Genomics and Metabolomics as Markers for the Interaction of Diet and Health: Lessons from Lipids[1]

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ABSTRACT Foods are not purified compounds acting on single molecular targets, but complex mixtures of molecules that modulate many biochemical pathways simultaneously. Diet affects the probability of developing various diseases. Nevertheless, specific recommendations for individual diets are not simple. Recommending nutrient intakes above and beyond those needed to provide adequacy requires scientific knowledge and regulatory scrutiny to ensure the efficacy and safety even of essential nutrients. Designing a diet to improve metabolic health is a bold and ambitious goal. It is possible to design foods that will alter metabolism, but what change will make everyone who is otherwise healthy even healthier? Changing one aspect of metabolism to lower the risk of one disease does not improve overall health if it comes at the expense of disrupting another aspect of metabolism that increases the risk of another disease. This issue has: *1*) frustrated nutritional recommendations that could provide benefits to the health of large subsets of the population, *2*) caused the recall of drugs with many beneficial effects and *3*) caused harm by implying that single nutrients/foods could be healthy for everyone. An individualized system for metabolic assessment would establish the efficacy and safety of nutrients such as amino acids or fatty acids when these are designed to be consumed at levels providing improved metabolic health. The need to document the lack of an adverse effect of a food or drug on physiology necessitates a global, i.e. metabolomic approach. J. Nutr. 133: 2078S–2083S, 2003.

KEY WORDS: • *genomics* • *individual health* • *metabolism* • *nutrition*

Now that the scientific knowledge of the nutrients that are the basis of nutrient deficiency diseases is established, determining the role of diet in metabolic regulation has become a key scientific objective of nutrition research. The importance of diet to health has become even more obvious with the realization that many of life's modern diseases are the result of subtle but chronic metabolic imbalances related in part to diet. Although many of the diseases that medicine has dealt with successfully over the past century have been those caused by exogenous toxins or pathogenic organisms, the metabolic diseases including atherosclerosis, obesity, hypertension, type 2 diabetes, osteoporosis and various inflammatory diseases are caused by chronic imbalances of normal metabolic pathways (1–3). These diseases thus pose a great challenge for all aspects of traditional public health intervention, including nutrition. Diets are a part of the problem and nutrition should play a vital role in metabolic disease prevention. Metabolic balance is responsive to not simply the presence of essential nutrients at the limits of their adequacy, but also to the proportion of essential nutrients and to the abundance of nonessential components in the diet. As dietary strategies seek to modify metabolism for health benefits, the importance of understanding precisely how much of each nutrient is optimal leads to the necessity to redefine safety of nutrients consumed in amounts beyond those necessary for adequacy. New strategies, technologies and knowledge must be established to evaluate both the efficacy and the safety of diets designed specifically to chronically influence metabolism.

Genomics technologies

Investigators of diagnostics, pharmaceuticals and nutrition are developing new approaches to recognize, prevent and reverse metabolic imbalances (4). New weapons in the scientific arsenal to support these approaches are the tools emerging from genomics and the technologies of global gene expression (5). In addition to providing new knowledge of genes and their functions, genomics has propelled a change in the perspective of biological scientists: "the systems approach." Until recently, rigorous studies to understand the biological reactions, structures, catalysts and signals of life were largely reductionist. Scientists reduced their biological interests to increasingly narrow spheres to understand molecular

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mechanisms. Nutritionists also focused greater attention on molecular details to understand how nutrients in the diet exert their effects on biology. These approaches, although successful in understanding specific mechanisms, lose the ability to examine the larger behavior of entire biological organisms. Genomics technologies, however, change the strategy of nutrition research. It is now possible to measure simultaneously thousands of biological events in molecular detail (6). With these principles in place, scientists are extending the analysis of gene expression and its global perspective to examine the other layers of biological organization, proteins and metabolites (7).

