Nicklaus Children's Hospital

MIAMI CHILDREN'S HEALTH SYSTEM

Pediatric Laboratory-Based Screening Methodology for Nutrition-based Disorders

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Assessment of Wellness vs. Disease

- The development of a nutritional status screening panel is a potentially important tool for assessing nutritional-based disorders in children and adults
- A further goal is a general assessment of wellness, not simply an identification of disease or disease risk, typical of most laboratory screens
- However, further delineating this process requires an understanding of wellness

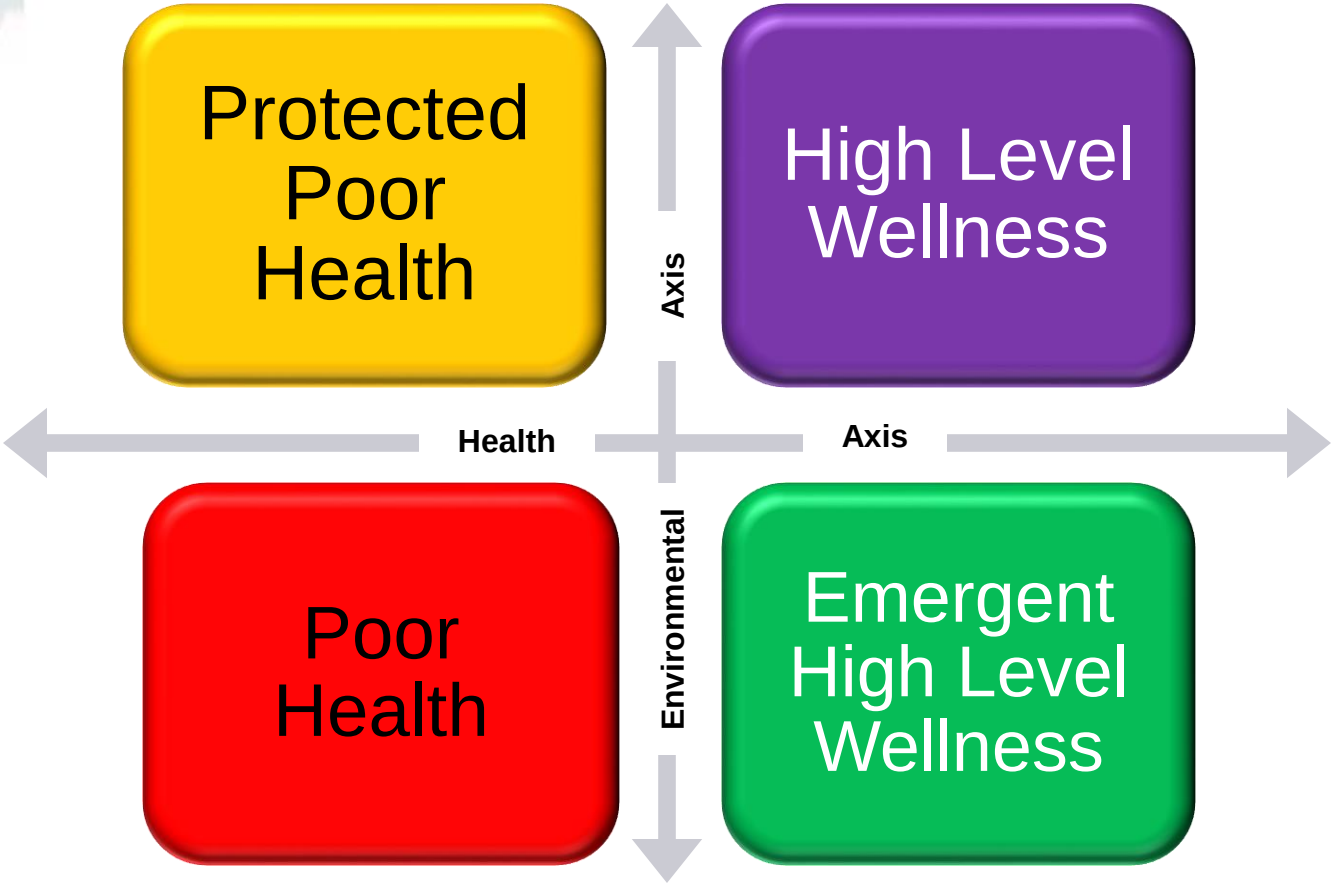
Definitions of Wellness

- "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity“-*WHO, 1948*
- “healthy balance of the mind, body and spirit that results in an overall feeling of well-being”- *Wikipedia*
- “Wellness is a multidimensional state of being describing the existence of positive health in an individual as exemplified by quality of life and a sense of well-being”-*Corbin and Pangrazi, Research Digest, Publ. of the President’s Council on Physical Fitness and Sports, 2001*
- “an integrated method of functioning which is oriented toward ***maximizing the potential*** of which the individual is capable. It requires that the individual maintain a ***continuum of balance and purposeful direction within the environment*** where he is functioning.”-*Halbert L. Dunn*

Halbert L. Dunn, M.D., Ph.D. (1896-1975)

- Regarded as the “Father of the Wellness Movement”
- 1929- the first biostatistician hired by the Mayo Clinic and established its computer coding system for deriving medical statistics
- 1935-1960- Chief of the National Office of Vital Statistics
- 1933- founder of the National Association for Public Health Statistics and Information Systems (NAPHSIS)
- Late 1950’s-Introduced the concept of wellness in a series of 29 lectures

The Health Grid



Dunn H. High Level Wellness for man and society. American Journal of Public Health 1959;49:786-792

Steps to Quantify Positive Health

- Refine incidence and prevalence rates distinguishing positive health from illness or disability
- Develop susceptibility indexes using biochemical and functional tests
- Establish precursors-of-disease indexes

"Once the concept of high-level wellness has been crystalized…the battle for wellness in man and society will be joined. There must be many points of engagement if the battle is to be won."

Key points from his 1959 paper "High Level Wellness for Man and Society"

- Both medicine and public health must be engaged in the elucidation of factors responsible for good health
- Disease and health should be regarded as a continuum, not a dichotomy.
- Wellness must take into account concerns of the body, mind and spirit; "*As if we could divide the sum total of man thus!*"
- ***Wellness must eventually become measurable***: "*If an objective yardstick of wellness can be calibrated in biochemical, physiological, and psychological terms, it would soon become a powerful new tool for the physician, enabling him to recognize low-level wellness and to develop therapies to raise lower levels to higher ones.*"

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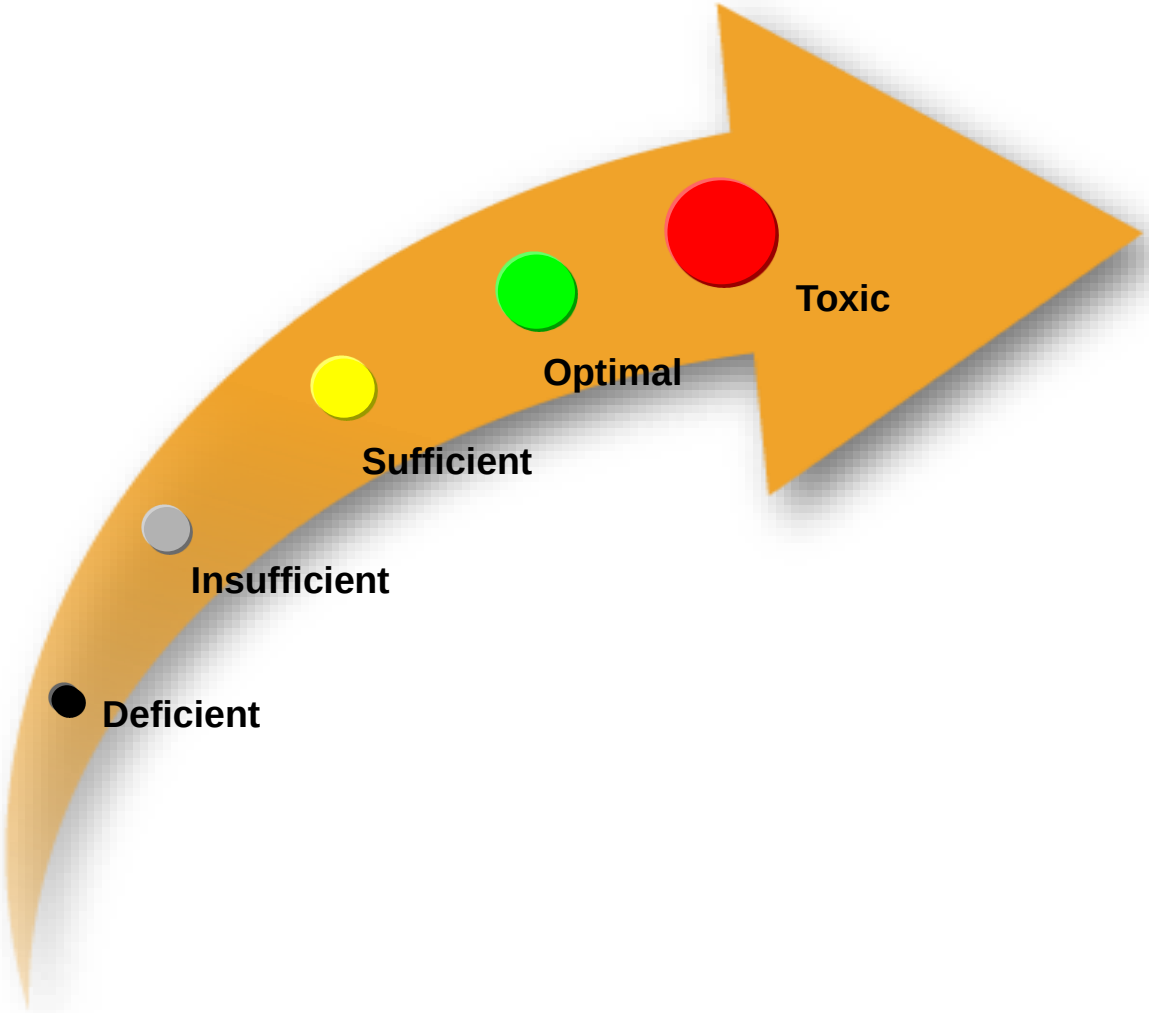
Nutritional Status Screening Panel

"More is missed by not looking than not knowing"

Thomas McCrae (1870-1935), Professor of Medicine at Jefferson Memorial College

Continuum of Nutritional Needs



Governing Principles: Screening for "Disease" Malnutrition

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a "once and for all" project.

