Environment is the sum of all external variables, including diet, lifestyle and not to be forgotten, coexisting organisms. Each of the environmental variables has interesting effects as sources for fuel, nutrients, metabolites and of course, inducers and attenuators of stress. The role of the various organisms that each individual lives with—from family and companion animals to the commensal and pathogenic bacteria that occupy intestine, skin and epithelial surfaces—is only now being studied scientifically. However, the striking correlation of noncommunicable diseases between family members and even extending to companion animals argues for a more quantitative approach of this aspect of environment and health (11). An important goal of nutrition is to bring these input variables of food, lifestyle, etc., into much more detailed and personally quantitative datasets (12).

Metabolic memory

The term Genotype Environment requires some additional definition as it applies to metabolism. Metabolism is not static. Throughout life, environment exerts a continuing and varying influence on the expression of an individual's genotype. The nutrition community is beginning to address the implications of this process and the persistence of phenotypic traits related to diet, and their largely deleterious consequences have led to the emergence of the fields termed nutritional imprinting (13,14) and nutritional programming (15). In point of fact, the biological plasticity of metabolism is not a rare effect related only to the detrimental imprinting of metabolism in response to inappropriate diets. Biological organisms possess myriad mechanisms to learn from the environment to which they are exposed, including diet, and adapt a variety of structural, biochemical and regulatory processes to improve their responses to this environment (16,17). Thus, this adaptation is much more appropriately referred to as metabolic memory, reflecting the fact that these metabolic adaptations are as varied, complex and valuable as other memory processes. Humans (as well as all animals to varying extents) enjoy a remarkable capacity for metabolic plasticity that allows them to adapt successfully to dietary and lifestyle niches. Again, while nutritionists tend to emphasize the potential of adverse diets to imprint an individual to a predisposition to particular diseases, metabolic memorization processes underlie such disparate but positive responses as athletic training and olfactory preference (18,19).

Phenotypic variation in humans

Phenotypic differences among humans are determined by extensive biological variation in each person's genotype, environment and metabolic memories. With such an extensive basis for human variation, it seems unlikely that dietary recommendations will remain similar across the entire population. As the vivid images captured in **Figure 1** (Sports Illustrated, 2002) illustrate, healthy humans are not all the same. This photographic depiction of many of the leading male and female athletes in various sporting events makes another important point—humans do not all want to be the same! It should not be the goal of nutrition to build a set of recommendations to constrain humans to be more the same. It is the goal of nutrition science to build a body of knowledge to enable humans to achieve the optimal health status within a lifestyle that each aspires to for themselves.

Assessment strategies for personalizing metabolic phenotype

The current interest in personalizing health stems from the breakthroughs emerging in genomics and the identification of genetic polymorphisms that are associated with variations in many aspects of health, including diet-related diseases (20). Eventually, the genetic variation in humans will be a vital dimension of all aspects of health and not just limited to disease prediction and intervention. However, it is not necessary to wait for a complete genetic understanding of personal variation to begin to intervene in personal health. In fact, as summarized above, genotype by itself cannot and never will completely predict health status. Therefore, more precise measures of the variation in metabolism must be developed and

