

REVIEW

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

Endobiogeny: 一种系统生物学的全球方法（第 2 部分，共 2 部分）

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

Jean-Claude Lapraz, MD, *France*; Kamyar M. Hedayat, MD, *United States*; Patrice Pauly, PhD, *France*

Editor's note: The following is the abridged version of part 2 of a two-part article. Part 1 of the article was published in the January 2013 issue of *Global Advances in Health and Medicine*. To read the unabridged version of this article, which includes a discussion of direct and indirect indexes, visit www.gahmj.com.

THE BIOMARKERS

Experimental and Clinical Basis for the Biomarkers Used in the Biology of Functions

Endobiogeny and the biology of functions are based on four scientific concepts that are known and generally accepted: (1) human physiology is complex and multifactorial and exhibits the properties of a system; (2) the endocrine system manages metabolism, which is the basis of the continuity of life; (3) the metabolic activity managed by the endocrine system results in the output of biomarkers that reflect the functional achievement of specific aspects of metabolism; and (4) when biomarkers are related to each other in ratios, it contextualizes one type of function relative to another to which is it linked anatomically, sequentially, chronologically, biochemically, etc.

As will be shown in this article, the relationship

between various hormones and particular biomarkers is a long and well-established fact based on modern physiology and scientific method. The indexes composed from these biomarkers have been derived through inductive reasoning and confirmed by more than 30 years of clinical practice. The indexes have not been individually validated in the peer-reviewed literature. However, it stands to reason that if the correlation of each biomarker to endocrine activity is sufficiently demonstrated, then it is possible that such a biological modeling system may be a more valid assessment of biological activity.

Bone Marrow: Complete Blood Count

Life is permanent dynamism, and the circulation of blood ensures this dynamism. Blood plasma is a conduit of information, a delivery system of nutrients, and a remover of metabolic waste, but it is the cellular elements—white blood cells (WBCs), red blood cells (RBCs), and platelets—that serve to deliver oxygen and defend, heal, and protect the body. The endocrine system as the manager of metabolism determines the rate of production of cellular elements from the bone marrow. Thus blood is the foundation of life, and the endocrine system as the manager of blood is the manager of this foundation. The evaluation of blood cells reveals how the endocrine system manages life.

Table 1 Biomarkers Used in the Biology of Functions

Origin	Biomarker	Value	Conversion
Bone marrow cellular products	Red blood cell	per μ L	$\pm 10^6$
	White blood cell, total	per μ L	$\pm 10^3$
	Neutrophil	%	None
	Lymphocytes		
	Eosinophils		
	Monocytes		
	Basophils		
	Hemoglobin	g/dL	None
	Platelets	per μ L	$\pm 10^3$
Bone marrow-serum interaction	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	± 2

Author Affiliations
Société internationale de médecine endobiogénique et de physiologie intégrative, Paris, France (Drs Lapraz and Pauly); American Society of Endobiogenic Medicine and Integrative physiology, San Diego, California, United States (Dr Hedayat).

Correspondence
Kamyar Hedayat, MD
acquamarine@mac.com.

Citation
Global Adv Health Med. 2013;2(2):32-44. DOI: 10.7453/gahmj.2013.013.

Key Words
Endobiogeny, systems biology, reductionism, functional medicine

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

In the biology of functions, more than 60% of biomarkers used are derived just from the cellular elements of blood, made in the bone marrow (Table 1). The complete blood count (CBC), then, is the basis of the biology of functions. Androgens and estrogens stimulate the proliferation of red and white blood cells, respectively. Thus, sex hormones are the foundation of the CBC—hence of life—and the initial point of study in the biology of functions. (The bone stroma, discussed below, plays three key roles: protection and nourishment the marrow, regulation and assistance in global energy management, and communication of the state of the peripheral terrain to the central nervous system).¹⁻³

The roles of androgens, and then later estrogens, as the basis of life are evident from the time of conception. For the first 17 days, it is the mother's hormones that the embryo shares. At day 18 of life, the yolk sac becomes the first endogenous source of RBCs.^{4,5} Rich in androgen receptors, the yolk sack stimulates erythropoietin, which itself plays a role in yolk sac maturation of RBCs,⁵ establishing the key role of androgens in the foundation of structure.⁶ The liver is an intermediate source of red blood cells,⁴ also under the management of androgens.⁷ By 34 weeks of gestation and throughout the remainder of life, the bone marrow, stimulated by androgens and estrogens, becomes the source of the majority of blood cells.⁸

In summary, the activity of androgens and estrogens is reflected in the output of red and white blood cells by the bone marrow. The evaluation of this activity, called the Genital Ratio, is used in the majority of indexes of the biology of functions. To accept the hypothesis that red blood cells are a biomarker of androgen activity and that total white blood cell count is a biomarker of estrogen activity at the level of the tissues is to accept the foundation of the majority of indexes of the biology of functions.

Red Blood Cells

Based on studies over the last 50 years, we postulate that RBCs are a biomarker of the *functional* role of androgens in metabolism. These studies demonstrate that the administration of androgens stimulate erythropoiesis, the development of RBCs.⁹⁻¹⁵ It is our belief that using RBCs as a marker of the functionality of androgens may prove to be more clinically relevant than quantitative measurements for four reasons: contradictory studies regarding serum levels of androgens and clinical effects, the complimentary nature of estrogens, the role of genomic vs non-genomic effects, and genetic variations in intracellular (IC) conversion of androgens.