Computer technologies

The massive increases in computer power are at the core of these scientific achievements (8).The expansion in the capabilities of computerized data management that has made genomics possible is also making it possible to see a new future for nutritional intervention: personalized health. Scientists of all biological disciplines are embracing computer-powered genomics as the ultimate key to understanding biodiversity. The differences between pathogenic and benign viruses and bacteria are emerging from genomic research (9–11). Interestingly, the field of nutrition is moving toward the complex issue of biodiversity, and the variation between individual humans (12). Genomics will not be the only, and probably not the most important, platform for nutrition. Nutrition scientists will use the downstream products of genes with -omic detail, proteomics and metabolomics.

Assessing efficacy and safety in the postgenomic era

Definition of healthy. In traditional public health, healthy is defined by the lack of disease. In effect, individuals are "diagnosed" for the presence of various diseases using a large battery of biomarkers whose presence reflect the explicit presence or consequences of pathogens, toxins, dysregulated cells or nutrient deficiencies. These diagnostics simultaneously identify the presence and physiological basis of disease. Individuals are judged to be healthy if they emerge from a complete battery of diagnostics without demonstrating the presence of any biomarkers of disease. This strategy has been very effective for decades, if not centuries. But as medical science has become successful in dealing with certain diseases, the remaining health issues have proven to be distinct in their properties, and to therefore require distinctly different strategies for detection, prevention and cure. Diseases that result after long-term chronic imbalances in metabolism do not necessarily produce biomarkers of damage until the disease is well established. Even more ominously, it is not possible to reverse chronic diseases by simply restoring normal balance to specific aspects of metabolism. Clearly, prevention is necessary as the restoration of optimal metabolism before damage has occurred. However, if imbalances in metabolism are to be reversed before explicit damage has occurred, it is necessary to be able to detect the metabolic imbalance itself, i.e., by requiring metabolic assessment. Furthermore, metabolic assessment must be relatively global to determine potential imbalances in any pathways that could lead to disease. Atherosclerosis is an example of a disease afflicting a large proportion of the population that is caused not by an acute exogenous agent, but by a chronic imbalance of the endogenous metabolism of cholesterol (13). Lessons learned from the battle against atherosclerosis are proving to be valuable in developing strategies to combat other metabolic problems with a significant nutritional component (14). Cholesterol is a very difficult molecule to analyze quantitatively, nevertheless, over decades scientists developed increasingly accurate methods to measure cholesterol in the blood compartment. With these technologies in place, clinical researchers adopted the routine measurement of cholesterol in blood to correlate these measures with the relative probabilities of developing atherosclerosis (15). History now attests to the success of these investigations, although it must be recognized that the scientific investment was massive and almost four dozen Nobel laureates received their prize for working on cholesterol. Because of their success, individuals are routinely monitored for their blood cholesterol in highly quantitative terms. Personal data on cholesterol concentrations are available to both clinicians and individuals. With this critical metabolic information available, various aspects of science and industry worked to understand how individuals could change their cholesterol concentrations through diet, drugs and excercise. This knowledge enables each individual to evaluate personal cholesterol information both as risk and, more importantly, how through drugs, diet and lifestyle they can change their metabolism to reduce their personal risk. The U.S. implemented this strategy as public policy by establishing a very ambitious countrywide project, the National Cholesterol Education Program (16). Reducing cholesterol became a public goal. When otherwise healthy individuals knew that they were at risk for heart disease because of their cholesterol levels the value of pharmaceuticals that would act to lower cholesterol became clear. This single prognostic marker is now the basis for a multibillion dollar pharmaceutical market bringing significant benefit to millions of people by lowering disease risk.

Now it is possible to merge the successful principles developed through cholesterol and atherosclerosis research with the new technologies of genomics and nutrition to move forward with a larger and more comprehensive strategy on metabolic health. Key elements of these new strategies are to distinguish biomarkers from quantitative metabolite profiles, to move from population recommendations to individual guidance, to combine isolated biochemical targets into integrated metabolism and to augment knowledge of essential nutrients to address overall diets. Nutrition is moving forward in this strategy, thus broadening its influence dramatically and providing an opportunity to improve public health through prevention of disease. In this new health paradigm, the toxicity and efficacy of foods and food components are also being reexamined.

