Personalised health preservation, precision medicine, precision nutrition and innovations in functional foods

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Review: Wie aus dem Titel des vorliegenden Manuskripts hervorgeht, handelt es sich dabei um eine thematisch umfangreiche Arbeit in den drei unterschiedlichen gesundheitsrelevanten Themen angesprochen werden. Diese Einschätzung wird durch das Inhaltsverzeichnis unterstrichen, wobei die Themen „Health aging, „functional food“ und „personalised health preservation“ als Hauptthemen in den Vordergrund treten. Allen genannten Themen kann eine wesentliche Bedeutung im Rahmen des Zusammenhanges zwischen Ernährung und Gesundheit zugesprochen werden. Die Thematik des Zusammenhanges zwischen Ernährung und Altern war immer schon Gegenstand des Interesses der Menschen, stehen doch dabei zwei grundsätzliche Fragestellungen im Mittelpunkt. Die eine dieser beiden Fragen betrifft die Möglichkeit bzw. den Wunsch, durch die Ernährung im Allgemeinen oder durch die Einhaltung bestimmter Ernährungsarten die individuelle Lebensspanne zu verlängern.

Die zweite Frage spricht die Salutogenese an, wobei es dabei darum geht, durch die Einhaltung bestimmter Ernährungsrichtlinien die Entstehung von chronischen Krankheiten präventiv zu beeinflussen oder durch die Einhaltung bestimmter diätetischer Maßnahmen den Verlauf solcher Erkrankungen in einer gesundheitlich erwünschten Richtung zu beeinflussen.

Wie im vorliegenden Manuskript zum Ausdruck kommt, stellt die Ernährung einen Umwelteinfluss dar, der im Gegensatz zu anderen Einflüssen doch in einem höheren Ausmaß der individuellen Disposition unterliegt. Damit ist auch die Thematik der Epigenetik angesprochen, der im vorliegenden Manuskript ein hoher Stellenwert zugeordnet wird. Die wissenschaftlich gesicherten Erkenntnisse der Epigenetik bilden eine solide Basis im Hinblick auf den Zusammenhang zwischen der Umwelt und der Gesundheit des Menschen. Sie relativieren die Bedeutung der Genetik und genetischer Manipulationen, wie sie in der konventionellen Medizin nicht selten als alleiniger Garant des wissenschaftlichen Fortschritts auf dem Gebiet der Diagnose und Therapie angesehen werden.

Der Abschnitt des Manuskripts welcher der Thematik „functional food“ gewidmet ist, befasst sich einerseits mit den Grundlagen dieses Begriffs und stellt andererseits einzelne Vertreter dieser Gruppe von Nährstoffen sowie Aspekte der gesetzlichen Regulation zur Diskussion.

Schließlich stellt der dritte Abschnitt des Manuskripts die Problematik der personalisierten Aspekte der Gesundheitsförderung in den Vordergrund. In diesem Abschnitt wird u.a. der Zusammenhang zwischen der Genetik, dem Genom und der Ernährung diskutiert.

Insgesamt ist festzuhalten, dass das vorliegende Manuskript verschiedene Aspekte der Beziehung zwischen bestimmten Ernährungsformen und der Gesundheit behandelt. Es geht dabei um sehr aktuelle gesundheitliche Probleme, die in der konventionellen Medizin nicht ausreichende Beachtung finden. Sachliche Bezüge zwischen den einzelnen thematischen Schwerpunkten des Manuskripts werden hergestellt. Alle im Manuskript getroffenen Aussagen sind nachvollziehbar und werden durch die vorhandene wissenschaftliche Literatur untermauert, die als repräsentativ zu beurteilen ist.

Insgesamt sind die im Manuskript behandelten Themen sowohl als innovativ, wissenschaftlich aktuell und praktisch relevant zu beurteilen.

Wien, 1.6.2020

Ao. Univ. Prof. Dr. W. Marktl

If someone is looking for health, ask him first whether he is also prepared in the future to avoid any causes of his disease – only then can you help him.