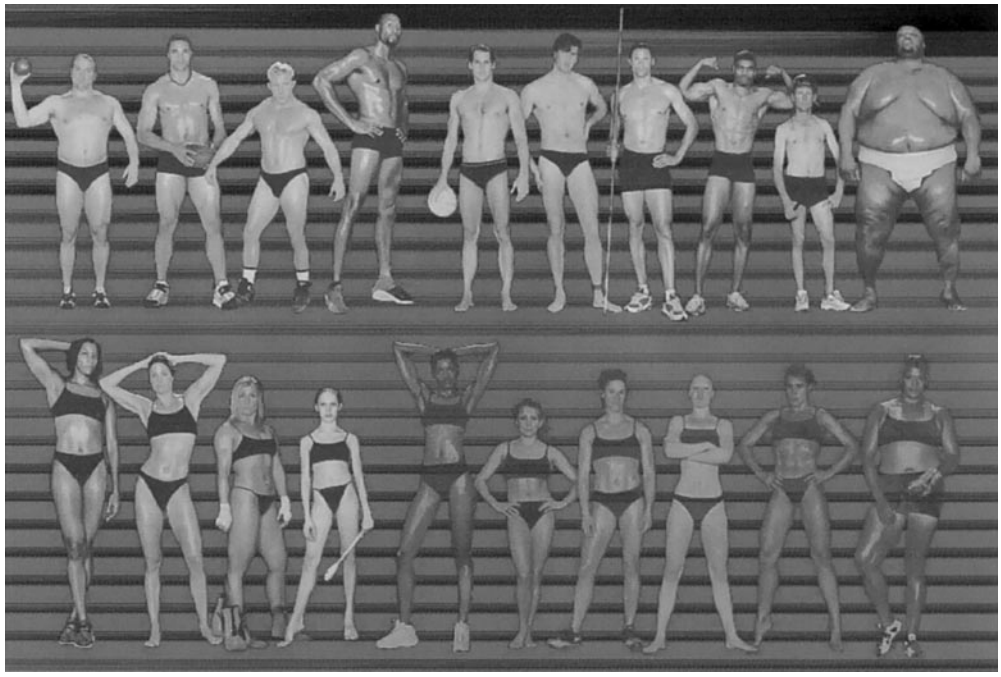

FIGURE 1 Some of the elite athletes of various sporting activities from Sports Illustrated 2002.

implemented. Personalizing metabolic health is not new and has been a core principle behind such programs as the National Cholesterol Education Program in the U.S. (21). Lessons from this national health program are to *1*) develop technologies that are capable of measuring blood levels of cholesterol in humans, *2*) use the analytical methods to measure a significant range of the population along with appropriate phenotypic endpoints, *3*) interrogate the resulting database for the quantitative relationships between levels of cholesterol and phenotypic outcome, and *4*) provide individual consumers and their health professionals with the technologies to measure their cholesterol. With this system in place, various industries were enabled to provide consumers the means to alter their cholesterol levels.

The principles of individual cholesterol measurement and action are applicable to all aspects of metabolic regulation. The basic steps are illustrated in **Figure 2**. Technologies for broad scale and parallel analysis of metabolites need to be developed. The technologies, once applied to large clinical assessments of populations, can produce databases that are simultaneously annotated according to various aspects of the effective phenotypes of the individuals involved. These databases provide information resources to explore the statistical relationship between concentrations of metabolites in individuals and their phenotypic outcomes. Scientists, with their access to all the tools of modern biology and genomics, will apply them to molecular, cellular or whole animal models of metabolism to establish the mechanisms that are responsible for these relationships. The knowledge that is being built from this process will become the guideposts to provide each indi-

