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# 3 Predictive Biomarkers in Personalized Laboratory Diagnoses and Best Practices Outcome Monitoring for Musculoskeletal Health

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## INTRODUCTION

Musculoskeletal disorders are not just conditions affecting bones and joints, but have a systemic impact on muscles, nerves, adjoining ligaments, tendons, and blood vessels. Pain and inflammation due to repair deficits are strong co-factors in these conditions. In fact in 2012, according to CDC statistics, more than 50% of American adults had musculoskeletal pain disorders [1].

### DEFINING THE TERM “BIOMARKER”

*National Institutes of Health:* “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic intervention.” [2]

*U.S. Food and Drug Administration:* “Any measurable diagnostic indicator that is used to assess the risk or presence of disease.” [3]

*Health Studies Collegium Working Group:* “A measurable functional indicator that accesses individual validated risk or change in risk over time.”

Chronic health issues, including those pertaining to the musculoskeletal system, precipitate from a myriad of origins. They share three common underlying causes:

- Cumulative repair deficits in essential nutrients and neurohormonal distress
- Oxidative damage due to antioxidant and buffering mineral unmet needs
- Metabolic acidosis due to cell mineral deficits that reduce cell energetics

This chapter presents eight biomarkers for these three causes of musculoskeletal dysfunction.

### BACKGROUND ON PREDICTIVE BIOMARKERS

The chapter’s predictive biomarkers (PBs) were developed on the premise that epigenetics influence 92% and genetics influence the remaining 8% of health [4,5]. Each PB covers an aspect of epigenetics and is an all-cause mortality predictor adding ten or more years of survival regardless of geographic, ethnic, and socioeconomic factors. Collectively the assays cover the 92% of conditions and diseases that are due to a lifetime of habits and lifestyle choices.

These PBs are different from most laboratory tests physicians would generally order. They are an interdependent suite of tests referenced to best outcome goal values rather than usual or normal statistical ranges. “Least risk, most gain” goal values for each test can be directly translated into quality years of life conserved. The chapter’s selected predictive biomarkers add 10 or more years of quality life to best outcome goal values or ranges.

The technologic advance for the treatment of musculoskeletal conditions is the interrelation of the assays. They are the first synergistic combination of tests that can result in a comprehensive, personalized lifestyle action plan based on quantitative risk reduction. This proactive approach is evidence-based and has been shown to lower costs while enhancing individual outcomes when compared to current best standards of care, reducing risks, and adding “years to life and life to years.”

These predictive biomarkers intended to better health and better care have also been demonstrated to lower projected medical costs through prevention and early treatment. Musculoskeletal diseases cost \$796.3 billion dollars in 2011, 5.7% of the annual U.S. gross domestic product (GDP) [6]. Table 3.1 illustrates mortality measured both by final diagnosis and by underlying fundamental cause [7].

## SELECTION CRITERIA FOR PREDICTIVE BIOMARKERS

Standards are rigorous for inclusion as a PB. Each PB is an all-cause morbidity and mortality indicator. Each is interdependent with the other predictive biomarkers in assessing or measuring aspects of epigenetics. All PBs are applicable to improving outcomes at lower costs based on a robust published literature of molecular and clinical outcome studies. This is a shift from comparing values

**TABLE 3.1**

### Mortality Measured both by Final Diagnosis and Underlying Fundamental Cause

Cause of Death (Final Diagnosis)	Annual Deaths	Underlying Fundamental Causes			
		Diabetes	Mood Disorder	Malnutrition	Environment or Other
Heart Disease	620,000	320,000	60,000	80,000	160,000
Cancer	600,000	15,000	150,000	100,000	435,000
Respiratory	150,000	10,000	30,000	20,000	90,000
Stroke	140,000	75,000		10,000	55,000
Accidents <sup>a</sup>	120,000	10,000	40,000	10,000	60,000
Alzheimer’s	85,000	10,000	30,000	5,000	25,000
Diabetes	70,000	70,000			
Kidney	50,000	10,000	20,000	5,000	15,000
Pneumonia	50,000	10,000	20,000	5,000	15,000
Suicide	40,000			15,000	25,000
Top 10 Causes	1,925,000	500,000	250,000	250,000	865,000
Other	675,000	Lives savable	Lives savable	Lives savable	unknown
Total Deaths	2,600,000				

*Source:* CDC Centers for Disease Control and Prevention, *National Center for Health Statistics*, “Table 18: Years of potential life lost before age 75 for selected causes of death, by sex, race, and Hispanic origin: United States selected years 1980–2014,” <https://www.cdc.gov/nchs/hsus/contents2015.htm#018>. [adapted] [7].

*Notes:* *National Center for Health Statistics data re-examined by fundamental cause by Health Studies Collegium Task Force on Sustainable Health.*; Legend: Predictive, proactive, personalized primary prevention saves lives at low cost compared to current best standards of disease care.

<sup>a</sup> Accidents (includes homicide)

to a statistical range to using best outcome values to assess individual risk. This shift turns concern into clinical opportunity to reduce risks and improve outcomes by meeting metabolic needs and enhancing restorative habits.

Every test has a standard deviation or range within which the value exists. Typical variance for most classic ELISA-based tests is 20% or more. This means that a value of 6 from a given specimen will cluster values around 6, with a large range of values from 4.8 to 7.2.

For predictive biomarker tests, a variance of 5% or less is desirable. The less the variance, the more predictive is the observed value. By example, if the “true” value is 6, and the test technology allows for better precision with a resulting 3% variance, a single test value *actually* exists between a narrow range of 5.92 and 6.18. In contrast, a 15+% variance offers limited individual applicability of results.

### **USUAL (STATISTICAL, “NORMAL”) TEST RESULTS VS. PREDICTIVE (BEST OUTCOME, ANTICIPATORY) GOAL VALUE RESULTS**

Conventional clinical lab tests provide information about “usual” or “normal” statistical ranges of a particular item analyzed. They are useful for population studies, yet are not individually predictive [8]. By contrast, specific PB tests provide information that allows the patient to implement individual habit changes based on the PB tests results that can bring about health improvements and changes in lab tests within just a few months. This approach builds upon the concept of “optimum” or “high-level health” reference ranges and the biochemical individuality concept pioneered by Roger Williams, Emanuel Cheraskin and colleagues [9], in which therapy, practice, and health standards must be tailored to individual’s metabolic requirements [10].

