

REVIEW

Endobiogeny: A Global Approach to Systems Biology (Part 1 of 2)

一种系统生物学的全球方法（第 1 部分，共 2 部分）

Endobiogenia: un enfoque global a la biología de los sistemas (Parte 1 de 2)

Jean-Claude Lapraz, MD, France; Kamyar M. Hedayat, MD, United States

Author Affiliations

Société internationale de médecine endobiogénique et de physiologie intégrative, Paris, France (Dr Lapraz); American society of endobiogenic medicine and integrative physiology, San Diego, California, United States (Dr Hedayat).

Correspondence

Kamyar Hedayat, MD
acquamarine.com

Citation

Glob Adv Health Med. 2013;2(1):64-78.

Key Words

Endobiogeny, systems biology, reductionism, functional medicine, endocrinology

Disclosure

The authors have completed the ICMJE Form for Disclosure of Potential Conflicts of Interest and disclosed that both have a financial stake in Endobiogenics Inc, Pocatello, Idaho.

Editor's note: *The following is part 1 of a two-part article. The second part of the article will be published in the March 2013 issue of Global Advances in Health and Medicine.*

Reductionism is the attempt to explain complex phenomena by defining the functional properties of the individual components that compose multicomponent systems . . . “naïve reductionism,” the belief that reductionism alone can lead to a complete understanding of living organisms, is not tenable. Organisms are clearly much more than the sum of their parts, and the behavior of complex physiological processes cannot be understood simply by knowing how the parts work in isolation.¹

—Kevin Strange, Department of Anesthesiology, Molecular Physiology and Biophysics and Pharmacology

INTRODUCTION

Endobiogeny is a global systems approach to human biology that may offer an advancement in clinical medicine based in scientific principles of rigor and experimentation and the humanistic principles of individualization of care and alleviation of suffering with minimization of harm. Endobiogeny is neither a movement away from modern science nor an uncritical embracing of pre-rational methods of inquiry but a synthesis of quantitative and qualitative relationships reflected in a systems-approach to life and based on new mathematical paradigms of pattern recognition.

Clinical medicine stands at a unique juncture in the history of science, philosophy and culture. Historically, medicine influenced and was influenced by these three branches of knowledge.² Through three phases of history over the last 500 years, a split has occurred. Medical inquiry has proceeded from holism to reductionism to “naïve reductionism.” What once resulted in fantastic insights and seemingly miraculous cures has reached a plateau and even devolution of disorders once believed to be controlled such as infection,³ cancer,⁴ and autoimmune disease.⁵ In contrast to this in the last 50 years, systems theory has reversed the reductionist trends in many fields of inquiry (except clinical medicine), returning to the Aristotelian observation that the “whole is greater than the sum of its parts.”

Reductionist experimentation can be a valuable tool in understanding the individual components of complex phenomenon. In fact, this approach has proved key not only in the foundation of modern medicine but also for the foundation of systems biology, which may one day replace the current reductionist approach. An isolated study of phenomena is neither an inherently problematic nor fundamentally flawed endeavor if it is used to create a global vision of how the organism works within its true dynamic state of function. Naïve reductionism, on the other hand, is contrary to the very existence of life as an experiential phenomenon.

The principles of reductionism originated in 17th century Europe. During this time, the focus of scientific inquiry shifted from “why” to “how,” from cause to mechanism and from understanding the whole to dissecting the parts.² Quantitative analysis supplanted qualitative analysis. The macrocosm and microcosm were characterized by three qualities: order, predictability and control, based on the works of three key thinkers. Newton's physics posited that objects follow defined, predictable rules of behavior. The French philosopher Laplace posited a type of determinism in which the past and future of all behaviors of objects could be precisely determined.⁶ The French philosopher Descartes first described the reductionist method of inquiry.⁷ In his first work, *A Discourse on the Method for Conducting Oneself With Reason, and Searching for Truth in the Sciences*, he writes,

The second [method I use] is to divide each difficulty . . . into the smallest components into which it can be divided in order to better resolve it. The third is to conduct my thoughts in an orderly manner, beginning with the objects that are most simple and easy to know, then progressing little by little, as if by degrees, to the knowledge of the most complex ones, even assuming an order between objects that do not logically precede one another in a natural way.⁷

It is worth noting that Descartes was not a “naïve reductionist”—merely a reductionist. His goal, as he explains, was to build back up to a global level of knowledge. The shortcoming of his thought process was in seeing the body as a collection of parts as opposed to a system.

Contemporary medicine, as Dr Strange notes, suffers from “naïve reductionism,” which can be characterized as follows: the body is a collection of organs composed of tissues, which are composed of cells, which are run by genes. Therefore, the object of study is genetics and the proteins and cellular activity that it guides. The true role and effect of each cell can be discovered only by studying each variable in isolation so as to rule out the effects of other variables. The sum of the effects of each individual variable is an accurate reflection of the function of the whole organism because it is merely a collection of parts. Because the cell is the ultimate unit of function and the genes contain the code that runs the cell, diseases arise from faulty information contained in genes or due to faulty translation of genetic information. Therefore, genes are the cause (not the mechanism) of disease.⁸⁻²³

According to this approach, symptoms express the loss of control and order within the body due to faulty genes because, as the 17th-century philosophers noted, order is the hallmark of perfection and functionality, and order must be restored. In order to restore order to the organism, symptoms must be controlled. Therefore, to control symptoms is to treat disease. The best treatment is the one that has the most precise control over the most specific variable of dysfunction. The best treatment will be predictable in action and non-competitive in its control. In this paradigm, only a single-compound drug, with a single mechanism of action on a single locus of activity can reliably control, and ergo, “treat” disease.

In the last 50 years, a shift has taken place in science and philosophy away from the reductionist trends of the last 500 years. More recent studies in physiology have revealed the existence of complex super-systems of physiologic regulation.²⁴⁻³⁶ Experimental and clinical studies have revealed the multifactorial nature of disease as well as the high degree of interrelatedness of physiologic factors and systems.^{1,37,38} The conclusion of a growing number of researchers is that the body functions like a system, not a collection of isolated parts, and therefore must be studied as a system, not in isolation. A paradigm shift in healthcare towards a systems analysis may offer a scientific approach to diagnosis and treatment that makes progress where the current paradigm has reached a plateau. The endobiogenic theory proposes such a paradigm shift.

SYSTEMS THEORY

*Aristotle’s statement “the whole is more than the sum of its parts” is a definition of the basic system problem which is still valid. Aristotelian teleology was eliminated in the later development of Western sciences, but the problem contained in it, such as the order and goal-directedness of living systems, were neglected and by-passed rather than solved. Hence, the basic system is still not obsolete.*³⁹

—Ludwig von Bertalanffy, PhD, biologist and founder of the General Systems Theory

A system is a collection of parts that form a whole. A system is self-generating, cohesive, closed unto itself but open to interaction with its environment. Its functionality is determined at four levels: (1) the individual units of activity in and of themselves, (2) their relationship to each other, (3) the global level of functionality of the system, (4) system’s relationship to its external environment. According to James Miller, MD, PhD, originator of the seminal “Living Systems Theory,” every system, from a cell to a supranational organization, must possess 19 properties divided into two general categories: matter-energy and information.⁴⁰

The properties of matter-energy are (1) ingestion of material, (2) distribution of material, (3) conversion of material into structure or energy, (4) production of material, (5) storage of material, (6) extrusion of waste, (7) movement of the system, (8) support and maintenance of spatial relationships of the sub-units, (9) reproduction, (10) maintenance of internal and external boundaries.

The properties of information are (1) external input transduction: conversion of light, chemical, tactile, and temperature information into a form recognized by the system, (2) internal input transduction of information about changes in one component or sub-system to other components or sub-systems, (3) channeling and distribution of information within the system, (4) decoding and translation of information, (5) learning and association (first stage of learning), (6) memory (second stage of learning), (7) decision making based on information from all sub-systems of the system (8) encoding information for external interpretation, and (9) output transduction.⁴⁰

In a system, the quantitative abilities alone do not determine the functionality of the system because each unit of activity depends on, influences, and is influenced by the activity of other units. While quantitative measurements determine the maximum potential of an individual unit of function in isolation from all the other parts, qualitative relationships determine the functional capabilities of the system and its various subsystems. Based on this understanding of systems dynamics, order and cohesiveness do not arise from rigid control but from permanent and dynamic management of the needs of the systems at the individual, regional, and global levels by the system itself.

The neurophysiologist P.K. Anokin, a student of Pavlov, was the first to describe feed-forward, feed-through, and feedback loops, which laid the foundation for the concept biological system *regulation*, as opposed to operation by reflex alone. In 1935, he published his theory of functional systems.⁴¹ The biologist Ludwig von Bertalanffy developed his general systems theory in 1937, which influenced the applications of systems theory across multiple disciplines. Miller published his theory of living systems in 1955.⁴² Since then, systems theory has been applied to biology,⁴³ social psychology,⁴³ resource management,⁴⁵ economics,⁴⁶ and other areas of material and human sciences, demonstrating the universal applicability of this concept.

Advances in cellular biology have demonstrated

how at every level—cell, tissue, organ and organism—the human being meets the criteria of being a system.²⁴⁻³⁶ With this new understanding of physiology, if medicine is to continue to progress, a similar paradigm shift will be critical. The shift starts by moving away from a quantitative, binary model of biochemistry that states,

Elevation in serum liver enzymes = liver dysfunction, therefore, if liver enzymes are not elevated, then there is no liver dysfunction.

to a qualitative evaluation of relationships that states,

Despite normal liver enzymes, there is hepatic strain due to a global insufficiency of oxidative activity relative to reductive activity,⁴⁷ which compromises glutathione recycling and hepatic detoxification pathways,^{48,49} which is rooted in an insufficiency of insulin sensitivity,⁵⁰ which is impairing mitochondrial respiration and ATP production,⁵⁰ which may explain the recent devolution in the patient's cardiac function and/or lipid metabolism, and/or neurocognitive status,⁵¹ all of which are related to oxidative impairment, which therefore will necessitate support of hepatic function despite normal liver enzymes.

The therapeutic approach in systems analysis is based not on control but on modification of physiology, on supporting the reengagement of endogenous mechanisms of management rather than a permanent substitutive approach. In order to apply such a therapeutic approach, the level of physiologic activity most responsible for the cohesiveness and integrity of the system must be determined.

DETERMINING THE LEVEL AND METHOD OF STUDY

Biological information is encoded in a multi-scale information hierarchy: DNA, RNA, proteins, interactions, biological networks, cells, tissues and organs, individuals and, finally, ecologies. The important point is that the environment impinges upon each of these levels of the hierarchy and modulates the digital informational output from the genome. Thus, systems-level investigations demand the collection of data at each relevant level of the hierarchy between the phenotypic measurement (features of the cell) and the core digital genome.³⁸

—Leroy Hood et al, Institute for Systems Biology

If the human organism is to be studied as a system, the level of study and the method of bioinformatics should be carefully considered so as not to confuse cause with mechanism of disease. There are three levels of activity within the system that can be studied in four ways:

1. The activity of an individual subsystem: ie, the cell, and the metabolic achievements of the cell in its structural and functional roles, including genetic transcription, production of proteins, enzymes, etc
2. The interaction of the various subsystems
3. The functionality of the global system
4. The interaction of the first three levels with its environment

For the first half of the 20th century, “naïve reductionism” focused primarily on the first level: the cell. The clinical result was the production and use of drugs that inhibit or stimulate individual pathways, enzymes, cellular products, etc (ie, antiinflammatory drugs, diuretics, beta-blockers, aromatase inhibitors, etc), and exogenous substitutes of physiologic products (ie, cortisol, estradiol, insulin, thyroxine, etc).