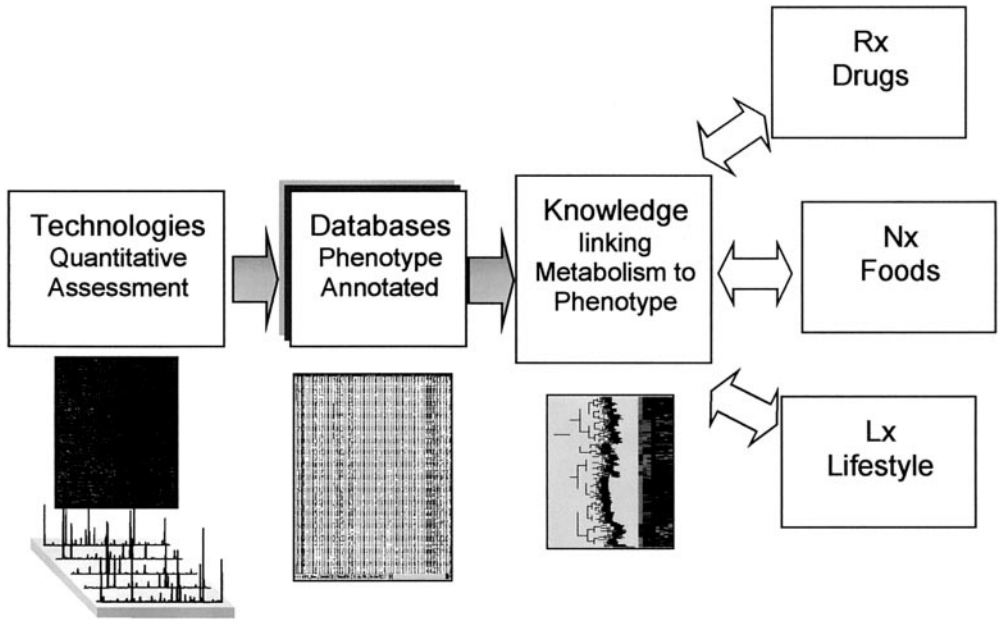

FIGURE 2 The steps to personalized assessment. The three steps that are needed to bring personalized assessment forward as the means to guide health are: first, to establish analytical technologies capable of measuring metabolites quantitatively in representative body fluids such as blood; second, to apply these technologies to the measurement of metabolites in representative populations of humans building databases that combine the individual metabolic measurements with various phenotypic endpoints; third, to develop the biochemical and metabolic knowledge to be able to alter metabolism in particular directions. Once in place these databases coupled to their annotation and metabolic knowledge will serve as predictive knowledge resources for the pharmaceutical industry (Rx), the food industry (Nx) and the leisure activity industry (Lx).

vidual actionable advice based on their own personal metabolic profile. The cholesterol program was successful for drug development but did not go far enough for foods by deciding to focus on a single metabolite and by assuming that the same mode of intervention would be successful for all individuals. Assuming that cholesterol could be acted upon in isolation was a mistake because the simple presence even of relatively high levels of cholesterol in blood does not constitute a disease. Therefore, any action designed to change cholesterol should ensure that metabolic regulation as a whole is not compromised. Various authors are beginning to argue that focusing health recommendations based solely on cholesterol has been a problem with recommending low fat diets to all individuals. While assisting many individuals to lower their cholesterol, such a dietary change precipitated a shift in metabolism that aggravated obesity and diabetes in others (22,23).

Assessment and health

The traditional tools of nutritional assessment that were designed to develop data on the relationships between diet and health have largely taken the perspective of understanding how single nutrients are associated with overt diseases. With such a perspective, single biomarkers of disease were adequate as outcome variables. Now, with the challenges of understanding metabolic health within individuals, it is necessary to take a more precise, yet broader approach. It is important to define both the input variables of foods as parts of complete diets and the outcome variables of integrated metabolism. This sounds like a daunting challenge, however, the technologies to obtain the data are available. To date, nutrition researchers have not addressed the acquisition of metabolism-wide data sets as output variables in nutrition clinical trials. This lack can now be addressed.

The experience with cholesterol reveals the importance of measuring a larger number of metabolites as metabolism assessment rather than a single biomarker or subset group of biomarkers as disease risk. While measuring cholesterol per se can provide a quantitative estimate of disease risk in a population and an individual, the simple measurement of cholesterol does not provide sufficient information to deduce why that individual accumulated the cholesterol nor does it suggest the appropriate intervention to resolve the problem. As an example, cholesterol can be high in the blood of an individual due to several mechanisms, but three are illustrative: *1*) the individual can absorb cholesterol inordinately well through the intestine, or *2*) they can produce too much through endogenous biosynthesis, or *3*) they can fail to convert cholesterol to bile acids sufficiently. The measurement of total blood cholesterol does not distinguish these three, but if the analytical measurements are simply expanded to include more sterol metabolites, it is possible to obtain sufficient information. Those who absorb excessive cholesterol absorb both cholesterol and phytosterols more than the average individual (24), and the levels of phytosterols in plasma reflect absolute absorption of sterols from the intestine. Individuals who hyperabsorb can be distinguished by including the quantitative analysis of phytosterols in cholesterol measurements. For these individuals, an intervention strategy that targets intestinal absorption of cholesterol is appropriate (25). Those individuals who produce excessive quantities of cholesterol via endogenous biosynthesis have increased levels of mevalonic acid in plasma as a direct quantitative reflection (26), and for these individuals, inhibitors of cholesterol biosynthesis are more appropriate. Finally, the causal mechanisms of insufficient bile acid conversion are detected in those individuals by the amounts of 7alpha-hydroxy-4-cholesten-3-one in plasma (27).

