# NEXT-GENERATION BIOMARKERS OF HEALTH

TNO innovation for life


## ABSTRACT

Current biomarkers used in health care and in nutrition and health research are based on quantifying disease onset and its progress. Yet, both health care and nutrition should focus on maintaining optimal health, where the related biology is essentially differing from biomedical science. Health is characterized by the ability to continuously adapt in varying circumstances where multiple mechanisms of systems flexibility are involved. A new generation of biomarkers is needed that quantifies all aspects of systems flexibility, opening the door to real lifestyle-related health optimization, self-empowerment, and related products and services.




## INTRODUCTION

Health care does not really focus on maintaining optimal health but rather on curing diseases. A large repertoire of tools, technologies, and treatments has been developed for this purpose, making disease care an enterprise that may soon become too costly. Also, within health care, citizens become patients in the literal sense of the word: patiently undergoing treatments instead of playing an active part in their own health. This needs to change and in theory should be simple as a huge health profit can be achieved if each person would adapt to an optimal lifestyle, including a proper diet, during their lifespan. Reviews suggest that major reductions in obesity, type-2 diabetes (T2D), cardiovascular disease, and cancers could be achieved [1]. Theory and practice differ and we face a multifactorial challenge, spanning economic, social, psychological, and biological aspects. Yet, from a biological viewpoint, a major breakthrough would be achieved if knowledge and technologies would become available that allow to understand and quantify the processes that maintain health. So far, efforts in biomarker development have mostly focused on quantification of disease states or development. This has been relatively easy, as disease biology significantly differs from health biology, and has also been rewarding because the health care economy provided major incentives for such biomarkers (diagnostics). Where diet and nutrition should aim at