Wilson JMG, **Jungner G** - Principles and practice of screening for Disease. WHO 1968

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Nutrition Model

Reductionism vs. Systems Approach
Clinical Features

Characteristic	Reductionism	Systems Approach
Principle	Behavior of a biological systems can be explained by the properties of its constituent parts	Biological systems possess emergent properties that are only possessed by the system as a whole and not by any isolated part of the system
Metaphor	Machine, magic bullet	Network
Approach	One factor is singled out for attention and is given explanatory weight on its own	Many factors are simultaneously evaluated to assess the dynamics of the system
Critical Factors	Predictors/associated factors	Time, space, context
Model Characteristics	Linear, predictable, frequently deterministic	Nonlinear, sensitive to initial conditions, stochastical, chaotic
Medical Concepts	Health is normalcy Health is risk reduction Health is homeostasis	Health is robustness Health is adaptation/plasticity Health is homeodynamics

Ahn AC, et. al. PLoS Medicine. 2006;3:956-60.

Reductionism vs. Systems Approach

Characteristic	Reductionism	Systems Approach
Optimal	Conditions where one or few components are responsible for the overall behavior of the system	Conditions where interaction between components are responsible for the overall behavior of the system
Disease types	Acute, simple diseases	Chronic, complex diseases
Examples	Urinary tract infections Appendicitis Aortic aneurysms	Diabetes Coronary artery disease Asthma
Theoretical limitations	Disregards component-component interactions and dynamics	Costly in resources in time (short term)

Ahn AC, et. al. PLoS Medicine. 2006;3:956-60.

Reductionist Approaches to Nutrition are Inadequate

- The early 20th Century nutrition paradigm (single-nutrient model) that explains disease based on micronutrient deficiency, is inadequate for complex forms of malnutrition:
  - A simple cause-effect relationship exists between a specific disease and a particular nutrient (François Magendie, Early 19th century).
  - Each nutrient deficiency disease can be explained physiologically in terms of the role played by the respective nutrient.
  - Providing the nutrient in the diet can prevent, and in many cases reverse, the disease
- Obesity is a complex multifactorial disorder that is a function of evolutionary trade-offs, socioeconomic, environmental and other factors
- A comprehensive model is required to address and monitor (screen) the entire spectrum of malnutrition

Classification of Malnutrition

- Primary nutrient deficiency/insufficiency/sufficient but suboptimal
- Secondary nutrient deficiency/insufficiency
- Primary metabolic disorders (inborn errors of metabolism)
- **Nutrition-acquired (secondary) metabolic disorders with/without primary or secondary nutrient deficiency/insufficiency**

Systems Biology Approach to Nutrition

- Systems biology is an emerging multidisciplinary field that bridges holistic and reductionist approaches to biology and medicine
- The goal is to develop predictive models that that describe how biological system act and change over time, respond to perturbations and how diseases manifest, can be diagnosed and treated
- Application of a systems approach to nutrition is ideally suited to understand complex disorders such a obesity and metabolic syndrome

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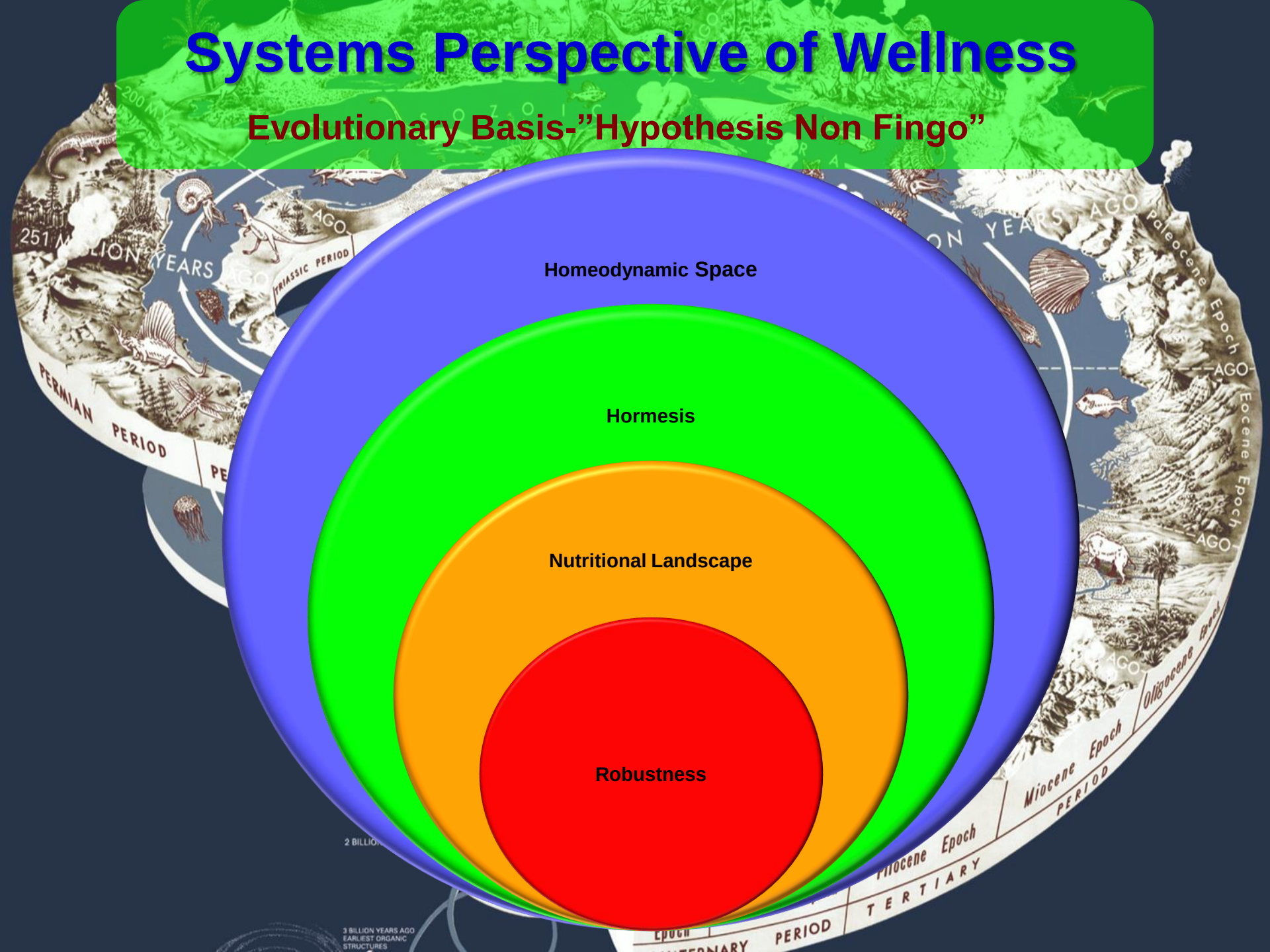
Framework for Systems Approach to Nutrition and Wellness

Systems Perspective of Wellness

Evolutionary Basis-"Hypothesis Non Fingo"



Robustness

A Fundamental Property of Biological Systems

- Robustness, a concept from Systems Biology is:
  - The property of active maintenance of the function of a system while exposed to internal/external environmental perturbations using an integrated network of regulatory controls
  - Robustness of the system is manifested as **adaptation** to the environment and as stability against external and internal disturbances
- The system exerts the capacity to “adapt” to the environment and maintain stability against perturbations or uncertainly (of variable duration and intensity) through:
  - Feedback control
  - Redundancy
  - Modularity
  - Structural stability

Characteristics of Robustness

- Robustness has meaning only through the relationship of a system, function and perturbation
- Implicit in Robustness are:
  - Feedback regulation that maintain homeostatic mechanisms (a function of robustness)
  - Functional redundancy of gene products as fail-safe mechanisms
  - Modularity that minimizes propagation of local perturbations from becoming system-wide (buffer)
    - Also minimizes drug effectiveness
  - Tight regulation of cellular sensing, signaling and metabolic processes that are highly conserved

In other words…

- Robustness reflects the ability of the body to respond to changes through coordination of multiple subsystems, organs, regulatory mechanisms at a biochemical and physiological level to ensure survival (somatic maintenance)

- Compromise of this capacity may correlate to disease while optimal somatic maintenance may relate to Wellness

Why Robustness?