Sokrates

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Summary

Health preservation and disease prevention are central objectives for establishing a healthy lifestyle and nutrition. Molecular mechanisms of aging, premature aging and the development of aging correlated complex diseases are step by step analysed by scientific disciplines addressing the hallmarks of aging. The concepts of healthy aging and attempts to increase longevity have resulted in remarkable innovations in precision medicine and precision nutrition.

The highly individually regulation of gene expression and DNA integrity by environmental factors and nutrition is shown in the developments of genetics and epigenetics. Also, the detailed characterization of personal aspects of the human microbiome shows the need for systemic OMIC approaches to understand pathological mechanisms and markers for them. The fields of nutrigenetics and nutriepigenetics are analysing mechanisms and markers in this area.

The use of molecular markers enables the detection of ongoing pathological mechanisms and interventions already before the onset of symptoms. Medical and nutritional- or dietary prevention and intervention are more often using personalised aspects. These developments will result into a preventive and personalised health care. Markers from the areas of genetics, epigenetics, microbiota, gene expression, and metabolomics are going to be integrated for the assessment of optimised personal pre- and intervention. Medical drugs, functional foods and nutrition are more and more used for personalised treatment of identified molecular mechanisms of concern.

A worldwide strongly increased awareness of ways for health preservation has boosted the development of functional foods and nutritional concepts. This includes science-based ways of caloric restriction and fasting. Selected bacteria, algae, cells, or plants and their metabolites or extracts are screened for health-promoting effects and developed into functional foods. Scientific literature shows an often-overwhelming flood of information on their activities in different health areas. Key areas of interest are cancerogenesis, metabolic and nervous diseases as well as immune functions.

A confusing array of terms for functional foods, food additives, dietary foods, medicinal foods and food with functional aspects such as epigenetic active foods, SIRT foods, senolytic foods, and many others developed. Regionally very different definitions, concepts for safety assessment, and regulations are complicating the scientifically sound uses of the possibilities of health preservation in this area. However, in the scenario of increasingly wealthy and aged societies, there will be a growing demand for holistic concepts of personalised health preservation. This is evidenced by the new, fast-growing trend for biohacking of technology interested consumers.

The importance to use OMICs and “system theorie” based information for a preventive, personalised health preservation should synergize with aspects of an integrative health care to stimulate “self-healing processes through regulative and informative processes in humans in order to promote and strengthen health” (salutogenesis). Such safe science-based holistic uses of a personalised health preservation needs improved, balanced information of citizens to enable an informed decision of consumers.

Zusammenfassung

Die Gesundheit zu erhalten und die Vorbeugung von Krankheiten sind zentrale Ziele für einen gesunden Lebensstil und eine gesunde Ernährung. Die molekularen Mechanismen des Alterns, die vorzeitiger Alterung und die Entwicklung komplexer, alterungskorrelierter Erkrankungen werden Schritt für Schritt analysiert. Konzepte des „gesunden Alterns“ und Versuche die Lebesspanne zu verlängern haben zu wissenschaftlich fundierten Innovationen in der Präzisionsmedizin und Präzisionsernährung geführt.

Das schnell wachsende Wissen der Genetik und Epigenetik zeigt die Bedeutung der individuellen Regulation der Genexpression und der DNA-Integrität durch Umweltfaktoren oder Ernährung. Darüber hinaus zeigt auch die detaillierte Charakterisierung persönlicher Aspekte des menschlichen Mikrobioms die Notwendigkeit systemischer OMIC-Ansätze zum Verständnis von Erkrankungsmechanismen und Markern für diese. Die Bereiche Nutrigenetik und Nutriepigenetik analysieren Mechanismen und Marker in diesem Bereich.