The goal values recommended here for each predictive biomarker are designed to improve precision in practice and are defined as the least risk or highest gain value or range for each PB. When predictive biomarker tests are at their goal value, all-cause morbidity and mortality are at their best outcome value; quality of life and lifespan are optimized; and net costs of care are reduced. Table 3.2 gives an overview of the selected predictive biomarkers with their clinical significance.

These biomarkers do not depend on age providing an advantage. *Usual*, or statistically *normal* ranges, for biomarkers are based on age and gender-calculated statistical ranges where higher proportions of unwell people are represented. This means that because there are more deficient and distressed people, and thus more unhealthy people in the population as it ages, statistical ranges are unhelpful in individual cases.

Age-conditional *usual* lab ranges drift toward the less well with advancing longevity. Age, however, is a contingent variable. The significant variable is how many unhealthy people are present at each age. Chronology is fixed, yet most of function is choice, based on habits of daily living that can be relearned particularly when appropriate incentives are applied.

### **PREDICTIVE BIOMARKER 1: GLYCOSYLATED HEMOGLOBIN (HEMOGLOBIN A1C)**

There is a known direct relationship with increased glycemia and increased musculoskeletal issues complicating metabolic syndrome and diabetes [12]. Similarly, high blood sugar levels are associated with increased pain [13]. Elevated blood glucose levels mean progressively increasing levels of advanced glycation end products (AGEs). AGEs result from an antioxidant deficit in granulocytes and oxidative free radical damage that harms the delicate endothelial lining of blood vessels. AGEs indicate cumulative repair deficits in turn due to immune defense and repair overload. The most common sources of increased immune defense work are digestive remnants, aero-allergens, and environmental chemicals [14].

Fasting and two-hour post-prandial glucose levels have long been measured to get information about levels at specific times of the day. Insulin and glucose/insulin ratios were developed more recently (HOMA, glycemic index).

**TABLE 3.2**  
**Predictive Biomarkers and Significance for Musculoskeletal Dysfunction**

Predictive Biomarker Test (PB)	Best Outcome Goal Value	Measures	Significance
<b>Hgb A1c, HbA1c</b> (Hemoglobin A1c)	< 5%	Blood sugar, diabetic risk, and insulin resistance	Highly predictive of certain aspects of physiology having to do with sugar metabolism and insulin functions, which in turn are linked to cell energy status, metabolism, weight and related chronic health conditions such as metabolic syndrome, diabetes, cardiovascular disease, and bone- and collagen-related conditions
<b>hsCRP</b> (high sensitivity C-reactive protein)	< 0.5 mg/L	Repair and inflammation status	Highly predictive of repair deficits, connected with pain in several musculoskeletal conditions
<b>Homocysteine</b> (high sensitivity homocysteine)	< 6 $\mu$ mol/L	Methylation, detoxification, cardiovascular risk	Helps to measure adequate methylation, sulfur metabolism, detoxification, and epigenetic modulation
<b>hs LRA by ELISA/ACT</b>	No delayed allergies	Immune tolerance to foods and chemicals	<i>LRA</i> measures immune defense and repair tolerance and intolerance across all delayed allergy pathways.
<b>First AM Urine pH</b>	6.5–7.5	Mineral need assessment and cellular acid/alkaline balance	Measurement after six hours rest reflects net acid excess and metabolic acidosis. Consequences of metabolic acidosis include hormonal changes, insulin resistance, loss of bone and muscle protein degradation
<b>Vitamin D</b> (25-OH cholecalciferol)	50–80 ng/mL	Cellular equilibrium and communication	Vitamin D pleiotropic functions dictate cellular function, physiology and proliferation—crucial for immune health, bone metabolism, neurological and cognitive function
<b>Omega-3 Index</b>	> 8%	Omega-3 level of oxidative stress	Reflects the relative amount of omega -3 fatty acids within red blood cell membranes and expressed as percentage of total fatty acids—useful in predicting oxidative stress
<b>DNA Oxidative Stress</b> (8-OHdG)	<5 ng/mg creatinine	Oxidative stress and nuclear antioxidant status	DNA oxidative stress marker that can be a predictor of repair deficit and risk of conditions like diabetes and atherosclerosis

Source: R Jaffe and J Mani, *Polyphenols in Human Health and Disease*, Academic Press, 2014 [11].

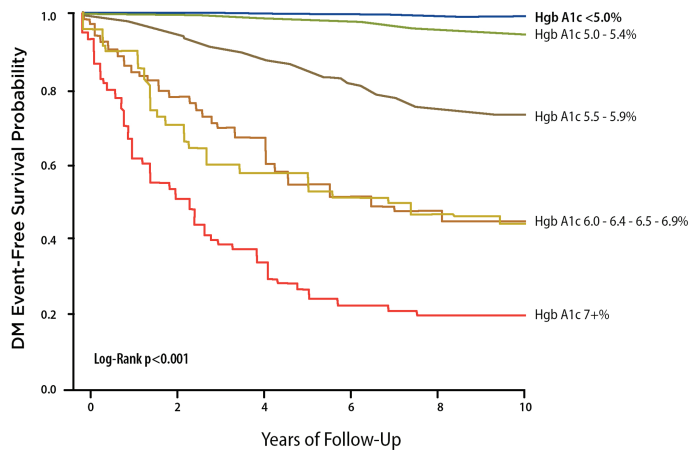
Hgb A1c is the current reference standard analyte to determine average “sugar” related risks. Of all measures of excess average sugar in the body, hemoglobin A1c is the best studied and the most validated and widely available.

Day-to-day blood sugar levels are influenced by exercise, meal timings, mood, and/or medications. In contrast, Hgb A1c provides the average blood sugar over the last three months and prevails over these variables, uncovering pre-diabetic states accurately and giving a more reliable indication of current and future sugar related risk [15, 16]. Hgb A1c/PB1 measures are also strongly linked to inflammation (hsCRP/PB2), immune tolerance (LRA/PB4), and oxidative stress (8-OHdG/PB8). Hgb A1C is a PB that anticipates chronic, degenerative, inflammatory, and autoimmune disease risks.