The mid-20th century gave rise to molecular biology and an intense study of the role of DNA. Advances in high-throughput assays and bioinformatics since the late 20th century have allowed for the complexity and systems-behavior of the cell to be clearly demonstrated. The response of many researchers has not been to move up to higher levels of organization to view how the body functions as a whole but to move to the lowest level of activity: the genome (genomics) and the attendant “-omics” that arise from such study: proteomics, transcriptionomics, metabolomics, etc.^{1,3,37,52,53}

Still, this approach can be seen as an improvement in the study of human physiology because it moves away from “naïve reductionism.” However, it does not represent a paradigm shift in the concept of how life is managed. It arose out of advances in reductionist molecular biology and it continues to be a gene-centered approach to disease. What is different is that the paradigm has expanded to look at many different proteins and metabolites related to a specific disease or cell. Thus, the paradigm still reads like this:

Gene “x” encodes for a single protein → that affects the cells metabolism → that affects the function of tissues and organs → that affects the whole system → that leads to disease y.
Ergo: gene “x” is the cause of disease “y.”

There are two compelling arguments against this paradigm, one nosologic, the other clinical. Nosology, the science of disease categorization, was traditionally based on classifying disease based on a group of symptoms (ie, fibromyalgia), pathologic appearance (ie, amyloidosis), or primary location of occurrence (ie, breast cancer). With the shift to genomics, diseases have been categorized by single-gene mutations or polymorphisms that are associated with single diseases.

New network-based classification methods, such as the human disease network, also known as “diseaseome”⁵⁴ (Figure 1), have found that disorders should be grouped neither by symptom nor single gene mutations but based on clusters of underlying physiologic

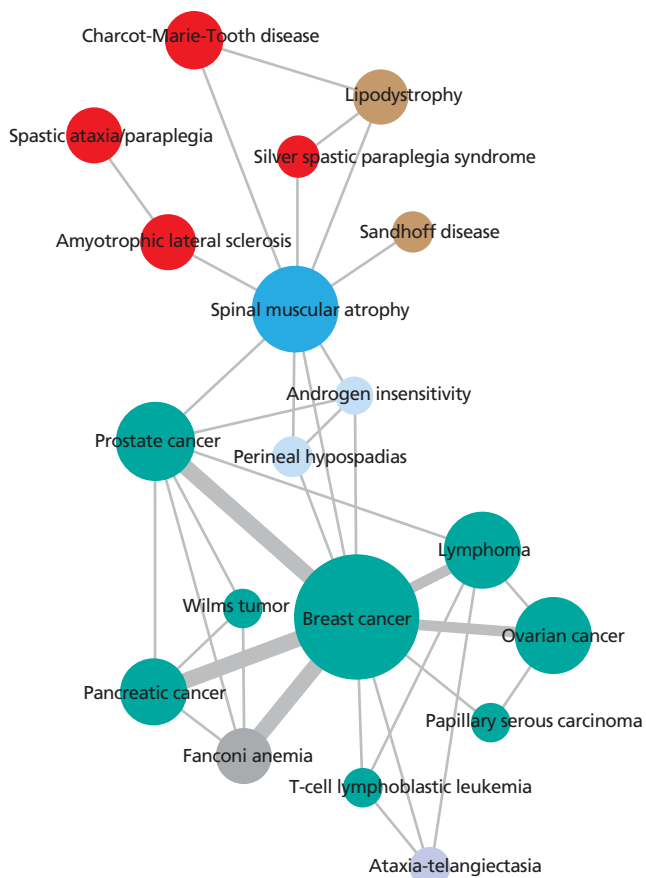


Figure 1 Human disease network and disease gene networks reveal the complex polygenetic basis of disease and propose a classification of disease based on complex physiologic activity rather than on phenotypic expression of symptoms. Reprinted with permission from: *Proc Natl Acad Sci U S A.* 2007;104(21):8685-90. Copyright 2007 National Academy of Sciences, USA.

dysfunction related to multiple simultaneous genetic polymorphisms and epigenetic changes.⁵⁴⁻⁶²

From the clinical perspective, this helps explain why two patients with an “identical” cancer—of the breast, for example—will have different responses to identical chemotherapy regimens. A landmark study of 2000 specimens of breast cancer suggests that it can be subclassified into 10 distinct groups based on various genomic and transcriptomic properties.⁶³ Even with a highly specific classification of breast cancer such as “triple negative,” there are sufficient variations in combinations of gene mutations that such a classification does not aid in evaluating drivers of growth or optimal therapy.⁶³ Likewise, the diseasesome concept offers intriguing concepts into how cancer of the breast and pancreas can be more similar physiologically due to shared genetic polymorphisms^{58,64} than two “identical” cancers of the breast based on staging methodologies.

In vitro, in vivo, epidemiologic, and small-scale clinical studies of single-gene polymorphisms yielded what appeared to be compelling evidence for the single-gene, single-disease view of physiology.⁶⁵ However, repeated clinical studies have failed, prospectively, to link single genes to the development of a single dis-

ease.⁶⁶⁻⁷⁴ The human disease network approach may help explain why: disease development is multifactorial, dependent on both multiple genetic and environmental factors as well as multiple neuroendocrine factors. Genes are the basis of the possibility of disease but do not appear to be the sole determinant of its probability.

A more holistic application of systems theory has been suggested in which the tripartite interaction between the genome, the cell, and the environment are evaluated as an ensemble.^{38,75,76} We agree with this approach but differ on the level of organization and management to be studied. If the inquiry is, “How did this disease develop?” the genome will contribute to a mechanistic understanding of pathophysiology. If our question is to be “why did this disease develop in *this* individual, and, *what* factors managed its appearance?” the level of study cannot be the genome. The genome is the *mechanistic* basis of life and of disease—the “how” of disease, not the “why.” Genes need to be told when, how often, and for how long to allow for transcription of their information. Therefore, there is something that manages why certain segments of the genetic code are transcribed or not.

At the microscopic level, the locus of management lies with the cell membrane. The membrane evaluates the internal functioning of the cell relative to the functioning of adjacent cells and vis-à-vis its external (ie, extracellular) environment (see Miller’s 19 sub-systems above to consider how this applies to a cell as well as human being as a whole). These data generate demands upon the nucleus to create new proteins to modify the functioning of the cell. Thus, the membrane manages the cell, not the nucleus, which is a respondent to the demands of the membrane. The membrane is *why* a gene is transcribed. The genome is *how* this management is executed.⁷⁷ At the macroscopic level, the global system is like the membrane: the system manages cells that manage genes, not vice versa. Thus, the development, expression, and continuation of disease are managed at the global level by non-genomic factors.

If we are to understand the person who has a disease and not just the disease a person has, as Sir William Osler said, we must understand what manages life and not limit ourselves only to its mechanisms. If, as evidence suggests, biology operates as a system and not a series of individual parts, then the future of medicine lies with a systems approach that studies the global manager of the system and not the individual mechanisms alone.

OVERVIEW OF THE ENDOBIOGENIC CONCEPT
Endobiogeny: A Global Systems Approach to Medicine

Endobiogeny is a theory of terrain. Conceived by Christian Duraffourd, MD, and developed with Jean-Claude Lapraz, MD, in the early 1980s,⁷⁸ endobiogeny seeks to explain how human life develops, maintains, and adapts itself as a dynamic, living system. The terrain consists of two aspects: structure and function. Structure

is the materialized constitutive elements of which an organism is composed, based on genetic heritage. Function refers to the expression of this constitution in the maintenance of structure, in basal functioning of structure, and the adaptive capacities of the organism in the face of exogenous and endogenous aggression.

If, as recent evidence suggests, the organism is a system and not merely a collection of parts, there must be a manager of this system, of this terrain. In order to ensure the integrity of the system, the manager must possess three qualities: (1) ubiquity of interaction with each structural element, (2) constancy of relationship with those elements, and (3) auto-regulation. This manager must function at every level of the system and within the four levels of interaction noted prior in the regulation each unit of function at the cellular level, tissue and organ level, as a global system, and in interaction with its external milieu.

A number of complex networks have been studied and proposed as managers of the organism.²⁴ The most widely studied are the autonomic nervous system (ANS), immune system, and endocrine system. A brief evaluation of these systems will reveal why the endocrine system is the manager of the terrain.

The ANS consists of the sympathetic and parasympathetic nervous systems. It calibrates the qualitative, quantitative, and chronologic duration of diverse areas of autonomic function from cardiac output to movement to digestion and sleep. The ANS is distributed throughout the body and synapses with every organ and tissue. Thus it meets the first criterion: ubiquity. However, it does not possess criterion 2: constancy of relationship. The ANS depends on other systems to solicit its activity because it acts as a means of calibration, not management. It also lacks criterion 3: auto-regulation. The ANS ends by autolysis or enzymatic degradation, not feedback.

The immune system participates in host defense against internal and external aggressions through the use of anti- and pro-inflammatory compounds, innate and learned immunologic activity, and various signaling molecules. It meets criterion 3: auto-regulation. In an optimal state, the anti- and pro-inflammatory aspects of the immune system are regulated through negative feedback. However, the immune system lacks the first two criteria: ubiquity and constancy of action. The immune system is not present throughout the entire body. It neither plays a managerial role in the formation of the structural elements of the cell nor in its basal functioning.

In contrast, the endocrine system meets all three criteria. Criterion 1: ubiquity. Hormones are excreted from glands into the circulation, where they are distributed to every cell in the body. Thanks to their paracrine and autocrine function, they are able to maintain precise local and regional management of the needs of specific sub-units of activity.

Criterion 2: constancy of relationship. The endocrine system manages all programmed phases of life

and is that which is necessary for the development of life. Long before the existence of the nervous or immune systems, the endocrine system manages the foundation of structure during fetogenesis and embryogenesis. It manages the evolution of structure during childhood, puberty, and genital pause, and it manages the dissolution of structure during the installation of death.

Recall that the terrain consists of function in addition to the materialization of structure. There are four levels of functional capacity related to metabolism, all of which are managed by the endocrine system. The first is the basal functioning of the structural elements of the cell for its own maintenance, growth, repair, and death. The second is its adaptive capacity: the momentary modification of the internal equilibrium of basal function. The third is the general adaptation syndrome of Selye, which refers to the programmed, chronologic response of the endocrine system to an unknown aggression, be it exogenous (ie, alimentation, infection, etc) or endogenous (ie, pregnancy, cancer, emotions, etc). The fourth and final level of functional regulation is adaptability: the ability of the organism to adapt the threshold of function of a specific aspect of the endocrine system without invoking the general adaptation syndrome. Adaptability occurs during anticipated but transitory states such as circadian changes in light, as well as unanticipated events, such as in Grave's disease, where there is an augmentation of thyrotropic activity.

Criterion 3: self-regulation. The endocrine system's use of feed-forward, feed-through, and feedback loops has been well established since Anokin's seminal work in 1935 and serves as the primary mechanism of self-regulation.

In summary, endobiogeny is a theory of terrain. The terrain assures its own functioning through permanent movement: a constant and unceasing adjustment of its internal equilibrium in the face of inductive and reactive elements. The manager of this terrain must similarly be dynamic, ubiquitous, constant in its association with every aspect of the organism, and self-regulating. The endocrine system is the only system that meets these criteria, thus it is the manager of the terrain. In conclusion, endobiogeny is the study of how the endocrine system manages the terrain.