Die Verwendung molekularer Marker ermöglicht die Erfassung einsetzender molekularer Mechanismen von Krankheiten und mögliche Interventionen bereits vor dem Auftreten von Symptomen. Medizinische und ernährungsphysiologische, diätetische Prävention und Intervention werden deshalb verstärkt personalisiert. Diese Entwicklungen führen zu einer vorbeugenden und personalisierten Krankheitsvorsorge. Marker aus den Bereichen Genetik, Epigenetik, Mikrobiota, Genexpression und Metabolomik werden zunehmend für eine optimierte, persönlichen Vorsorge und Intervention integriert. Medikamente, funktionelle Lebensmittel und Ernährung werden zur personalisierten Behandlung identifizierter, molekularer Mechanismen eingesetzt.

Ein verstärktes Bewusstsein für Möglichkeiten zur Erhaltung der Gesundheit hat die Entwicklung von funktionellen Lebensmitteln und speziellen Ernährungskonzepten vorangetrieben. Dies schließt auch die verstärkte Nutzung wissenschaftlich anerkannter Konzepte der Kalorienreduzierung und des Fastens ein. Ausgewählte Bakterien, Algen, Zellen oder Pflanzen sowie deren Metaboliten oder Extrakte werden auf gesundheitsfördernde Wirkungen untersucht und zu funktionellen Lebensmitteln entwickelt. Die wissenschaftliche Literatur zeigt die oft überwältigende Informationsflut über ihre Aktivitäten in verschiedenen Gesundheitsbereichen. Hauptinteressensgebiete sind Krebsentstehung, Stoffwechsel- und Nervenkrankheiten sowie Immunfunktionen.

Eine verwirrende Reihe von Begriffen für funktionelle Lebensmittel, Lebensmittelzusatzstoffe, diätetische Lebensmittel, medizinische Lebensmittel, Lebensmittel mit funktionellen Aspekten wie epigenetischen aktive Lebensmitteln, SIRT-Lebensmittel, senolytische Lebensmittel und viele anderen entwickelten sich rasch. Regional sehr unterschiedliche Definitionen, Konzepte zur Sicherheitsbewertung und Vorschriften erschweren die wissenschaftlich fundierte Nutzung der vielversprechenden Möglichkeiten zur Erhaltung der Gesundheit in diesem Bereich. Im Szenario einer zunehmend wohlhabenden und gealterter Gesellschaft besteht eine wachsende Nachfrage nach ganzheitlichen Konzepten zur personalisierten Erhaltung der Gesundheit. Dies zeigt auch der neue, schnell wachsende Trend zum Biohacking von Technologie interessierten KonsumentInnen.

Die Notwendigkeit der Verwendung von „OMICs“ und Konzepten der Systemtheorie sollten mit dem Verständnis einer integrativen Krankheitsvorsorge zusammenwirken, um „Selbstheilungsprozesse durch regulative und informative Prozesse beim Menschen zu stimulieren, um die Gesundheit zu fördern und zu stärken“ (Salutogenese). Die Nutzung derartiger wissenschaftlich fundierter Möglichkeiten zur personalisierten Erhaltung der Gesundheit bedarf jedenfalls auch einer verbesserten, ausgewogenen Information für KonsumentInnen.

Contents

Introduction, objectives

WHO has developed the definition of human health as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. Health preservation and disease prevention are central objectives for establishing a healthy lifestyle and nutrition.

In parallel, healthcare has changed from focusing on intervention after the observation of the development of symptoms of diseases to a **preventive, personalised health care** and the use of **markers** which indicate the development of pathologies that allow intervention already before the development of symptoms. The need for a preventive, personal healthcare has been seen since the seventies of the last century (Merchant, 1978)

This development was also reflected by an upcoming understanding of **salutogenesis**, a health approach focusing on factors that support human health and well-being, rather than on factors that cause disease (pathogenesis). More specifically, the "salutogenic model is concerned with the relationship between health, stress, and coping" (Lindström & Eriksson, 2005) and strongly relates to nutrition, dealing with challenges to healthy eating in a health-promoting manner (Swan et al., 2015).

Preventive personal health care needs the development of analytical methods and markers for upcoming diseases as well as products for prevention which address the results from analytical concepts.