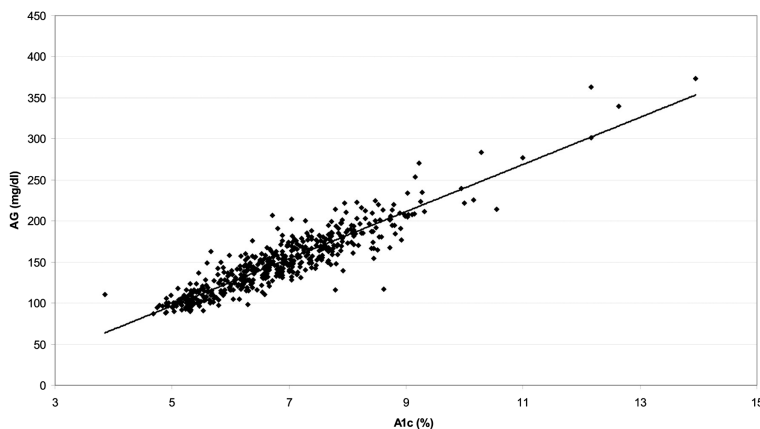
Insulin has been known to contribute to the bone remodeling process for a number of years [17]. But when insulin is present in excessive amounts (as in insulin resistance and type 2 diabetes), bone resorption and circulating levels of osteocalcin both decrease within hours of an insulin surge, according to a recent study [18]. Collagen renewal is reduced when average blood sugar is increased, resulting in weaker bones and higher fracture incidences.

The following graphs (Figures 3.1 and 3.2) represent the correlation between Hgb A1c levels, blood glucose levels, and 10+ year survival probability.

When Hgb A1c levels and blood glucose levels are above goal value, an immunotolerant diet of whole foods enriched with nutrient-dense super foods is appropriate. In addition, advanced supplements with enhanced uptake and chaperoned delivery of essential nutrients are shown to improve stability of energy, reduce average blood glucose, and improve insulin sensitivity. Being active physically and mentally is an integral part of this approach to evoke healing responses.



**FIGURE 3.1** Hgb A1c levels and extent of 10-year survival probability. (From Peiyao Cheng, et al., *Diabetes Care*, 34, 610–15, 2011 [19].) Source: \*DM – Diabetes Mellitus. Diabetes is linked to accelerated loss of bone, joint, and lean muscle. This graph highlights the link between the HgbA1c level and 10+ year all cause morbidity and mortality risk.



**FIGURE 3.2** Correlation of Hgb A1c levels with average blood glucose. (From David M. Nathan, et al., *Diabetes Care*, 31, 1473–8, 2008 [15].) Source: Each dot represents one individual. The linear regression analysis represented in this graph confirms that HgbA1c correlates tightly with average glucose (AG).

For example, since researchers have found that constant sitting has as much disease risk as smoking, an exercise routine is critical. Walking for an aggregate of 45+ minutes per day or being physically active for five minutes for each hour at a desk can dramatically reduce risk as documented by improved Hgb A1c. Practicing abdominal breathing and relaxation response mindfulness practice also favorably affects this and other PB measures.

A Hgb A1c of <5% is the desired or goal value and reflects a 99% probability of living ten+ years and also reflects efficient use of sugar to regulate cell growth and energy.

## PREDICTIVE BIOMARKER 2: HIGH SENSITIVITY C-REACTIVE PROTEIN (hsCRP)

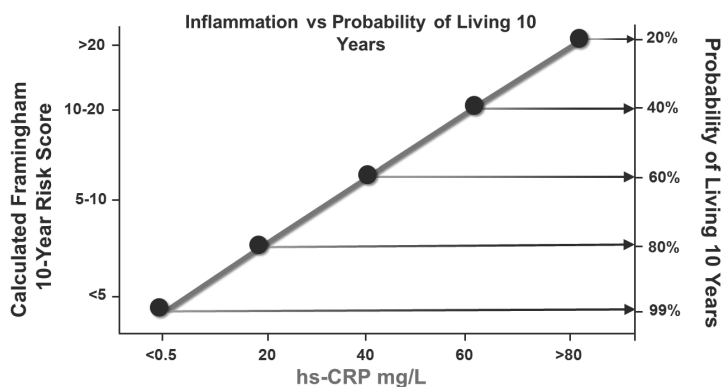
A healthy body repairs itself from injury or from wear and tear promptly, efficiently, and effectively. When health is compromised, the body's attempt to heal or repair itself falls short, resulting in a repair deficit, otherwise known as inflammation. The process of defense and repair is a complex one involving many cells and enzymes. Inducible proteins for example, are a family of mostly liver-derived glycoproteins that, in aggregate, are the body's cry for help to induce cells and systems to effect repair and heal.

When repair deficits persist, inflammation puts a metabolic burden on the body's organ systems, especially the immune system. Unmet repair needs and too much defense work slowly wear down immune functions, taking a toll on daily quality of life, increasing risk for disease, and reducing survival [20].

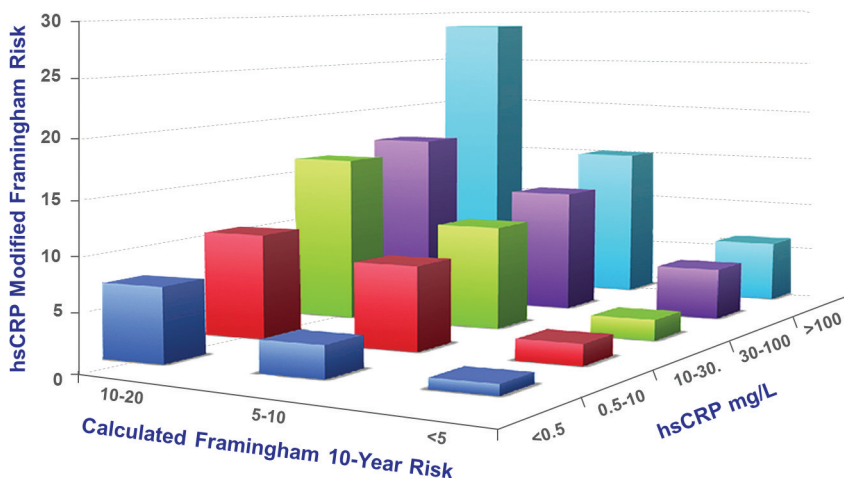
The musculoskeletal system is especially vulnerable to inflammation. Inflammation is connected to increased oxidative stress, which produces Reactive Oxygen Species (ROS). ROS plays a crucial role in cartilage homeostasis [21] and has been implicated in bone loss even while having a role in bone remodeling [22].