- Robustness is a powerful concept because it is capable of reflecting a highly dynamic system such as a biological organism
- Robustness is a universal feature in biological systems (evolutionary adaptation) that is highly conserved
- All biological systems are in constant motion, the behavior of which arises from the interactions of components
- While the dynamic system is complex it rests on three basis principles
  - ***Context***-the components that participate in the process
  - ***Time***-the temporal variations of components
  - ***Space***-the topographical relationship between and among the components

Robustness and Disease

- There is an important balance between robustness and fragility in biological systems
  - The cost of improved robustness is fragility against unusual perturbations
  - Feedback mitigates this to a point but a cost elsewhere in the system-fragility to unexpected perturbations
  - Redundancy and modularity also help but at the cost of increased resources (redundancy) or ineffective therapy (modularity of system reduces drug effectiveness)
- That which ensures survival can be co-opted

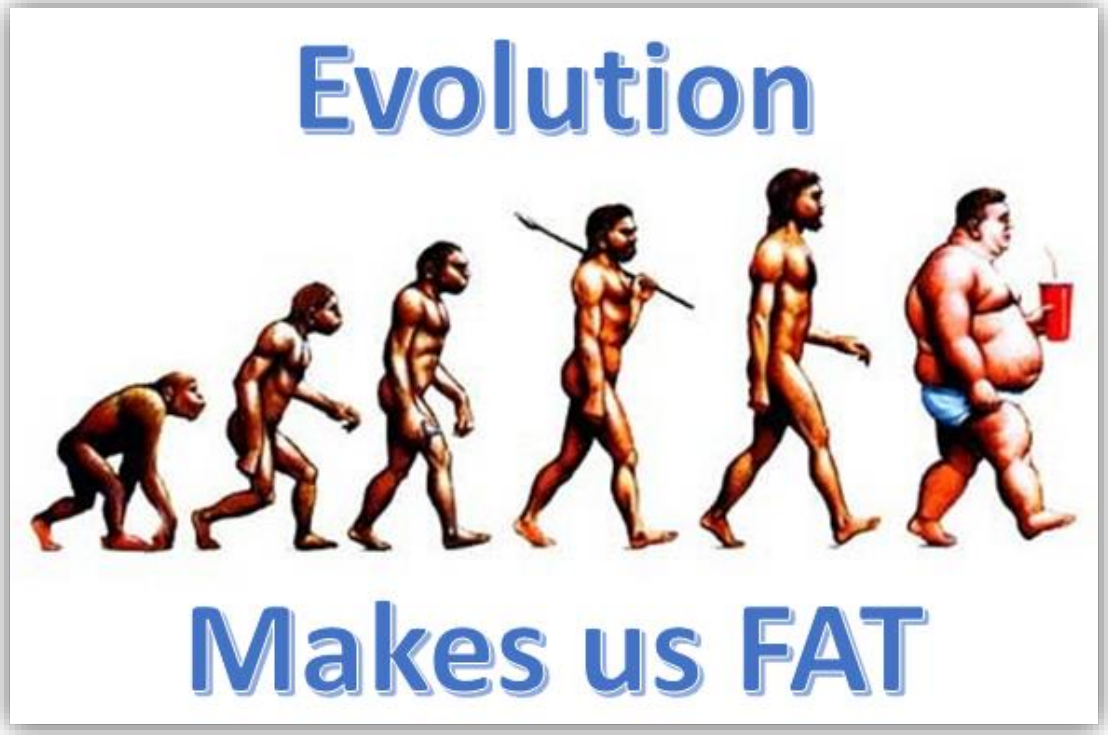
Clinical Trade-offs

In Nature there is no Free Lunch

- Through evolution, humans have developed robustness against near starvation, a high energy demand lifestyle and risk of infection but the system is susceptible to the unusual perturbation of over nutrition and low energy demand
  - Type 2 diabetes
  - Metabolic syndrome
- The system is relatively tolerant to the removal of some compounds or cells because of alternative mechanisms (redundancy) but vulnerable when components or mechanisms are not recognized (hijack the system)
  - Cancer
  - HIV

Evolutionary Basis of Obesity

Humans evolved to resist starvation, not over-nutrition

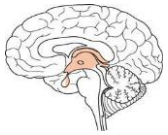


Nutritional Landscape and the Ames Triage Hypothesis of Micronutrients and Chronic Diseases

- Natural Selection favors short-term survival at the expense of long-term health
- Short-term survival is achieved by allocating scarce micronutrients by triage
- Micronutrient deficiencies that trigger the triage response accelerate aging, cancer, neural degeneration, etc. sparing critical metabolic functions such as energy metabolism (ATP) at the expense of “less essential” functions
- Micronutrient malnutrition accelerates late onset diseases; damage from inflammation, oxidative stress and other stressors that increase the risk of age related diseases and disorders
- Triage can be extended to macronutrients-Protein Leverage Hypothesis

Ames PNAS 2006; McCann and Ames FASEB J 2011

Role of Protein Leverage



High: AA/CHO+FA

Nutrients

Low: AA/CHO+FA

- Calorie restriction
- Exercise
- Phytochemicals

Insulin Resistance: Inhibition of autophagy and repair

mTOR

AMPK

Insulin Sensitivity: Promotes autophagy and repair

Under eat

Anorexic

Orexigenic

Over eat: Low protein and high glycemic foods

Anabolic Responses:
- Protein synthesis
- Lipogenesis
- Cell proliferation
- Growth
- Reproduction

Catabolic Responses:
- Cell cycle arrest
- Inhibition of growth
- Inhibition of reproduction
- Lipolysis
- Proteolysis

Anorexia/Cachexia

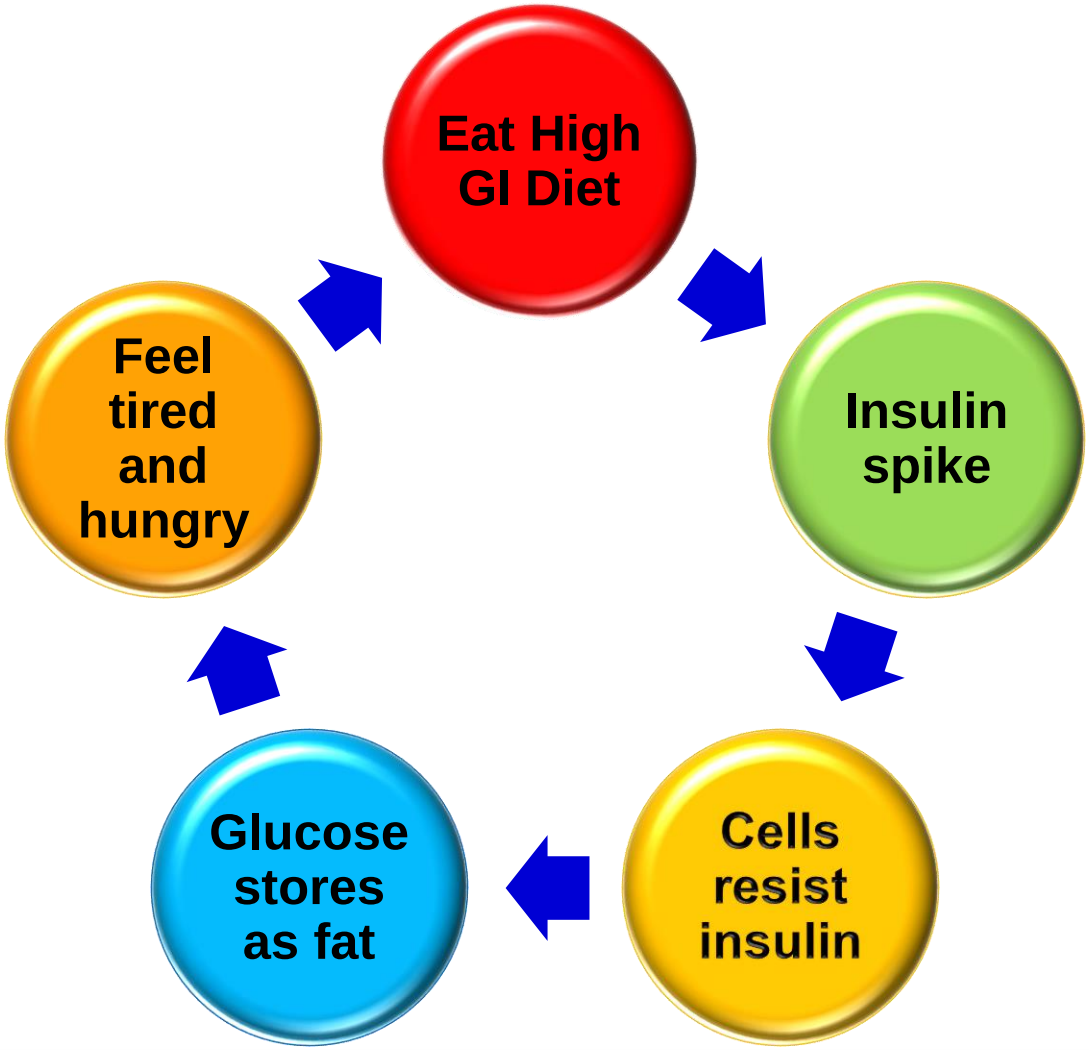
Increased Lifespan

Shortened Lifespan

Obesity and insulin resistance

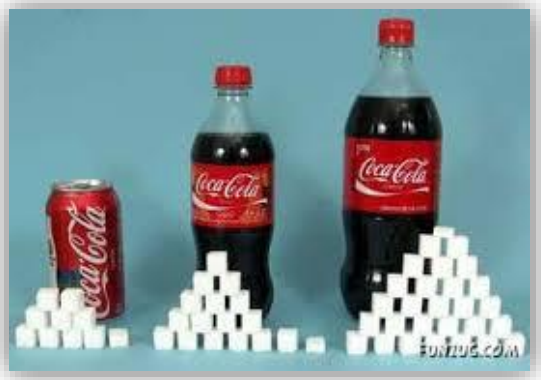
Simpson SJ and Raubenheimer D, The Nature of Nutrition Princeton University Press 2012

Cycle of Insulin Resistance



Increases in Added Sugar and Refined Carbohydrates

- **Sugar consumption:** 152 lbs/yr up from 40 lbs in 1980
- **Flour consumption:** 146 lbs/yr
- **Added sugars:**
  - 600,000 products and 80% with added sugar
  - 8-15% calories from soda
  - HFCS biggest source of calories in diet
  - Children sugar consumption facts: ***34 tsp/day***



Chair of American Beverage Association in testimony to Congress: "In a well balanced diet we need two liters of liquids a day. Soft drinks can be a healthy part of that intake. I would reject any argument that they are in any way Harmful."