Comprehensive metabolomic analysis of plasma lipids

The goal of metabolomics is to prepare a comprehensive dataset of every metabolite within a given biological sample. Such an ambitious goal is already possible for gene expression transcriptomics via microarrays and at least conceptually possible for proteomics via 2-D electrophoresis-mass spectrometry. Unfortunately, given the wide dynamic and chemical range of small metabolites within biological samples, it is not yet possible to address all metabolites within a single analytical platform. However, it is possible to divide metabolites into specific classes, analyze these and then reassemble the data electronically. By modifying straightforward analytical tools for structural lipids in blood and tissues to higher throughput capabilities (28), all lipid classes in blood, for example, can be quantified according to the mass of each fatty acid constituent. The technologies that are available at present provide the necessary qualitative and quantitative precision. That is, each and every fatty acid can be identified by its retention on defined chromatographic systems and mass spectrometry. The use of a flame-ionization detection system provides a highly quantitative output that is linearly proportional to compound mass over a wide dynamic range. The use of judicious internal standards provides the means to combine separated lipid classes into an overall highly quantitative analysis related to the original biofluid sample. These technologies provide absolute masses of each lipid class and fatty acid as a function of blood weight. These technologies are well developed, have been widely commercialized by various instrument manufacturers and the costs of acquisition and implementation of such systems is a small fraction of modern 'omic technologies. Nevertheless, because the technologies are mature, they are readily converted to automated relatively high throughput systems, and their inherent low cost makes it possible to run multiple analytical stations in parallel thus providing genuinely high throughput.

The technologies that are available to address most of the metabolite classes are equally as available as those for fatty acids and complex lipids. Thus, no significant technological hurdle stands in the way of using these technologies to assemble metabolite databases of humans and experimental animals for amino acids and small peptides, sterols, organic acids, sugars and alcohols, vitamins, nucleotides, etc. So long as the data are qualitative and quantitative, such data from various human and animal investigations are directly comparable, i.e., capable of producing legacy databases and appropriate for subsequent data mining. Studies conducted in separate laboratories, using entirely different analytical technologies years apart, will produce directly comparable data if the data are qualitative and quantitative. These data are thus analytical platform independent, and as the technologies needed for higher throughput are developed, they will build upon existing databases and not obsolesce them. An obvious consideration that such an approach raises is the importance of annotating subjects and animals that are the sources of the metabolic data. It will be possible to relate metabolism only to those physiological, health and behavioral endpoints that are measured coincident with the metabolic data. Researchers should be considering how to convert these endpoints to quantitative and comprehensive analyses to improve the ability of bioinformatics to mine the relationships between endpoint and metabolism. Rather than simply bias the outcome by including only disease endpoints, the databases should include multiple

biologic (physiological, neurological, genetic, anatomical, etc.), behavioral, cognitive and other dimensions of health that can then be used for examining specific hypotheses, and perhaps more importantly, for generating new hypotheses in a wide range of health.

Bioinformatics of metabolite profiles

Driven by rapid progress in genomics, the enormous task of cataloging, crossreferencing and organizing data for massive biological datasets is now being tackled by both academia and the private sector. These advances in information management have enabled a conceptual shift in the scientific study, from single object (e.g., a gene, a protein) to system studies, aimed at capturing the true complexity of biological systems through global analysis. While extremely important within this context, metabolic pathway information has been discouragingly underdeveloped relative to information on genes, proteins and regulatory elements (29) The primary causative factors in disease are often the altered biochemical composition of cells and tissues. Thus, the link between the gene regulatory control and the primary causative factors will be crucial for application in drug development, medicine, nutrition and other therapeutic courses of action. The identification of relationships between genes, transcripts, proteins and metabolites are essential components to understand integrative metabolism (30,31). The annotation of genes with such information has been attempted by a number of public efforts, most notably from Kyoto Encyclopedia of Genes and Genomes (KEGG) (32,33,34). Software is now available to superimpose analytical data onto said pathways, providing a powerful means to identify biological regulation of metabolism through the coexpression of gene data obtained from microarrays. GenMAPP is a particularly useful tool for such purpose, allowing the user to link pathway information to gene expression data (35).