Definition of nutrient toxicity. Toxicity has always been recognized to be a combination of a chemical and its exposure: "The dose makes the poison." Now health research is expanding its influence from considering only acute toxicity to addressing chronic metabolic imbalance. Toxicity is similarly being considered more broadly and especially more quantitatively. Toxicity has become associated not simply with acutely damaging molecules, but with molecules and foods that perturb metabolic regulation to cause sustained unbalances. When imbalance occurs, dose is even more important because essential nutrients are by definition necessary up to certain levels; yet at very high levels, the same nutrients can become damaging, particularly by altering metabolism in a net destructive direction. Detecting such "damage" in biological samples using a single biomarker strategy has been unsuccessful from both a biological and analytical perspective. Biologically, a clear link must be established between the biomarker and a human physiopathological outcome. Although possible for a disease with a straightforward cause and effect relationship, such links are difficult to establish for complex multifactorial and multistep metabolic diseases. The problem is also analytic.

The changes induced in metabolic biomarkers by food ingredients are small, not in the range of the variations induced by drugs. Nutrition research in the past attempted to resolve this problem with increasingly accurate and reproducible techniques applied to a small number of surrogate biomarkers within a normal human population. This strategy is not succeeding. As a scientific field, nutrition is dedicated to understanding and successfully intervening in the integrative metabolism of humans and animals (17). Nutrition is now bringing together the principles of modern analytics with genomics and nutritional biochemistry into an integrative approach to understand and influence the variations in human metabolism. We can apply the power of modern analytics not to focus intensively on a few surrogate biomarkers but to quantify, in parallel, a broad range of metabolic determinants of health.

Efficacy and toxicity testing of nutrients needs metabolic assessment. A biomarker can be viewed as a molecule or group of molecules whose simple presence is an indicator of a problem, state or condition. The presence of antibodies to specific pathogens is an example of a biomarker. It is necessary only to detect its presence to imply underlying processes of disease. Such biomarkers have proven to be of immense value in disease diagnosis. Both in managing diseases in populations and in individuals, one of the great successes in the history of public health has been the development of diagnostic biomarkers. However, single biomarkers are less appropriate for metabolic diseases because there is rarely a molecule whose simple presence indicates the metabolic imbalance. The mere presence of normal metabolic intermediates does not discriminate metabolically balanced from imbalanced individuals. The key difference between disease and health for metabolic disease is in the amounts of specific metabolic intermediates. The basis for metabolic disease is an imbalance in the concentrations among metabolites, not in the appearance or disappearance of any single intermediate. Hence, only quantitative and comprehensive metabolite measurements can identify metabolic imbalances. A quantitative metabolite profile is a more appropriate term for the approaches that will be necessary for assessing such imbalances. The key example of a quantitative metabolite profile that predicts health is blood cholesterol. All blood contains cholesterol. Simply reporting that blood contains cholesterol provides no information as to metabolic regulation and health. Only when the specific quantity of cholesterol per unit blood is determined, is it possible to relate the measurement to established variations within a population and to corresponding variations in health. Further, now that the complexity of cholesterol metabolism is becoming clear, it is necessary to measure more than just cholesterol (LDL, HDL, triacylglycerides, etc) to gain a more complete picture of an individual's risk due to imbalances in cholesterol metabolism. The obvious question is, "Why not measure everything?" This, in fact, is the goal, and although it may be impossible to imagine the full application of this ambition, in principle, nutritional sciences are getting closer than one would think.