The Need to Evaluate the Effects of the Endocrine System

Because the endocrine system is the true manager of the organism, it is the ideal object of study. However, serum levels of hormones are not sufficient to determine functionality. The primary significance of serum hormone levels is their indication of the central-peripheral feedback relationships, and quantitative organ output. Altered receptor binding and intracellular messenger activity,⁷⁹ epigenetic changes,⁸⁰ and persistent environmental pollutants⁸¹ can all affect the true impact of serum hormones on management of cellular metabolism.

Serum measurements of a hormones does not indicate where within the normal range is optimal for the individual at that particular homeostatic state. It also does not indicate what the proper level of a hormone should be relative to other hormones that facilitate, augment, diminish or inhibit its activity. Hormones have complex relationships to each other that need to be captured simultaneously in order to determine the true functionality of an individual. Probability profiles based on normal ranges of each individual hormone are not sufficient.

It is fallacious to assume that if each individual hormone is within normal limits, the entire system is functioning adequately.⁸²⁻¹⁰⁴ Because direct measurement of circulating hormones is not sufficient to measure functionality, what are needed are direct and indirect biomarkers of endocrine function that can reflect the functional efficiency of endocrine management of the terrain at every level of the system.

The Need to Use Blood Tests

Blood tests are an important diagnostic tool in modern medicine. The advantages of blood tests are many. They are objective, accurate, and reproducible. They are minimally invasive yet allow for evaluation of complex physiology. They are easily repeated, offering longitudinal assessment of the evolution and devolution of physiologic processes and treatments.

The primary shortcoming of modern lab studies is the binary nature of interpretation. Like the zeroes and ones of digital code, lab results are viewed as having two values and two interpretations:

o: lab test within normal range → no abnormality, ergo: no dysfunction

r: lab test outside the normal range → abnormality, ergo: dysfunction

This algorithm is repeated for each individual lab value assuming, in the reductionist model, that each lab value can be viewed in isolation from other lab values.

Routine lab testing creates a quandary for the clinician in two situations. The first is a symptomatic patient with normal lab values.¹⁰⁵⁻¹¹⁰ The second is an asymptomatic patient with abnormal lab values.¹¹¹⁻¹¹³ Both situations call into question the sufficiency of the reductionist model to explain the correlation between individual symptoms and individual biochemical data. This is typically the case for electrolytes; hepatic enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), and gamma-glutamyl transferase (GGT); and other common tests.

Such a binary system reads with an “error” message for both of these situations. The clinician must either ignore abnormal values, ignore symptoms, do further testing without clear guidance of what or how to test, or empirically medicate the patient in hopes that the problem will go away.

Other tests, such as antibodies associated with autoimmune disease, require more complex evaluation and decision making but pose problems themselves. They have a high degree of specificity but a low degree of sensitivity. Specificity is the percentage of patients who have a negative test and do not have a disease. Sensitivity is the number of patients who have a positive test and have the disease.¹¹⁴ When evaluating a patient for lupus, for example, anti-Smith antibodies (anti-Sm) have a sensitivity of 25% to 30% but a high specificity.¹¹⁵ In other words, the presence of anti-Sm antibodies does not rule in disease, but their absence makes it less likely that lupus is present.

If a patient is tested for anti-Sm in order to make a diagnosis of lupus in the presence of two clinical symptoms and the patient is positive for anti-Sm, will he or she be denied treatment because a total of four criteria have not been met? More than 70% of patients with positive anti-Sm antibodies will not have lupus, but if they do not have lupus, what does it mean that the test was positive? A binary test cannot answer this question. If a patient meets four or more of the criteria associated with lupus, including anti-Sm, how does that advance an understanding of why they have lupus or how they will be treated? Regardless of the test results, they will be treated symptomatically based on the organ(s) involved and the intensity of inflammatory or autoimmune manifestations.¹¹⁶

Because the human body operates as a system, a method of evaluation is needed that can reflect this complexity while still using serum values as the foundation of its assessment. Such a method should reflect all the properties of a system. The method should be dynamic and individualized, characterizing the function of a single unit of activity in and of itself, relative to other units and relative to the global functioning of the organism in a quantitative and qualitative fashion. If the object of study and method of interpretation are based in a systems approach, serum lab values can be viewed in a nonbinary format, reclaiming their key role in analytical and objective medical practice.

The Necessity of Using Serum Biomarkers and Their Shortcomings

A biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”¹¹⁷ Biomarkers are used to screen, diagnose, and prognosticate.¹¹⁸ All blood analytes are biomarkers in that they are markers of some biologic process. However, the ability to “screen, diagnose, or prognosticate” arises from a proper analysis of biomarkers that is accurate, valid and clinically relevant.

Numerous biomarkers have been proposed over the years only to be discredited or discarded later. The fundamental shortcoming of these biomarkers is that they continue to be based in reductionist biology rather than systems biology.

With respect to the selection and use of biomarkers, there are four common errors that have limited their clinical utility: (1) selection based on an animal model that does not realistically replicate human illness, (2) selection based on taking a clinical problem a priori and using statistical pooling to find an a posteriori correlative relationship, (3) use of biomarkers that are specific but not sensitive, and (4) mistaking downstream effects of pathology with upstream causes.

1. Selection Based on an Animal Model of Disease.

Animal models of human illness have long been used to determine single-causative agents of disease. Creating disease in a previously healthy animal is not always a realistic assessment of how the disease develops over time in humans because it fails to replicate the multiple factors within the terrain that are involved. Hepatic encephalopathy and the role of ammonia is a good example. Ammonia was long considered to be the primary cause of hepatic encephalopathy because (a) hepatic injury reduced the metabolic conversion of ammonia to urea, (b) humans with hepatic encephalopathy often had elevated serum ammonia, and (c) ammonia was shown to cause encephalopathy when infused in large amounts in otherwise healthy primates.^{119,120} Clinical studies have demonstrated that neither the presence nor severity of encephalopathy could be predicted solely by the serum ammonia level, nor was improvement in encephalopathy proportional to the reduction in ammonia levels. Currently, most experts agree that there are multiple variables that play a role in the development of hepatic encephalopathy, of which ammonia is but one.^{121,122}

2. Selection Based Statistical Pooling. Another common method of selecting a biomarker is through epidemiologic studies. In these studies, a clinical condition is selected a priori and numerous biomarkers and epidemiologic data are collected. Patterns of abnormalities in biomarkers are then correlated with the specific condition. Gamma-glutamyl transferase (GGT) is a good example of this with dozens of epidemiologic studies showing strong correlation with various clinical conditions.

GGT is an enzyme that transfers glutamyl residues. An elevation in serum GGT *above the norm* is seen in hepatobiliary disease, biliary obstruction, and intrahepatic cholestatic disorders. GGT also plays a key role in glutathione recycling most notably in the liver but also in bile ducts, small bowel, kidney, brain, pancreas, spleen, and breast. Retrospective analysis of epidemiological studies have associated normal GGT in the upper quartile of normal (40-60; normal = 0-60 IU/L), with the bioaccumulation of heavy metals,¹²³ persistent organic pollutants,¹²⁴⁻¹²⁷ dementia,¹²⁸⁻¹³⁰ hepatic insulin resistance,¹³¹ type 2 diabetes mellitus,^{126,127,132-140} hypertension,^{132,134,135,138,140-151} and dyslipidemia¹⁴⁰ independent of body mass index, lifestyle risk factors, or gender.

While GGT has been noted to be elevated in a wide variety of disorders, all these disorders could be best described as having a component of oxidative stress, which would explain the elevation of GGT (even within the upper quartile of the normal range). Oxidation of glucose to make ATP is fundamental to human physiology. A disturbance in oxidation will be implicated in so many disorders that we wonder how it could be predictive of a specific disease state prospectively.

If one finds a GGT in the upper range of normal, can one determine from this alone which patient has or will develop diabetes vs hypertension vs hyperlipidemia or a combination of these disorders? In a patient with hepatobiliary disease, with a GGT many-fold above the norm, GGT can no longer be used to predict the presence of the disorders noted above. How can the risk of these various disorders be evaluated in such patients?

There are numerous steps in the oxidation of glucose and in cellular respiration. How does a GGT in the upper quartile of normal guide the clinician in choosing the point of intervention, say, between insulin sensitization vs. oxidants vs antioxidants vs the Krebs cycle vs. mitochondrial support with L-carnitine, CoQ10, D-ribose, etc? All it says is that somewhere in the body, there is or may be an insufficiency of glutathione, without clarifying if it is due to a deficiency in glutathione production, an insufficiency in glutathione recycling, or an excess of glutathione consumption.

3. Application of a Biomarker With High Specificity When It Has a Low Sensitivity.

Prostate specific antigen (PSA) is the most widely used screening test for prostate cancer in the United States and Europe. In the US alone, over \$3 billions is spent annually on the test. Discovered in 1970, it was approved by the US Food and Drug Administration (FDA) in 1994 to detect cancer even though its success rate is only 3.8%.¹⁵² PSA is a good screening tool in evaluating the efficacy of treatment of known prostate cancer and in the surveillance of men with a history of prostate cancer post-treatment.¹⁵³

In other words, the PSA test is specific for known, active prostate cancer but not sensitive for cancer, much less predictive of cancer risk. It distinguishes neither benign nor malignant growth. It simply implicates dysregulated growth.¹⁵⁴ Dysregulated prostate growth is seen in prostate cancer but also in non-cancer-related events, such as benign prostatic hypertrophy, injury, use of certain medications, and infection. PSA levels are low in some men with malignant cancer and elevated in other men without cancer. Thus, PSA alone is not a good biomarker in screening for prostate cancer.

This is not a purely academic discussion because with an abnormal PSA the number-needed-to-treat is 48:1, meaning that in order to save 1 man's life, 47 men will undergo unnecessary biopsies with loss of sexual and urinary function based on a test that is being used as an indicator of a specific pathology when it is simply a nonspecific indicator of a disturbed pathophysiological state within the prostate.¹⁵⁵

4. Studies That Mistake the Result of a Pathologic Event as the Cause of That Event.

Dysregulation of the immune system is implicated in chronic, low-grade microbial infection and in altered inflammation/anti-inflammation pathways. There was a large body of evidence in the 1980s and 1990s that strongly associated titers of *Chlamydia pneumoniae* with myocardial infarction.¹⁵⁵⁻¹⁶⁴ It was observed that patients who had myocardial infarction had a greater incidence of chlamydial infection compared to those who did not. Chlamydia was found in biopsies of atherosclerotic tissue, and chlamydia was found to be atherogenic in vitro. It was hypothesized that treating chlamydia with the antibiotic clarithromycin would lower the risk of myocardial infarction. Smaller studies supported this hypothesis¹⁶⁵ but larger studies and meta-analysis found no benefit.^{166,167}

Both chlamydial infections and arterial disease occur in an environment of immune dysregulation. Thus, they are both downstream effects of altered immunity. The error here was to consider the downstream effects to be sequential, where chlamydia caused arterial disease, or, contributed in a significant enough way that it warranted treating with antibiotics (Figure 2).

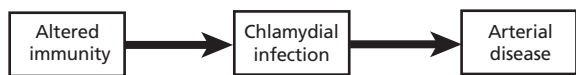


Figure 2 Downstream effects: attributing causality between related but unlinked events.

Later studies found that chlamydial titers and arterial disease also correlated with an elevation of c-reactive protein, a nonspecific indicator of acute inflammation.¹⁶⁸ Thus, it is more accurate to conclude that while all these elements are downstream events of altered immunity, they are neither sequentially nor causally related. That is to say, the same dysregulation in immune activity that favors inflammation creates a terrain that also favors the installation of chronic, low-grade infections. The greater the inflammatory milieu, the greater the risk of atherosclerotic disease (Figure 3).