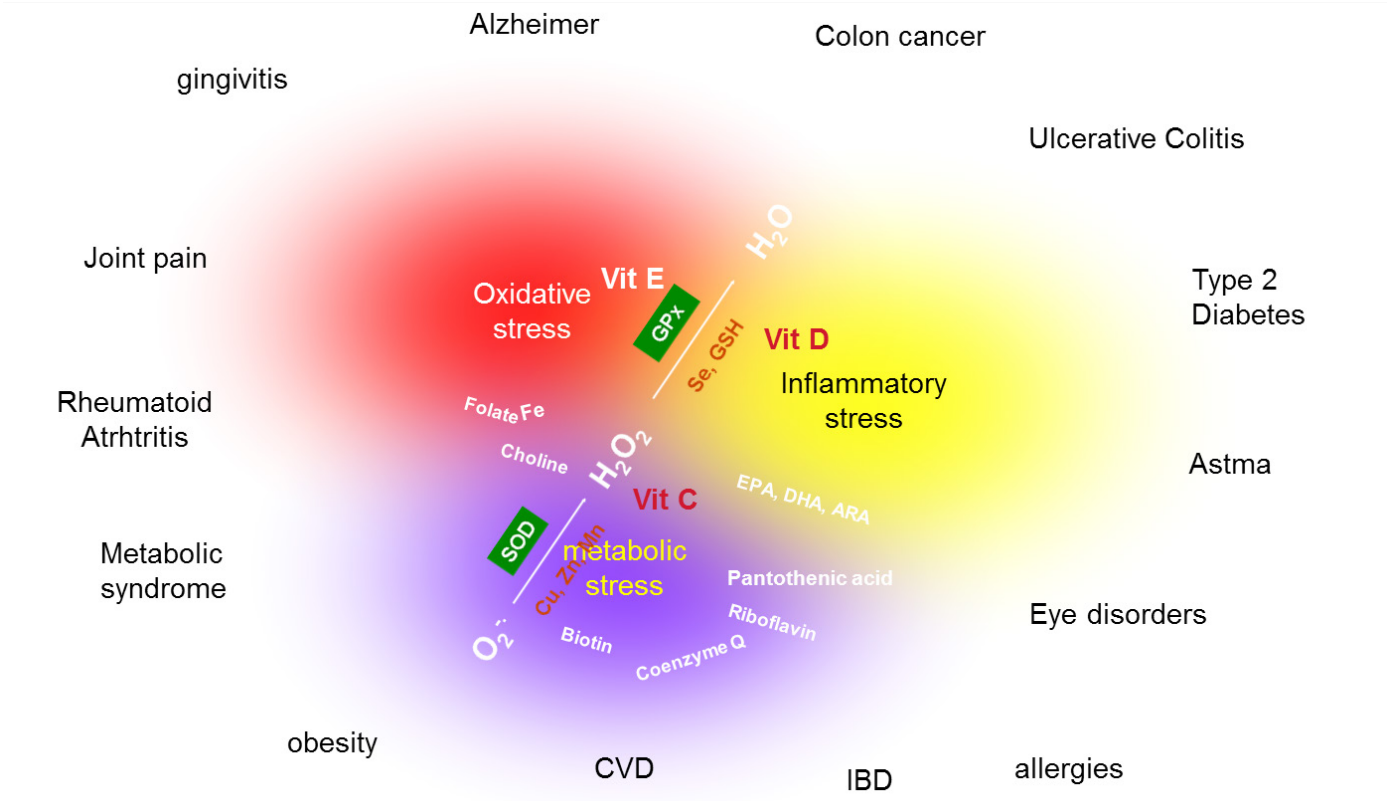


*Fig. 1. Many diseases (the outer circle) have a 'lifestyle-related component', yet the mechanisms of disease progression are essentially different from the processes that maintain optimal health. Here, three crucial processes are described, i.e. the capacity to maintain flexibility in metabolic, oxidative, and inflammatory stress. Flexibility in this context is described as the capacity to contain the stress response reactions within 'healthy limits' (both in amplitude and time), and thus the capacity to maintain optimal homeostasis. Interestingly, many essential nutrients function in this area [19] . CVD = Cardiovascular disease; IBD = inflammatory bowel disease; IR = insulin resistance; SOD = superoxide dismutase.*

maintaining optimal health, research in this area was hampered because abundant use was made of 'disease biomarkers'. This created tension in regulation and food industry, and drawbacks for (funding in) nutrition and health research.

What is needed is a complete refocusing of health research, starting with (re) defining health and its related mechanisms, understanding that integrated personalized health optimization strategies are needed, redesigning the methods to quantify health, and from there onwards building a new generation of health biomarkers. These biomarkers have to serve two crucial goals: to report on health improvement or health maintenance instead on disease progression and to empower the individual to achieve this. Each of these aspects is further detailed below.

## SYSTEMS FLEXIBILITY AS A CHARACTERISTIC OF OPTIMAL HEALTH

Human health is based on a complex network of interactions between pathways, mechanisms, processes, and organs. Many of these processes have to function in a continuously changing environment (diet, infections, stress, temperature, and exercise, for example) and thus strive to maintain internal homeostasis by adapting to these changes. We call this phenotypic flexibility [2] and realize that disease onset occurs when and where these adaptive processes fail. Importantly, diet plays both a positive and negative role here. Many nutrients serve specifically to optimize these 'flexibility processes' (fig. 1).

Shifting the focus from disease biomarkers towards the development of nextgeneration biomarkers of (optimal) health needs a different approach to quantify health and different strategies of testing. Health is maintained by a complex interaction of processes, each maintaining 'homeostasis', elasticity, and robustness. This well-orchestrated physiologic machinery (fig. 2) to adapt to the continuously changing environment is termed 'phenotypic flexibility' [2]. A suboptimal health condition becomes apparent under situations of temporary stress, like physical exercise, infections, or mental stress. Also, dietary habits, e.g. excess intake of sugars or fats, present temporary stress to the body. In various systems (e.g. transfer of people, goods, finances, and energy), optimal performance is achieved only when logistics and infrastructure function well and are capable of dealing with temporary overload or stress. Disturbances in these systems lead to traffic jams, shortages, or damages. Stress tests are applied to test the flexibility of such systems in unexpected situations. Similarly, proper management of calories and nutrients by our body requires the optimal metabolism and condition of the organs. When this is the case, the body's flexibility is able to cope with temporary distortions, a condition which can be qualified as 'healthy'.