Lustig, R, Nature, Feb 2012, Volume 482, 27-29

Consequences of Added Sugar and HFCS

- Empty calories that replace foods containing nutrients
- HFCS (no physiologic role for fructose) is exclusively metabolized in the liver-NAFLD/NASH
- Induces IR and can result in obesity and metabolic syndrome in children and adults
- Associated with a higher risk of cancer
- Stimulates hunger and fatigue
- Raises cholesterol increasing CVD risk
- Cause massive dopamine release-addictive

Physiologic Impacts of Obesity

- **Assimilation (intestine)**
  - Changes in microbiome
  - Leaky Gut: metabolic endotoxemia
- **Defense and Repair**
  - Chronic inflammatory state
  - Immune dysfunction
- **Energy**
  - Mitochondrial dysfunction
- **Communication**
  - Neuroendocrine dysregulation: cortisol, insulin, leptin, ghrelin, appetite
  - Metabolic inflexibility
- **Secondary malnutrition**

Not Only Food Composition and Quality but Loss of Agency

- Commercial organizations maximize profits through making consumer decisions for them
  - **Behavioral level:** advertising, price manipulation, restriction of choice
  - **Physiological level:** Enhancement of addictive properties of foods
- "Loss of agency characterizes not only individuals but also governments and other organizations promoting health. In the 21st century, the food-industrial complex has become so powerful that efforts to redress the scenario have proven futile and each of obesity and malnutrition is increasing" Wells, JCK. Am J Hum Biol, 2012 DOI 10.
- Governments are also complicit

Traditional and modern shops in Jimma, Ethiopia

Hormesis-Response to Stress

Capacity to Improve Somatic Maintenance and Resist Stress

- **Toxicology** - biphasic dose response to an environmental agent characterized by a low dose stimulation or beneficial effect and a high dose inhibitory or toxic effect
- **Biological systems** - biologically adaptive process whereby nonlethal improves robustness of an organism to resist it and improve somatic maintenance
  - Exercise
  - Dietary energy restriction
  - Exposures to low doses of certain phytochemicals
- **Hormesis is integral to the normal physiological function of cells and organisms**
  - **Implicitly understood for millennia:** “if you don’t use it, you lose it”; “that which does not kill you makes you stronger”
  - Conditioning by exogenous/endogenous stressors contribute to enhancing cells capacity to respond to/resist stress (stress tolerance/reduced stress response mediators)
  - This conditioning is mediated by molecular signaling pathways and cellular processes
  - Interventions including nutritional or botanical therapy (adaptogens) may support the stress response capacity

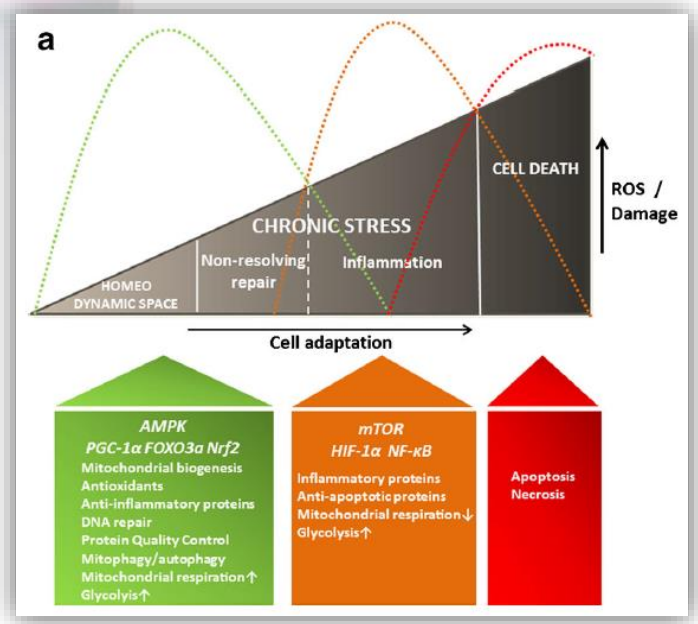
Homeodynamic Space

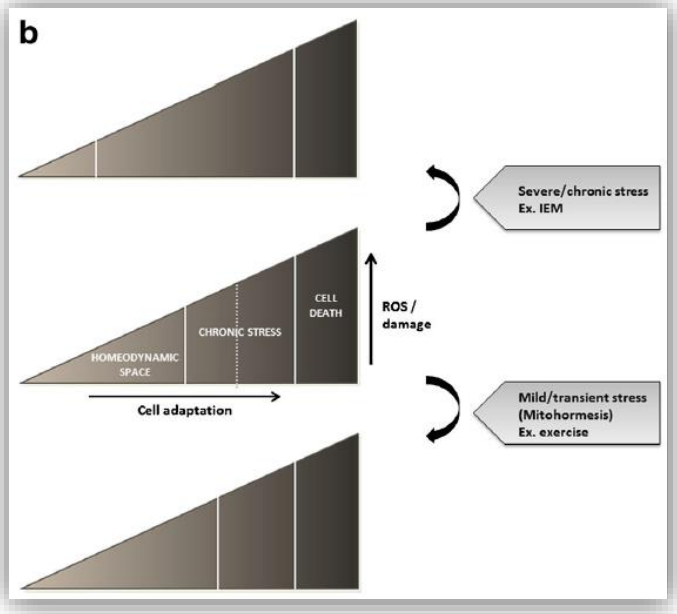
A Broad Indicator of General Wellness

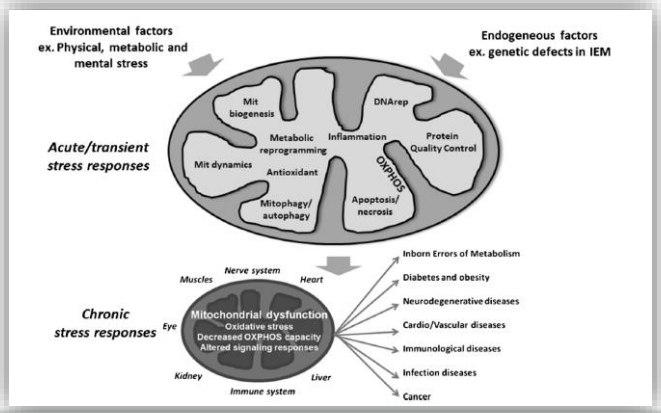
- The dynamic range of implicit integral network of cellular and system metabolic processes involved in somatic maintenance and repair of a biological system that ensure survival
- Property of biological systems that reflects the capacity to respond to stress; a measure of the system's robustness
- The ability of the living systems to respond and counteract stress, to repair and remove the damage, and to undergo constant remodeling and adaptation
- Genetic polymorphism and epigenetic factors establish a personalized homeodynamic space during growth, development and maturation, within the evolutionary constraints of essential lifespan
- Imperfections of the maintenance and repair systems reflect an always existing vulnerability zone even at a young aging
- **The ultimate determinant of an individuals state of wellness**

Rattan SIS. Current Pharmaceutical Design, 2014, 20, 3036-3039

Relationship of Hormesis and Homeodynamic Space







Olsen RKJ, Cornelius N, Gregersen N. J Inherit Metab Dis (2015) 38:703–719

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Probing the Homeodynamic Space

Role of the TOP™ Screening Panel

Markers of the Homeodynamic Space

- **Micronutrient Status**-Nutritional landscape
  - Nutrients that ensure the potential for optimal metabolic and physiologic function
  - Insufficiency results in triage favoring short term survival and resulting in long term nutrition-acquired diseases
- **Energy Metabolism**
  - Quantitative and qualitative features of macronutrients
  - Protein, carbohydrate and lipid metabolism
- **Stress Response Capacity**
  - The capacity to respond to environmental stress (internal, external) as reflected by the oxidative and inflammatory states
  - Metabolic flexibility

Shrinking of the Homeodynamic Space and Chronic Disease

- There is an important balance between robustness and fragility in biological systems.
- The cost of improved robustness is fragility against unusual perturbations
- That which ensures survival can be co-opted
- Reduced stress response capacity-risk of decompensation (catastrophe)

Biological trade-off (in Nature there is no free lunch)

- Through evolution, humans have developed robustness against near starvation, a high energy demand lifestyle and risk of infection but the system is susceptible to the unusual perturbation of over-nutrition and low energy demand
  - Type 2 diabetes
  - Metabolic syndrome

Hormesis: Expansion of the Homeodynamic Space

- The universal biological principle where mild, non-lethal stress **improves somatic maintenance and robustness**
- Principle that it has been viewed as essential for life and evolution since natural selection requires the ability of adaptation to ever-changing and uncertain environmental influences
- Explains the health benefits of periodic calorie restriction, activity/exercise and plant phytochemicals since they produce a mild stress that stimulates vital underlying processes that ensure improvement of organism's maintenance

"Adaptability and resistance to stress are fundamental prerequisites for life, and every vital organ and function participates in them."-Hans Selye 1950.