The same type of error was made with respect to vitamin D and heart disease. Low vitamin D levels have been associated with an increased incidence of cardiovascular disease (CVD)¹⁶⁹⁻¹⁷¹ due to its association with a pro-inflammatory state. However, normalizing vitamin D levels does not change the clinical outcome of

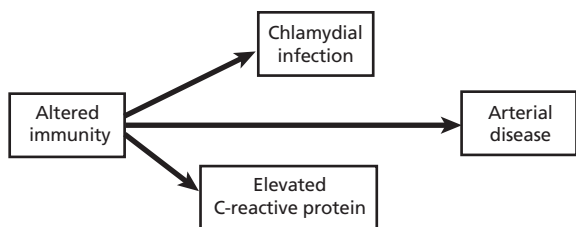


Figure 3 Downstream effects with a common upstream dysregulation.

patients with CVD even when it improves the inflammatory terrain.¹⁷² Because diseases are multifactorial, altering one factor does not necessarily reverse the course of disease.

In general, most biomarkers are single variables used to evaluate complex, multisystem disorders. From the endobiogenic perspective, there are very few cases of “single variable disorders” because the body is a system containing many variables that affect each other’s function. Relying on single biomarkers to screen or diagnose or prognosticate ultimately has limited benefit for the clinician and the patient. Most often, it results in indiscriminate treatment, as in the case of PSA, where men receive potentially harmful biopsies and are prescribed the use of 5 α -reductase inhibitors “just to be safe.” Or it results in excessive treatment because a single biomarker does not allow for the pathophysiologic individuality of the patient (ie, the terrain) to be determined. For example, in CVD, there is a trend to use vitamin D (for low vitamin D), aspirin, and fish oil (for elevated CRP), and a statin (for hyperlipidemia) because it is not possible from the current methods of evaluation to determine which aspect(s) of dysregulation is most responsible for the current state of disease.

An exception to the trend of using single biomarkers has been the use of multiple markers simultaneously in critical illness, such as septic shock or multi-system organ failure. Some examples include the Pediatric Risk of Mortality (PRISM-III revised) and the Acute Physiology, Age, Chronic Health Evaluation (APACHE IV).^{173,174} These scores use dozens of serum biomarkers and vital signs, such as serum glucose, respiratory rate, heart rate, etc, as well as clinical classifiers, such as surgical status, use of mechanical ventilation on admission, etc.

There are two key shortcomings of these multifactor tests with respect to clinical applicability. First, and most fundamentally, these evaluations represent retrospective attempts to find variables that predict mortality in order to stratify patients in research studies. Even within this narrow focus of interest, there is not an attempt to integrate these various factors into a coherent understanding of illness.

These scores do not truly integrate physiologic abnormalities in a way that reflects the patient’s terrain. Even if the scores are valid, they do not provide clinical guidance on determining which system(s) is most responsible for the current disorder or to what degree or in what order interventions should be administered, ie, cortisol, vasopressors, ventilation, dialysis, etc.

In summary, biomarkers are indicators of normal or pathologic activity. Biomarkers are commonly used in medicine and can be useful, but their ability to describe why an abnormality occurred or predict future imbalances is limited by the binary nature of the test. The ideal use of biomarkers would be based on a systems biology approach. In such a system, multiple factors are evaluated simultaneously, relative to each

other, describing human physiology in a dynamic fashion. Such an approach can offer specific areas and methods of intervention tailored to the terrain of each individual patient. Endobiogeny offers such a system: the biology of functions.

The Biology of Functions: A Biological Modeling System

*The new mathematics . . . is one of relationships and patterns. It is qualitative rather than quantitative and thus embodies the shift of emphasis that is characteristic of systems thinking—from objects to relationships, from quantity to quality, from substance to pattern.*⁴³

—Fritjof Capra

The biology of functions (BoF) is a biological modeling system developed by Dr Duraffourd, based on the theory of endobiogeny. As with other biological models, it simulates biological activity based on variables assumed to be most representative of the system, and is not a measurement of actual function. It differs from other biological models in three key ways. First, it simulates biological activity using biomarkers related to the direct and indirect effects of neuroendocrine activity. Second, it evaluates quantitative as well as qualitative function. Finally, it evaluates both the potential and functional achievements of the organism.

Current research in systems biology is focused on genomic and cellular activity. Many of these mathematical methods have proven to be robust, accurate and predicative in examining narrow areas of physio-

logic activity.¹⁷⁵⁻¹⁷⁷ However, due to conceptual limitations, they can neither describe the terrain that brought about disease nor suggest the optimal treatment within the context of the global functioning of the individual organism and serve largely as research tools.

As a model of the global functioning of the organism, the biology of functions evaluates factors both in and of themselves, in relationship to other units of activity, and in relationship to the system as a whole. There are over 150 indexes evaluating neuroendocrine activity in the biology of function. They are derived from 17 serum biomarkers that are linked to the various aspects of this activity, without directly measuring serum hormone levels except for thyroid stimulating hormone (Table 1).

The biomarker norms are the normative data of the adult, premenopausal female, which are considered to be the null state of human physiology in endobiogeny. The values of postmenopausal women, of men, and of children are compared against this normative data. Some exceptions include particular indexes that have grossly different values in various phases of childhood (unpublished data) and well-characterized sexual dimorphisms noted between men and women and their corresponding variations in serum biomarker values.¹⁷⁸ A current shortcoming of the algorithm is the exclusive reliance on normative data from a Western European population. This will need to be addressed to broaden the applicability of the biology of functions *vis-à-vis* men and women,^{178,179} non-European populations,^{180,181} and children.¹⁸²

Four biomarkers have a high degree of variability in their normative values from lab to lab and during particular phases of life: osteocalcin, total serum alka-

Table 1 Biomarkers Used in the Biology of Functions

Origin	Biomarker	Value	Conversion
Bone marrow cellular products	Red blood cell	per μ L	$\div 10^6$
	White blood cell, total	per μ L	$\div 10^3$
	Neutrophil	%	None
	Lymphocytes		
	Eosinophils		
	Monocytes		
	Basophils		
	Hemoglobin	g/dL	None
Bone marrow-serum interaction	Platelets	per μ L	$\div 10^3$
	Erythrocyte sedimentation rate	mm/h	None
Bone stroma enzymes	Osteocalcin	ng/mL	Proprietary
	Alkaline phosphatase bone isoenzyme	%	Proprietary
General enzymes	Lactate dehydrogenase	IU/L	Proprietary
	Creatine phosphokinase		
Endocrine	Thyroid-stimulating hormone	μ IU/mL	None
Electrolytes	Potassium	mmol/L	None
	Calcium, total serum	mmol/L	$\div 2$

line phosphatase, lactate dehydrogenase, and creatine phosphokinase. The normative values determined by each lab are standardized to an internal consistency in the following manner:

$$(a \times b) / (x + y)$$

where “a” is the lab value of the patient, “b” is a proprietary adjustment factor, “x” is the high, and “y” is the low value reported by the laboratory for the given biomarker. The adjustment factor varies for each of the four biomarkers noted above.

The indexes evaluate relative neuroendocrine functionality and are derived from 16 direct ratios of the 17 biomarkers. The remaining indexes are indirect ratios: indexes of indexes. Nearly 90% of the indexes describe relative and qualitative function. In other words, they describe the physiologic capability of the organism in a contextual manner. The relativity of the indexes ensures global internal consistency and reproducibility across patients and diseases with reliable norms.

The normal range of each index is determined by two methods. First, the general range is determined from high and low values of each biomarker of which an index is composed. The specific normative range is based on retrospective analysis of unpublished data from clinical practice.

The use of such a limited number of biomarkers to derive a large amount of information about human physiology can only be achieved under two conditions. The first is if the body functions as a system and the effects of one event affect other events. The second is if the level of evaluation is sufficiently upstream that a small number of factors are linked to a wide variety of downstream events, having a profound effect on multiple lines of biologic activity. Hormones are secreted in extremely low concentrations (10^{-9} - 10^{-12} g/dL) yet have a profound impact on global physiology at the nuclear, cytoplasmic, cellular, tissue, organ and system levels. Each subsystem of activity that the endocrine system manages is amplified at the level below it because each system manages or influences increasingly complex subsystems of activity. Thus, small changes at the endocrine level can have a profound and wide-ranging impact on the ensemble of metabolic processes. This is why we believe that such a small number of biomarkers can be used to generate such a large number of indexes.

The Logic Behind the Biology of Functions Indexes

Three basic observations are the foundation of this elegant and simple biologic model: (1) The endocrine system is the manager of the terrain, of the biologic system; (2) certain biomarkers are produced as a result of this endocrine management; and (3) the true functionality of any system is based on the relative activity of one factor to another. Because these biomarkers are an indicator of endocrine management, indexing biomarker values as ratios provides an assessment of relative functionality of the endocrine management of the terrain.

1. Endocrine management. We have established that the endocrine system is the true manager of the terrain, and that the effects of endocrine activity cannot be accurately evaluated by direct serum measurement.

2. Biomarkers and the endocrine system. It has been known for nearly 100 years that changes in common biomarkers were associated with specific endocrinopathies.¹⁸³⁻¹⁸⁶ Through elegant experiments, it has been clarified that the changes in these biomarkers are the result of endocrine management of metabolism. For example, it was observed in the 1950s that androgens cause a proliferation of red blood cells.¹⁸⁷⁻¹⁹³ Thus, red blood cell levels in the serum are a marker of a certain aspect of androgen function. It was also observed that estrogens cause a proliferation of white blood cells and the same can be said about white blood cells and estrogen activity.^{194,195}

3. Systems analysis and relative relationships. As noted above, newer evidence suggests that the body is a true system, composed of various subsystems that act independently of each other but in coordination with each other. Because the functioning of each unit is integrated and interrelated to the functioning of the others units and to the whole, it is the relative activity of one unit to another that determines the true state of functionality. The appreciation of relative changes in biomarkers has been present for nearly 100 years and is gaining increasing appreciation once again.^{184-186,196-198}

The value of relative changes of biomarkers in and of themselves and with respect to other markers is paramount in a systems approach. For example, in and of themselves, normal red and white blood cell counts do not offer actionable information about the state of androgens or estrogens. However, if you relate one to the other, you have a general evaluation of the global activity of androgens relative to estrogens regardless of the absolute value of red or white blood cells or the quantitative serum level of androgens or estrogens.

The relative imbalance of androgens and estrogens can be clinically significant. Numerous studies have shown that even with normal serum levels of androgens and estrogens, one can develop fibroids, polycystic ovarian disease, infertility, or hair loss.¹⁹⁶⁻²⁰¹ The ratio of red to white blood cells, called the “genital ratio” (described below) is a necessary but not sufficient evaluation of gonadotropic activity. However, it lays the foundation for increasingly complex evaluations with respect to these and other disorders.