Changes of lifestyle and nutrition regimes as well as **functional foods or nutraceuticals** are a possibility to address these expectations. However, the area of nutraceuticals, phytoceuticals, food additives, functional foods, dietary foods, and medicinal foods is highly diverse in regional regulations and standard setting which complicates science-based developments in this area. Also, in the present paper often terms in this area are overlapping.

In the medical field **precision medicine** is an emerging approach for disease treatment and prevention that considers individual variability in genes, environment, and lifestyle for each person. This approach will allow doctors and researchers to predict more accurately which treatment and prevention strategies for a particular disease will work in which group of people.

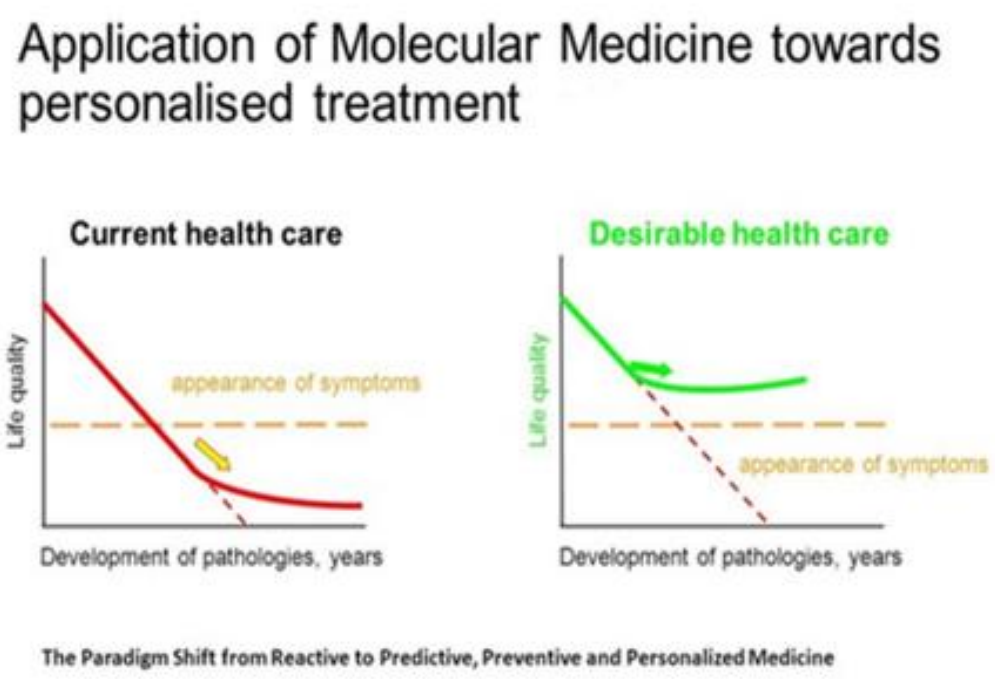

Fig Medicine in the early twenty-first century: Paradigm and anticipation - EPMA position paper 2016 (Golubnitschaja et al., 2016), European Association for Predictive, Preventive and Personalised Medicine.

Also, in the area of nutrition the term **precision nutrition** is evolving where tools of nutrigenetic, nutriepigenetics, metabolomics or the analysis of microbiota is used to design **personalised diets** or selection of functional foods for health preservation.

Ways of intervention and prevention followed the concept of healthy aging (Woo, 2017) and later in economically relevant aspects of longevity claiming that there is no limit to longevity, reviving the human lifespan debate (Dolgin, 2018a)(Dolgin, 2018b)

The meaning and objectives of healthy aging, decelerated aging or longevity need a better common understanding as longevity research is often seen with the goal of the principle increase of lifespan. In contrast e.g. the American Federation of Aging Research defined as primary (composite) endpoints "the incidence of any one of several age-related diseases: myocardial infarction, congestive heart failure, stroke, most cancers, dementia, and death", (but not diabetes) (S B Kritchevsky & Espeland, 2018).

Interventions for healthy aging comprise ways of the adaptation of lifestyle, exercise, caloric restriction, nutrition, and functional foods.