"Stress is the salt of life… total elimination of stress (cessation of demands made upon any part of the body) would be equivalent to death."- Hans Selye 1976

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Rationale for Analytes

Technical Principles of the TOP™ Panel

- The panel must be supported by evidence-based documentation
  - Valid for screening (non-fasting) purposes in children (or adults)
- Minimal blood volume requirement
- Not intended to be a comprehensive assessment of nutrition
  - Initial assessment for common nutritional deficiencies and insufficiencies
  - Potential for reflecting other nutritional disorders or health problems requiring a secondary evaluation
- Ideally performed on a single platform capable of high throughput and rapid turnaround time
- The laboratory report will include:
  - Information about the significance of each analyte
  - Interpretation of each result or combination of results with appropriate disclaimers and recommendations for follow-up

Governing Principles of the TOP™ Panel

- Non-fasting: fasting may artificially bias or mask underlying state of insufficient or non-optimal stress response capacity
- Avoid analytes that are highly dynamic and do not afford a picture over a broad range in time
  - e.g; fasting glucose vs. HbA1c
- Include analytes that reflect nutritional status and chronic disorders (malnutrition) at early and late stages
  - e.g.; Fe and ferritin
- Secondary evaluation (not in TOP™ panel)-Functional assay to probe homeodynamic space

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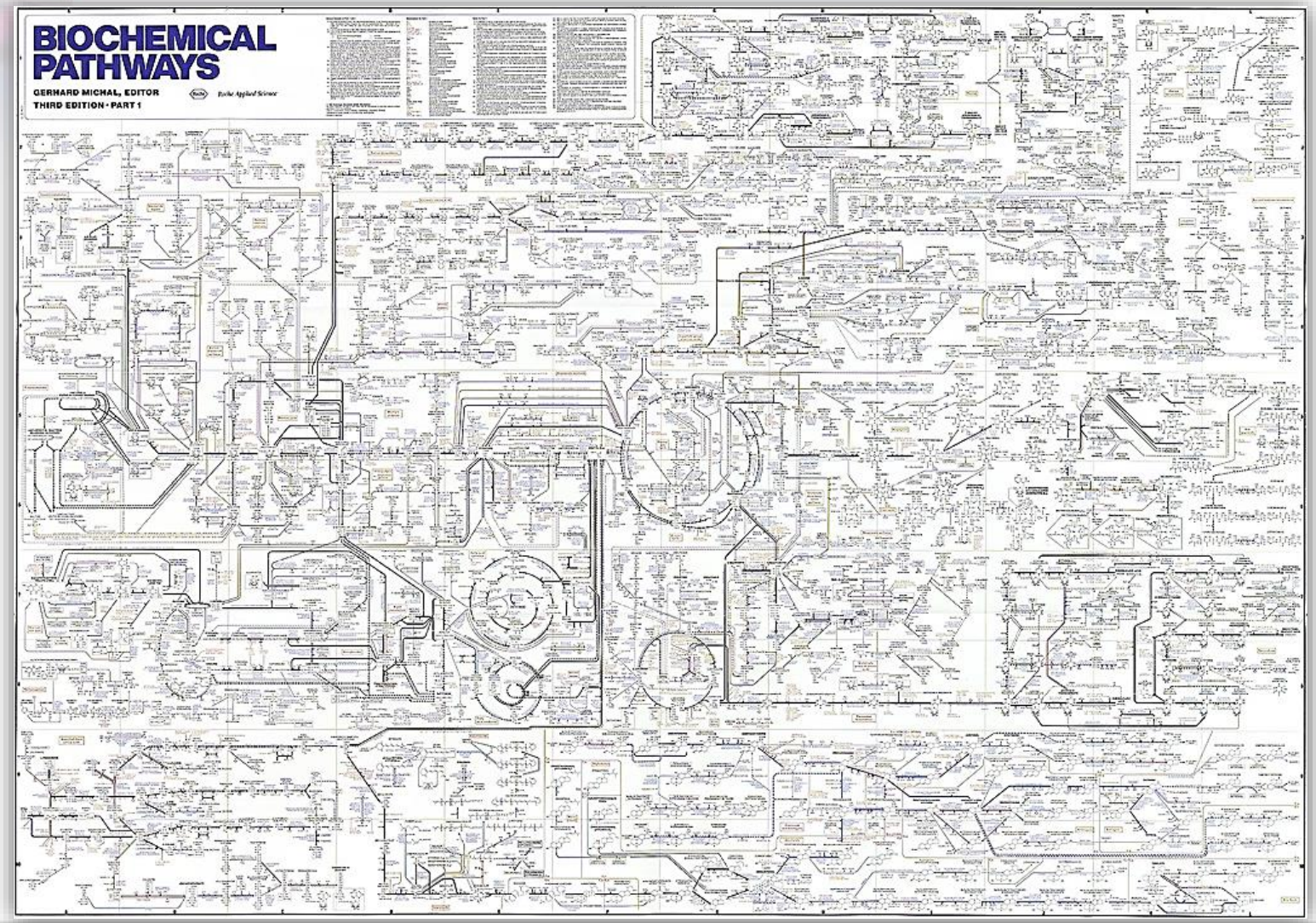
Consideration of Analytes

Complex Reductionist Description of Biochemical Pathways



Essential Micronutrients

- **Vitamins**
  - Vitamin A
  - Vitamin B1 (thiamine)
  - Vitamin B2 (riboflavin)
  - Vitamin B3 (niacin)
  - Vitamin B5 (pantothenic acid)
  - Vitamin B6
  - Vitamin B9 (folic acid)
  - Vitamin B12
  - Biotin
  - Vitamin C
  - Vitamin D
  - Vitamin E
  - Vitamin K
  - Choline
- **Amino Acids**
  - Isoleucine
  - Leucine
  - Lysine
  - Methionine
  - Phenylalanine
  - Threonine
  - Tryptophan
  - Valine
  - histidine
- **Minerals**
  - Calcium
  - Chloride
  - Chromium
  - Cobalt
  - Copper
  - Iodide
  - Iron
  - Magnesium
  - Manganese
  - Molybdenum
  - Potassium
  - Selenium
  - Sodium
  - Zinc
- **Fatty Acids**
  - alpha-Linolenic acid/DHA (omega -3)
  - Linoleic acid (omega-6)

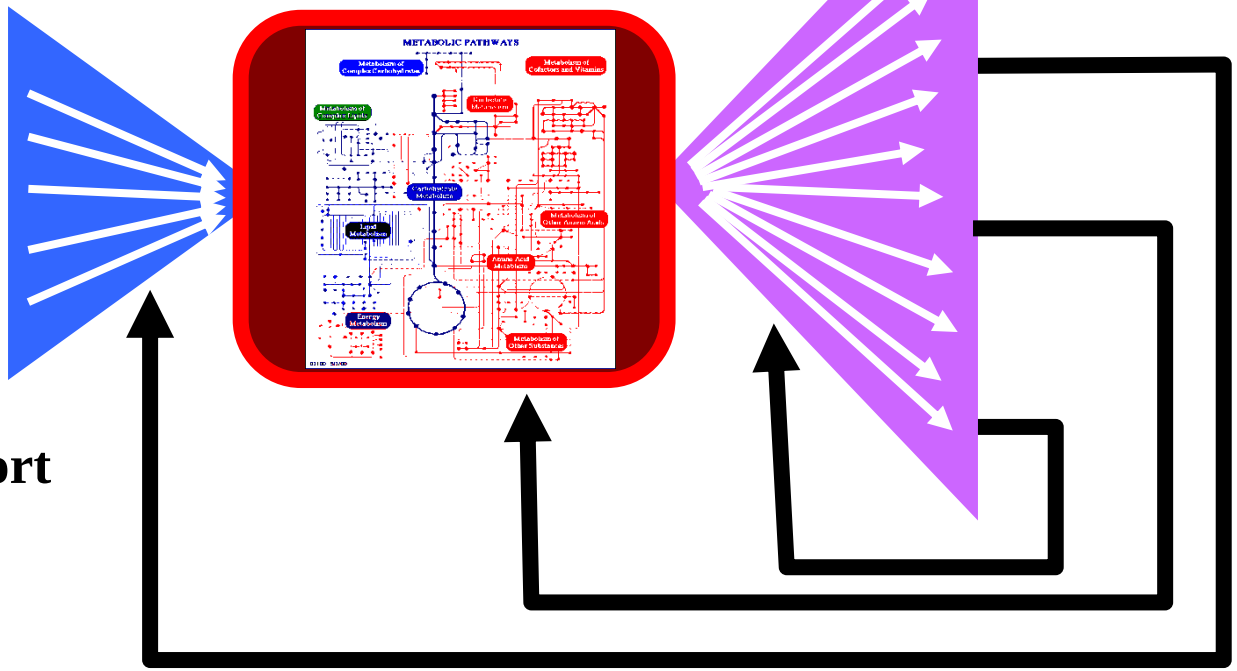
Typical Screening Biomarkers

- **Hematologic**
  - WBC
  - Hemoglobin
  - Hematocrit
  - Iron
  - Ferritin
- **Lipids**
  - Total Cholesterol
  - LDL-cholesterol
  - HDL-cholesterol
  - Non-HDL-cholesterol
  - Triglycerodes
  - Lipoprotein particle numbers
- **Vascular/Inflammation**
  - ApoB 100
  - Lp(a)
  - Hs-CRP
  - Homocysteine
- **Hepatic**
  - AST
  - ALT
  - GGT
- **Glucose metabolism**
  - Glucose
  - Insulin
  - HbA1c
- **Endocrine**