Until recently, most nutrition scientists were obliged to study molecules one at a time. The development of genomics and metabolomics transforms the biomarker concept from a reductionist pursuit of one ideal biomarker to a holistic concept in which a significant fraction of all regulated genes and metabolites are quantified simultaneously. The technologies of separation, detection and computing are simultaneously merging to produce new tools to study the complex interactions that occur in biological systems. Vivid examples of the merging of high throughput analytics with genomic knowledge are DNA arrays (18). In addition to new technologies, several techniques developed decades ago to measure metabolites (e.g., fatty acids, amino acids, hormones, etc.) will remain invaluable as they are modernized for high throughput. Automation capabilities of separation techniques coupled to mass spectrometry make it possible to measure these metabolites in healthy subjects before and after nutritional disease and intervention. Because the numbers of genes and activated enzymes that can be involved in each metabolic situation are large, access to end-point metabolite measurements will be the easiest way to understand gene expression and functions. Just as in genomics, bioinformatics will be crucial to the success of functional metabolomics. Fortunately, bioinformaticians are already recognizing and addressing the challenges of linking genomics and metabolic databases (19). Global gene-expression analysis was made possible by a single invention: the gene array for which bioinformatic tools are emerging to manage, annotate and interpret the massive data sets that are created. Metabolomics has neither the luxury of a single class of analyte nor a single mode of analysis. As a result, global analyses are impossible on a single analytical platform for all metabolites. Several technologies are used, including Nuclear Magnetic Resonance (20), chromatography (21) and mass spectrometry (22). This approach is already in practice as researchers are examining the effect of environment, toxins and nutrition on entire biochemical pathways (23,24). Highly accurate quantitative analysis of an entire class of metabolites, lipids, has been demonstrated (25). With the technologies and perspectives of genomics, it is now possible to measure metabolism in the -omic era as metabolomic assessment.

Foods and food ingredients for health, efficacy and safety

Foods are not purified compounds acting on single molecular targets, but complex mixtures of molecules that enter various biochemical pathways. The basic principle that the quality of a particular diet affects health, defined as the probability of developing various diseases, is accepted after centuries of observation and modern epidemiology. But the challenges posed by attempting to convert epidemiology data to specific molecular recommendations to individuals remain substantial. An example from a modification in the amounts and composition of essential dietary lipids illustrates vividly this complexity (26).

Polyunsaturated fatty acids: consequences of diet on gene expression. The transcriptional responses in liver and hippocampus were compared in mice fed 18 carbon polyunsaturated fatty acids (PUFA) with mice fed the same diets plus long-chain (n-6) PUFA or mice fed the same diets plus long-chain (n-3) PUFA. All animals received essential fatty acids exceeding the nutritional requirements. Thus, the experiment addressed whether fatty acids, if fed in varying amounts and compositions, would influence any aspect of metabolism and genetic regulation.

The livers and hippocampus of the mice fed the described diets were examined for their differential gene expression using commercial gene arrays (Affymetrix, Sacramento, CA) and livers were examined for variations in lipid composition (26). This experiment revealed many of the issues that nutrition research is now struggling with as it addresses the problems of understanding how diet interacts with metabolism, and especially the complexity of addressing the question of how diet can be modified to improve health. The total number of discrete biological data points generated by such an experiment and the range in response across various aspects of the genome is illustrated by a plot of the fold expression changes plotted as a function of the absolute expression (**Fig. 1**). These