Limits of Quantitative Measurements of Androgens. Multiple studies have positively associated elevated levels of serum androgens, RBCs, or both in hypertension,¹⁶⁻¹⁹ thrombus formation,²⁰⁻²⁸ impaired insulin sensitivity,^{29,30} and insulin resistance.³¹ However, low serum levels of androgens have also been positively associated with the same disorders.³²⁻³⁵ For

example, while androgens are positively associated with dyslipidemia, they have also been associated with a reduction in triglycerides and LDL.²¹ Thus, evaluating serum androgen levels may be misleading.

Protective Role of Estrogens? For years, it was believed without strong evidence that delayed cardiovascular mortality in women was due to a protective effect of estrogens. Prospective studies of estrogen supplementation demonstrated not only that supplemental estrogens offered no benefit but that they elevated the risk of cardiovascular events.³⁶⁻³⁸ The lack of definitive protective effects of estrogens and the harmful effects of elevated and low serum levels of androgens in some men and not others suggests to us that it is the relative ratio of androgens to estrogens that is clinically relevant, not the absolute quantitative value of either in isolation.

Are Androgens Harmful in and of Themselves?

Studies suggest that androgens alone are not predictive of life span or risk of death from cardiovascular disease in men³⁹⁻⁴¹ or women.⁴²⁻⁴⁵ Rather, androgens appear to be but one of many factors in a complex interplay of endocrine drivers of metabolism that influence the development, progression, and severity of a wide range of disorders from vascular disease^{16,46} to Alzheimer's disease.⁴⁷ This may be one reason that assessments relying on serum androgens measurements alone have been inconsistent or contradictory.

Determining Androgen Function: Genomic and Non-genomic Effects. Androgens, like most other steroid hormones, have genomic and non-genomic effects.⁴⁸ The ability to evaluate the relative impact of non-genomic vs genomic affects in a particular individual may help solve the conundrum of whether high or low androgen activity is protective or harmful.

The genomic of effects of androgens are what have been associated with serum levels of androgens. In contrast to the non-genomic effects, these effects take hours to occur and are linked to many of the classic effects associated with androgens deemed to be harmful when dysregulated. These effects include smooth muscle proliferation, migration, and vasorelaxation; increased monocyte migration and foam cell production; and increased apoptosis.⁴⁸

Non-genomic effects occur within seconds. Mechanisms of action are believed to include a novel membrane-bound receptor, second messenger activation, and sex-hormone binding globulin receptors. Many of the non-genomic effects of androgens are physiologically beneficial and explain the protective effects of androgens observed in studies. They include relaxation of smooth muscle, increased neuromuscular signal transmission by calcium regulation, improved neuroplasticity, cellular proliferation and migration, and modulation of the transcriptional effects of classic androgen receptors.^{49,50} What is clinically relevant is that these non-genomic effects cannot be blocked by drugs that block androgen receptor activity. This may explain two observations: (1) the

variability of responsiveness to androgen blockers and (2) factors of risk and protection from disease cannot be reliably assessed by quantitative measurement of serum androgens, sex hormone binding globulin, or free androgen levels—because their effects do not rely solely on receptor activity.

Determining Androgen Function: Metabolic Pathways. There are a number of other factors adding to the difficulty of equating quantitative levels of testosterone (free or total) with androgen functionality. Recent studies have demonstrated in vitro and in vivo sex-based variability in androgen receptor sensitivity and concentration in various tissues.⁴³ Approximately 5% of testosterone is converted within the cell to either dihydrotestosterone (DHT) or estrogens.

In summary, the individual effects of testosterone on the body can vary based on (1) genomic effects, (2) non-genomic effects, (3) receptor concentration, and (4) IC conversion tendency between DHT and estradiol. The net effect can be an amplification of genomic or non-genomic effects (DHT) or a counter-balancing effect (estrogens). Therefore, we believe that RBCs may be a useful biomarker reflecting the global degree of tissue functionality of androgens when evaluated relative to other factors.

White Blood Cells

WBCs, also known as leukocytes, are blood elements that mature in the bone marrow then enter the circulation. Leukocytes consist of five types of cells that arise from a common hematopoietic precursor. White cells differentiate into neutrophils, monocytes, eosinophils, basophils, and lymphocytes. Estrogen stimulates a proliferation of leukocytes in the bone marrow.⁵¹ Leukocytosis is associated with high estrogen states such as pregnancy⁵² and autoimmunity,⁵³ as well as during the acute phase of infections. Thus, we believe that total WBC count can be considered to reflect the basic tissue effect of estrogens throughout the body.

Limits of Quantitative Measurements of Estrogens. The challenges of evaluating the role of estrogens in human physiology are far greater than for androgens, which is why specific aspects of estrogen activity requires more than a single biomarker.

