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**Commentary**

# The revolution in microanalytic chemistry: a macro-opportunity for clinical nutrition[1,2]

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For many decades, clinical scientists have used special biochemical markers that reflect disease states and their reversal. Increasingly, scientists have searched for similar markers that are predictive of the risk of future disease. In clinical nutrition especially, the search has been to identify markers that reflect the nutritionally responsive aspects of health and disease. To prevent disease, ie, maintain health, which by necessity involves screening large numbers of subjects, markers of nutritional status must be not only highly sensitive and accurate but measurable by fast and easy-to-use analytic devices that are minimally invasive. Recently, researchers in analytic chemistry and microelectronic engineering have developed a host of new miniaturized technologies that will address many such needs in the clinical chemistry community (1). These new technology platforms have been dubbed with evocative titles like "micro and nano-technology," "biochips," "lab on a chip," and "micro-total analytic systems" (µTAS). These technologic innovations can be split into 2 broad categories: array-based assays (receptor-ligand binding, DNA-DNA hybridization, or both) and microanalytic separations, each of which will be mentioned here with respect to their ability to affect nutritional science.

The term *µTAS* indicates the future of analytic instrumentation, ie, an entire analytic process from sample preparation to detection could be put onto a small, diskette-style card that would perform a completely automated analysis when inserted into a reader. Although current µTAS need further development to reach widespread use, the hand-held diagnostic device is a tangible, attractive goal because it incorporates the ideas of automated sample preparation, compound identification, use of computerized data management for diagnosis, and understandable presentation of results. What is potentially revolutionary about µTAS is the fact that implicit in its concept and design is that it is a semidisposable and low-cost technology (2). This means that sophisticated instrumental analysis, once the domain of expensive central laboratories, will now be much more generally accessible. Hence, it would be possible to screen large numbers of healthy individuals to ascertain risk, not just to follow disease in ill patients.

Like all biological sciences, advances in clinical nutrition can be empowered by analytic innovation. A recent review lists advantages in microanalytic systems that will soon affect clinical analysis, including low costs in manufacturing and operation, submicroliter sample consumption, reduced waste streams, increased automation, and parallelism, among others (3). All microanalysis inherently occurs in small diffusional spaces, and because of scaling laws, real advantages in the resolution and efficiency of solute separations are realized. Receptor-ligand binding assays will approach equilibrium much faster, which can lead not only to greater speed but to better assay performance (3). The consequences of miniaturization will be to enable improvement of orders of magnitude in, again, not only analytic speed but in capacity, giving rise to the notion of so-called *parallelism*. In practice, *parallelism* means that multiple analytes are measured simultaneously.

Arguably, it is the responses of many metabolites that reflect perturbation of health, not just one. The capability to measure many analytes should dramatically enable nutrition research to eliminate, if nothing else, introduction into the literature of so-called "ideal single biomarkers" that claim to reflect an entire physiologic state in a single number. In fact, it has been the frankly illogical search for a single marker of nutritional status that has held back progress in many fields of nutrition (eg, antioxidants, inflammation, and energy metabolism). Capabilities will emerge from these new microanalytic approaches that were not conceivable 5 y ago: highly parallel bioassays, separation times fast enough to approach real-time sensors, and data capture and analyses that constitute such broad and comprehensive analysis of the system variables that they approach a global description of the biological response. The new generation of microinstrumentation promises more than just faster and cheaper data. Limits to the availability of information will change dramatically. The resulting increase in information will profoundly extend investigative powers in both breadth and time. In breadth, nutritionally relevant data will become truly systemic, and in time it will be truly continuous.

In terms of measurement science and data handling, few leaps of scale could rival that of modern genomic analysis. One microtechnology that has emerged as a result is the DNA array or biochip. Through the use of microspotting technologies (4) or light-directed syntheses (5), it is possible to place large numbers of oligonucleotide sequences (≤400 000/array, depending on the technology) on solid supports with micrometer-level precision. A recent review lists 22 companies that have significant biochip development programs (6). Several of these have

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bioarray products on the market that cover large numbers of genes and even some entire genomes (eg, companies such as Affymetrix, Incyte, Hyseq, and Packard Instrument Co). On a more individual basis, proprietary expressed sequence tag (EST) arrays are now being produced under contract to biochip companies; the final chips themselves are used for mass hybridization experiments at the customer's site. Therefore, for the first applications of genomic information—gene expression analysis—the state of the art is already customized global analysis.

DNA chips are influencing our understanding of cellular gene expression in a complex and interactive or systemic sense (7). When 8300 ESTs were assembled onto a single activated glass slide (8), the differences in expressed genes between control and treated human fibroblasts revealed many more unsuspectedly active gene clusters than when assayed separately and compared under the same experimental conditions. Such a comprehensive comparison in cellular response analysis shows clearly that global analysis has truly arrived for gene expression studies.