## QUANTIFICATION OF SYSTEMS FLEXIBILITY: STRESS RESPONSE BIOMARKERS

Due to a wide variety of reasons (e.g. genetic and epigenetic factors, exposure, diet, stress, and exercise), individuals differ in their 'wiring' of phenotypic flexibility,

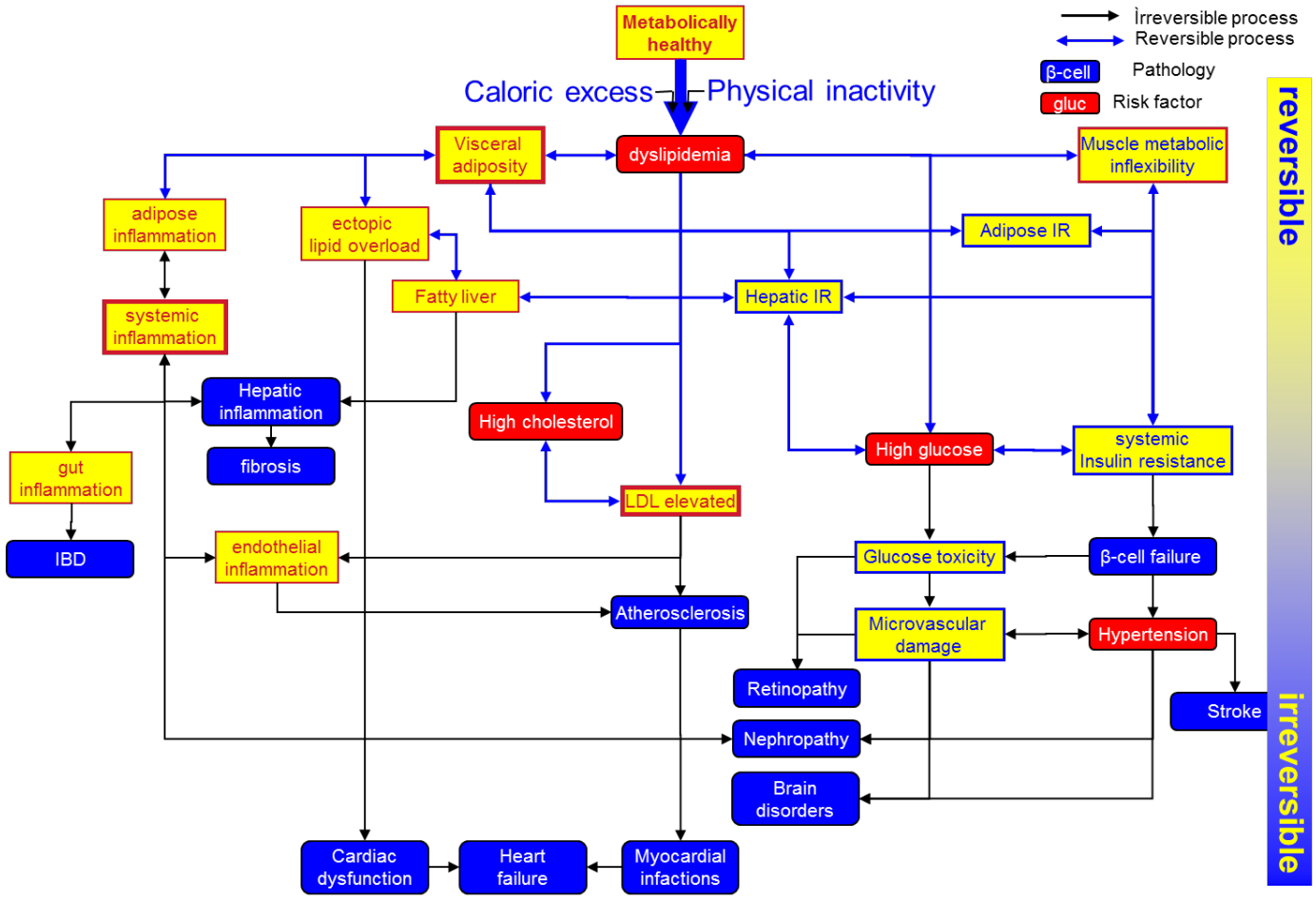


*Fig. 2. The system of phenotypic flexibility where multiple processes spanning multiple organs interact in maintaining optimal metabolic-inflammatory health, and where caloric imbalance eventually leads to a series of disorders, developing in a personal manner depending on genotype, previous exposure, and lifestyle (reproduced with authors permission from van Ommen et al. [2] ). Gluc = Glucose; IBD = inflammatory bowel disease; IR = insulin resistance; SOD = superoxide dismutase.*

will react differently to acute and chronic stressors, and develop a personal trajectory of metabolic-inflammatory health and disease. Thus, personalized diagnosis of the phenotypic flexibility system needs to reveal the 'weak spots' in this flexibility network. For one person, this may be impaired triglyceride storage in adipose tissue resulting in a fatty liver, for another the impaired excretion of VLDL particles from the liver due to a shortage in choline, which results in a fatty liver. A third person may accumulate liver fat due to a shortage of carnitine, which causes inadequate fatty acid oxidation, for example. Each of these processes needs to be diagnosed and require a specific (food-based) therapy [3]. To better quantify these conditions, the development and application of standardized metabolic stress tests have been suggested to quantify health and health effects from diets or treatments [4]. For the different organs and processes shown in figure 2, markers are needed where the 'amplitude' and the 'duration' of disturbance (time needed to get back to homeostatic conditions) are taken as readout. These are 'multibiomarker' panels representing defined and