Estrogen activity is complex, varied, and fundamental to human life. It involves endocrine and metabolic functions, both genomic and non-genomic in nature. Of all sex steroids, metabolically estrogens require the greatest number of metabolic conversions, being derived as such: cholesterol → progesterone → androgens → estrogens. Estrogens can be produced in the ovaries, in the adrenals, and by peripheral conversions in various tissues.^{54,55} The pattern of estrogen production (central vs peripheral, adrenal vs gonadal vs hepatic) varies based on hereditary factors, age, and parturition status and is affected by endocrine disruptors.⁵⁴⁻⁵⁷

There are multiple active forms of estrogens (estrone, estradiol, and estriol) as well as varying degrees of activity of estrogen metabolites. There are

two types of estrogen receptors (alpha, beta), which have opposing activity with respect to cellular proliferation and various metabolic function. There are genetic polymorphisms in p450 metabolism of estrogens and polymorphisms with respect to receptor sensitivity, concentration, and rate of aromatase activity as well as non-genomic effects, which in sum all impact the effects of estrogens.⁵⁸⁻⁶⁴ In their review of estrogen metabolism, Zhu and Conney conclude,

Studies that identify genetic and environmental factors influencing estrogen metabolism at or near estrogen receptors in target cells may be of considerable importance since these factors could profoundly modify the biological effects of estrogens in complex manners depending on the pathways of metabolism that are affected and the biological activities of the metabolites that are formed. Such effects need not be associated with an altered profile of estrogen metabolites in the blood or urine.⁶¹

Estrogens: Beneficial or Harmful? As with androgens, clinical trials are conflicting with respect to the beneficial or harmful role of estrogens in the body. The protective role of estrogens in cardiovascular disease has come under question, as we have discussed above.^{36-38,54} With respect to cancer, estrogens can promote or lower the risk for cancer in and of themselves and in conjunction with other hormones.⁶⁵⁻⁶⁸ The contradictory nature of estrogen's effects on telomere length and the role of telomere length in cancer serve as other good examples of the limitations of both quantitative hormone measurement and single-cause theories of disease. Estrogens increase telomere length. Women have the longest telomere length when follicle-stimulating hormone and estrogen peak during the menstrual cycle.⁶⁹ Telomere length is positively correlated with the rate of apoptosis and inversely associated with the risk of cancer. However, estrogens also cause leukocytosis, which is associated with shorter telomere length, less apoptosis, and greater risk of cancer.^{69,70} Telomere length alone, like quantitative levels of estrogen, does not appear to be a sufficient indicator of the global effects of estrogens on the terrain.

The Case for Multiple Biomarkers of Estrogen. In conclusion, estrogens have various sources of origin, various rates of metabolism, and changing concentrations and receptor densities throughout life and can be affected by and affect other hormones in the body, as well as being disrupted by endocrine disruptors. Mounting evidence suggests that serum and urinary levels of estrogen and their metabolites may not be sensitive or specific enough measures of the effects of estrogens.

We hypothesize, based on experimental evidence and clinical studies, that specific functional effects of estrogens can be inferred through the evaluation of particular serum biomarkers in and of themselves, as well as in conjunction with other biomarkers in increasingly

complex ratios. In the biology of functions, this assessment of estrogen function is accomplished by evaluating six different biomarkers: (1) total WBC count, (2) percent neutrophil count, (3) percent monocyte count, (4) percent lymphocyte count, (5) thyrotropin-stimulating hormone (TSH), and (6) serum osteocalcin. Of these, WBC count is used as a general marker of global estrogen effects on tissues and is the most foundational. Through the use of the genital ratio or its variation, the corrected genital ratio (see "Indirect Indexes"), WBC count can be used to evaluate the structural, functional, and adaptive role of estrogens in the body.

Neutrophils

Neutrophils are a type of leukocyte that arise from granulocytes in the bone marrow. While the total leukocyte count (WBC count) reflects global tissue effects of estrogens, we hypothesize that neutrophils can be used to assess particular aspects of estrogen activity, namely immune regulation and anabolism of tissue.

The Direct role of neutrophils is to participate in the immunologic response of the organism to aggressors. This can occur through inflammation⁷¹ or phagocytosis of microbes and cellular debris.⁷² Neutrophilia, absolute or relative, is associated with the anabolism of tissue, such as during pregnancy,⁷¹ wound healing,⁷³ autoimmune disease,⁷⁴⁻⁷⁶ and cancer.⁷⁷⁻⁸¹ Estrogens are associated with these same events: preeclampsia,⁷¹ autoimmune diseases,⁸² and cancer,⁶⁰ as well as wound healing.⁸³⁻⁸⁸ The majority of patients suffering from autoimmune disease are women, which implies a role for estrogens in the etiology of these disorders. New-onset autoimmune disease is frequently diagnosed in the peripartum period, and flare-ups of existing disease often occur during pregnancy as estrogen levels increase up to 100-fold from nonpregnant levels.^{89,90}

Neutrophils ordinarily exhibit a short half-life of 3 to 6 hours, requiring a constant production by bone marrow to maintain normal circulating levels. Estrogens affect neutrophil populations in two ways. They increase the total production of neutrophils in bone marrow,⁹¹ and they inhibit apoptosis of circulating neutrophils, which increases the relative percentage of neutrophils in the total leukocyte differential, even when the leukocyte count is within normal limits, ie, in noninfectious states.⁹² Estrogens manage the production and maintenance of neutrophils; thus, estrogens manage a particular aspect of immunity related to inflammation, host-defense, autoimmunity, and cancer. Therefore, neutrophils may be considered as a biomarker of the role of estrogens in immunologic, inflammatory, and anabolic activity within the body.

Monocytes

Monocytes are WBCs derived from monoblasts in the bone marrow. They play an important role in the immune system, combating foreign organisms in the blood through phagocytosis and the release of proinflammatory cytokines. After 24 to 72 hours of

circulation, they migrate into extravascular tissue where they differentiate into macrophages or dendritic cells (histiocytes).