A next step for integrating nutrition is to annotate the metabolome in order to relate changes in metabolites within specific pathways to genetic and environmental influences. The science is in many cases already well underway and only needs to be applied to this annotation task. For example to annotate the products of lipid metabolism, each metabolite and its absolute amounts in biofluids would be linked to mechanistic information about the various endogenous and exogenous inputs that alter its level in these same biofluids. The absolute amount of arachidonic acid is an example of a metabolite that illustrates the extent of the scientific information that already exists to annotate the biology of this molecule as a part of the metabolome. The dietary, genetic, hormonal, pharmacologic and toxicologic influences on gene expression of the lipid metabolic enzymes that produce and remove arachidonic acid have been studied from various perspectives. Growth hormone stimulates the expression of the delta 5 and delta 6 desaturase enzymes and growth hormone leads to increased abundance of the metabolic products of these enzymes, including arachidonic acid (36,37). Pharmaceutical intervention data will also be useful in these same annotations and again, such information has been the subject of pharmaceutical research. To continue using the same example of arachidonic acid, statin drugs affect the delta 6 and delta 5 desaturase enzymes that produce arachidonic acid causing a preferential increase in delta 5 desaturase (38). The effects of environmental toxicants on the metabolites and the pathways that produce them will provide further annotation information. Once again, many studies have produced data on the effects of toxins on arachidonic acid's metabolic pathways as illustrated by the research demonstrating the effects of PCBs on inhibiting the desaturase enzymes and reducing arachidonic acid levels in plasma (39).

Software tools will need to be developed to examine metabolites in a broad scope rather than as individual biomarkers. A first generation approach has been developed for lipid metabolites (28). This approach uses a straightforward calculation and visualization tool adapted from gene array analysis tools to take advantage of the growing familiarity of scientists with such three-dimensional visualization strategies. Visualization algorithms can be used to present fatty acid data quantitatively according to various criteria of lipid family, lipid class, tissue of origin and metabolic origin. The format of difference maps enables scientists to examine differences between samples across as many criteria as possible. Informatic software can visualize quantitative differences in various formats, such as color coding of changes between samples much as is used to advantage in visualizing gene array data. Additionally, extensive bioinformatic efforts will continue to target metabolic data via statistical clustering and quality analyses and ultimately will directly link metabolic pathways to all other phenotypic determinants as systems biology (40,41,42).

Examples of metabolic analysis and interpretation

Various laboratories are now collaborating—using the tools of modern biology including transgenic models, gene expression, metabolic pathway modeling, etc.—to propel scientific understanding of the metabolism of lipid classes in order to be able to interpret specific differences in blood lipids from humans and experimental animals. The goal ultimately is to provide predictive knowledge of potential interventions using food, drugs and lifestyle to improve lipid metabolism. Perhaps the most important lesson learned to date is that there is a need for the scientific community to come together to produce centralized metabolomics databases as the means to unify scientists around this aspect of biology.

A number of studies have now taken steps to observe the metabolic consequences of diet using 'omic approaches (43,44,31,45). In one study, the effects of PUFA, either alone or in combination, were studied using a combined genomic–metabolomic approach (31). This study showed clear evidence of both correlated gene function and lipid metabolite level, as well as other instances of disconcordance, where apparent gene expression changes were not reflected by predicted changes in lipid metabolites. Both cases are interesting, but it is the discordance that most likely points to a lack of understanding for the basic cellular and physiological controls on metabolism. New studies using a quantitative metabolic analysis combined with other 'omic techniques will continue to aid our understanding and complete our grasp of integrative metabolism.

The inappropriateness of relying on the traditional biomarker approach of disease diagnostics for metabolic dysregulation is shown in various recent studies. First, the use of a metabolic intervention may alter one target appropriately, but compromise metabolism elsewhere. Second, individuals may vary with respect to a particular dietary intervention. Third, the metabolic state of an individual can significantly affect the net metabolic consequences, and an individual in one metabolic state may be benefited by a particular intervention, while in another metabolic state—i.e., overweight—the same intervention may be net deleterious.