This paper, therefore, is **structured** in aspects of

- Healthy aging as a major objective of personalised health preservation, precision medicine and innovation in functional foods
- Additives, functional foods and nutraceuticals in healthy aging
- Personalisation in medicine and mainly nutritional intervention in healthy aging

Legal aspects are not a main scope of this study and overlaps between chapters shall enable the reading of single aspects of the study.

Healthy aging

Aristotle has been one of the first philosopher to show a serious interest in health and aging. He considers it a natural process, and so within the purview of his philosophy of nature. Aging is a process that living things undergo. And life is essentially tied up with soul. Aristotle recognizes many different psychic powers, but he sees the power of nutrition as in some sense the most fundamental since it alone can be separated from the others, while the other powers are always held in conjunction with nutrition. (Adam Woodcox, 2018)

In recent times healthy aging was defined as a major objective and WHO defines *Healthy Aging* "as the process of developing and maintaining the **functional ability** that enables **wellbeing** in older age" https://www.who.int/aging/healthy-aging/en/

Aging is a complex multifactorial biological process shared by all living organisms. It is manifested by a gradual decline of normal physiological functions in a time-dependent manner. Organismal aging holds significant importance for human health because it increases susceptibility to many diseases, including cancer, metabolic disorders, such as diabetes, cardiovascular disorders, and neurodegenerative diseases. On the other hand, cellular senescence, also called replicative senescence, is a specialized process, considered to be a potential endogenous anticancer mechanism, during which there is irreversible growth arrest in response to potentially oncogenic stimuli. It is also considered a potential trigger to cause tissue remodelling during embryonic development and following tissue damage, which also requires a proliferation arrest. Cellular senescence bears many similarities to the aging process but also shows distinct features. Although the causes of aging are poorly understood, there are continued efforts to delineate longevity pathways conserved among all eukaryotes (Campisi, 2013)

Hallmarks of aging

Modern molecular biology has summarized major molecular mechanisms, hallmarks of aging, which determine biological aging. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (López-Otín et al., 2013).

Fig Hallmarks of Aging. (López-Otín et al., 2013)

Genetics and healthy aging

Organisms that do not live very long are a famous way to study lifespan. Over the last several decades, C. elegans has been the subject of many published studies. Cynthia Kenyon and her associates showed that C. elegans with a specific single-gene mutation lived twice as long as members of the species that lacked this mutation. (Braeckman & Vanfleteren, 2007; Kenyon et al., 1993) The responsible gene is called **daf-2** (Kimura et al., 1997). Scientists were surprised to find that the protein coded for by this gene (designated DAF-2) looked much like the receptor protein within humans that responds to the hormone insulin. It turned out that daf-2 normally controls many other genes, which in turn regulate a variety of physiological processes at different stages in life. For example, in their studies of C. elegans, researchers have found a large set of genes that are either "turned on" or "turned off" in worms that carry two copies of the daf-2 mutation. (Pal & Tyler, 2016a)

There is good evidence that **insulin signaling** is critically involved in longevity because the hormone pathways are conserved through evolution and insulin pathway plays a role in diseases like diabetes and cancer. For example, insulin resistance at the cellular level is a key feature of type II diabetes. Similarly, mutations along the pathway that insulin/IGF-1 receptors put into motion have been associated with the dysregulation of growth that characterizes cancer. The disease idea is especially tantalizing, as the risk of both diabetes and cancer increases with age. “So, how does one gene control life span? Possibly because it acts by controlling a lot of other genes that just happen to coordinate the survival system within” (Adams, 2008).

Epigenetics and healthy aging

Among the hallmarks of aging epigenetic alterations represent one crucial mechanism behind the deteriorated cellular functions observed during aging and in age-related disorders. By definition, epigenetics represents the **reversible heritable mechanisms that occur without any alteration of the underlying DNA sequence** (Fymat, 2017).