accepted health-related processes that need to be combined with a standardized stress test or challenge test that preferably modulates these defined and accepted healthrelated processes. In covering the metabolic health arena, flexibility quantification should focus on the 'overarching processes' that are oxidative stress, metabolism, and inflammation ( ig. 1), since these processes are important for maintaining health, and disturbances can cause the switch from healthy towards the development of chronic metabolic diseases. Multibiomarker panels will emerge that act as composite descriptors of physiological processes. In the example of vascular health, such a composite biomarker could be composed of flow-mediated dilation, a functional marker of endothelial function and blood pressure, resilience markers for endothelial damage after a metabolic challenge test such as VCAM, ICAM, and E-selectin responses, and total cholesterol or specific single nucleotide polymorphisms related to an increased risk for cardiometabolic disease development. By combining this information into an integrated readout such as the 'vascular health index' [5] or as a 'health space' [6], a flexibility marker for vascular health can be obtained that has broader value, both for product development and health care. It is important that a standardized stress test or challenge test will be developed that modulates most phenotypic health processes. A standardized challenge test should be characterized in how it modulates the different processes of phenotypic flexibility and how well it differentiates between health states in the sequel from optimal healthy to suboptimal healthy to diseased, including heterogeneity, subpopulations, and different stages of the disease. Finally, variation in the response to a challenge should be related to established markers of disease or to long-term health outcomes (disease risk/longevity) in cohort studies for their validation.