C-reactive protein (CRP) is a measure of inflammation—the liver produces more CRP when inflamed. High sensitivity C-reactive protein (hsCRP), however, is more precise and predictive than regular CRP [23] and is known as a predictive marker of inflammation systemically, particularly in connection with the cardiovascular risks.

Figures 3.3 and 3.4 illustrate the correlation between hsCRP, Framingham 10-year CVD risk scores, and 10-year survival probability.



**FIGURE 3.3** High sensitivity C-reactive protein (hsCRP) measurements and Framingham risk score. (From Paul Ridker, et al., *Arch Intern Med*, 166, 1327–8 [24].) Source: Inflammation is repair deficit. The hsCRP test correlates best with degree of repair deficit/inflammation and has been validated as an all cause morbidity/mortality parameter. Repair of the musculoskeletal system depends upon essential nutrients to prevent hsCRP from being induced by repair deficit. In this linear regression analysis, it is evident that the probability of living longer is indirectly proportional to the calculated cardiovascular Framingham risk score and increased hsCRP levels.



**FIGURE 3.4** 3-D presentation of Framingham data demonstrates the combined effect of high sensitivity C-reactive protein (hsCRP) measurements and 10-year survival. (From PM Ridker, PWF Wilson, and SM Grundy, *Circulation*, 109, 2818–925, 2004 [25].)

HsCRP correlates with clinical activity of other inflammatory, autoimmune diseases, from rheumatoid to juvenile arthritis, lupus (SLE), diabetes, vasculitis, psoriasis, eczema, asthma, and multiple sclerosis. Levels of hsCRP rise in proportion to the need for repair. Persisting elevations of hsCRP indicate overload in the innate immune system, fatigue in phagocytic functions, and increased host hospitality to chronic infection or autoimmune self-attack.

Higher levels of hsCRP are associated with increased fracture risk. The SWAN (Study of Women's Health Across the Nation) study [26] showed that bone strength significantly declines with increased inflammation. Higher hsCRP measurements also indicate disease progression in osteoarthritis, reflecting synovial inflammation in such patients, perhaps by means of increased synovial IL-6 production [27].

#### GOAL VALUE FOR HsCRP OF <0.5 MG/L

An hsCRP of less than 0.5 mg/L indicates the individual has tamed inflammation risk. Recommended nutrients to accomplish this goal include antioxidants like fully buffered l-ascorbate, polyphenolics (particularly quercetin dehydrate and soluble OPC), magnesium and choline citrate and ubiquinone (CoEnzyme Q10) that can dramatically alter the state of inflammation and improve hsCRP levels by potentiating much-needed cellular repair.

### PREDICTIVE BIOMARKER 3: HOMOCYSTEINE (HCY)

A healthy body requires the correct ratio of methionine to homocysteine since HCY is an important predictor of long-term survival for all-cause morbidity and mortality. The usual lab values for HCY range from 5–15  $\mu\text{mol/L}$ , and most practitioners take action when values are above 15  $\mu\text{mol/L}$ . To predict better lifetime health, the goal value for HCY is to be less than 6  $\mu\text{mol/L}$ . At this homocysteine level, the methionine to homocysteine ratio favors healthy methylation, robust, self-renewing vascular and tissue infrastructure, and sulfur-based detoxification.

Methylation controls many aspects of cell function, including DNA and RNA expression of genes and helps to transport or deposit proteins as needed. When methylation is impaired, e.g., when methylation regulators like C complex, magnesium, and trimethylglycine (TMG) are deficient,

homocysteine levels increase, and methionine decreases, reflecting defects in sulfur metabolism, detoxification, and methyl group transfer.

Elevated homocysteine levels result in oxidative stress that is linked to increased risk of heart attacks, strokes, senility, systemic inflammation, osteoporosis, and autoimmune diseases [28]. For example, in osteoporosis and osteoarthritis (two epigenetically modulated conditions) it is important to note how impaired methylation of the DNA of bone cells and cartilage is part of the disease process [29].

In osteoporosis, elevated homocysteine levels alter the structure of collagen cross-linking, thus affecting stability and mineralization occurring in bone tissue [30]. Similarly, elevated homocysteine levels have been shown to cause corresponding decreases in bone mineral density [31].

As a measure of all-cause morbidity and mortality, homocysteine is definitely an important predictor of long-term survival. Healthier people have adequate dietary sulfur sources to produce higher MET and lower HCY as indicated in Figures 3.5 and 3.6.

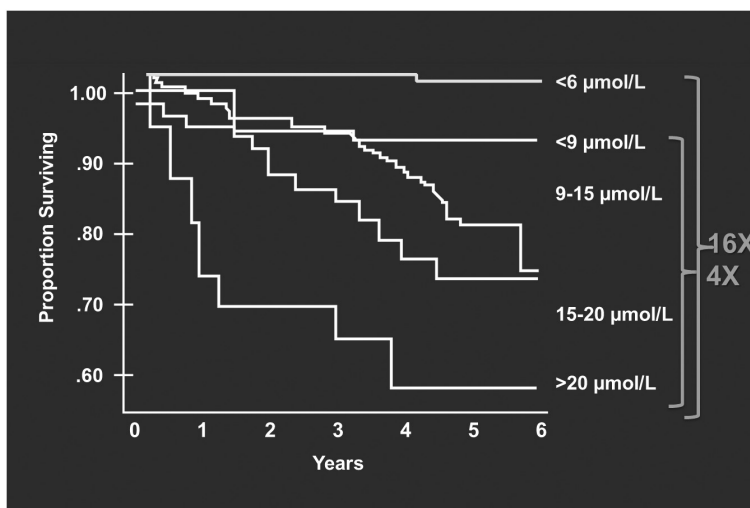
If homocysteine levels are above the goal value, our recommendation includes an immunotolerant whole foods diet, with an emphasis on sulfur rich super foods such as ginger, garlic, onions, brassica sprouts, and eggs. It is important to include sufficient intake of buffered ascorbate, vitamin B12 (hydroxocobalamin) natural folate, vitamin B6 (natural forms preferred), magnesium, and trimethylglycine (TMG; betaine HCl) [34].