Futhermore, such a strategy was recently used to study the gene expression profile of aging in mice fed a control or energy-restricted diet (9). This experiment used commercially available oligonucleotide DNA arrays to simultaneously measure 6347 genes over time in the 2 diet groups. Approximately 113 genes with significant age-related changes in gene expression were observed, many of which could be correlated with known aging mechanisms from the literature. The systemic nature of analysis was then able to show that energy restriction led to increased protein and energy metabolism, increased fatty acid and nucleotide precursor synthesis, and could indicate reduced macromolecular damage due to the expression of detoxification and DNA repair systems. The globality of genomic information observed in this study clearly foreshadows more experimentation in nutrition.

Approximately 1.7 million ESTs, representing 80000–100000 unique human genes, now exist in the public database and can now be arrayed onto biochips. Chips will soon be used to monitor gene expression in response to a wide variety of nutritional events, ie, global analysis has come to nutrition. These chips, especially when low in cost, will give the field of nutrition the means to reformulate diets for specific metabolic and physiologic objectives by using sound, molecular-based knowledge and then to show their metabolic actions. Imagine the benefits to fields such as nutritional epidemiology with such standardized systemic information. The biochips, in particular, represent a prime opportunity for the field of nutrition. Nutritional intervention in health is inherently multiparametric in both inputs and outputs of the biological system. To truly understand the complexity represented by even the simplest of nutritional interventions, such as management of plasma glucose, global analysis was inevitable—now it will be possible.

Speed enhancement is another immediate advantage of the miniaturization of analytic instrumentation, especially in separation science. Almost all modes of molecular separations, from HPLC to field-flow fractionation to capillary electrophoresis, have now been performed on customized chip-based instruments (3, 10). Procedures that historically required sequential extractions, purifications, and separations, each necessitating multiple transfers, are being replaced by single-stage immobilized platforms that take advantage of miniaturization in fluidics, separation, and detection. These platforms are already beginning to perform entire analyses on a single wafer with key performance elements of analysis: enhanced efficiency of separation (separations per unit time) and improved detection limits (1, 11–14). By examining the basic components of μTAS, inherent advantages in the technology have been shown. These advantages include automated dilution, filtration, and reagent addition (15, 16); rapid mixing and reaction chemistry because of the constrained diffusional environment (17); extremely rapid free-zone immunoassay screening (15-s assay times) (16, 18); rapid, high-efficiency, and high-resolution electrophoretic separations (>800 plates/s) (19, 20); highly parallel separations that dramatically increase throughput over conventional capillary electrophoretic separations (21); integrated polymerase chain reactions on a chip (1, 22); and even whole-cell transport and manipulation (23). All of the above components can be thought of as analytic circuits integrated in various combinations to deliver a device with custom-made performance characteristics, just like circuits are assembled into electronic devices. Researchers will one day sit at their computers and draw out the circuits for a particular experimental series, e-mail this to the manufacturer, and then receive a series of wafers in the mail. These wafers will be inserted into a docking station to perform all control operations and load reagent reservoirs as needed. With such speed of analysis, routine, sensor-like capabilities are in hand. Nutritionally relevant metabolites in a clinical situation will be monitored much as pulse rate is monitored now.

The ability of microtechnology to accelerate research is broad reaching. μTAS devices can be made that perform analytic separations on the same time scale as sensors (24). Free-solution immunoassy measurements of circulating drug residues in human plasma have been performed with fast (2–3 min) capillary electrophoresis (25, 26). μTAS chips have been used to perform this same type of analysis in <15 s per data point (20). Furthermore, the immunoreactor required before separation has now been incorporated into a μTAS format, thereby allowing the addition of human blood to the device, which then carries out all necessary steps to obtain a useable measurement value at the end (16). The speed of these electrophoresis-based immunoassays makes them functional sensors while maintaining the information quality of separation-based methods. Such an increase in the speed of analysis will make it possible for the field of nutrition to study biological systems at unprecedented time intervals, enabling routine investigations of the entire time course of the responses to nutrient intakes.

The impact of an innovation is not felt instantly. The first applications of microanalytic technology are simply to do what was done before but cheaper and faster. The implications of the methodologies mentioned above go far beyond the idea that the data are being obtained faster and cheaper simply because they are being gathered in parallel. The advantage is that the knowledge gained from global testing is greater than the sum of the individual tests because a relational database is generated in every single experiment.

The future of clinical nutrition is in new approaches to addressing previously unsolvable problems. The micro revolution portends remarkable advances for chemical analysis, including pharmaceutical development and analysis, toxicologic assessment, environmental surveillance, biosciences (3), and potentially, clinical nutrition. However, history is clear: clinical nutrition must play an active role by providing attractive targets and applications for microanalytic technology to be included in the first generation of instruments developed.