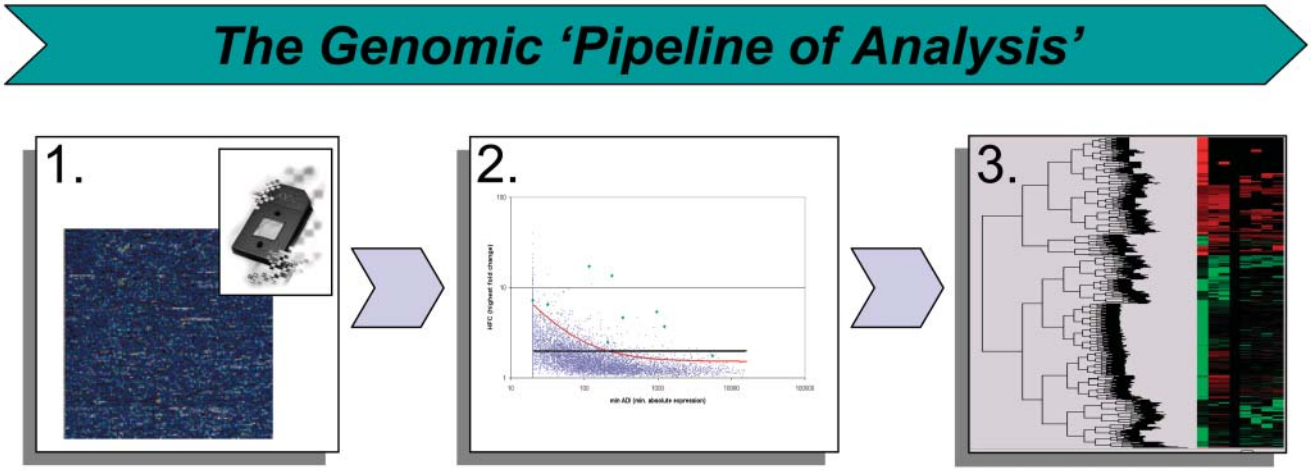

FIGURE 1 The genomic pipeline of analysis. *1.*) Microarray data stemming from a single Affymetrix GeneChip produces a large volume of data, with the statistically and biologically significant data hidden within. Therefore, the goal is to extract the significant information relating to the experimental conditions. *2.*) Gene selection is performed using a variety of methods attempting to resolve several problems including that lowly expressed genes have both an increased biological and measurement variability. The limit fold change (LFC) model develops a variable fold change cutoff that is more strict for lowly expressed genes than it is for highly expressed genes (reference LFC paper). The LFC systematically selects genes across the range of gene expression values in a uniform manner, rather than predominantly selecting lowly expressed genes. *3.*) Once those genes determined by statistical criteria to be differentially expressed have been selected, one can use the many bioinformatic tools to further explore the differential gene-expression profile stemming from the experimental conditions. Various methods to cluster and visualize the data can reveal global patterns of gene expression that would not have been recognized should single-gene analysis have been performed. (adapted by D. M. Mutch from 26 with permission).

data allow a direct quantitative comparison in the abundance of specific mRNAs (expression) in the same tissues from animals fed the different diets. The effects on gene expression were considerable and broadly distributed across the biochemical spectrum. By rigorous and conservative screening and selection criteria for biologically important changes in gene expression (27), the number of genes that were differentially expressed in the livers and brains of these animals due to consuming different dietary polyunsaturated fatty acids was determined. Of the 12,000 discrete genetic elements of the murine genome whose abundance is measurable on these arrays, over 300 genes in both liver and hippocampus differed in their expression.

The power of genomics tools to explore biological functions was also explored in the study. Bioinformatic tools, which now routinely access the world's entire electronic biological knowledge pool, are able to process the gene-expression data and compare the results with the known or suspected functions of genes, proteins and pathways. By clustering the responsiveness of the genes according to the experimental design, and comparing these clusters with biological clustering of genes according to known functions by gene ontogeny (GO), it was possible to classify the various genes influenced by diet and for example establish that fish oils and arachidonic acid-rich oils exert their functions in part through the activation of multiple nuclear transcription factors (**Fig. 2**). That is, long-chain PUFA