In addition, any system that brings together movement and conscious breathing is recommended to evoke healing responses, including meditation, Hatha Yoga, Qigong, or even walking. Alternating practices that involve gentle stretching exercises and cardio or weight-bearing activities are also highly beneficial, since muscles and bones equally benefit from exercise to evoke healing responses.

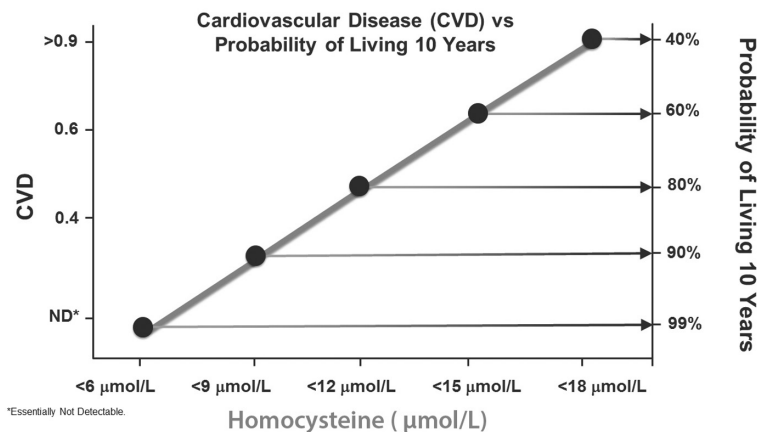
The good news is that as homocysteine levels come back into a more normal range and methylation functions normally, risk for serious health conditions can be reduced in a few short months.

### GOAL VALUES FOR HCY

The goal value of less than 6  $\mu\text{mol/L}$  for HCY predicts better lifetime health. While the usual lab values range for HCY is 5–15  $\mu\text{mol/L}$ , most practitioners take action when values are above 15  $\mu\text{mol/L}$ . When the patient achieves an HCY under 6  $\mu\text{mol/L}$ , the methionine to homocysteine



**FIGURE 3.5** Homocysteine <6  $\mu\text{mol/L}$  is Predictive Biomarker. (From O Nygård, et al., *NEJM*, 337, 230–6, 1997 [32].) Source: This is a survival plot and shows the percentage of survival (proportion surviving) starting from 100 and declining as homocysteine levels rise.



**FIGURE 3.6** Homocysteine level, cardiovascular disease, and 10-year survival probability. (From AG Bostom, et al., *Arch Intern Med*, 159, 1077–80, 1999 [33].) Source: This regression analysis illustrates the probability of living 10+ years, increasing with decreasing homocysteine levels and lower incidence of cardiovascular disease, confirming the value of homocysteine as an important all cause morbidity/mortality parameter.

ratio favors methionine to support healthy methylation, robust, self-renewing vascular and tissue infrastructure, and sulfur-based detoxification.

#### PREDICTIVE BIOMARKER 4:

##### IMMUNE TOLERANCE CELL CULTURES LYMPHOCYTE RESPONSE ASSAY (LRA)

The lymphocyte response assay (LRA) determines individual reactive foods and/or chemicals that can impair immune system functions. A healthy immune system is tolerant. This means that the innate immune first-line cells are able to handle defense, repair, and deletion of abnormal cells. When the immune system attacks rather than defends *and* repairs, a condition called inflammation and/or autoimmunity (AI) is present.

AI conditions are widely prevalent. Autoimmunity means loss of immune tolerance and self-restoring homeostasis. Examples range from diabetes to thyroiditis and arthritis, to migraine headaches, eczema, and psoriasis. Concurrent presence of multiple AI syndromes is common [35], and while it may not seem immediately apparent, the immune system is closely involved in so many conditions associated with the musculoskeletal system, especially rheumatic conditions such as rheumatoid arthritis, multiple sclerosis, polymyalgia rheumatica, temporal arteritis, and systemic lupus [36].

Highlighting the importance of the immune system is a recent study by stem cell scientists from Harvard University who have shown that regulatory T cells (Tregs) that temper immune responses increase in muscular dystrophy cases and actually could be utilized to heal wounds and improve disease outcome [37].

When routine wear-and-tear is *not* repaired, the integrity of the extracellular connective tissue scaffolding is impaired [38]. Increase in tissue permeability ensues, setting the stage for AI. This can be provoked by a variety of external or internal antigens perceived as foreign that preoccupy the immune system with excess defense burden [39]. Initially, this increase in tissue permeability results in the entry of larger plasma proteins and platelets, dendritic cells, and lymphocytes all seeking to induce repair, i.e., to “put things right” [40]. Fibromyalgia is a classic example. Leaky gut syndrome is another common clinical term for incomplete repair. Too often, the lack of essential nutrients and/or excess defense burdens on the immune system prevent repair of cells from being completed [41].

Causes of repair deficit, also known as inflammation, can be summarized as:

1. Chronic deferral of necessary routine repair due to distress (neurohormone imbalance) [42–44], toxin excess (impaired detoxification), or lack of essential nutrients needed for the immune defense and repair systems to function;
2. Depletion of buffering reserve (particularly magnesium) resulting in intracellular acidosis, loss of the proton gradient, and mitochondrial shutdown [45];
3. Immunologic overload from repeated digestive, respiratory, and/or auto-antigen exposure resulting in loss of tolerance and development of B and T cell delayed allergy responses;
4. Adequate activity patterns to move fluids and help maintain a healthy digestive transit time (since long-term sitting can lead to as much disease as smoking); and
5. Lack of learned stress resilience based on relaxation response practices.

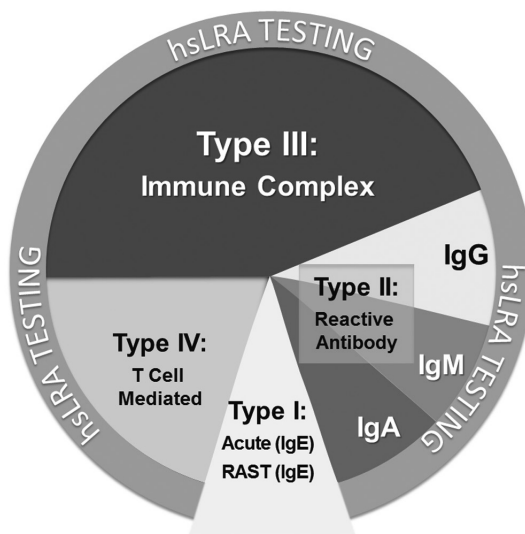
The LRA measures all three delayed allergy pathways *ex vivo* while avoiding false positives that are common in other types of delayed allergy tests according to the Gel and Coombs organization of immune responses:

- Type II: Reactive antibody (IgA, IgM, and IgG) meaning the distinction between protective, neutralizing, and harmful symptom-provoking antibodies
- Type III: Immune complexes: IgM anti-IgG-antigen complex
- Type IV: Direct T-cell activation: no antibodies, yet direct activation

Figure 3.7 illustrates the immune response mechanism.