Typically, monocytes represent 3% to 8% of the total leukocyte population. Follicle-stimulating hormone (FSH) stimulates estrogen production and estrogens suppress monocyte production.⁹³ During adaptation, as FSH and estrogen levels rise, monocyte levels should fall, indicating an anabolic response commensurate to the initial anti-anabolic activity of cortisol. The lower the monocyte count, the greater the influence of FSH and estrogen on the adaptation response, but this needs to be evaluated relative to the eosinophil count, which reflects the role of adrenocorticotrophic hormone (ACTH) on adrenal stimulation, as well as other factors.

Conversely, monocytosis is inversely related to the relative efficiency of FSH in stimulating estrogen production. In menopause, monocytosis is observed.⁹⁴ Monocytosis also reflects a relative or absolute insufficiency of estrogen's activity during adaptation⁹⁵ and is associated with increased risk of mortality in multiple diseases marked by dysregulation of the immune system such as lupus,⁹⁶ autism,⁹⁷ asthma,⁹⁸ sepsis,⁹⁹ atherosclerosis,¹⁰⁰ myocardial infarction,¹⁰¹ myeloproliferative disorders, and leukemia.¹⁰²⁻¹⁰⁷ Thus, monocytosis implicates a terrain that is more favorable to inflammation and altered immune states: in other words, a terrain of dysadaptation of estrogen activity.

As the bioavailability of estrogens and androgens are inversely related to each other due to the activity sex hormone binding globulin,¹⁰⁸ and as monocytosis reflects a relative insufficiency of estrogens during adaptation, monocytosis also reflects a more predominant peripheral androgen activity relative to that of estrogens.¹⁰⁹

Eosinophils

Eosinophils are a subpopulation of white blood cells. Fundamentally, the role of the eosinophil is to serve as an indirect method of adaptation and congestion when the adrenal cortical response is not sufficiently adapted to the needs of the organism.

While estrogens, as noted above, have a general effect on the proliferation of all leukocytes within the bone marrow, it is ACTH and cortisol that affect the circulating levels of eosinophils. The degree and intensity of ACTH activity on the adrenal cortex is proportional to the level of circulating eosinophils. Thus, the greater the ACTH solicitation of adrenal activity is, the greater the rise in eosinophils.^{110,111} Eosinophilia, relative or absolute, is proportional to the degree of adrenal insufficiency, which is proportional to the demand for ACTH and inversely proportional to the efficiency of cortisol.^{112,113}

On the other hand, cortisol is inversely related to the eosinophil count because it reduces circulating eosinophils in three ways: (1) suppression of eosinophil maturation, recruitment, and survival¹¹⁴; (2) sequestration of mature eosinophils in lymphoid organs¹¹⁵; and (3) stimulation of eosinophil apoptosis

through transcriptional up-regulation.¹¹⁶ The greater the degree of circulating cortisol, the lower the eosinophil count. The lower the circulating cortisol activity, the higher the eosinophil count.

While eosinophils cannot replace the complex roles that cortisol plays in the body, they can compensate in part for some of the adaptive functions of cortisol with respect to immune modulation. Eosinophils have direct antimicrobial effects through the production of RNase enzymes¹¹⁷⁻¹²⁶ and the generation of reactive oxygen species and are immunomodulatory through antigen presentation to T-cells.¹²⁷⁻¹³³ Indirectly, they are an indirect source of histamine, which modulates the immune system.^{134,135}

In summary, eosinophil count is used in the biology of functions to assess the intensity of the ACTH solicitation of adrenal activity (positively correlated) and the relative efficiency of cortisol activity (inversely correlated). The less efficient the adaptation response is, the lower the circulating cortisol levels, the greater the role of ACTH in re-stimulating the adrenal cortex, and the higher the circulating eosinophil count will be. Eosinophils also contribute to the evaluation of inflammation, thrombosis, and immune and other activities.

Basophils

Basophils are the least populous of all white cells. Basophils have been likened to circulating mast cells and play a role in the innate immune response, particularly against allergens¹³⁶ and parasites.¹³⁷ Basophils share similar receptors to eosinophils, such as eotaxin, and may serve as a tertiary method of adapting the adrenal response to aggressors in the face of inadequate cortisol response and insufficient eosinophil response. They are found in high concentration in the circulation and extracellular (EC) spaces of the skin and lungs in patients with atopic disease.¹³⁸ The percent basophil count on differential is used in only one index in the biology of functions but indirectly in all indexes in which the total WBC count is used.

Lymphocytes

Lymphocytes are a subset of leukocytes that are the direct mediators of immunity. The lymphocytes count is the sum of all three subsets of lymphocytes: natural killer (NK), T, and B cells. NK cells are part of the innate immune system. They survey and directly attack viruses and tumors. T and B cells comprise the adaptive immune system. T cells manage cell-mediated immunity through the secretion of cytokines and regulate the activity of other immune cells and lyse cells infected by viruses. T cells also play a role in immunoregulation. B cells form antibodies specific to a unique aggressor and retains a memory of the aggressor in case of future aggression. Lymphocytes play a role in cancer surveillance, immunity, and autoimmunity. The concentration of total circulating lymphocytes can be related to three factors: cortisol, estrogen, and TSH.

Cortisol is inversely related to lymphocyte counts.