The effects of alternative pharmaceutical strategies on the metabolic condition of diabesity (the comorbidities of obesity and diabesity) illustrate the problems of ignoring disparate

metabolic consequences of a single intervention. The animal model (NZOxNO)F1 male mouse (46) provides a genetically modified experimental animal with many of the metabolic dysregulations of humans with these conditions. In affected animals, the three diagnostic markers typically used in humans (serum glucose, insulin and triglycerides) are all elevated at the same time that the animals become obese on specific diets. The pharmaceuticals roziglitazone, a PPAR agonist, is a successful drug for type 2 diabetes. Animals treated with high doses of roziglitazone responded with changes to the three biomarkers (glucose, insulin and triglycerides). These three biomarkers are considered to be the hallmark of a therapeutic benefit. The metabolic consequences to these animals, at the doses of roziglitazone applied, was to exacerbate hepatic lipidosis in the model. Metabolomic analysis (quantitative fatty acid analysis of all lipid classes) in liver, blood and muscle identified significant metabolic differences and highlighted the lipid metabolic abnormalities by which hepatic lipidosis was caused by roziglitazone (28).

Summary

Humans differ in their metabolic regulation, and the optimal diet for one individual is not necessarily the optimum for another. How can we know what is best for each? Personalized assessment will be necessary. As we move towards the goal of individualizing health, the traditional reliance on single biomarkers, a valuable strategy for disease diagnosis for decades, is inadequate for accurate surveillance and intervention in problems of metabolic regulation in healthy individuals. Measuring entire metabolic pathways is the ultimate scientific goal, and modern analytic technologies are in a position to deliver such capabilities. The challenge to biological research is to build the metabolic knowledge to understand metabolism as a whole and provide guidance to individuals to change their diets and lifestyles to affect metabolism in a net positive direction. One other aspect of the challenge would seem daunting. What is the scientific basis of a good diet? While many of our current ideas of optimal diets are based on epidemiological observations and anecdote, the results are not particularly compelling scientifically. The mediterranean diet is consistent with the longevity and relative health of individuals in this region; nevertheless, the confounding variables that may contribute to health of the peoples living in the Mediterranean area and that yet may be unrelated to diet are numerous. Also, an empirical approach examining a population's dietary habits yields neither mechanistic targets nor molecular solutions. What diets should serve as experimental models for scientific research to progress rapidly along molecular pathways? There is an existing alternative that is not based on existing dietary habits but evolution of biological purpose for nourishment: milk. Milk evolved as a biofluid to selectively nourish mammalian offspring. The lessons of this molecular evolution provide the means to establish mechanisms and targets for foods with net positive nutritional impact and scientific strategies to understand the relationship between diets and sustained health (47).

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LITERATURE CITED

1. Barr, S. I., Murphy, S. P. & Poos, M. I. (2002) Interpreting and using the dietary references intakes in dietary assessment of individuals and groups. J. Am. Diet. Assoc. 102: 780–788.

2. Jensen, G. L. & Binkley, J. (2002) Clinical manifestations of nutrient deficiency. J. Parenter. Enteral. Nutr. 26(Suppl): S29–S33.

3. Berner, L. A., Clydesdale, F. M. & Douglass, J. S. (2001) Fortification contributed greatly to vitamin and mineral intakes in the United States, 1989–1991. J. Nutr. 131: 2177–2183.

4. Puska, P. (2002) Nutrition and global prevention on non-communicable diseases. Asia Pac. J. Clin. Nutr. 11(Suppl): S755–S758.

5. Alberti, G. (2001) Noncommunicable diseases: tomorrow's pandemics. Bull. World Health Organ. 79: 907.

6. World Health Organization Process for a Global Strategy on Diet, Physical Activity and Health February (2003) World Health Organization, Geneva, Switzerland (www.bookorders@who.int).

7. Chagnon, Y. C., Rankinen, T., Snyder, E. E., Weisnagel, S. J., Perusse, L. & Bouchard, C. (2003) The human obesity gene map: the 2002 update. Obes. Res. 11: 313–367.

8. Barsh, G. S., Farooqi, I. S. & O'Rahilly, S. (2000) Genetics of bodyweight regulation. Nature 404: 644–651.

9. Muller, M. & Kersten, S. (2003) Nutrigenomics: goals and strategies. Nat. Rev. Genet. 4: 315–322.

10. van Ommen, B. & Stierum, R. (2002) Nutrigenomics: exploiting systems biology in the nutrition and health arena. Curr. Opin. Biotechnol. 13: 517–521.