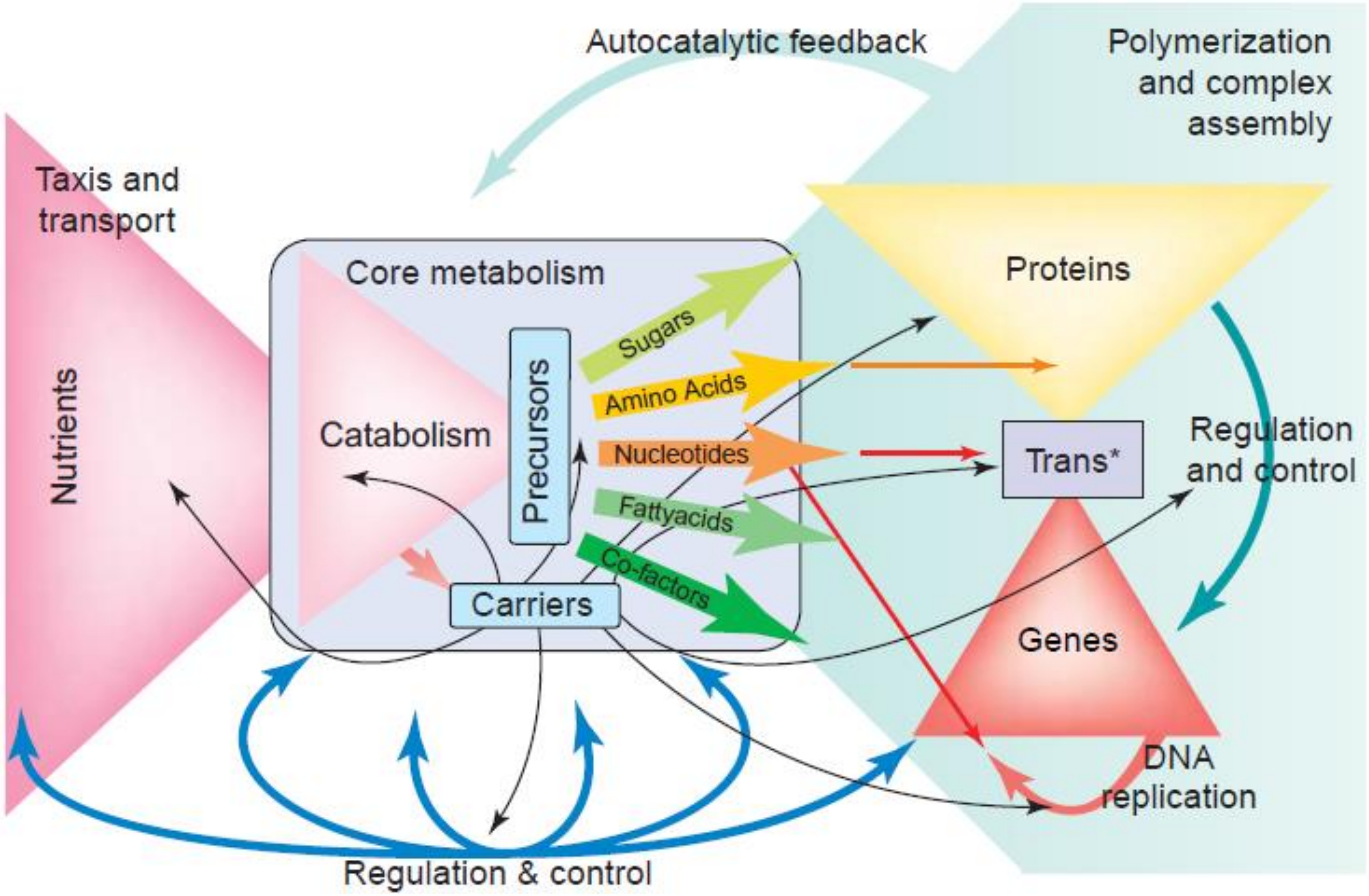
  Thyroid hormones
  - Free T3, T4
  - Total T4
  - rT3 - Reverse T3
  - TSH
  - Anti-TG
  - Anti-TPO
  - TG
  - TBG

  Sex steroid hormones
  - DHEAS (Dehydroepiandrosterone sulfate)
  - Androstenedione
  - Testosterone
  - Estradiol
  - Estrone
  - Estriol, unconjugated
  - Progesterone
- **Antioxidant**
  - Alpha Lipoic Acid
  - Coenzyme Q10
  - Glutathione

Whole Cell Metabolism



Biosynthesis Bow Tie Structure (and nested bow ties)



Structural proteins, enzymes, Co-factors, hormones, etc.

Csete M and Doyle J Trends in Biotechnology 2004;22:446-450

TOP Panel™

Probe of Homeodynamic Space

- **Energy Metabolism Status**
  - **Lipid metabolism**
    - Non-HDL cholesterol
    - HDL-cholesterol
    - Triglycerides
  - **Carbohydrate metabolism**
    - HbA1c
  - **Protein metabolism**
    - Transthyretin (prealbumin)
- **Nutrition-associated endocrine status**
  - Thyroid stimulating hormone (TSH)
- **Micronutrient Status (sensitive points in metabolic cycles)**
  - Vitamin B_{12}
  - Folate (Vitamin B_9)
  - Vitamin D
  - Iron (Fe)
  - Ferritin
  - Zn
- **Inflammation/Oxidative stress (biomarkers of stress response)**
  - Homocysteine
  - hs-CRP
  - GGT

Vitamin B12/Folate (Vitamin B9) Rationale (Nexus of cycles)

- Key interactions occur between folate and vitamin B12 in the folate cycle
  - Nucleotide, DNA and RNA synthesis
  - Neurotransmitter synthesis
  - Coupling with Methylation cycle
    - Homocysteine and glutathione synthesis
    - Epigenetic regulation of gene expression
- Vitamin B12 and folate deficiency/insufficiency in infants and young children may result in a variety of clinical manifestations; hematologic, neurologic, and gastrointestinal that may be ameliorated with administration of vitamin B12 and/or folate therapy
- Prevalence of vitamin B12 deficiency in children and adolescents in the US- <1% (<200pg/ml); <4 years- <3%

Vitamin B9 and B12 Insufficiency

- The prevalence of Vitamin B9 and B12 deficiency is low in the US
- However, the prevalence of insufficiency and associated conditions has not been well established
- Abnormal Methylation is a potential consequence of Vitamin B9 and B12 (and other micronutrients) insufficiency with or without MTHFR gene polymorphisms such as C677T or A1298C
  - Either may be reflected by elevated homocysteine levels
- Disorders associated with deficient methylation include:
  - Cancer
  - Cardiovascular risk
  - Dementia

Vitamin D [25(OH)D] Rationale

- Vitamin D [25(OH)D] deficiency (≤ 20 ng/ml) is a highly prevalent condition among infants, children, and adolescents in the USA and worldwide
- ***Evidence links vitamin D deficiency in early childhood and specific conditions that manifest more frequently in adults***; osteoporosis, multiple sclerosis, obesity, type 2 diabetes, cancer, immune suppression, asthma and cardiovascular disease
- Prevalence of vitamin D deficiency (< 15 ng/ml) is as high as 24% in healthy adolescents and 14% in toddlers and infants
- The highest prevalence of deficiency seen in the African American and Hispanic populations
- **The prevalence of "healthy" children and adolescents with insufficiency is even higher**

Iron/Ferritin Rationale

- **Clinical manifestations of iron deficiency:** iron deficiency anemia, impaired psychomotor and/or mental development, cognitive impairment, susceptibility to infection, impaired immunity, decreased exercise capacity (even without evidence of anemia)
- Prevalence of iron deficiency in the US
  - 9% percent of toddlers (one to three years old) have iron deficiency
  - 2% to 3% percent of toddlers have iron deficiency anemia
  - Rates decrease with advancing age until adolescence
    - ≤16% percent of girls develop iron deficiency
    - 3% of girls develop iron deficiency anemia
- The prevalence of iron deficiency is higher among children living at or below the poverty level, and in African American and Hispanic children
- **Inflammation of obesity is becoming recognized as an important cause of iron deficiency**
- Serum ferritin is the earliest marker of iron deficiency and thus a sensitive marker for this condition