It reduces the circulating concentration of all three subtypes of lymphocytes and augments destruction of lymphocytes.¹³⁹⁻¹⁴²

Estrogens are also inversely related to lymphocytes. There are several lines of evidence and clinical observations related to this. Estrogens directly inhibit the proliferation of lymphocytes.¹⁴³ In high-estrogen states, such as pregnancy, there is a relative suppression of lymphocyte proliferation in order to reduce immune attack by the mother against the fetus.⁵² Autoimmune disorders occur disproportionately in females who tend to have higher levels of estrogen activity and estrogen variability.¹⁴⁴ There is an additional risk of developing autoimmune disease in the peripartum state when there is a terrain of hyperestrogenism and thyroid overstimulation.^{145,146} Estrogens augment the infiltration of lymphocytes into various tissues, reducing the level of circulating lymphocytes.¹⁴⁴

The relationship between serum TSH and peripheral lymphocytes is positively correlated to the metabolic needs of the body and the degree to which TSH is used to modulate thyroid activity.^{147,148} When the lymphocyte counts are elevated, serum TSH levels tend also to be elevated, and the body tends to be in a state of increased need of thyroid activity. For example, in subclinical hypothyroidism, there is an increased appeal to TSH to stimulate the thyroid. These patients have lymphocyte counts that are elevated relative to euthyroid patients and/or in an absolute sense. When the body's demand for thyroid hormones have been met by exogenous administration of thyroxine, lymphocyte counts reduce from their pre-intervention levels.¹⁴⁹

In disorders of thyroid overactivity, such as Grave's disease or autoimmunity, there is diminished appeal by the thyroid to TSH for stimulation. One does find diminished peripheral blood lymphocytes in these patients, though not consistently.¹⁵⁰ As we will demonstrate later in this article, other assessments of thyroid function (see the sections on lactate dehydrogenase and creatine phosphokinase) help further contextualize thyroid efficiency.

In summary, lymphocytes are inversely related to the degree of cortisol and estrogen activity in adaptation and tissue anabolism. The greater the degree of cortisol expression and/or the greater the predominance of estrogen activity, the lower the lymphocyte levels. Lymphocytes are directly related to the degree of appeal to TSH to regulate thyroid function. The higher the lymphocyte count, the greater the appeal to TSH is and often the greater the degree of thyroid insufficiency. Conversely, the lower the lymphocyte count, the more successful TSH has been in modulating thyroid activity regardless of the serum TSH level.

Platelets

Platelets are circulating blood cells that arise from megakaryocytes in the bone marrow. Platelets have four direct functions in the body: hemostasis, repair and growth of connective tissues, transport of various factors,

and modulation of inflammation. The hemostatic function of platelets has been observed for more than 120 years and is well characterized.¹⁵¹ Platelets secrete numerous growth factors for the regeneration of connective tissue once hemostasis has been achieved, including platelet-derived growth factor, insulin-like growth factor 1, fibroblast growth factor, and others.^{152,153}

In general, platelets are adsorbers of numerous factors in the blood, such as clotting factors and calcium, which allows them to participate in immediate hemostatic activity.¹⁵⁴ In addition, platelets serve as the direct transporter of serotonin from the enteric cells where they are produced. Serotonin aids in intestinal motility and carbohydrate absorption.¹⁵⁵ Serotonin also plays constitutive roles in the regulation of bone density.^{1,156-158} Thus, platelets contribute to these physiologic activities as a serotonin transporter.

Platelets participate in proinflammatory activity, adapting innate and adaptive immune mechanisms through the expression of chemokines and cytokines and receptor-receptor interaction with leukocytes.¹⁵⁹ Platelets also contain histamine, which is secreted before aggregation occurs.¹⁶⁰

In the biology of functions, after the total WBC and RBC count, platelets are the most important biomarker derived from the bone marrow. Through the starter index (discussed below), they are used to correct the genital ratio (RBC:WBC) in order to evaluate the role of genital hormones during adaptation. The genital ratio corrected is used in more than 50% of the indexes of the biology of functions. Platelets, along with other factors, are used to assess histamine activity, risk of thrombosis, thromboembolic phenomena, adrenaline activity, and peripheral serotonin activity.

Hemoglobin

Hemoglobin (Hg) is a metalloprotein found within RBCs. Each red blood cell contains four Hg subunits with an iron molecule in the center of each Hg subunit. The direct role of Hg is to bind and deliver oxygen from the lungs to the tissues and bind and deliver carbon dioxide from the tissues back to the lungs. Thus, Hg plays a role in acid-base balance as well as oxygen delivery.

Hg is an important determinant of the oxygen content of arterial blood, based on the equation of the calculation of arterial oxygen content (C_A):

$$C_A = [\text{Hg (g/dL)} \times 1.34 \times \text{arterial saturation of blood (percent)}] + [0.0032 \times \text{partial pressure of oxygen (torr)}]$$

For a given saturation of blood and rate of consumption of oxygen, the lower the hemoglobin content is, the lower the oxygen content will be. Thus, the more the cardiac output must increase in order to maintain an equivalent rate of oxygen delivery. This can be expressed in the following equation, based on a rearrangement of the Fick equation:

$$Q = (\text{VO}_2 / (C_A - C_V)) \times 100,$$

where Q = cardiac output, VO_2 = oxygen consumption, C_A = arterial oxygen content, and C_V = venous oxygen content.

In vivo and clinical studies demonstrate that in both children and adults, iron-deficiency anemia up-regulates alpha-sympathetic activity regardless of the origin of the anemia (genetic, hemorrhagic, renal, acute or chronic) resulting in cardiovascular diseases such as cardiac remodeling and coronary ischemia.¹⁶¹⁻¹⁶⁶ Anemia appears to alter the normal adaptive response to stressors, resulting in overadaptation.¹⁶⁷

Based on these observations, we hypothesize that Hg can be viewed as a marker of the degree of alpha-sympathetic activity in adaptation. Because the general adaptation syndrome is initiated by alpha-sympathetic discharge (ie, noradrenaline), Hg comes to play an important and pervasive role in the biology of functions.