11. Butterwick, R. F. & Hawthorne, A. J. (1998) Advances in dietary management of obesity in dogs and cats. J. Nutr. 128(Suppl.): 2771S–2775S.

12. Pelto, G. H. & Freake, H. C. ASNS Long Range Planning Committee (2003) Social research in an integrated science of nutrition: future directions. J. Nutr. 133: 1231–1234.

13. Levin, B. E. (2000) Metabolic imprinting on genetically predisposed neural circuits perpetuates obesity. Nutrition 16: 909–915.

14. Waterland, R. A. & Garza, C. (1999) Potential mechanisms of metabolic imprinting that lead to chronic disease. Am. J. Clin. Nutr. 69: 179–197.

15. Singhal, A., Wells, J., Cole, T. J., Fewtrell, M. & Lucas, A. (2003) Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? Am. J. Clin. Nutr. 77: 726–730.

16. Minsky, A., Shimoni, E. & Frenkiel-Krispin, D. (2002) Stress, order and survival. Nat. Rev. Mol. Cell. Biol. 3: 50–60.

17. Reddy, J. K. & Hashimoto, T. (2001) Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system Annu. Rev. Nutr. 21: 193–230.

18. Decombaz, J., Schmitt, B., Ith, M., Decarli, B., Diem, P., Kreis, R., Hoppeler, H. & Boesch, C. (2001) Postexercise fat intake repletes intramyocellular lipids but no faster in trained than in sedentary subjects. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281: R760–R769.

19. Zhang, J. J., Okutani, F., Inoue, S. & Kaba, H. (2003) Activation of the cyclic AMP response element-binding protein signaling pathway in the olfactory bulb is required for the acquisition of olfactory aversive learning in young rats. Neuroscience 117: 707–713.

20. Mooser, V. & Ordovas, J. M. (2003) Editorial comment: 'Omic' approaches and lipid metabolism: are these new technologies holding their promises? Curr. Opin. Lipidol. 14: 115–119.

21. Grundy, S. M. (2002) National Cholesterol Education Program (NCEP)-The National Cholesterol Guidelines in 2001, Adult Treatment Panel (ATP) III. Approach to lipoprotein management in 2001 National Cholesterol Guidelines. Am. J. Cardiol. 90: 11i–21i.

22. Brehm, B. J., Seeley, R. J., Daniels, S. R. & D'Alessio, D. A. (2003) A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. J. Clin. Endocrinol. Metab. 88: 1617–1623.

23. Samaha, F. F., Iqbal, N., Seshadri, P., Chicano, K. L., Daily, D. A., McGrory, J., Williams, T., Williams, M., Gracely, E. J. & Stern, L. (2003) A low-carbohydrate as compared with a low-fat diet in severe obesity N. Engl. J. Med. 348: 2074–2081.

24. Lutjohann, D., Bjorkhem, I., Beil U. F. & von Bergmann, K. (1995) Sterol absorption and sterol balance in phytosterolemia evaluated by deuterium-labeled sterols: effect of sitostanol treatment. J. Lipid Res. 36: 1763–1773.

25. Mussner, M. J., Parhofer, K. G., Von Bergmann, K., Shwandt, P., Broedl, U. & Otto, C. (2002) Effects of phytosterol ester-enriched margarine on plasma lipoproteins in mild to moderate hypercholesterolemia are related to basal cholesterol and fat intake Metabolism 51: 189–194.

26. Yoshida, T., Honda, A., Tanaka Net Matsuzaki, Y., He, B., Osuga, T., Kobayashi, N., Ozawa, K. & Miyazaki, H. (1993) Simultaneous determination of mevalonate and 7α-hydroxycholesterol in human plasma by gas chromatography-mass spectrometry as indices of cholesterol and bile acid biosynthesis J. Chromatogr. 14: 185–193.

27. Shoda, J., Miyamoto, J., Kano, M., Ikegami, T., Matsuzaki, Y., Tanaka, N., Osuga, T. & Miyazaki, H. (1997) Simultaneous determination of plasma mevalonate and 7alpha-hydroxy-4-cholesten-3-one levels in hyperlipoproteinemia: convenient indices for estimating hepatic defects of cholesterol and bile acid syntheses and biliary cholesterol supersaturation. Hepatology 25: 18–26.