Cholesterol/Non-HDL-c Rationale

- ***American Academy of Pediatrics guidelines strongly recommend universal cholesterol screening between the ages of 9 and 11 and between 17 and 21 years***
  - Selective screening fasting lipid profile (FLP) was expanded to include children with conditions that increase risk of cardiovascular disease with or without a family history
- The new guidelines are based on research:
  - Early atherosclerosis exists in young patients with elevated cholesterol
  - Early treatment of cardiovascular risk factors in youth is effective
  - Lipid disorders are common in children and increasing coincident with childhood obesity
  - 30-60% of children with dyslipidemias are missed using the traditional selective screening methods
- ***The universal screening lipid profile can be done non-fasting***
- NON-high density lipoprotein cholesterol fraction (known as non-HDL-c) has comparable predictive risk to the low density lipoprotein cholesterol (LDL-C) calculation on a standard FLP
- The prevalence of abnormal lipid levels in youths (12-19 years) is at least 20%
- ***Approximately 32% of youths are overweight or obese and are considered to be candidates for lipid screening***

HbA1c Rationale

- ***The American Diabetes Association position***: HbA1c measurements are supported for the diagnosis of diabetes with values ≥6.5% considered diagnostic with a range of 5.7%-6.4% considered pre-diabetic
- Glycated hemoglobin values reflect the 2-to-3-month average endogenous exposure to glucose, including postprandial spikes in the blood glucose level with low intra-individual variability
- HbA1c testing has been recommended by International guidelines for the first-line screening and diagnosis of type 2 diabetes in Europe and other countries
- HbA1c has several advantages over these tests for the majority of patients:
  - There is no need for fasting.
  - People are often non-compliant with the requirement for fasting, thereby reducing the accuracy of fasting plasma and oral glucose tolerance tests.
  - HbA1c is less affected by day to day variation in plasma glucose (exercise, medicines, diet, etc.)
- HbA1c has simpler sampling and analysis requirements and is very stable
  - Glucose levels can be misleading if the sample is not processed immediately, due to pre-analytical instability since glucose consumption continues to occur in blood after sampling
  - The pre-analytic variability of fasting plasma glucose testing is approximately 5-10% compared to the pre-analytic variability of HbA1c which is negligible

HbA1c Rationale

Drugs and Therapeutics Committee of the Pediatric Endocrine Society

"Conclusions that dismiss HbA1c use for the diagnosis of diabetes in children are based on incomplete data. Considering that the demographics of Type 2 diabetes skew towards disadvantaged populations, we should not dismiss a valuable, flexible tool that, put into widespread use, may in fact increase, not decrease, early detection of this disease."

Kapadia C and Zeitler P. Hemoglobin A1c measurement for the diagnosis of Type 2 diabetes in children. International Journal of Pediatric Endocrinology 2012; 2012:31-34.

Homocysteine Rationale

- Screening of homocysteine levels (traditionally for newborn screening for homocystinuria attributable to cystathionine β-synthase deficiency) has expanded role for screening in adults and children
- Screening may play a role in the risk assessment and disease diagnosis of other conditions
  - Folate, vitamins B2, B6 and B12 deficiencies
  - Renal failure, hypothyroidism and osteoporosis
  - Psychiatric disorders and cognitive impairment
  - Pregnancy complications and birth defects
  - Genetic factors such as the MTHFR 677C→T polymorphism
- **A causal relationship has been established between hyperhomocysteinemia and certain nutritional deficiencies; folate, vitamins B2, B6, B12 and Zn deficiency or insufficiency**
- Homocysteine has been shown to induce oxidative stress (inefficient conversion to glutathione) resulting in damage to cholesterol, inhibition of eNOS and apoptosis of endothelial progenitor cells, contributing to atherosclerosis, impaired function of immune cells and induction of inflammation
- **In general there exists a strong relationships between high levels of homocysteine and disease and between low levels of homocysteine and health**

Transthyretin (Prealbumin) Rationale

- ***Currently, transthyretin (prealbumin) testing is used in nutrition assessment and monitoring which is now one of the most utilized nutritional marker worldwide***
  - Low transthyretin levels suggest a risk for malnutrition (status of the body's metabolic nitrogen pool)
  - The half-life of transthyretin is approximately 1.9 days, making this a more sensitive marker of protein status that albumin and other markers of nutritional status
  - Transthyretin is also depressed by anti-inflammatory response; consequently, it is a negative acute phase reactant (complement to hs-CRP)
- Typically, transthyretin screening is used in the hospital setting where studies have shown substantial benefits;
  - Reduced lengths of stay
  - Decreased morbidity/mortality through earlier nutritional intervention
  - Decreased costs
- Measurement of transthyretin is a simple, rapid, inexpensive and accurate with well established normal ranges in children and adults
- Transthyretin screening as a compliment to other markers in the community setting should be useful in uncovering children at risk for nutritional deficiencies and may be an important tool to consider (along with the panel) in the inpatient setting to address early or undocumented nutritional deficiencies

hs (high sensitivity)-CRP Rationale

- hs-CRP is a nonspecific acute phase reactant or marker of the inflammatory state that is clinically important in clinical context
- Serum hs-CRP level increases with
  - mild chronic infection
  - tissue damage
  - chronic diseases such as cancer and cardiovascular disease
  - bacterial or fungal infection (marked elevation)
- hs-CRP is an excellent predictor of cardiovascular disease in children and adolescents who are overweight or obese, have metabolic syndrome or have type 2 diabetes since ***studies in obese children have demonstrated early functional and morphologic vascular changes***
- The combination of hs-CRP + total cholesterol/HDL is an even more powerful predictor of cardiovascular disease risk and may serve as a monitor to assess the effect of therapeutic intervention
- **While in some cases the elevated inflammatory state may be a reflection of acute disease, it may also be induced by external; factors such as poor nutrition which can lead to obesity, metabolic syndrome or type 2 diabetes**
- **hs-CRP can also serve as a marker to assess the effect of nutritional and lifestyle intervention designed to correct behaviors that lead to disease states**

Gamma glutamyltransferase (GGT) Rationale

- Ubiquitous enzyme: liver and other organ tissues; kidney, lung, pancreas, heart, brain and blood vessels
- Traditional Biomarker of liver dysfunction; however…
  - GGT is fundamental to glutathione metabolism and indicator of pro-oxidant activity (decreased antioxidant defenses) resulting in cell, tissue and DNA damage via oxidative and nitrosative stress-dysfunction of somatic maintenance
- **Growing evidence suggesting GGT is a predictive biomarker for the risk or onset chronic diseases aside from liver disease; CVD, T2D, MetS, hypertension, cancer and all-cause mortality**

Zinc Rationale

- Manifestations of nutritional deficiency of Zn (first identified in 1969) are fairly prevalent throughout the developing world
- **Zn deficiency is associated with increased oxidative stress and correlated to elevated levels of hs-CRP**
- Manifestations of severe Zn deficiency
  - alopecia, diarrhea, weight loss, intercurrent infections
  - Male hypogonadism, neurosensory disorders and delayed wound healing
- Manifestations of moderate Zn deficiency
  - growth retardation, male hypogonadism, poor appetite, mental lethargy,
  - delayed wound healing, cell-mediated immune dysfunctions, abnormal neurosensory changes
- Manifestations of mild Zn deficiency remain difficult
  - Immune dysfunction
  - Atherosclerosis
  - Diabetes
  - Dementia
- Manifestations of conditioned Zn deficiency
  - Gastrointestinal, Liver and Renal disorders

Prasad AS. Adv. Nutr. 4: 176–190, 2013

Nicklaus Children's Hospital

MIAMI CHILDREN'S HEALTH SYSTEM

Healthy Lifestyle Program Data

Healthy Lifestyles Program Data

- The population consisted of 4,190 participants in a Healthy Lifestyles program
- Program, managed by Interactive Health* routinely includes an assessment involving a range of laboratory analytes, clinical measures and prescribed interventions based on an algorithm used by the company called Active Engine
- In this study, 50.4% of the participants were ≥ 40 years; 34% male, average age 44 years and 66% female; average age 41 years
- Based on the usual evaluation, approximately 58% of individuals screened were referred to a physician because of laboratory findings
- With the addition of the TOP™ panel, this number increased to about 77%

Interactive HEALTH

*

TOP™ Panel Findings (Fasting Levels)

TOP™ Panel		
Biomarker	**Decision Point**	**% individuals exceeding decision point**
Vitamin B12*	<200 pg/ml (deficiency)	1% (42)
Folate (Vitamin B9)*	<2.0 ng/ml (deficiency)	0
Vitamin D	**<30 nmol/L (deficiency)**	**65.6% (2,750)**
Iron*	<15 mcg/dL (deficiency) >300 mcg/dL (overload)	0.21% (9) 0.05% (2)
Ferritin*	**<12 ng/ml**	**5.5% (230)**
Non-HDL-c*	≥ 190 mg/dL	6.3% (265)
HDL-c*	<40 mg/dL	11.5% (481)
Triglycerides*	>200 mg/dL	8.8% (368)
HbA1c*	**5.7-6.4% (prediabetes) or >6.4% (diabetes)**	**40.8% (1,710)** **7.0% (294)**
Homocysteine	>13 mcmol/L	9.0% (379)
hs-CRP*	**≥ 3.0 mg/dL**	**30.4% (1,274)**
GGT*	>40 U/L	11.3% (472)
TSH*	>5.1 mIU/L ≤0.01 mIU/L	4.3% (182) 0.02% (1)
Zinc*	<60 mcg/dL	0
Prealbumin (transthyretin)	<20 mg/dL	N/A