Bone Stroma-derived Enzymes

Two key stroma-derived enzymes are osteocalcin and alkaline phosphatase bone isoenzyme. In addition to their bone-related activity, they have direct effects on non-bone metabolic activity. These biomarkers in particular and the skeletal system in general inform the central nervous system of the state of the internal milieu, helping it modulate basal and adaptive capacities to meet the needs of the organism.¹⁻³

Osteocalcin. Osteocalcin is a noncollagenous protein. Within the skeletal metabolism, it plays an important role in osteoblasts, fixing ionized calcium to hydroxyapatite crystals. In its nonskeletal role, osteocalcin plays a key role in global energy regulation and adaptation in at least three ways:

1. **Glucose regulation:** It improves the production and secretion of and cellular sensitivity to insulin, as well as the rate of glucose metabolism.^{2,3,168-170}
2. **Fat regulation:** It increases the metabolism of adipocytes.^{2,3,170}
3. **Adenosine triphosphate (ATP) production:** It augments the number and efficiency of mitochondria both in part from its role in glucose regulation and independent of this role.²

Serum osteocalcin measures the inactive carboxylated form. When osteocalcin is decarboxylated to its active form, it enters the tissues. The less active osteocalcin is, the higher the serum levels. The more active a role it plays in global metabolism, the lower the serum level.

Osteocalcin regulates and is subject to regulation by various anabolic hormones. Serum osteocalcin is inversely related to insulin-like growth factors (IGFs)¹⁷¹ and estrogen activity. Estrogens stimulate osteoblasts to fix calcium, which requires active, carboxylated osteocalcin, which results in a decrease in serum decarboxylated osteocalcin.¹⁷²⁻¹⁷⁴ TSH levels vary inversely with serum osteocalcin levels.¹⁷⁵⁻¹⁷⁷ Serum osteocalcin is directly correlated with tumor growth in both hormone independent and hormone-dependent tumors.^{178,179}

The wide-ranging impact of osteocalcin on the structure (bones) and function (metabolism) of the body cannot be overstated, thus its key role in the biology of functions, where it is involved in over 60% of the indexes.

Alkaline Phosphatase Bone Isoenzyme (APBi).

Alkaline phosphatases are hydrolytic enzymes that work in an alkaline environment. They hydrolyze phosphates to be (re)used in the formation of proteins and nucleotides and in the mineralization of bone. Though present in all tissues, they are concentrated in the liver and bile ducts, bone, intestine, and placenta, for which isoenzymes have been identified.¹⁸⁰

APBi is present in the plasma membrane of osteoblasts. It is an indicator of bone mineralization¹⁸¹ and bone turnover. APBi is influenced by thyrotropic hormones in managing bone density.¹⁷⁶ APBi is inversely associated with the efficiency of IGFs,^{182,183} but the strength of this association depends on other factors as well. APBi's relationship to IGFs implies a relationship between serum APBi and all the activities in which the IGF family plays a role, such as energy production through regulation of glucose entry into the cell, membrane permeability, free radical production, ATP production, inflammation, etc. APBi is also an indicator of dysregulated growth and is associated with acute lymphocytic leukemia, Paget's disease, and metastasis of cancer to the bone.¹⁸⁰

Systemic Enzymes

Creatine Phosphokinase. Creatine phosphokinase (CPK) is an enzyme that manages the ultra-acute energy needs of the body. It manages the homeostatic state between ATP and adenosine diphosphate (ADP) and the reservoir of phosphate between creatine and phosphocreatine. Based on computer modeling paradigms and in vitro experiments, phosphocreatine, not ATP, carries the majority of energy produced by oxidative phosphorylation out of the mitochondria into the cytoplasm.¹⁸⁴

When the cell has sufficient ATP, it donates a phosphate to creatine, creating phosphocreatine and ADP. Phosphocreatine is a stable reservoir of phosphate. When the cell needs an immediate augmentation of ATP, phosphocreatine donates a phosphate to ADP, which then becomes ATP. During periods of sudden increases in metabolic demand throughout the body¹⁸⁵ and in tissues with chronically elevated energy requirements, there is increased demand for CPK to

transfer phosphate from ADP back to ATP. This allows for instantaneous availability of energy without de novo ATP production.¹⁸⁴ The enzyme CPK catalyzes both reactions (Figure 1).

Skeletal and cardiac muscles contain the greatest concentration of CPK as they have the greatest needs for ultra-acute adaptation of energy. In general, when there is insufficient response to a metabolic demand, cells die, either by apoptosis or necrosis,¹⁸⁶ resulting in either case in elevated amounts of CPK in the serum. This is classically observed during¹⁸⁷ and rhabdomyolysis.¹⁸⁸ Thus, serum CPK is proportional to the rate of muscle turnover and the metabolic role of androgens (which anabolise muscle) but not in a strictly linear way nor as the sole determinant of these functions.¹⁸⁹

Elevated CPK levels in the serum are also associated with myocardial infarction¹⁹⁰ but lack sensitivity and specificity as a sole biomarker of acute myocardial infarction.¹⁹¹ Biomarkers such as total white count, total neutrophil count, and platelets increase the sensitivity of the diagnosis and risk of mortality, which is consistent with the endobiogenic posit that multiple biomarkers are required to accurately assess complex physiologic events.^{192,193}