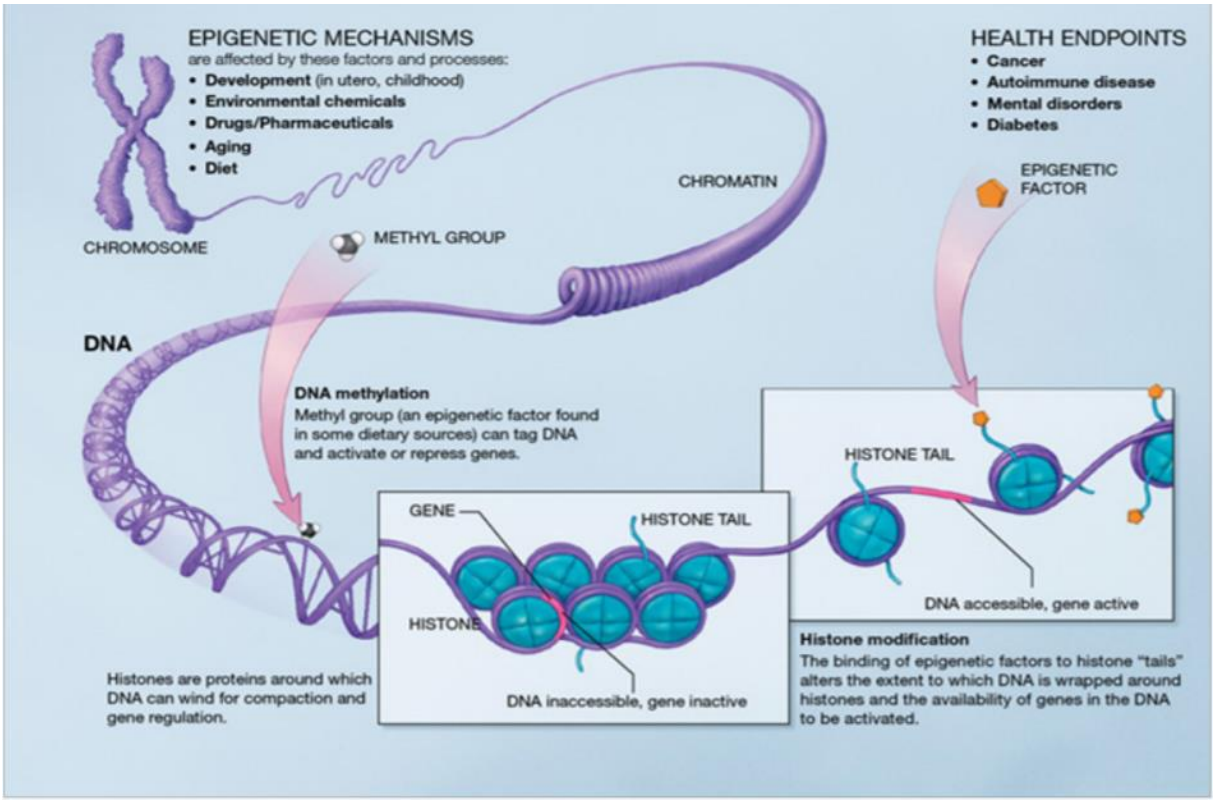

Fig Epigenetic mechanisms (Fymat, 2017)

Although the chromosomes in our genome carry the genetic information, the epigenome is responsible for the functional use and stability of that valuable information; that is, it connects the genotype with the phenotype. These epigenetic changes can either be spontaneous or driven by external or internal influences. Epigenetics potentially serves as the missing link to explain why the pattern of aging is different between two genetically identical individuals, such as identical twins, or, in the animal kingdom, between animals with identical genetic makeup, such as queen bees and worker bees (Brunet & Berger, 2014a; G. M. Martin, 2005; Sargent, 2010). Although longevity studies on the human population have shown that genetic factors could explain a fraction (20 to 30%) of the differences observed in life spans of monozygotic twins, the majority of the remainder of variation is thought to have arisen through epigenetic drift during their lifetime (Herskind et al., 1996; Muñoz-Najar & Sedivy, 2011; P. Poulsen, M. Esteller, A. Vaag, n.d.).