Identifying the patient's specific sensitivities and delayed allergies that burden the immune system can result in a clinical breakthrough. Patients often experience sustained remission when using an alkalinizing, immune tolerant diet and targeted supplementation, as well as changes in lifestyle habits and attitude.

Tolerance can be restored as part of a proactive prevention lifestyle. If reactions are found by LRA tests, substitution of reactive foods can be initiated. Eating whole foods that can be digested,



**FIGURE 3.7** Wheel of immune response mechanism. (From PG Gel, et al., *Clinical Aspects of Immunology*, Hoboken, NJ: Wiley-Blackwell, 1975 [46].) Source: hsLRA- high sensitivity Lymphocyte Response Assay; Ig- Immunoglobulin of E, G, A and M types.

assimilated, and eliminated without immune burden is recommended. The plan includes high-nutrient-dense, easily digested super foods and targeted supplementation to promote repair and rebalance mental as well as physical reserves, while evoking healing responses.

LRA by ELISA/ACT cell cultures are reproducible [47] with a variance of less than 3% for over 30 years on consecutive blind split samples such as the consecutive data presented at the American Society for Investigative Pathology (ASIP) conference in 2016 (Table 3.3).

Common conventional practice today includes therapies assessing immune intolerance based only on antibodies. The assumption is that *any* antibody is harmful, and some combination of immune suppressive therapies results. This approach lacks solid research support and clinical evidence.

### LRA GOAL VALUE

The *healthy goal value is immune tolerance, with no LRA reactions*, which confirms innate and adaptive immune tolerance and functional resilience in the immune defense and repair system. Using LRA tests and developing an associated immune enhancement program can help restore immune competence, tolerance, and resilience.

**TABLE 3.3**  
**Consecutive Blind Split LRA Samples Showing High Reproducibility**

# Items Tested	# Items Matched	# Items Unmatched	% Items Matched	% Items Unmatched	Time (Years)
4138	4050	88	97.60 ± 3.00	2.25 ± 2.75	2011–14
Split variance					

*Source:* AE Lynch and R Jaffe, Lymphocyte response assay: Report on precision of novel cell culture test, experimental biology, *Poster presented at the American Society for Investigative Pathology Conference*, San Diego, CA, 2016 [47].

Legend: Test precision is measured best by consecutive blind split samples analyzed over at least two years as shown in this table. By contrast other cell culture methods that assess lymphocytic white blood cell function report 15%–30% variance. LRA by this method is a documented advance in precision, sensitivity, specificity and predictive significance.

### PREDICTIVE BIOMARKER 5: URINE PH AFTER 6+ HOURS REST

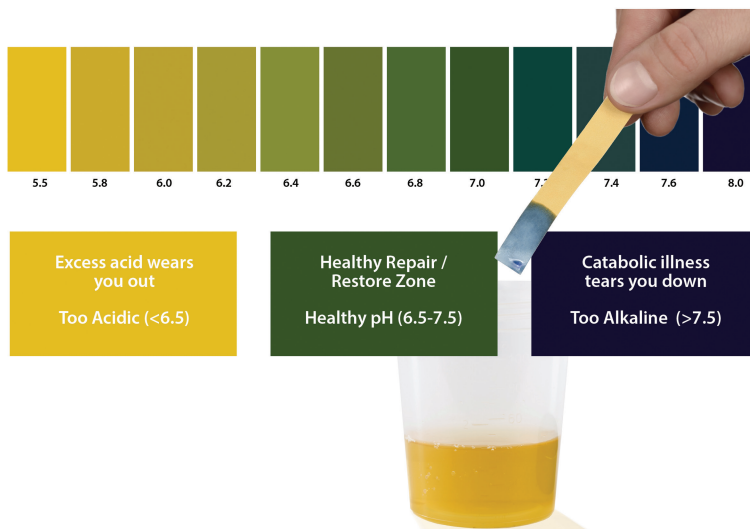
Diet-induced cell acidosis has been shown to create changes in the body that have fundamental effects on the degree of oxidative stress, free radical activity, cell energetics (ATP/ADP ratio), and osteoclastic activity in bone, and on possible tumor progression [48].

The pH of urine after six hours of rest reflects equilibration with kidney and bladder cells. Levels below 6.5 indicate metabolic acidosis inside cells, as is indicated in Figure 3.8. Low pH suggests mineral deficits. Minerals tend to get pulled from bone and body fluids to neutralize metabolic acids that can form faster than the intake of buffering minerals and alkalinizing nutrients. [49]

Tiny changes in cell pH have deep implications for metabolism. Life exists poised exquisitely just above the pH neutral point of 7.0. Levels of urine pH above 7.5 can indicate presence of catabolic illness in which amino acids are used as energy sources. When net acid excess is expressed in urine, changes in diet, supplements, activity, attitude (mental and emotional health) are needed to restore acid-alkaline balance.

Figure 3.9 outlines foods based on their acidifying and alkalinizing capabilities.

Checking the pH level each day provides ongoing monitoring to see whether acid-alkaline balance is present.



**FIGURE 3.8** First morning urine pH levels and health status. (From R Jaffe and S Brown, *Intl J Integrative Med*, 2, 7–18, 2000 [49].)

### GOAL VALUE FOR URINE pH

The pH value in urine is more accurate when the urine is fresh. Cells and bacteria in urine shift pH over time due to their metabolic products.

The predictive *goal value range for urine pH* is 6.5–7.5 after six or more hours of rest, typically checked first thing in the morning.

### PREDICTIVE BIOMARKER 6: VITAMIN D

It is estimated that anywhere from 30% to 50% of Americans, depending upon their age and community living environments, are deficient in Vitamin D. Vitamin D is a neurohormone. Functionally, this nutrient is important in regulating mineral uptake and regulating cell proliferation.