CPK levels correlate with the degree of ATP flux due to insufficiency of oxidative phosphorylation, ie, mitochondrial strain but again, not in a strictly linear way. As a method of assessing oxidative deficiencies, serum CPK levels alone are neither necessary nor sufficient, but one of many associated factors,¹⁸⁶ as is evidenced in cases of chronic fatigue syndrome where patients have normal cytochrome enzyme activity.¹⁹⁴ Subclinical thyroid dysfunction (SCTD) has been associated with elevated morbidity and mortality in diabetes and cardiovascular disease, both of which are disorders of deranged redox states.¹⁹⁵⁻¹⁹⁷

CPK is inversely related to thyroid metabolic activity^{198,199} and may be elevated in hypothyroidism and SCTD. CPK has been shown to be inversely related to free T₃ and free T₄ levels in both the diagnosis and treatment of hypothyroidism.^{200,201} However, in any particular patient, the correlation is not linear, which supports the endobiogenic theory that quantitative expression of thyroid hormones is neither sufficiently precise nor reliable to determine the actual metabolic impact of thyroid hormones on cellular metabolism.

Lactate dehydrogenase. Lactate dehydrogenase

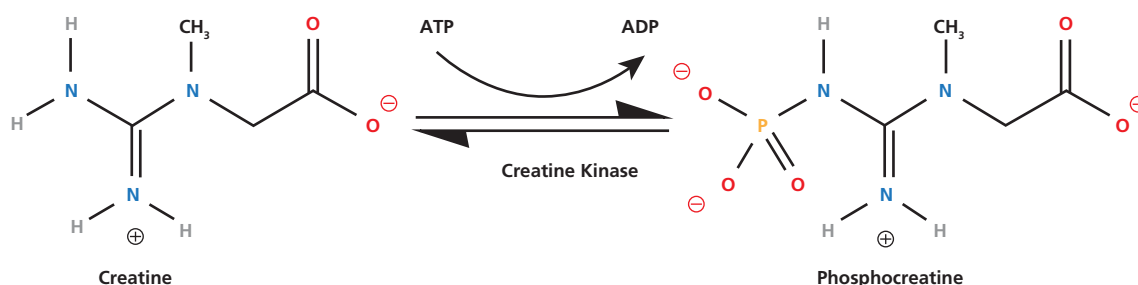


Figure 1 Interconversion of adenosine diphosphate (ADP) and adenosine triphosphate (ATP).

(LDH) is an enzyme that catalyzes the inter-conversion of pyruvate and lactate (Figure 2). Aerobic respiration, using glucose as a substrate, is the most efficient manner of ATP production in the cells. The preferential pathway in the cell is to metabolize glycogen to glucose to pyruvate. Pyruvate is then converted to acetyl-CoA, which enters the Krebs cycle. When there is an insufficiency of coenzymes in the Krebs cycle and/or oxidative stress, LDH activity increases in order to convert pyruvate to lactate. Lactate generates ATP by anaerobic metabolism but at a much lower yield than is attained with aerobic metabolism of glucose. LDH also converts lactate back into pyruvate to produce glycogen as energy storage for future use.

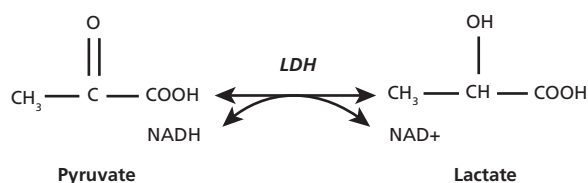


Figure 2 Lactate dehydrogenase (LDH) conversion of pyruvate and lactate.

LDH is contained in large amounts in the liver (the direct storage site of glycogen), as well as cardiac muscle (a major consumer of glucose) and in certain tissues and red blood cells but is found in the serum at low levels. An elevation of LDH in the serum represents a state of impaired oxidation of glucose relative to demands of the organism, as seen in cardiac ischemia,^{202,203} muscle turnover,¹⁸⁶ rapid cell and tissue growth,²⁰⁴ hemolysis,^{205,206} and cancer.²⁰⁷⁻²⁰⁹

Endocrine

Thyroid-stimulating hormone. TSH is a glycoprotein created in and secreted from the anterior pituitary gland. In clinical medicine, it is considered strictly within its intrathyroid activity of stimulation of thyroxine (T₄) and triiodothyronine (T₃), ie, merely as a barometer of thyroid function. Based on more current studies and the endobiogenic theory of terrain, serum TSH levels have key intra- and extrathyroid implications that should also be considered if the clinical significance of a serum TSH level is to be properly contextualized.

Euthyroidism is defined as normal thyroid function that occurs with normal serum levels of TSH and T₄. It has been assumed that TSH and serum levels of T₄ have an inverse linear relationship based on classic feedback loops and that this relationship is a reliable indicator of the sufficiency of thyrotropic regulation of metabolism.

There are a sufficient number of anomalies to this assumption that raise questions about its validity. For example, euthyroid sick syndrome is defined as a clinical condition with normal thyroid function with a normal TSH levels but low serum T₄ and T₃. Subclinical hypothyroidism is a condition in which there is a functional hypothyroid state based on an elevated serum TSH but a normal serum T₄. Subclinical hyperthyroidism is a functional hyperthyroid state based on a serum

TSH value below the normal limit but normal T₄. Finally, patients with normal serum levels of TSH, T₄, and T₃ may present with symptoms consistent with hypo- or hyperthyroidism. See the section on creatine phosphokinase for a further discussion of the functional evaluation of thyroid metabolic activity.