28. Watkins, S. M., Reifsnyder, P. R., Pan, H-J., German, J. B. & Leiter, E. H. (2002) Lipid metabolome-wide effects of the peroxisome proliferator-activated receptor gamma agonist rosiglitazone. J. Lipid Res. 43: 1809–1817.

29. Anderle, P., Duval, M., Kuklin, A., Dragarichi, S., Medrano, J., Littlejohn, T., Vilanova, D. & Roberts, M. A. (2003) Gene expression databases and data mining. Biotechniques 34: S36–S44.

30. Roberts, M. A., Mutch, D. & German, J. B. (2001) Genomics in food and nutrition. Curr. Opin. Biotechnol. 12: 516–522.

31. Berger, A., Mutch, D. M., German, J. B. & Roberts, M. A. (2002) Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression. Lipids Health Dis. 1: 2.

32. Kanehisa, M., Goto, S., Kawashima, S. & Nakaya, A. (2002) The KEGGdatabases at GenomeNet. Nucleic Acids Res. 30: 42–46.

33. Kanehisa, M. & Goto, S. (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 28: 27–30.

34. Ogata, H., Goto, S., Sato, K., Fujibuchi, W., Bono, H. & Kanehisa, M. (1999) KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 27: 29–34.

35. Dahlquist, K. D., Salomonis, N., Vranizan, K., Lawlor, S. C. & Conklin, B. R. (2002) GenMAPP, a new tool for viewing and analyzing microarray dataon biological pathways. Nat. Genet. 31: 19–20.

36. Nakamura, M. T., Phinney, S. D., Tang, A. B., Oberbauer, A. M., German, J. B. & Murray, J. D. (1996) Increased hepatic Δ6-desaturase activity with growth hormone expression in the MG101 transgenic mouse. Lipids 31: 139–143.

37. Murray, J. D., Oberbauer, A. M., Sharp, K. R. & German, J. B. (1994) Expression of an ovine growth hormone transgene in mice increases arachidonic acid in cellular membranes. Transgen. Res. 3: 241–248.

38. Rise, P., Ghezzi, S. & Galli, C. (2003) Relative potencies of statins in reducing cholesterol synthesis and enhancing linoleic acid metabolism. Eur. J. Pharm. 467: 73–75.

39. Grandjean, P. & Weihe, P. (2003) Arachidonic acid status during pregnancy is associated with polychlorinated biphenyl exposure. Am. J. Clin. Nutr. 77: 715–719.

40. Taylor, J., King, R. D., Altmann, T. & Fiehn, O. (2002) Application of metabolomics to plant genotype discrimination using statistics and machine learning. Bioinformatics 18(Suppl): S241–S248.

41. Fiehn, O. (2003) Metabolic networks of Cucurbita maxima phloem. Phytochemistry 62: 875–886.

42. Steuer, R., Kurths, J., Fiehn, O. & Weckwerth, W. (2003) Observing and interpreting correlations in metabolomic networks. Bioinformatics 19: 1019–1026.

43. Narayanan, B. A., Narayanan, N. K. & Reddy, B. S. (2001) Docosahexaenoic acid regulated genes and transcription factors inducing apoptosis in human colon cancer cells. Int. J. Oncol. 19: 1255–1262.

44. Narayanan, B. A., Narayanan, N. K., Simi, B. & Reddy, B. S. (2003) Modulation of inducible nitric oxide synthase and related proinflammatory genes by the omega-3 fatty acid docosahexaenoic acid in human colon cancer cells. Cancer Res. 63: 972–979.

45. Puskas, L. G., Kitajka, K., Nyakas, C., Barcelo-Coblijn, G. & Farkas, T. (2003) Short-term administration of omega 3 fatty acids from fish oil results in increased transthyretin transcription in old rat hippocampus. Proc. Natl. Acad. Sci. USA 100: 1580–1585.

46. Reifsnyder, P. C., Churchill, G. & Leiter, E. H. (2000) Maternal environment and genotype interact to establish diabesity in mice. Genome Res. 10: 1568–1578.

47. German, J. B., Dillard, C. J. & Ward, R. E. (2002) Bioactive components in milk. Curr. Opin. Clin. Nutr. Metab. Care 5: 653–658.