| T2D subgroups (see fig. 2, based on processes involved) | Diagnosis (i.e. parameters of the P4 biopassport) | Potential interventions |
|---|---|---|
| (1) Pancreatic β-cell function (impaired IR) | OGTT: I/ΔG and DI(0), PYY, Arg, His, Phe, Val, Leu | β-cell-protective nutrients (MUFA) and drugs (TZD, GLP-1 analogs, and DPP-4 inhibitors) |
| (2) Muscle IR (decreased glucose uptake) | OGTT: muscle IR index, insulin secretion/IR index, Val, Ile, Leu, γ-glutamyl derivates, Tyr, Phe, Met | PUFA/SFA balance; physical activity; weight loss; TZD (e.g. PPAR-γ) |
| (3) Hepatic IR with decreased glucose uptake but increased production and release | Hepatic IR index, OGTT, hepatic IS index, ALAT, ASAT, bilirubin, GGT, ALP, CK-18 fragments, lactate, α/β-hydroxybutyrate | Decrease in SFA and n-6 PUFA, and increase in n-3 PUFA; weight loss; metformin; TZD; exenatide (GLP-1 analog); DPP-4 inhibitors |
| (4) Adipocyte IR and lipotoxicity | Basal adipocyte IR index, FFA platform, glycerol | α-Lipoic acid; PUFA/SFA balance; n-3 FA; chitosan/plant sterols; TZD; acipimox |
| (5) Gastrointestinal tract (incretin deficiency/resistance) | i.v. GTT vs. OGTT, GLP-1, GIP, glucagon, bile acids | MUFA; dietary fiber (pasta/rye bread); exenatide |
| (6) Pancreatic α-cell hyperfunction | Fasting plasma glucagon | Glucagon receptor antagonist; exenatide; DPP-4 inhibitors |
| (7) Chronic low-grade inflammation | CRP, total leukocytes VCAM, ICAM, oxylipids, cytokines | Fish oil/n-3 fatty acids; vitamin C/E; carotenoids; salicylates; TNF-α inhibitors |
| Currently, 7 processes involved in T2D are identified, and for each of them a biomarker approach to quantify the process, as well as an intervention strategy to optimize/restore health, is suggested. ALP = Alkaline phosphatase; CK = cytokeratin; CRP = C-reactive protein; DDP-4 = dipeptidylpeptidase-4; DI = disposition index; FA = fatty acids; FFA = free FA; GGT = γ-glutamyltransferase; GIP = glucose-dependent insulinotropic polypeptide; GLP = glucagon-like peptide; IR = insulin resistance; IS = insulin sensitivity; MUFA = monounsaturated FA; OGTT = oral glucose tolerance test; PPAR-γ = peroxisome proliferator-activated receptor-γ; PUFA = polyunsaturated FA; PYY = peptide YY; SFA = saturated FA; TZD = thiazolidinedione. | | |

*Table 1. T2D subgroup (process)-dependent diagnosis/intervention strategies.*

## SYSTEMS INTERVENTIONS – OPTIMIZING EACH PROCESS INVOLVED IN SYSTEMS FLEXIBILITY

Systems diseases require systems diagnoses based on quantifying 'phenotypic flexibility' as described in the previous paragraph, revealing the underlying disease cause(s) within a complex network of nonlinear metabolic and inflammatory processes which drive most systems diseases. For optimal phenotypic flexibility, each process needs to function optimally. In T2D, several organs can contribute to disruption of (glucose) metabolism [7]. The degree of insulin sensitivity of the three main organs, pancreas, muscle tissue, and liver, can be assessed by measuring glucose and insulin at 30-min intervals during an oral glucose tolerance test. It is known that the severity of insulin resistance can differ between the various tissues, and that different interventions may have organspecific effects related to increasing insulin sensitivity [8], as demonstrated by the example of treatment of T2D patients with a very low caloric diet (VLCD) or physical exercise. Research showed that T2D patients react differently to these treatments. When insulin resistance is mainly localized in muscles, physical exercise has a higher and faster improvement in health as compared to VLCD. In patients with insulin resistance mainly located in the liver, VLCD can normalize the glucose metabolism already within 8 weeks. However, when β-cell capacity is not sufficient enough it is known that the patient has neither benefit from VLCD nor from physical exercise [9–12]. This allows possibilities for systems interventions based on the diagnosis of decreased flexibility of specific health-related processes. In taking this concept further, towards all aspects of phenotypic flexibility, table 1 gives an example how systems diagnosis and related interventions can be created for T2D. A first beautiful example of such an approach is given in a recent publication on the reversal of cognitive decline by Bredesen [13]. This report describes a novel, comprehensive, and personalized therapeutic program that is based on the underlying pathogenesis of

Alzheimer's disease, where 9 out of the 10 early Alzheimer patients displayed sustained cognitive improvement.