More recent studies demonstrate that serum TSH lacks a log-linear relationship to thyroid output of free T₄ (fT₄) and free T₃ (fT₃) (Figure 3). In their evaluation of 3223 untreated patients referred for thyroid testing, Hoermann et al found poor correlation ($R^2=0.236$) between TSH and fT₄. For example, a serum TSH of 1.0 mU/L (0.4-4.1 mU/L) was associated with a fT₄ anywhere between 4pmol/L and 28 pmol/L (9.5-25 pmol/L). Conversely, a free T₄ of 14.5 pmol/L was associated with TSH between 0.1 mU/L and 100 mU/L.¹⁴⁸

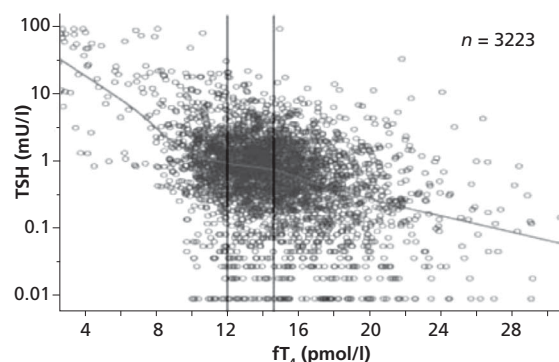


Figure 3 Thyroid-stimulating hormone (TSH) relationship to thyroxine (T₄).

Regression analysis of fT₄ vs log TSH. Fitting data set 1 with the use of SPSS and cut-off values estimated from Figure 1 indicated three distinct regression lines, which were significantly different from each other and described by the following equations: a (fT₄<12): $\text{TSH} = -0.13 \times \text{fT}_4 + 1.46$; b ($12 \leq \text{fT}_4 \leq 15$): $\text{TSH} = -0.06 \times \text{fT}_4 + 0.05$; c (fT₄>15): $\text{TSH} = -0.07 \times \text{fT}_4 + 0.89$; a vs b: $P < .0001$; b vs c: $P = .0002$; and a vs c: $P = .0001$.

From Hoermann R, Eckl W, Hoermann C, Larisch R. Complex relationship between free thyroxine and TSH in the regulation of thyroid function. *Eur J Endocrinol.* Jun 2010;162(6):1123-1129. Reprinted with permission.

In our opinion, the serum level of TSH only reflects the responsiveness of the thyroid to stimulation without determining the final degree of metabolic efficiency of T₄ or T₃ or the degree to which thyroid catabolic activity has been adapted to anabolic demands from estrogen.

TSH has a number of extrathyroid relationships and functions independent of T₄ or T₃. TSH receptors are found in divergent tissues throughout the body.²¹⁰ TSH activity is augmented by estrogen.²¹²⁻²¹⁴ TSH is suppressed by somatostatin.²¹¹ TSH helps regulated bone density as well.^{175,176}

In summary, in the theory of endobiogeny, serum TSH is used to evaluate intra- and extrathyroid activity. Serum TSH is not a sufficient indicator of the efficiency of thyroid regulation of metabolism but can help contextualize thyroid function relative to the demands of the body.

Electrolytes. Potassium and calcium are the only two electrolytes used in the biology of functions.

Potassium. Potassium is the direct IC ion in the body and serves to maintain the resting membrane potential. IC levels are around 140 mmol/L and EC levels, 4 mmol/L. It is not the quantitative concentration per se but the ratio of IC to EC potassium (35:1) that maintains the resting membrane potential and neuromuscular stability. Serum potassium levels are regulated closely in order to maintain neuromuscular stability. A quantitative increase in serum potassium of 1 mmol/L can have a significant impact on neuromuscular activity.²¹⁵

One source of EC potassium augmentation is glutamate. The most prominent neurotransmitter in the brain, glutamate is involved in neural plasticity and augments neuronal excitability.²¹⁶ The egress of potassium from the cell changes the resting membrane potential, allowing for neurons to be more excitable.

Calcium. While potassium is the element of membrane and cell stability, calcium is the element of action, movement, and variability. Calcium is the most predominant element in the human body because of its role in skeletal formation. Of total body calcium, 99% is in bones and 1% is bioavailable. Of the 1% that is bio-available, 99.9999% is in the EC space, maintaining an EC:IC ratio of 12000:1. Calcium reserves are extremely important to ensuring the proper adaptability of the organism during aggression and programmed changes. Approximately 50% of serum calcium is ionized and bioavailable, and 50% is bound to proteins remaining in reserve. While cytoplasmic calcium levels are kept low, the mitochondrion and endoplasmic reticulum store calcium and make it available to calibrate cell function.

Within the blood, calcium is the essential cofactor in the coagulation cascade. Within the interstitium, it is essential as a second messenger in muscle contraction. Calcium augments the rate of neuronal signal transduction and neurotransmitter secretion through up-regulation of vesicle fusion. Within the IC space, calcium serves as a key signal transducer.

In summary, both potassium and calcium concentrations are finely regulated at the extra- and intracellular levels. Potassium is the direct IC element and maintains membrane stability. Calcium is a key element of adaptation and stimulates excitation, movement, and activity, both extra- and intracellularly. These two elements have opposing actions and overlapping factors that increase or diminish their serum concentration. Our interest in these elements with respect to the biology of functions is how they regulate or are regulated by the adaptation response.

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