Vitamin D levels play a significant role in numerous systems in the body, including immune and neurological regulation and bone health. Less than healthy levels increase risks of obesity, cancer, heart disease, inflammatory, autoimmune disorders, and psychiatric and mood disorders [50].

Healthy vitamin D levels:

- Are protective against musculoskeletal disorders (muscle weakness, falls, fractures), multiple sclerosis, rheumatoid arthritis, type 1 and type 2 diabetes, cardiovascular diseases, neurocognitive dysfunction, and others;
- Allow vitamin D to function successfully as a hormone, moderating cell division, providing vital communication links between cells, normalizing cell growth, and avoiding aggressive cell production;
- Improve immune status and protect against autoimmune disorders; and
- Reduce inflammation in the brain and nervous system—this is particularly important since brain repair depends on an energetic, tolerant immune system.

Vitamin D deficiency/insufficiency is associated with all-cause mortality [51].

### GOAL VALUE RANGE FOR VITAMIN D

Knowing the status of vitamin D levels in the body is helpful to determine if supplementation is required. Less than 5% of Americans have adequate vitamin D levels. The preferred test to assess vitamin D levels involves the measurement of 25 hydroxycholecalciferol (25 OH-D). The best outcome *goal value range for 25-OH D is 50–80 ng/ml*.

### PREDICTIVE BIOMARKER 7: OMEGA 3 TEST

Most Americans have low omega-3 levels and excess omega-6 fat intake. People pay a substantial metabolic price for this imbalance in essential oils. Omega-3 fats are an integral part of cell membranes throughout the body and affect the function of the cell receptors in these membranes. They provide the starting point for making hormones that regulate blood clotting, contraction and relaxation of artery walls, and inflammation. By greatly tuning down inflammatory signaling, omega 3 fats send osteoclasts home, preventing excessive bone loss. They also increase the action of osteoblasts and give them enough time to lay down new bone. The attainment of peak bone mass in adolescence, increased bone mineral density and the prevention of age-related osteoporosis are positive effects of omega 3 fatty acids [52].

The omega-3 test is a measure of the active omega-3 fatty acids, eicosapentanoic acid (EPA) + docosahexanoic acid (DHA), in red blood cells. Levels of omega-3 are predictive of coronary heart disease (CHD) risk and are important for brain and joint health [53]. Low levels of omega-3s are also related to increased risk for fatal heart attack, depression, and possibly dementia [54, 55].

While deficiency in omega-3 fats is too common, excess of omega-6 fats is equally prevalent. In essence, omega-3 fats enhance, repair, and soothe the body, while omega-6 fats stimulate and activate the body. We need both in balance. Recent studies, such as NHANES IV [56] suggest typical Americans take in 20 to 100 times more omega-6 than omega-3, mostly due to the high consumption of vegetable oils and practices like frying and high-heat cooking. Cooking with broth and wine and minimizes the use of edible oils. Salads can be enjoyed with a drizzle of vinegar, fresh ground pepper, and sea salt rather than with added oils.


Whole foods like deep sea, cold water fish, nuts and seeds, and greens have balanced omega-3 to omega-6 fats. Whole foods also contain protective antioxidants so that easily damaged fats do not become rancid, a common problem when oils are isolated and separated from their source.

An omega-3 index of <8% indicates an increase in the intake of EPA and DHA through food and supplements is needed. The amount of EPA/DHA needed to raise the omega-3 index to the target range is individual and is based on current levels of active omega-3 fatty acids, EPA + DHA, and how much the individual takes in through their diet.

An index of 4% or less (common in the US) indicates the highest risk [57]. *The goal value for the omega-3 test is 8% and above*, a level associated with the lowest risk of death from coronary heart disease.



### PREDICTIVE BIOMARKER 8: 8-OXOGUANINE OR 8-OHDG (8-HYDROXYGUANINE, 8-OXO-GUA, OR OH<sup>8</sup>GUA)

Oxidative stress describes the injury caused to cells resulting from increased formation of free radicals and/or decreased antioxidant reserve. Oxidative stress can define bone cell behavior [58] and is seen to play a crucial role in the development of conditions like osteopenia [59], diabetes, and cardiovascular disease. Testing for 8-oxoguanine provides important information about oxidative



**MORE ACID**  
(Consume Less)

Food Category	Acidic (4 icons)	Acidic (3 icons)	Acidic (2 icons)	Alkaline (1 icon)
Citrus Fruit Fruit		Cranberry Pomegranate	Plum Prune Tomato	Coconut Fig Guava Persimmon Juice Cherimoya Date Dry Fruit
Bean Vegetable Legume Pulse Root	Soybean Carob	Pea Green Snow  Peanut Legumes (other) Carrot Chick Pea/Garbanzo	Bean Pinto White Navy/Red Aduki Lima or Mung  Chard Split Pea	Bean Fava Kidney Black-eyed String/Wax  Spinach Zucchini Chutney Rhubarb
Grain Cereal Grass	Barley <i>Processed Flour</i>	Corn Rye Oat Bran	Wheat Semolina Spelt, teff Kamut White Rice Buckwheat	Triticale Brown Rice Millet Kasha
Fowl	Pheasant	Chicken	Goose/Turkey	Wild Duck
Meat Game Fish/Shell Fish	Beef Shell Fish (Processed) Lobster	Pork/Veal Mussel/Squid	Lamb/Mutton Game Meat Shell Fish (Whole)	Gelatin/Organs Venison Fish
Egg				Egg, Chicken
Processed Dairy Cow/Human Soy Goat/Sheep	<i>Processed Cheese</i> Ice Cream	Casein Cottage Cheese Milk, Soy	Milk; Goat, Cow, Sheep	Cream/Butter Yogurt Cheese; Goat, Sheep
Oil Seed/Sprout Nut	<i>Cottonseed Oil/Meal</i> <i>Fried Food</i> Hazelnut Walnut Brazil Nut	Oil Chestnut Palm Kernel  Lard Pistachio Seed Pecan	Oil Almond Sesame Safflower  Tapioca Seitan or Tofu	Oil Canola Pumpkin Seed Grape Seed Sunflower  Pine Nut
Beverage Preservative Sweetener Vinegar	<i>Beer</i> <i>"Soda"</i> <i>Table Salt</i> Yeast/Hops/Malt Sugar/Cocoa White/Acetic Vinegar	Coffee Aspartame Saccharin Red Wine Vinegar	<i>Alcohol</i> Black Tea Benzoate Balsamic Vinegar	<i>Kona Coffee</i> MSG Honey/Maple Syrup Rice Vinegar
Spice/Herb	Pudding/Jam/Jelly	Nutmeg	Vanilla Stevia	Curry
Therapeutic	<i>Antibiotics</i>	<i>Psychotropics</i>	<i>Antihistamines</i>	

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**FIGURE 3.9** Food and chemical effects on acid/alkaline body chemical balance.