## NEED FOR A TIMELINE OF THE HEALTH TRAJECTORY

Ideally, phenotypic flexibility biomarkers develop into two dimensions. Firstly, from a single process to the complete system of flexibility ('systems flexibility biomarker', described above), and secondly, along the timeline of an individual's health trajectory, building the life story of systems flexibility, a 'biopassport'. Loss of phenotypic flexibility is a process that develops over the time span of many years. Interventions are most successful in early stages, when full reversal is possible.

The storage and availability of biomarkers have been common practice in longitudinal cohorts, but the translations of its results into health care is a tediously slow process. On the other hand, (personal) health care data are collected in a fragmented (case-by-case) manner and usually not available in a structured and understandable manner for the citizen to valorize for his personal health.

Since lifestyle-related health is primarily dependent on self-management and self-empowerment, it is vital that the citizen/consumer/patient has access to all relevant health data and information [14] . If biomarkers of phenotypic flexibility are the key in optimizing metabolic health, and in the prevention and treatment of metabolic diseases, they need to be measured at regular intervals. At this moment, this is neither practical nor affordable, and, moreover, most health care systems do neither focus on nor reimburse preventive diagnostics. Therefore, new diagnostic applications need to be developed which are cost-effective and minimally invasive and preferably suitable for 'do-it-yourself' applications. Developments, both in personal health portals, e.g. ITC (Information Communication Technology) for Health, and in diagnostics, e.g. 'gadgets' or dried blood spot diagnostics, are rapidly elucidating this area. The 'Nutrition Researcher Cohort' https://humanstudies.tno.nl/nrc/ [15] aims at professionalizing this movement.

This biopassport is the ideal starting point for the design of both (food-based) personal health optimization and self-empowerment strategies.

## CONCLUSION: FROM PRODUCTS TO SERVICES

The food and nutrition market faces major challenges. The Western world suffers from too much and relatively cheap food with low nutrient, but high caloric density, mostly derived from low-cost ingredients like vegetable fat and sugars. This is a trend rapidly adapted by the developing world [16]. The food industry finds difficulty in providing scientific evidence that their products are healthy or have added health value [17]. The two key solutions here are the availability of foods with substantiated health benefits and the facilitation of personal healthy food choices. Biomarkers of phenotypic flexibility, which refocus on the assessment of health instead of disease, can help in the design and performance of science-based nutritional interventions that allow to evaluate health improvement in (apparently) healthy consumers.

A sustainable shift in eating habits towards healthier diets will not be easy to achieve. Clearly, individuals themselves are directly responsible for what they eat. However, in a complex interplay, many external agents (regulators, industrial sectors, medical professionals, the media, and social networks) influence the choices individuals make [18]. Making healthier choices is critical for the future health of our bodies and our societies. An essential part in this process is the individual's self-empowerment in making these choices, by having access to reliable information on food products and on one's personal health status through personal access to longitudinal systems flexibility diagnostics, as described above. In shaping this new reality, self-empowerment needs to be embedded in, and possibly even become the driver of, a new health care economy based on personal data ownership [14].

The development of systems diagnosis with preventive and personalized interventions may create and trigger a series of commercial service-based health industry activities in the area of diagnostics, personal food solutions, food-pharma combinations, and health advice systems, for example. Food companies may shift their product portfolio from product branding to product-service combinations (personalized products connected to a diagnostic service), food services may be integrated into a health-based personal portfolio, and ICT services will emerge based on a personal biopassport (interpretation of an individual's health data and relate this to nutrition and lifestyle advice). All of this

needs to be developed based on evidence-based science and within adequate regulatory-ethical frameworks. In other words, there is some work to be done. Yet, the next generation of biomarkers of health is not only urgently needed but will also open the door to a new cost-effective model of health and health care.

## DISCLOSURE STATEMENT

The authors declare that no financial or other conflict of interest exists in relation to the contents of the chapter.

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