Laboratory assays used in clinical nutrition have always, in essence, piggybacked on the analytic chemistry of diagnostic medicine and pharmaceuticals. An important decision made by

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investigators in these latter fields, that ironically had a great effect on clinical nutrition, was to measure blood primarily in the fasted condition. The basis for the decision is discouragingly simple. The changes in metabolites induced by the fed state posed a significant problem. The most effective means to eliminate what emerges as variation in measurable components because of diet (even nutrient-responsive metabolites) is to analyze blood in the fasted state. This decision was based not on the quality of information that would be obtained but on expediency. As a result of the decision, however, most variables of nutritional interest that have emerged as predictive of health are those that persist for prolonged periods, ie, during fasting. The most obvious example is blood cholesterol, in which the concentrations of LDL and HDL cholesterol are influenced by diet over the long term but are relatively insensitive to feeding and fasting in the immediate term. Now it is possible to change this decision. With the expanding possibilities in microanalytic chemistry, indexes need not be measured only once. Technologies can be developed to measure blood indexes repeatedly so as to describe the entire postprandial response. But a convincing argument must be made if technologies are to be developed.

It can be argued that a future role of foods and nutrition in health and preventive medicine will depend on being able to analyze the responses of an individual to the food or nutrient as a function of time. Only then will it be possible to understand the relation between nutrient intakes and health or risk of disease. The need to describe the myriad responses to a nutrient is important to prevention but is not vital for curative intervention. For curative medicine, the average or chronic response in the fasted condition is acceptable as an endpoint (in the antibiotic elimination of pathogens, for example). In contrast, it is the variation of an individual's metabolism in response to food consumption that will predict nutritional effects on chronic and degenerative diseases.

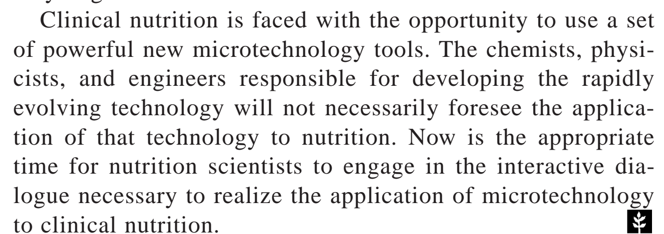
Some examples of what is now known for a few metabolic indexes clearly make the case for performing several analyses over time. Blood glucose and insulin are clearly illustrative of the value of real-time measures of metabolites in blood. Similarly, the technologic development of sensor devices that rapidly measure blood glucose has enabled a substantial improvement in health for persons with diabetes by providing the tools to time nutrient intakes according to metabolic status. This technology is so advanced that glucose sensors are now worn by diabetic patients (27). But glucose is not the only example. The relative rate at which triacylglycerol is cleared from blood after a meal is now recognized as highly predictive of individual predisposition to atherosclerosis (28). The concentration of blood triacylglycerol is an index for which the decision to measure it at a single time point has slowed the appreciation of its importance to prediction of health. It is highly likely that the importance of variations in delivery of all fat-soluble nutrients because of delayed clearance of triacylglycerol-rich lipoproteins will emerge only when such variations are measured routinely.

Not only do nutrients vary in blood after food intake but many hormonal and signaling molecules vary as well. Circulating amounts of leptin and cholecystokinin vary in response to food intakes and differ among individuals and in response to meal composition (29). The genetic and metabolic bases for these differences are believed to be contributors to the dysfunctions in control of food intake that underlie obesity (30).

Examples of how the immediate response to particular foods is important to their effects on health clearly abound, but what is more important is that these examples have emerged from research that has lacked effective tools for examining variables of interest repeatedly over time. Postprandial studies are difficult to undertake and have provided information only about the experimental hypotheses for which they were specifically used to test. Many more compelling aspects of the relation between the postprandial state and health will emerge when more data become available. Without a significant investment, the means to acquire such data simply will not happen. What then is necessary?

To realize the potential of microtechnology to benefit research and its applications to clinical nutrition, several technologies must be developed; however, none need be discovered. The inherent speed, sensitivity, and volume requirements for microassays eliminate the most important hurdle: blood volume. However, blood sampling methods must be developed that are both painless and nondestructive. Specific analytic protocols for analytes will need to be assembled, but the foresightedness of nutritional researchers must provide the spectrum of candidate analytes and protocols. Finally, rapid information packages will be necessary to handle the substantial increase in volume of data generated. Given the size of the task of the human genome project, however, and the bioinformatics emerging to deal with this project, it is not necessary to invent software systems to handle very large data sets.

Clinical nutrition is faced with the opportunity to use a set of powerful new microtechnology tools. The chemists, physicists, and engineers responsible for developing the rapidly evolving technology will not necessarily foresee the application of that technology to nutrition. Now is the appropriate time for nutrition scientists to engage in the interactive dialogue necessary to realize the application of microtechnology to clinical nutrition.



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