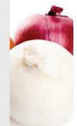


# MORE ALKALINE

(Consume More)



+	++	+++	++++	Food Category
Orange Banana Blueberry Raisin, Grapes Currant Strawberry	Lemon Pear Avocado Apple Blackberry Cherry Peach	Grapefruit Cantaloupe Honeydew Olive Mango Citrus Loganberry	Lime Nectarine Raspberry Watermelon Tangerine Pineapple	Citrus Fruit Fruit
Brussel Sprout Beet Chive/Scallion Celery/Cilantro Squash Artichoke Lettuce Jicama Turnip Greens	Potato/Bell Pepper Mushroom/Fungi Cauliflower Cabbage Eggplant Pumpkin Collard Greens	Kohlrabi Parsnip/Taro Garlic Asparagus Kale/Parsley Endive/Arugula Jerusalem Artichoke Ginger Root Broccoli	Lentil Broccoli Seaweed Noril/Kombu Wakame/Hijiki Onion/Miso Daikon/Taro Root Sea Vegetables Burdock/Lotus Root Sweet Potato/Yam	Bean Vegetable Legume Pulse Root
Quinoa Wild Rice Oat				Grain Cereal Grass
				Fowl
				Meat Game Fish/Shell Fish
Egg, Duck	Egg, Quail			Egg
Ghee Human Breast Milk				Processed Dairy Cow/Human Soy Goat/Sheep
Oil Avocado Coconut Olive/Macadamia Linseed/Flax Seeds (most)	Oil Cod Liver Primrose Sesame Seed Almond Sprout	Poppy Seed Pepper Chestnut Cashew	Pumpkin Seed	Oil Seed/Sprout Nut
Ginger Tea Sulfite Sucanat Umeboshi vinegar	Green or Mu Tea Rice syrup Apple Cider Vinegar	Kombucha Molasses Soy Sauce	Mineral Water Sea Salt	Beverage Preservative Sweetener Vinegar
White Willow Bark Slippery Elm Artemesia Annua	Herbs Aloe Vera Nettle	Spices/Cinnamon Valerian Licorice Agave	Baking Soda	Spice/Herb
Algae, Blue Green	Sake		Umeboshi Plum	Therapeutic



Italicised items are NOT recommended

stress and its effects on DNA and the genetic sequence. The test is well validated as a measure of nuclear and mitochondrial DNA oxidative stress and is well supported in the research literature [60].

This predictive biomarker focuses on the acceleration of age-related decline due to DNA status, particularly telomere length, and is an effective way to evaluate the success of an intervention, whether it involves dietary change or essential nutrients. When antioxidant levels are sufficient, oxidative damage from free radicals does not occur.

Tracking the results of this test provides an indication of:

1. Risks due to oxidative stress in the DNA of both mitochondria and cell nuclei that particularly increase cardiovascular disease; and
2. Benefit or lack of benefit from therapies designed to reduce DNA oxidative distress to reduce future disease risks.

### GOAL VALUE FOR 8-OXOGUANINE

The *goal for 8-oxoguanine we suggest is a value of <5 ng/mg of creatinine*, indicating adequate DNA antioxidant protection and efficient DNA repair from oxidative stresses. To achieve goal values, we recommend antioxidants such as ascorbate, polyphenolics, micellized ubiquinone, mixed natural carotenoids and tocopherols, which stimulate efficient energy production.

## DISCUSSION AND CONCLUSION

When one biomarker is no longer at best outcome goal value, the entire organism is distressed, less resilient, and more at risk. Homeostasis is reduced; impaired functions are likely. This loss of homeostasis is captured by the biomarkers because they are *interdependent*. When all eight are interpreted together, their predictive power increases, together covering the 92% of lifetime health conditions and diseases determined by choice and habit. And though each biomarker is a separate marker of specific aspects of physiology, human systems are interdependent.

The biomarkers are *responsive*. Changes in daily lifestyle habits, diet, and stress levels return markers back to or toward the goal or best outcome value. The biomarkers are *not age dependent*. Healthy people at any age have best outcome values for these predictive biomarker tests; outcome goal values for PB are *not* dependent on age or gender. What is known as aging is more accurately understood as having more unwell people as age advances.

Each biomarker has biologic plausibility and elucidated metabolic pathways. At times for clinical efficiency physicians may wish to select biomarkers to evaluate a suspected disturbance in one particular pathway. Four essential predictive biomarkers that should make the list are HgbA1c, homocysteine, hsCRP and Lymphocyte Response Assay (Table 3.2). These biomarkers provide the practitioner with a window into glucose, inflammation, methylation, and immune status of an individual.

The PBs are most meaningful when pre-analytic variables can be improved. A superior assay is one that provides accurate measurement. For that, it is important to reduce any interfering substances used in the assay, e.g., glass versus plastic. The conditions involved in the analysis are equally crucial and any compromise there can skew the assay accuracy. Last of all, the curve of the assay is critical in making sure that the assay conveys an accurate measurement.

As a result, the predictive significance of any specific value becomes much greater if the analysis is scientifically sound. Predictive biomarkers are timely. An increasing number of healthcare consumers are interested in predictive, proactive, personalized primary prevention. They are careful of information found on health websites and books (and even from their healthcare providers) and are more inclined to verify that tests and procedures are effective and applicable to themselves. Those of us who are evidence-based clinicians have an obligation to integrate advances in informatics,

human behavior, technology, and mind and body techniques into our practices, with a focus on predictive biomarkers.

Lastly, predictive biomarkers can help healthcare practitioners fulfill an inner calling. We can help our patients avert the tragic cycle explained by the 14th Dalai Lama [61].

Sometimes people sacrifice their health to gain wealth only to later sacrifice their wealth to regain their health. You can help those whose health forces them to live in the past or in the future. As health care practitioners, we are uniquely placed to help return individuals to vibrant living in the present moment.

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