Normally, the diagnosis of and decision to treat “endocrine” disorders is based solely on quantitative serum concentrations of hormones. If levels are normal, there will be no justifiable basis for treatment and the patient is condemned to suffer. If an empirical treatment is started out of compassion, there rests no objective reason for the choice of treatment nor a manner in which to understand why the treatment failed if it does not work. In such cases, the patient is consid-

Table 2 Ratios in Medicine

System	Ratio	Composition	Indication	Shortcoming
Renal	Blood urea nitrogen/creatinine	Blood urea nitrogen/creatinine	Evaluates the rate of renal perfusion relative to renal clearance	Does not indicate why perfusion or clearance is impaired, if it is due to structural or functional impairments, or both
	Microalbumin/creatinine	Microalbumin/creatinine	Evaluates resorptive integrity of kidney relative to its clearance ability	Does not indicate functional reasons for disruption in renal tubule integrity
Immune	Albumin/globulin ratio	Albumin/Globulin	Evaluates the risk of autoimmunity vs cancer vs liver failure	Does not evaluate endocrine, gastrointestinal factors related to protein uptake, distribution, or utilization
	CD4+/CD8+	Subsets of lymphocytes based on specific cell determinates (CD)	Used to assess relative strength of immune system in HIV seropositive patients; CD4 counts vary day to day, so they are indexed relative to the CD8 count	Does not evaluate the factors related to generation, mobilization, and regulation of immune cells
Hematologic	Hematocrit	Red blood cells/whole blood volume	Evaluates the density of blood relative to intravascular volume by indexing the number of red blood cells produced relative to the total blood volume	Does not evaluate the factors influencing red blood cell production or demargination from the spleen

ered to have an “idiopathic” disorder, often deemed untreatable. We believe that using ratios of biomarkers may be a more accurate and valid method of determining physiologic functionality not only in cases of idiopathic disorders but more broadly when evaluating various disorders, even common ones with atypical courses or unexpected response to treatment.

Precedence of Using Ratios in Clinical Medicine

The practice of relating biomarker to each other is not new in medicine and there are many examples used on a daily basis (Table 2).

While these tests are dynamic—they are derived from circulating blood analytes—they do not indicate the relationship of individual units to each other or to the whole system, which is why we do not consider them to be candidates for use in a systems approach to biology.

The biology of functions is composed of a series of direct and indirect indexes. Direct indexes are composed of individual biomarkers directly related to each by various mathematical relationships. Indirect indexes are composed of direct indexes, indirect indexes, and/or individual biomarkers in various permutations that can contain up to six levels of indexes within indexes.

An example of a direct index is the genital ratio, which looks at the impact of androgens relative to estrogens at the tissue level (Figure 4). It is a ratio of two of the 17 biomarkers: red blood cells and white blood cells.

$$\begin{aligned} \text{Genital ratio} &= \text{Red blood cells} / \text{White blood cells} \\ \text{Genital ratio} &= \text{Androgens} / \text{Estrogens} \end{aligned}$$

Figure 4 Direct index in the biology of functions.

An example of an indirect index is the Thrombotic index (Figure 5), which expresses the risk of sudden thromboembolic phenomenon. Acute ischemic events that result in sudden cardiac death occur in arteries that

$$\begin{aligned} \text{Thrombotic index} &= \\ &(\text{Thrombogenic index} \times \text{Evoked Histamine index} \times \text{Genital Ratio})/10 \\ &[\text{Thrombogenic index} = 10 \\ &(\text{Bone remodeling} \times \text{Apoptosis} \times \text{Necrosis})/\text{Metabolic yield}] \\ \text{Thrombotic index} &= \\ &\text{Bone remodeling index} \times \text{Apoptosis} \times \text{Necrosis} \times \\ &\text{Evoked Histamine index} \times \text{Genital Ratio} \\ &\text{Metabolic yield index} \end{aligned}$$

Figure 5 Indirect index in the biology of functions: Thrombotic index.

often have mild coronary artery luminal occlusion and minimal plaque calcification. Neither calcium score by computed tomography scan nor angiography will be able to identify patients most at risk. Typically these events occur in patients under the age of 60, with minimal classic risk factors, thus general screening factors will not identify them either.²⁰² The ability to aggregate the primary known factors related to thrombus formation and plaque rupture may help identify patients most at risk for sudden cardiac death or acute ischemia based on functional factors rather than structural factors.

The mathematical relationships in the index express that the risk of thromboembolism is the result of a triad of factors that are necessary but not sufficient on their own: (1) risk of thrombus formation, which can occur due to necrosis²⁰³⁻²⁰⁵ or apoptosis,²⁰² (2) histamine activity,²⁰⁶⁻²⁰⁸ and (3) elevated androgens²⁰⁹⁻²¹⁷ represented by the genital ratio (androgens/estrogens) in the numerator, which is consistent with known pathophysiologic mechanisms of thromboembolic phenomenon.

In summary, evaluating biologic activity relative to each other has precedence in medicine. It helps contextualize the relevance of one finding to another. The biology of functions is composed of direct indexes where individual biomarkers are related to each other and indirect indexes where direct indexes and various biomarkers are indexed against each other to express increasingly com-

plex biological activity that is multi-factorial in nature. The majority of indexes in the biology of functions are indirect indexes that evaluate the function of units of activity relative to other units of activity.

Part 2 of this article, which will be published in the March 2013 issue of *Global Advances in Health and Medicine*, addresses the experimental and clinical basis for the biomarkers used in the biology of functions.

REFERENCES

- Strange K. The end of "naive reductionism": rise of systems biology or renaissance of physiology? *Am J Physiol Cell Physiol*. 2005;288(5):C968-74.
- Lyons AS, Petrucelli R. *Medicine: An illustrated history*. New York: Harry N. Abrams; 1987.
- Fang FC, Casadevall A. reductionistic and holistic science. *Infect Immun*. 2011;79(4):1401-4.
- Hanin L. Why victory in the war on cancer remains elusive: biomedical hypotheses and mathematical models. *Cancers*. 2011;3:340-67.
- Jackson Nakazawa D. The autoimmune epidemic: bodies gone haywire in a world out of balance—and the cutting-edge science that promises hope. 1st ed. New York: Touchstone; 2008.
- Laplace P-S. *Essai philosophique sur les probabilités*, p.4: éd. Bachelier; 1840.
- Descartes R. *Discours de la méthode: pour bien conduire sa raison, et chercher la vérité dans les sciences* (A discourse of the method for conducting oneself with reason, and searching for truth in the sciences), Vol Cinquième: éd. Cousin; 1637:142.
- Verduijn M, Jager KJ, Zoccali C, Dekker FW. Genetic association studies: discovery of the genetic basis of renal disease. *Nephron Clin Pract*. 2011;119(3):c236-9.
- Woods MO, Younghusband HB, Parfrey PS, et al. The genetic basis of colorectal cancer in a population-based incident cohort with a high rate of familial disease. *Gut*. Oct 2010;59(10):1369-77.
- Moller CC, Pollak MR, Reiser J. The genetic basis of human glomerular disease. *Adv Chronic Kidney Dis*. 2006;13(2):166-73.
- Day IN, Gu D, Ganderton RH, Spanakis E, Ye S. Epidemiology and the genetic basis of disease. *Int J Epidemiol*. 2001;30(4):661-7.
- Partridge M. Oral cancer: 1. The genetic basis of the disease. *Dent Update*. 2000;27(5):242-8.
- Tsipouras P, Silverman DI. The genetic basis of aortic disease. Marfan syndrome and beyond. *Cardiol Clin*. 1999;17(4):683-96.
- Tay A. Genetic basis of disease: studies of eicosanoids and some complex disease traits. *Clin Sci (Lond)*. 1996;91(3):264-6.
- Johnson MC, Payne RM, Grant JW, Strauss AW. The genetic basis of paediatric heart disease. *Ann Med*. Jun 1995;27(3):289-300.
- Arnold A. Genetic basis of endocrine disease 5. Molecular genetics of parathyroid gland neoplasia. *J Clin Endocrinol Metab*. Nov 1993;77(5):1108-12.
- McPhaul MJ, Marcelli M, Zoppi S, Griffin JE, Wilson JD. Genetic basis of endocrine disease. 4. The spectrum of mutations in the androgen receptor gene that causes androgen resistance. *J Clin Endocrinol Metab*. Jan 1993;76(1):17-23.
- Fagin JA. Genetic basis of endocrine disease 3: Molecular defects in thyroid gland neoplasia. *J Clin Endocrinol Metab*. Dec 1992;75(6):1398-1400.
- Wadia RS. Another genetic basis for vascular disease. *Natl Med J India*. 1992;5(2):71-2.
- White PC, New MI. Genetic basis of endocrine disease 2: congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 1992;74(1):6-11.
- Taylor SI, Cama A, Accili D, et al. Genetic basis of endocrine disease. 1. Molecular genetics of insulin resistant diabetes mellitus. *J Clin Endocrinol Metab*. 1991;73(6):1158-63.
- Grant DA. Genetic polymorphism of the alcohol metabolising enzymes as a basis for alcoholic liver disease. *Br J Addict*. 1988;83(11):1255-9.
- Kidson C, Chen P. DNA damage, DNA repair and the genetic basis of Alzheimer's disease. *Prog Brain Res*. 1986;70:291-301.
- Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev*. 2000;52(4):595-638.
- Madsen R, Lundstedt T, Trygg J. Chemometrics in metabolomics—a review in human disease diagnosis. *Anal Chim Acta*. 2010;659(1-2):23-33.
- Spratlin JL, Serkova NJ, Eckhardt SG. Clinical applications of metabolomics in oncology: a review. *Clin Cancer Res*. 2009;15(2):431-40.
- Wang H, Tso VK, Slupsky CM, Fedorak RN. Metabolomics and detection of colorectal cancer in humans: a systematic review. *Future Oncol*. 2010;6(9):1395-406.
- Heazell AE, Brown M, Worton SA, Dunn WB. Review: The effects of oxygen on normal and pre-eclamptic placental tissue—insights from metabolomics. *Placenta*. 2011;32 Suppl 2:S119-24.
- Galvao ER, Martins LM, Ibiapina JO, Andrade HM, Monte SJ. Breast cancer proteomics: a review for clinicians. *J Cancer Res Clin Oncol*. 2011;137(6):915-25.
- Buczynski MW, Dumlaio DS, Dennis EA. Thematic review series: proteomics. An integrated omics analysis of eicosanoid biology. *J Lipid Res*. 2009;50(6):1015-38.
- Schiffer E, Mischak H, Zimmerli LU. Proteomics in gerontology: current applications and future aspects—a mini-review. *Gerontology*. 2009;55(2):123-37.
- Gast MC, Schellens JH, Beijnen JH. Clinical proteomics in breast cancer: a review. *Breast Cancer Res Treat*. 2009;116(1):17-29.
- Conrad DH, Goyette J, Thomas PS. Proteomics as a method for early detection of cancer: a review of proteomics, exhaled breath condensate, and lung cancer screening. *J Gen Intern Med*. 2008;23 Suppl 1:78-84.
- Drake TA, Ping P. Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Proteomics approaches to the systems biology of cardiovascular diseases. *J Lipid Res*. 2007;48(1):1-8.
- Rice GE, Georgiou HM, Ahmed N, Shi G, Kruppa G. Translational proteomics: developing a predictive capacity—a review. *Placenta*. 2006;27 Suppl A:S76-86.
- Patel PS, Telang SD, Rawal RM, Shah MH. A review of proteomics in cancer research. *Asian Pac J Cancer Prev*. Apr-Jun 2005;6(2):113-117.
- Ideker T, Galitski T, Hood L. A new approach to decoding life: systems biology. *Annu Rev Genomics Hum Genet*. 2001;2:343-72.
- Hood L, Rowen L, Galas DJ, Aitchison JD. Systems biology at the Institute for Systems Biology. Briefings in functional genomics and proteomics. 2008;7(4):239-248.
- Von Bertalanffy L. The history and status of general systems theory. *Acad Manage J*. 1972;15(4):407-26.
- Miller JG. *Living systems*. New York: McGraw-Hill; 1978.
- Red'ko VG, Prokhorov DV, Burtsev MB. Theory of functional systems, adaptive critics and neural networks. Paper presented at: Proceedings of International Joint Conference on Neural Networks, 2004; Budapest.
- Miller JG. Toward a general theory for the behavioral sciences. *Am Psychol*. 1955;10(9):513-531.
- Capra F. *The web of life : a new scientific understanding of living systems*. 1st Anchor Books ed. New York: Anchor Books; 1996.
- Tubbs S. *A systems approach to small group interaction*. 11th ed. New York: McGraw-Hill Humanities/Social Sciences/Languages; 2011.
- Clayton T, Radcliff N. *Sustainability: a systems approach*. 1st ed. London: Routledge; 1996.
- Capra F, Pauli GA. *Steering business toward sustainability*. Tokyo; New York: United Nations University Press; 1995.
- Dryden GW, Jr, Deaciuc I, Arteil G, McClain CJ. Clinical implications of oxidative stress and antioxidant therapy. *Curr Gastroenterol Rep*. 2005;7(4):308-16.
- James SJ, Rose S, Melnyk S, et al. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. *FASEB J*. 2009;23(8):2374-83.
- Lee DH, Gross MD, Steffes MW, Jacobs DR Jr. Is serum gamma-glutamyltransferase a biomarker of xenobiotics, which are conjugated by glutathione? *Arterioscler Thromb Vasc Biol*. 2008;28(4):e26-8; author reply e29.
- Morino K, Petersen KF, Shulman GI. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes*. 2006;55 Suppl 2:S9-15.
- Dhanasekaran M, Ren J. The emerging role of coenzyme Q-10 in aging, neurodegeneration, cardiovascular disease, cancer and diabetes mellitus. *Curr Neurovasc Res*. 2005;2(5):447-59.
- Auffray C, Adcock IM, Chung KF, Djukanovic R, Pison C, Sterk PJ. An integrative systems biology approach to understanding pulmonary diseases. *Chest*. 2010;137(6):1410-6.
- Taylor BS, Varambally S, Chinnaiyan AM. A systems approach to model metastatic progression. *Cancer Res*. 2006;66(11):5537-9.
- Barrenas F, Chavali S, Holme P, Mobini R, Benson M. Network properties of complex human disease genes identified through genome-wide association studies. *PLoS One*. 2009;4(11):e8090.
- Bosco A, McKenna KL, Firth MJ, Sly PD, Holt PG. A network modeling approach to analysis of the Th2 memory responses underlying human atopical disease. *J Immunol*. 2009;182(10):6011-21.
- Chavali S, Barrenas F, Kanduri K, Benson M. Network properties of human disease genes with pleiotropic effects. *BMC Syst Biol*. 2010;4:78.
- Dudley JT, Butte AJ. Identification of discriminating biomarkers for human disease using integrative network biology. *Pac Symp Biocomput*. 2009:27-38.
- Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabasi AL. The human disease network. *Proc Natl Acad Sci U S A*. 2007;104(21):8685-90.
- Jiang JQ, Dress AW, Chen M. Towards prediction and prioritization of disease genes by the modularity of human phenome-genome assembled network. *J Integr Bioinform*. 2010;7(2).
- Lee DS, Park J, Kay KA, Christakis NA, Oltvai ZN, Barabasi AL. The implications of human metabolic network topology for disease comorbidity. *Proc Natl Acad Sci U S A*. 2008;105(29):9880-5.
- Loscalzo J. Systems biology and personalized medicine: a network approach