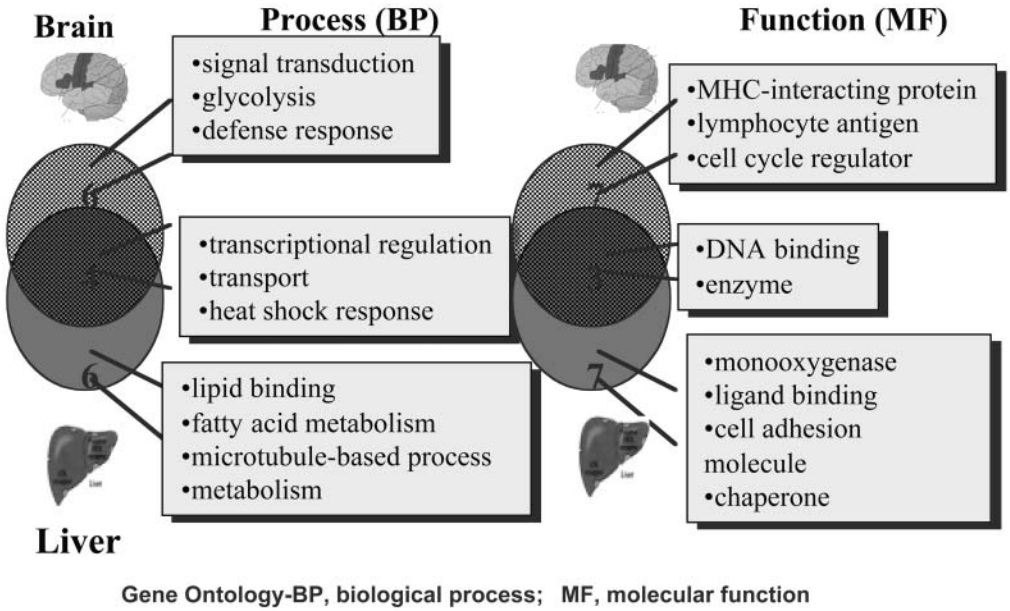

FIGURE 2 Gene ontology for the major genes that were changed by feeding dietary polyunsaturated fatty acids that were identified in liver and brain and their functions assigned by bioinformatic analysis. [adapted from (26) with permission].

or their products bind to and act as agonists for proteins whose functions are to translocate to the nucleus and influence the expression of multiple genes.

The approach of using global gene-expression arrays to examine the effects of dietary change also illustrates the ability to screen for all consequences of diet, not just those hypothesized. From a gene-expression perspective, at least in those tissues examined, no unintended effects of the diets would have gone undetected. Such confidence is precisely what is necessary when healthy individuals are provided dietary ingredients designed to improve their health; what are all the consequences of consuming the ingredients? However, gene expression is not the same as metabolism. Whereas the expression of genes is a clear indication of the potential of a tissue to carry out particular aspects of metabolism, confidence in the efficacy and safety of agents designed to alter metabolism ultimately is the quantitative effect on metabolism. Studies exploring the metabolic consequences of diet and competing drugs illustrate the same principles of genome expression and further document the investigative power of global strategies (25).

Drug responses: consequences of drugs on metabolic profiles. The difference between approaching health with biomarkers and approaching health with a broad metabolic perspective was captured in the study of the effects of rosiglitazone for the treatment of diabesity, (the comorbidities of obesity and type 2 diabetes). The signature biomarkers of type 2 diabetes are elevated serum glucose, insulin and triacylglyceride concentrations. The targeted benefits of the thiazolidinedione drugs, such as rosiglitazone, are to reduce the problems associated with diabesity and in particular to lower blood concentrations of glucose, insulin and triglycerides. Animals and humans differ in various aspects of metabolism, particularly as it relates to diabesity, thus, animals are in general inadequate in providing an experimental model to test the development and reversal of diabesity in humans. Recently, the group at The Jackson Laboratory made a significant breakthrough by developing a mouse line that exhibits many of the metabolic features of human diabesity (25). Although the administration of rosiglitazone to the diabetic mice improved plasma glucose, insulin and triacylglyceride concentrations, a more detailed phenotypic investigation and metabolomic-type analyses, i.e., on the fatty acid subset of the hepatic and plasma metabolomes, revealed the metabolic actions of the drug. Although rosiglitazone lowered plasma triglyceride concentrations, the treatment led to an increase in overall body weight and fat mass. The basis for this apparent contradiction was that the drug induced an increase in fatty acid synthesis, while simultaneously impairing the export of the synthesized lipid into blood. Thus, there was an overall increase in fat content in liver and adipose, but the plasma concentrations of triacylglycerides dropped as a result of the treatment. Although this effect was thought to be specific to the mouse model described in the study it demonstrated that metabolomic analyses, when focused on analyses of pathway biochemistry, can determine the difference between appropriate and inappropriate metabolic responses to drug intervention.

The road ahead

Human genome information is increasingly accessible as a single database resource that is growing in size and value with time. A similar integrated and open resource is not yet available for the human metabolome. Nevertheless, scientists are assembling increasingly broad and annotated databases of lipid metabolites whose structure will eventually lead to a universally accessible quantitative database resource of lipid metabolites.