Percentage of individuals with ≥ 1 abnormality (indicated by *) - 71% (2,975)

Percentage of individuals with ≥2 abnormalities of any type - 35.5% (1,488)

Sobering Statistics:
The Cost of Obesity

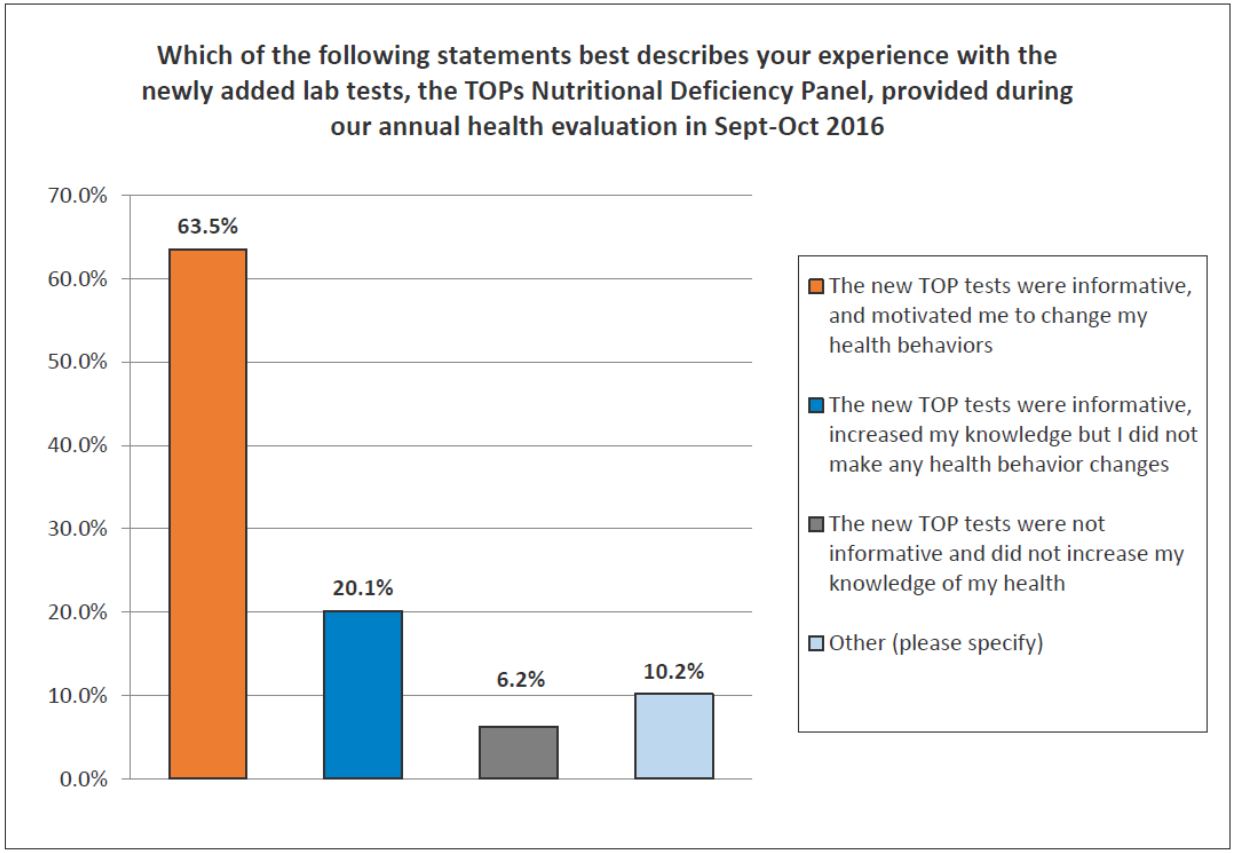
- 2012 estimated costs attributable to obesity (including T2D) in the US-$147B (9.1% of all US health expenditures)
- 2030 projected costs attributable to obesity-$1T (18% of all US health expenditures)*
- AMA estimates 38% of Americans are living with pre-diabetes
- TOP™ panel finding on HbA1c alone
  - % Pre-Diabetes: A1c: 39.0% identified
  - **With fasting blood glucose only 10% identified**
- The value of stopping pre-diabetes from progressing to diabetes is conservatively estimated at $1,095 per individual** (>$100B)

* Not included are the impacts of lost productivity or other societal costs

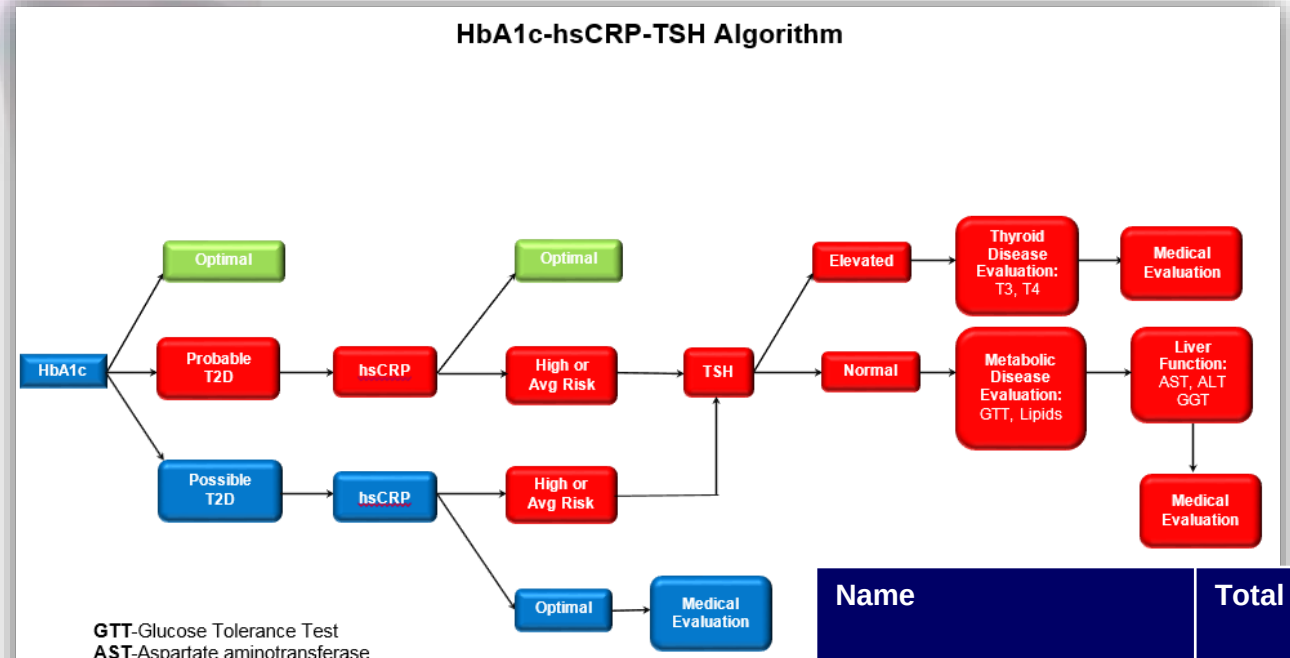
** Interactive Health analysis

Participant Responses

TOPs Survey Results



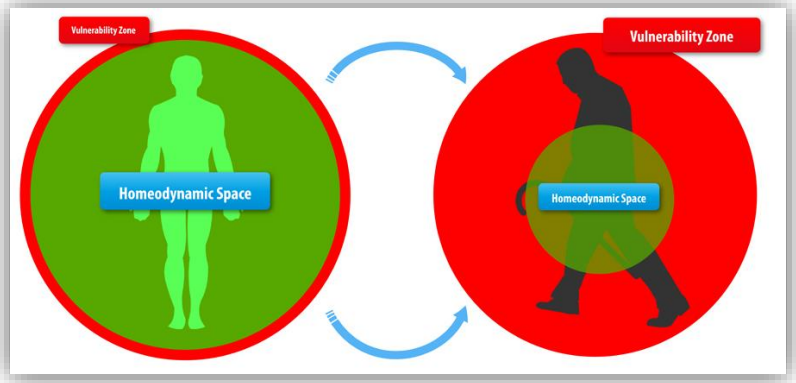
TOP™ Qualifier Algorithms



Name	Total	% of population
Optimal HbA1c	2181	52.2%
Probable T2D and optimal hs-CRP	20	0.50%
Possible T2D and optimal hs-CRP	566	13.5%
Thyroid Evaluation	84	2.0%
Metabolic Disease Evaluation	1061	25.3%

Trends-Linkages?

- Increasing number of abnormality factors are associated with:
  - ↑HbA1c
  - ↑hs-CRP
  - ↑GGT
  - ↑non-HDL-c
  - ↓HDL-c
  - ↑Triglycerides
  - Homocysteine and Vitamin D not analyzed
- There at least appears to be a correlation if not linkage possible related to:
  - Compromised somatic maintenance; chronic oxidative stress and inflammation
  - Micronutrient insufficiency (nutritional landscape)
  - Suboptimal energy metabolism
- In other words…



TOP™ OVERVIEW

A PROGRAMMATIC APPROACH TO WELLNESS

MIAMI CHILDREN'S HEALTH SYSTEM

TOP™ Test. Optimize. Perform.