- to human disease. *Proc Am Thorac Soc.* 2011;8(2):196-8.
62. Zanzoni A, Soler-Lopez M, Aloy P. A network medicine approach to human disease. *FEBS Lett.* 2009;583(11):1759-65.
 63. Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature.* 2012;486(7403):395-9.
 64. Urbach D, Moore JH. Mining the diseasome. *BioData Min.* 2011;4:25.
 65. Iakoubova OA, Robertson M, Tong CH, et al. KIP6 Trp719Arg polymorphism and the effect of statin therapy in elderly patients: results from the PROSPER study. *Eur J Cardiovasc Prev Rehabil.* 2010;17(4):455-61.
 66. Assimes TL, Holm H, Kathiresan S, et al. Lack of association between the Trp719Arg polymorphism in kinesin-like protein-6 and coronary artery disease in 19 case-control studies. *J Am Coll Cardiol.* 2010;56(19):1552-63.
 67. Mathew J, Narayanan P, Sundaram R, et al. Lack of association between Glu(298) asp polymorphism of endothelial nitric oxide synthase (eNOS) gene and coronary artery disease in Tamilian population. *Indian Heart J.* 2008;60(3):223-7.
 68. Tsantes AE, Nikolopoulos GK, Bagos PG, Vaiopoulos G, Travlou A. Lack of association between the platelet glycoprotein Ia C807T gene polymorphism and coronary artery disease: a meta-analysis. *Int J Cardiol.* 2007;118(2):189-96.
 69. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Lack of association between the P2Y12 receptor gene polymorphism and platelet response to clopidogrel in patients with coronary artery disease. *Thromb Res.* 2005;116(6):491-7.
 70. Schmoelzer I, Renner W, Paulweber B, et al. Lack of association of the Glu298Asp polymorphism of endothelial nitric oxide synthase with manifest coronary artery disease, carotid atherosclerosis and forearm vascular reactivity in two Austrian populations. *Eur J Clin Invest.* 2003;33(3):191-8.
 71. Sigusch HH, Surber R, Lehmann MH, et al. Lack of association between 27-bp repeat polymorphism in intron 4 of the endothelial nitric oxide synthase gene and the risk of coronary artery disease. *Scand J Clin Lab Invest.* 2000;60(3):229-35.
 72. Anderson JL, Muhlestein JB, Habashi J, et al. Lack of association of a common polymorphism of the plasminogen activator inhibitor-1 gene with coronary artery disease and myocardial infarction. *J Am Coll Cardiol.* 1999;34(6):1778-83.
 73. Garg UC, Arnett DK, Folsom AR, Province MA, Williams RR, Eckfeldt JH. Lack of association between platelet glycoprotein IIb/IIIa receptor PLA polymorphism and coronary artery disease or carotid intima-media thickness. *Thromb Res.* 1998;89(2):85-9.
 74. Fujimura T, Yokota M, Kato S, et al. Lack of association of angiotensin converting enzyme gene polymorphism or serum enzyme activity with coronary artery disease in Japanese subjects. *Am J Hypertens.* 1997;10(12 Pt 1):1384-90.
 75. Halanynch KM, Goertzen LR. Grand challenges in organismal biology: The need to develop both theory and resources. *Integr Comp Biol.* 2009;49(5):475-9.
 76. Schwenk K, Padilla DK, Bakken GS, Full RJ. Grand challenges in organismal biology. *Integr Comp Biol.* 2009;49(1):7-14.
 77. Lipton B. *The biology of belief: unleashing the power of consciousness, matter, & miracles.* 13 ed. New York: Hay House; 2011.
 78. Duraffourd C, Lapraz JC. *Traite de Phytotherapie Clinique: Medicine et Endobiogenie.* Masson ed. Paris: Masson; 2002.
 79. Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am J Psychiatry.* 2003;160(9):1554-65.
 80. Chiam K, Tilley WD, Butler LM, Bianco-Miotto T. The dynamic and static modification of the epigenome by hormones: A role in the developmental origin of hormone related cancers. *Biochim Biophys Acta.* 2009;1795(2):104-9.
 81. Gerhard I, Waibel S, Daniel V, Runnebaum B. Impact of heavy metals on hormonal and immunological factors in women with repeated miscarriages. *Hum Reprod Update.* 1998;4(3):301-9.
 82. Atkinson SA. Vitamin D status and bone biomarkers in childhood cancer. *Pediatr Blood Cancer.* 2008;50(2 Suppl):479-82; discussion 486.
 83. Chlebna-Sokol D, Rusinska A. Serum insulin/like growth factor I, bone mineral density and biochemical markers of bone metabolism in children with idiopathic osteoporosis. *Endocr Regul.* 2001;35(4):201-8.
 84. Delmas PD, Chateelain P, Malaval L, Bonne G. Serum bone GLA-protein in growth hormone deficient children. *J Bone Miner Res.* 1986;1(4):333-8.
 85. Nimptsch K, Hailer S, Rohrmann S, Gedrich K, Wolfram G, Linseisen J. Determinants and correlates of serum undercarboxylated osteocalcin. *Ann Nutr Metab.* 2007;51(6):563-70.
 86. Tarle M. Plasma osteocalcin values and related hormonal parameters in patients subjected to a variety of prostate anticancer agents. *Urol Res.* 1991;19(1):39-44.
 87. Vietri MT, Sessa M, Pilla P, et al. Serum osteocalcin and parathyroid hormone in healthy children assessed with two new automated assays. *J Pediatr Endocrinol Metab.* 2006;19(12):1413-9.
 88. Banu KS, Aruldas MM. Sex steroids regulate TSH-induced thyroid growth during sexual maturation in Wistar rats. *Exp Clin Endocrinol Diabetes.* 2002;110(1):37-42.
 89. Cuenca E, Serrano MI, Gibert-Rahola J, Galiana J. Enhancement of noradrenaline responses by thyrotropine-releasing hormone. *J Pharm Pharmacol.* 1975;27(3):199-200.
 90. Gidron Y, Ronson A. Psychosocial factors, biological mediators, and cancer prognosis: a new look at an old story. *Curr Opin Oncol.* 2008;20(4):386-92.
 91. Grimm Y, Reichlin S. Thyrotropin releasing hormone (TRH): neurotransmitter regulation of secretion by mouse hypothalamic tissue in vitro. *Endocrinology.* 1973;93(3):626-31.
 92. Morley JE. Extrahypothalamic thyrotropin releasing hormone (TRH) – its distribution and its functions. *Life Sci.* Oct 29 1979;25(18):1539-1550.
 93. Morley JE, Klein C, Kalk WJ, et al. Pituitary responsiveness to thyrotropin releasing hormone in insulin-dependent diabetes mellitus. *Horm Metab Res.* 1978;10(3):231-7.
 94. Mortimer CH, Besser GM, McNeilly AS, Tunbridge WM, Gomez-Pan A, Hall R. Interaction between secretion of the gonadotrophins, prolactin, growth hormone, thyrotrophin and corticosteroids in man: the effects of LH FSH-RH, TRH and hypoglycaemia alone and in combination. *Clin Endocrinol (Oxf).* Oct 1973;2(4):317-26.
 95. Sekulic M, Susic-Jurjevic B, Filipovic B, Manojlovic-Stojanoski M, Milosevic V. Immunoreactive TSH cells in juvenile and peripubertal rats after estradiol and human chorionic gonadotropin treatment. *Acta Histochem.* 2006;108(2):117-23.
 96. Shah N, Evans WS, Veldhuis JD. Actions of estrogen on pulsatile, nocturnal, and entropic modes of growth hormone secretion. *Am J Physiol.* 1999;276(5 Pt 2):R1351-8.
 97. Joosten KF, de Kleijn ED, Westerterp M, et al. Endocrine and metabolic responses in children with meningococcal sepsis: striking differences between survivors and nonsurvivors. *J Clin Endocrinol Metab.* 2000;85(10):3746-53.
 98. Goeters C, Mertes N, Tacke J, et al. Repeated administration of recombinant human insulin-like growth factor-I in patients after gastric surgery. Effect on metabolic and hormonal patterns. *Ann Surg.* 1995;222(5):646-53.
 99. Radetti G, Paganini C, Gentili L, Barbin F, Pasquino B, Zachmann M. Altered adrenal and thyroid function in children with insulin-dependent diabetes mellitus. *Acta Diabetol.* 1994;31(3):138-40.
 100. Bugaresti JM, Tator CH, Silverberg JD, et al. Changes in thyroid hormones, thyroid stimulating hormone and cortisol in acute spinal cord injury. *Paraplegia.* 1992;30(6):401-9.
 101. Kjellman BF, Ljunggren JG, Beck-Friis J, Wetterberg L. Reverse T3 levels in affective disorders. *Psychiatry Res.* 1983;10(1):1-9.
 102. Kahana L, Keidar S, Sheinfeld M, Palant A. Endogenous cortisol and thyroid hormone levels in patients with acute myocardial infarction. *Clin Endocrinol (Oxf).* 1983;19(1):131-9.
 103. Koono N. Reciprocal changes in serum concentrations of triiodothyronine and reverse triiodothyronine between summer and winter in normal adult men. *Endocrinol Jpn.* 1980;27(4):471-6.
 104. Banos C, Tako J, Salamon F, Gyorgyi S, Czikkely R. Effect of ACTH-stimulated glucocorticoid hypersecretion on the serum concentrations of thyroxine-binding globulin, thyroxine, triiodothyronine, reverse triiodothyronine and on the TSH-response to TRH. *Acta Med Acad Sci Hung.* 1979;36(4):381-94.
 105. Boomershine CS. A comprehensive evaluation of standardized assessment tools in the diagnosis of fibromyalgia and in the assessment of fibromyalgia severity. *Pain Res Treat.* 2011;2012:653714.
 106. Simonsick EM, Newman AB, Ferrucci L, et al. Subclinical hypothyroidism and functional mobility in older adults. *Arch Intern Med.* 2009;169(21):2011-7.
 107. Baek JH, Chung PW, Kim YB, et al. Favorable influence of subclinical hypothyroidism on the functional outcomes in stroke patients. *Endocr J.* 2009;57(1):23-9.
 108. Akcakoyun M, Kaya H, Kargin R, et al. Abnormal left ventricular longitudinal functional reserve assessed by exercise pulsed wave tissue Doppler imaging in patients with subclinical hypothyroidism. *J Clin Endocrinol Metab.* 2009;94(8):2979-83.
 109. Sell MA, Schott M, Tharandt L, Cissewski K, Scherbaum WA, Willenberg HS. Functional central hypothyroidism in the elderly. *Aging Clin Exp Res.* 2008;20(3):207-10.
 110. Tramon E, Aubert-Khalifa S, Guye M, Ranjeva JP, Felician O, Ceccaldi M. Hypo-retrieval and hyper-suppression mechanisms in functional amnesia. *Neuropsychologia.* 2009;47(3):611-24.
 111. Verslype C. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *Acta Clin Belg.* 2004;59(5):285-9.
 112. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med.* Apr 27 2000;342(17):1266-71.
 113. Theal RM, Scott K. Evaluating asymptomatic patients with abnormal liver function test results. *Am Fam Physician.* 1996;53(6):2111-9.
 114. Altman DG, Bland JM. Diagnostic tests. 1: Sensitivity and specificity. *BMJ.* 1994;308(6943):1552.
 115. Colglazier CL, Sutej PG. Laboratory testing in the rheumatic diseases: a practical review. *South Med J.* 2005;98(2):185-91.

116. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997;40(9):1725.
117. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69(3):89-95.
118. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation.* 2006;113(19):2335-62.
119. Cole M, Rutherford RB, Smith FO. Experimental ammonia encephalopathy in the primate. *Trans Am Neurol Assoc.* 1970;95:82-5.
120. Cole M, Rutherford RB, Smith FO. Experimental ammonia encephalopathy in the primate. *Arch Neurol.* 1972;26(2):130-6.
121. Bernal W, Hall C, Karvellas CJ, Auzinger G, Sizer E, Wendon J. Arterial ammonia and clinical risk factors for encephalopathy and intracranial hypertension in acute liver failure. *Hepatology.* 2007;46(6):1844-52.
122. Haussinger D, Schliess F. Pathogenetic mechanisms of hepatic encephalopathy. *Gut.* 2008;57(8):1156-65.
123. Lee DH, Lim JS, Song K, Boo Y, Jacobs DR, Jr. Graded associations of blood lead and urinary cadmium concentrations with oxidative-stress-related markers in the U.S. population: results from the third National Health and Nutrition Examination Survey. *Environ Health Perspect.* 2006;114(3):350-4.
124. Shi M, Gozal E, Choy HA, Forman HJ. Extracellular glutathione and gamma-glutamyl transpeptidase prevent H₂O₂-induced injury by 2,3-dimethoxy-1,4-naphthoquinone. *Free Radic Biol Med.* 1993;15(1):57-67.
125. Lee DH, Jacobs DR, Jr. Association between serum concentrations of persistent organic pollutants and gamma glutamyltransferase: results from the National Health and Examination Survey 1999-2002. *Clin Chem.* 2006;52(9):1825-7.
126. Lee DH, Steffes MW, Jacobs DR, Jr. Can persistent organic pollutants explain the association between serum gamma-glutamyltransferase and type 2 diabetes? *Diabetologia.* 2008;51(3):402-7.
127. Lee DH, Silventoinen K, Jacobs DR, Jr., Jousilahti P, Tuomilehto J. gamma-Glutamyltransferase, obesity, and the risk of type 2 diabetes: observational cohort study among 20,158 middle-aged men and women. *J Clin Endocrinol Metab.* 2004;89(11):5410-4.
128. Nemes Z, Devreese B, Steinert PM, Van Beeumen J, Fesus L. Cross-linking of ubiquitin, HSP27, parkin, and alpha-synuclein by gamma-glutamyl-epsilon-lysine bonds in Alzheimer's neurofibrillary tangles. *FASEB J.* 2004;18(10):1135-7.
129. Yates CM, Butterworth J, Gordon A. gamma-Glutamyl transpeptidase in dementia. *Neurobiol Aging.* 1989;10(1):107-8.
130. Butterworth J, Yates CM, Reynolds GP. Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase and gamma-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. *J Neuro Sci.* 1985;67(2):161-71.
131. Loomba R, Rao F, Zhang L, et al. Genetic covariance between gamma-glutamyl transpeptidase and fatty liver risk factors: role of beta2-adrenergic receptor genetic variation in twins. *Gastroenterology.* 2010;139(3):836-45, 845 e831.
132. Bidel S, Silventoinen K, Hu G, Lee DH, Kaprio J, Tuomilehto J. Coffee consumption, serum gamma-glutamyltransferase and risk of type II diabetes. *Eur J Clin Nutr.* 2008;62(2):178-85.
133. Lee D, Jacobs Jr DR, Gross M, et al. Gamma-Glutamyltransferase is a predictor of incident diabetes and hypertension: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Clin Chem.* 2003;49(8):1358-66.
134. Lee DH, Ha MH, Kim JH, et al. Gamma-glutamyltransferase and diabetes—a 4 year follow-up study. *Diabetologia.* Mar 2003;46(3):359-364.
135. Lee DH, Jacobs DR Jr, Gross M, Steffes M. Serum gamma-glutamyltransferase was differently associated with microalbuminuria by status of hypertension or diabetes: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Clin Chem.* Jul 2005;51(7):1185-1191.
136. Lim JS, Lee DH, Park JY, Jin SH, Jacobs DR Jr. A strong interaction between serum gamma-glutamyltransferase and obesity on the risk of prevalent type 2 diabetes: results from the Third National Health and Nutrition Examination Survey. *Clin Chem.* 2007;53(6):1092-1098.
137. Shin JY, Hwang JH, Jeong JY, et al. [The association of central obesity with type 2 diabetes among Koreans according to the serum gamma-glutamyltransferase level: Korean genome and epidemiology study]. *J Prev Med Public Health.* Nov 2009;42(6):386-391.
138. Song SH, Kwak IS, Kim YJ, et al. Can gamma-glutamyltransferase be an additional marker of arterial stiffness? *Circ J.* Nov 2007;71(11):1715-20.
139. Targher G, Zoppini G, Lippi G, Guidi GC, Muggeo M. Effect of serum gamma-glutamyltransferase and obesity on the risk of dyslipidemia and poor glycemic control in type 2 diabetic patients: cross-sectional findings from the Verona Diabetes Study. *Clin Chem.* 2007;53(10):1867-69; author reply 1869-70.
140. Kawamoto R, Kohara K, Tabara Y, Miki T, Otsuka N. Serum gamma-glutamyl transferase levels are associated with metabolic syndrome in community-dwelling individuals. *J Atheroscler Thromb.* 2009;16(4):355-62.
141. Hwang JH, Shin JY, Chun B, et al. Association between gamma-glutamyltransferase and hypertension incidence in rural prehypertensive adults. *J Prev Med Public Health.* 2010;43(1):18-25. [Korean.]
142. Lee DH, Ha MH, Kim JR, Gross M, Jacobs DR Jr. Gamma-glutamyltransferase, alcohol, and blood pressure. A four-year follow-up study. *Ann Epidemiol.* 2002;12(2):90-6.
143. Lee DH, Ha MH, Kim KY, Jin DG, Jacobs DR Jr. Gamma-glutamyltransferase: an effect modifier in the association between age and hypertension in a 4-year follow-up study. *J Hum Hypertens.* 2004;18(11):803-7.
144. Kotani K, Shimohiro H, Adachi S, Sakane N. The association between an increased level of gamma-glutamyl transferase and systolic blood pressure in diabetic subjects. *Tohoku J Exp Med.* 2008;214(4):321-5.
145. Kotani K, Shimohiro H, Adachi S, Sakane N. Changes in serum gamma-glutamyl transferase and blood pressure levels in subjects with normal blood pressure and prehypertension. *Clin Chim Acta.* 2008;389(1-2):189-90.
146. Yamada Y, Ikai E, Tsuritani I, Ishizaki M, Honda R, Ishida M. The relationship between serum gamma-glutamyl transpeptidase levels and hypertension: common in drinkers and nondrinkers. *Hypertens Res.* 1995;18(4):295-301.
147. Miura K, Nakagawa H, Nakamura H, et al. Serum gamma-glutamyl transferase level in predicting hypertension among male drinkers. *J Hum Hypertens.* 1994;8(6):445-9.
148. Ikai E, Noborizaka Y, Tsuritani I, Honda R, Ishizaki M, Yamada Y. Serum gamma-glutamyl transpeptidase levels and hypertension in non-drinkers: a possible role of fatty liver in the pathogenesis of obesity related hypertension. *Obes Res.* 1993;1(6):469-74.
149. Yamada Y, Ishizaki M, Kido T, et al. Alcohol, high blood pressure, and serum gamma-glutamyl transpeptidase level. *Hypertension.* 1991;18(6):819-26.
150. Yamada Y, Ishizaki M, Kido T, Honda R, Tsuritani I, Yamaya H. Relationship between serum gamma-glutamyl transpeptidase activity and blood pressure in middle-aged male and female non-drinkers. *J Hum Hypertens.* 1990;4(6):609-614.
151. Henningsen NC, Ohlsson O, Mattiasson I, Trelle E, Kristensson H, Hood B. Hypertension, levels of serum gamma-glutamyl transpeptidase and degree of blood pressure control in middle-aged males. *Acta Med Scand.* 1980;207(4):245-51.
152. Ablin RJ. Prostate-specific antigen: chronology of its identification. *Oncology (Williston Park).* 1998;12(7):1016.
153. Ablin RJ. A retrospective and prospective overview of prostate-specific antigen. *J Cancer Res Clin Oncol.* 1997;123(11-12):583-94.
154. Gupta S, Camm AJ. Chlamydia pneumoniae and coronary heart disease. *BMJ.* 1997;314(7097):1778-9.
155. Otto SJ, Moss SM, Maattanen L, et al. PSA levels and cancer detection rate by centre in the European Randomized Study of Screening for Prostate Cancer. *Eur J Cancer.* 2010;46(17):3053-60.
156. Gura T. Chlamydia protein linked to heart disease. *Science.* 1999;283(5406):1238-9.
157. Mendall MA, Carrington D, Strachan D, et al. Chlamydia pneumoniae: risk factors for seropositivity and association with coronary heart disease. *J Infect.* 1995;30(2):112-8.
158. Miettinen H, Lehto S, Saikku P, et al. Association of Chlamydia pneumoniae and acute coronary heart disease events in non-insulin dependent diabetic and non-diabetic subjects in Finland. *Eur Heart J.* 1996;17(5):682-8.
159. Miyashita N, Toyota E, Sawayama T, Matsushima T. An association of an antibody against Chlamydia pneumoniae and coronary heart disease observed in Japan. *Eur Heart J.* 1998;19(6):971.
160. Naidu BR, Ngeow YF, Kannan P, et al. Evidence of Chlamydia pneumoniae infection obtained by the polymerase chain reaction (PCR) in patients with acute myocardial infarction and coronary heart disease. *J Infect.* 1997;35(2):199-200.
161. Nieto FJ, Folsom AR, Sorlie PD, Grayston JT, Wang SP, Chambless LE. Chlamydia pneumoniae infection and incident coronary heart disease: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol.* 1999;150(2):149-56.
162. Saikku P, Leinonen M, Mattila K, et al. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet.* 1988;2(8618):983-6.
163. Saikku P, Leinonen M, Tenkanen L, et al. Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Intern Med.* 1992;116(4):273-8.
164. Taylor-Robinson D, Thomas BJ. Antibodies to cytomegalovirus or Chlamydia pneumoniae and coronary heart disease. *Lancet.* 1998;351(9096):142; author reply 143.
165. Quinn TC. Does Chlamydia pneumoniae cause coronary heart disease? *Curr Opin Infect Dis.* 1998;11(3):301-7.
166. Song Z, Brassard P, Brophy JM. A meta-analysis of antibiotic use for the secondary prevention of cardiovascular diseases. *Can J Cardiol.* 2008;24(5):3391-5.
167. Stassen FR, Vainas T, Bruggeman CA. Infection and atherosclerosis. An alternative view on an outdated hypothesis. *Pharmacol Rep.* 2008;60(1):85-92.
168. Jafarzadeh A, Esmaeli-Nadimi A, Shariati M. High sensitivity C-reactive protein and immunoglobulin G against Chlamydia pneumoniae and chla-