The comprehensive study of metabolites combines the benefits of quantitative assessment of metabolism with the -omic perspective and informatics tools of genomics, and the integrated knowledge of modern biochemistry (28). To build metabolomic data resources into the transparent and practical tool set that they should be, metabolites must be measured quantitatively as complete sets or subsets of metabolic pathways or molecular classes. Although the bioinformatic tools needed to mine metabolomic databases are lagging behind those developed for genomics, much of the classical statistical repertoire is applicable to metabolomic datasets. The large biochemical knowledge base is also directly applicable making it possible to interpret changes in metabolite profiles as the consequence of specific alterations in specific reactions (21). Metabolomic approaches are being recognized as valuable in the following two discrete perspectives.

Unintended effects of drugs, foods, genes and toxins. The goal of many drugs, foods, etc. is to influence a single biochemical target. In practice, however, no drug or food ingredient that is active on one biological structure is without effects on any other. In particular, drugs and foods consumed over long periods of time may exert small effects that are cumulative. Even when the intended benefit is achieved acutely, it is not certain that there is a net positive outcome at a longer time scale if there are alterations in other aspects of metabolism. Any benefits due to alterations in targeted metabolites in the short term could be offset by ultimately catastrophic but minor alterations in unintended metabolic sites. Unintended effects can only be discovered by measuring all metabolic intermediates, i.e., by metabolomics. The use of metabolomics for estimating unintended effects is already the focus of research to define the hazards of genetically modified organisms as food (29). This approach has been effective in identifying the nature of toxicity in which the complex multiple consequences of individual toxins on biological systems can provide a diagnostic footprint even after the toxin is no longer detectable (30).

Individual variation. The genomics fields have demonstrated that single-point mutations can account for variations in individuals within the population. However, the complexity of the interactions between several polymorphic differences in related genes means that genetic analysis will be able to predict individual variation in phenotype for only a few selected outcomes for which a strong effect of a particular polymorphism can be demonstrated. If the desired effect of a pharmaceutical intervention is a chronic shift in metabolic regulation, it will be necessary to measure all of the metabolic consequences in an individual to ensure the safety of the treatment, hence metabolomics. It is a simple, but true, statement that accurate assessment of benefit or risk to an individual requires a measurement of their metabolic response. This may appear to be a prohibitively expensive and invasive undertaking. However, pharmaceuticals are evolving into therapeutics that are more tailored to individual variations and to more lifestyle-based interventions that call for prolonged drug intervention. In both directions, more individualized drugs and more chronic administration of drugs for benefits that are targeted at quality-of-life issues and not related to life-threatening diseases are enabled by broad-based metabolic diagnostics. Because metabolomics will be necessary to optimize health management in individuals who vary in their response to drugs, health benefits can be delivered through nutrition using parallel individual metabolomic approaches. Eventually, individual metabolic knowledge will be an integral part of the public health system. Importantly, this change will not come immediately, nor will it come all at once. Cholesterol is being used as a quantitative

assessor of lipid metabolism, estimating health risk and identifying individuals for whom particular drugs are suitable. Triglycerides, glucose and insulin are continuing this trend, and as scientific evidence supports additional metabolic knowledge sufficient for successful intervention, they will merge into the clinical tool set.

The power of broad metabolic approaches was shown by the example of the multiple effects of the rosiglitazone (discussed above). In obese mice showing symptoms of type 2 diabetics, elevations in blood triglycerides, glucose and insulin are the signature biomarkers of disease. Although successful in improving all three measures in a particular animal model, drugs can induce unintended metabolic and pathophysiological side effects, for example, hepatic lipidosis. From a comprehensive quantitative analysis of all of the lipid metabolites in plasma and liver of control and drug-treated mice, i.e., a lipid metabolome, it was possible to observe the myriad metabolic effects of the drug on lipid metabolism and eventually to establish the metabolic sites of those effects. Similarly, the wide responses in the expression of hundreds of genes were seen in mice provided varying amounts of polyunsaturated fatty acids in their diet. The metabolic and gene expression responses to varying levels of amino acids in the diet can be considered to be similar to the responses to fatty acids in the diet. Amino acids represent a similar metabolite class in that their presence in the diet is essential, but varying diet, drugs and amino acid concentrations themselves can produce highly complex responses in the metabolism of amino acids. The lessons we have learned from fatty acids can provide a model for pursuing amino acids and various other essential and nonessential metabolites that are both consumed in the diet and constitute important metabolites in vivo.

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