- mydial heat shock protein-60 in ischemic heart disease. *Iran J Immunol.* 2008;5(1):51-6.
169. Anderson JL, May HT, Horne BD, et al. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol.* 2010;106(7):963-8.
 170. Judd SE, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. *Am J Med Sci.* 2009;338(1):40-4.
 171. Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation.* 2008;117(4):50311.
 172. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr.* 2006;83(4):754-9.
 173. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. *Crit Care Med.* 1996;24(5):743-52.
 174. Zimmerman JE, Kramer AA, McNair DS, Malila FM. Acute Physiology and Chronic Health Evaluation (APACHE) IV: hospital mortality assessment for today's critically ill patients. *Crit Care Med.* May 2006;34(5):1297-1310.
 175. Chen BS, Wang YC, Wu WS, Li WH. A new measure of the robustness of biochemical networks. *Bioinformatics.* 2005;21(11):2698-705.
 176. Hwang D, Rust AG, Ramsey S, et al. A data integration methodology for systems biology. *Proc Natl Acad Sci U S A.* 2005;102(48):17296-301.
 177. Hwang D, Smith JJ, Leslie DM, et al. A data integration methodology for systems biology: experimental verification. *Proc Natl Acad Sci U S A.* 2005;102(48):17302-17307.
 178. Bain BJ, England JM. Normal haematological values: sex difference in neutrophil count. *Br Med J.* 1975;1(5953):306-9.
 179. Molloy EJ, O'Neill AJ, Grantham JJ, et al. Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone. *Blood.* 2003;102(7):2653-9.
 180. Bain B, Seed M, Godsland I. Normal values for peripheral blood white cell counts in women of four different ethnic origins. *J Clin Pathol.* 1984;37(2):188-93.
 181. Bain BJ. Ethnic and sex differences in the total and differential white cell count and platelet count. *J Clin Pathol.* 1996;49(8):664-6.
 182. Chan PC, Hayes L, Bain BJ. A comparison of the white cell counts of cord blood from babies of different ethnic origins. *Ann Trop Paediatr.* 1985;5(3):153-5.
 183. Daughaday WH, Williams RH, Daland GA. The effect of endocrinopathies on the blood. *Blood.* 1948;3(12):1342-66.
 184. Carryer HM, Berkman JM, Mason HL. Relative lymphocytosis in anorexia nervosa. *Proc Staff Meet Mayo Clin.* 1959;34:426-32.
 185. Le Marquand HS, Tozer FH. Fragilitas ossium in a family showing both thickening and rarefaction of bones, relative lymphocytosis and raised serum phosphatase with absence of blue sclerotics and otosclerosis. *Proc R Soc Med.* 1935;28(12):1640-1.
 186. Menkin V. Relative lymphocytosis in hyperthyroidism. *Arch Intern Med.* 1928;42:419-24.
 187. Gardner FH, Pringle JC, Jr. Androgens and erythropoiesis. I. Preliminary clinical observations. *Arch Intern Med.* 1961;107:846-62.
 188. Naets JP, Wittek M. The mechanism of action of androgens on erythropoiesis. *Minerva Nucl.* 1965;9(5):281-4.
 189. Gardner FH, Nathan DG. Androgens and erythropoiesis. 3. Further evaluation of testosterone treatment of myelofibrosis. *N Engl J Med.* 1966;274(8):420-6.
 190. Naets JP, Wittek M. Mechanism of action of androgens on erythropoiesis. *Am J Physiol.* 1966;210(2):315-20.
 191. Fisher JW, Samuels AI, Malgor LA. Androgens and erythropoiesis. *Isr J Med Sci.* 1971;7(7):892-900.
 192. Gardner FH, Gorshein D. Regulation of erythropoiesis by androgens. *Trans Am Clin Climatol Assoc.* 1973;84:60-70.
 193. Evens RP, Amerson AB. Androgens and erythropoiesis. *J Clin Pharmacol.* 1974;14(2):94-101.
 194. Cruickshank JM, Morris R, Butt WR, Crooke AC. The relationship of total and differential leukocyte counts with urinary oestrogen and plasma cortisol levels. *J Obstet Gynaecol Br Commonw.* 1970;77(7):634-9.
 195. Cruickshank JM, Morris R, Butt WR, Corker CS. Inter-relationships between levels of plasma oestradiol, urinary total oestrogens and blood haemoglobin and neutrophil counts. *J Obstet Gynaecol Br Commonw.* 1972;79(5):450-4.
 196. Riedel-Baima B, Riedel A. Female pattern hair loss may be triggered by low oestrogen to androgen ratio. *Endocr Regul.* 2008;42(1):13-6.
 197. Chang RJ, Katz SE. Diagnosis of polycystic ovary syndrome. *Endocrinol Metab Clin North Am.* 1999;28(2):397-408.
 198. Goudas VT, Dumesic DA. Polycystic ovary syndrome. *Endocrinol Metab Clin North Am.* 1997;26(4):893-912.
 199. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. *J Urol.* 2002;167(2 Pt 1):624-9.
 200. Fejes I, Koloszar S, Zavacki Z, Daru J, Szollosi J, Pal A. Effect of body weight on testosterone/estradiol ratio in oligozoospermic patients. *Arch Androl.* 2006;52(2):97-102.
 201. Faerstein E, Szklo M, Rosenshein N. Risk factors for uterine leiomyoma: a practice-based case-control study. I. African-American heritage, reproductive history, body size, and smoking. *Am J Epidemiol.* 2001;153(1):11-10.
 202. Farb A, Burke AP, Tang AL, et al. Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. *Circulation.* 1996;93(7):1354-63.
 203. Shimomura K, Manda T, Mukumoto S, Kobayashi K, Nakano K, Mori J. Recombinant human tumor necrosis factor-alpha: thrombus formation is a cause of anti-tumor activity. *Int J Cancer.* 1988;41(2):243-7.
 204. Yacoub D, Hachem A, Theoret JF, Gillis MA, Mourad W, Merhi Y. Enhanced levels of soluble CD40 ligand exacerbate platelet aggregation and thrombus formation through a CD40-dependent tumor necrosis factor receptor-associated factor-2/Rac1/p38 mitogen-activated protein kinase signaling pathway. *Arterioscler Thromb Vasc Biol.* 2010;30(12):2424-33.
 205. Olmarker K, Rydevik B. Selective inhibition of tumor necrosis factor-alpha prevents nucleus pulposus-induced thrombus formation, intraneural edema, and reduction of nerve conduction velocity: possible implications for future pharmacologic treatment strategies of sciatica. *Spine (Phila, Pa 1976).* 2001;26(8):863-9.
 206. Ohtsu H. Progress in allergy signal research on mast cells: the role of histamine in immunological and cardiovascular disease and the transporting system of histamine in the cell. *J Pharmacol Sci.* 2008;106(3):347-53.
 207. Ozben B, Erdogan O. The role of inflammation and allergy in acute coronary syndromes. *Inflamm Allergy Drug Targets.* Sep 2008;7(3):136-144.
 208. Tanimoto A, Sasaguri Y, Ohtsu H. Histamine network in atherosclerosis. *Trends Cardiovasc Med.* 2006;16(8):280-4.
 209. D'Ascenzo S, Millimaggi D, Di Massimo C, et al. Detrimental effects of anabolic steroids on human endothelial cells. *Toxicol Lett.* 2007;169(2):129-36.
 210. Dickerman RD, McConathy WJ, Zachariah NY. Testosterone, sex hormone-binding globulin, lipoproteins, and vascular disease risk. *J Cardiovasc Risk.* 1997;4(5-6):363-6.
 211. Dickerman RD, McConathy WJ, Schaller F, Zachariah NY. Cardiovascular complications and anabolic steroids. *Eur Heart J.* Dec 1996;17(12):1912.
 212. Winkler UH. Effects of androgens on haemostasis. *Maturitas.* Jul 1996;24(3):147-55.
 213. Halushka PV, Masuda A, Matsuda K. The Gordon Wilson Lecture. Regulation of thromboxane A2 receptors by testosterone: implications for steroid abuse and cardiovascular disease. *Trans Am Clin Climatol Assoc.* 1994;105:95-103.
 214. Noh JY, Lim KM, Bae ON, et al. Procoagulant and prothrombotic activation of human erythrocytes by phosphatidic acid. *Am J Physiol Heart Circ Physiol.* 2010;299(2):H347-55.
 215. Santos MT, Valles J, Lago A, et al. Residual platelet thromboxane A2 and prothrombotic effects of erythrocytes are important determinants of aspirin resistance in patients with vascular disease. *J Thromb Haemost.* 2008;6(4):615-21.
 216. Valles J, Santos MT, Aznar J, et al. Erythrocyte promotion of platelet reactivity decreases the effectiveness of aspirin as an antithrombotic therapeutic modality: the effect of low-dose aspirin is less than optimal in patients with vascular disease due to prothrombotic effects of erythrocytes on platelet reactivity. *Circulation.* 1998;97(4):350-5.
 217. Santos MT, Valles J, Aznar J, Marcus AJ, Broekman MJ, Safier LB. Prothrombotic effects of erythrocytes on platelet reactivity. Reduction by aspirin. *Circulation.* 1997;95(1):63-8.