Similarly, different environmental stimuli, including diet, cause differential alterations of stored epigenetic information to create a striking contrast in physical appearance, reproductive behaviour, and life span of queen and worker honeybees, despite their identical DNA content. In turn, the resulting variability in the pattern of epigenetic information within individual cells in the population during aging leads to transcriptional drift and genomic instability. Being established by enzymes, epigenetic information is reversible. Hence, epigenetics holds great prospects for targeting by

therapeutic interventions, as opposed to genetic changes, which are currently technically irreversible in humans. Accordingly, delineating and understanding the epigenetic changes that happen during aging is a major ongoing area of study, which may potentially lead the way to the development of novel therapeutic approaches to delay aging and age-related diseases. (Pal & Tyler, 2016b)

Types of epigenetic information: There are different types of epigenetic information encoded within our epigenome, including but not limited to the presence or absence of histones on any particular DNA sequence, DNA methylation, chromatin remodelling, posttranslational modifications of the histone proteins, structural and functional variants of histones, and transcription of noncoding RNAs (ncRNAs) (Brunet & Berger, 2014b; Feser & Tyler, 2011; Gelato & Fischle, 2008; Lazarus et al., 2013; O'Sullivan & Karlseder, 2012).

Epigenetic modifications

DNA methylation is defined by the addition of a methyl group ($-CH_3$) at the position 5 of the pyrimidine ring of cytosine in specific CpG islands. CpG island are DNA regions, mostly found in promotors, rich in repetitive guanin and cytosine nucleotides that are linked to each other through a phosphate group.

A methylated CpG site "closes" the chromatin structure at the promotor site and inhibits the binding of the transcription machinery and thus hinders gene expression. Methylation is therefore mostly associated with no gene expression or gene silencing, but whether this has positive or negative effects is dependent on the gene being methylated.

Certain enzymes called DNA methyltransferases (DNMTs) are responsible for the methylation patterns of genes; each DNMT has its own function and uses SAM as a methyl donator. DNMT1 is active during replication, it copies the methylation pattern from one generation to the next to maintain the exact methylation patterns. DNTM2 is responsible for embryonic stem cell methylation while DNMT3a and 3b create new methylation patterns on CpG islands. In the human genome 60-80% of CpG islands are methylated.

Histone modifications are post translational modifications that occur on the tails (N or C terminal amino acid residues) of the histone octamer. Histones are the packing components that allow to compress DNA into chromatin. The DNA is wrapped around the histones and forms chromatin, a compacted and "closed" version of DNA.

Histone modifications allow chromatin to "open" and "close", giving or denying access to the DNA strand and transcription. There are different chemical mechanisms that modify the histone structure so that it releases or tightens the DNA around it; these include acetylation, phosphorylation, methylation, simulation, and ubiquitination of different amino acid residues at the histone tails. Such mechanisms are guided by different enzymes, for example histone acetylation is performed by histone acetyltransferases (HAT) and histone deacetyl transferases (HDAC). An acetylated histone usually indicates relaxed chromatin, allowing access to the DNA and thus transcription is possible. In contrast deacetylation leads to transcriptional silencing.

RNA silencing is a post transcriptional gene regulating mechanism that involves **microRNAs (miRNA) and small interfering RNAs (siRNAs)**, which are small (20-25 bps) non-coding RNAs. These non-

coding RNAs regulate translation by binding to the target mRNA. Mostly the consequence is translational silencing through mRNA degradation, inhibition of translation by blocking the promotor or by recruiting histone methyltransferases (HMT), which close the chromatin structure.

Together, these different types of epigenetic information comprise our epigenome and are important determining factors behind the function and fate of all cells and tissues, in both unicellular and multicellular organisms. Invariably, each of these different types of epigenetic information is functionally significant for the process of aging

DNA methylation, health and aging

DNA methylation is one of the most extensively studied and best characterized epigenetic modifications during aging (Deaton & Bird, 2011; Jung & Pfeifer, 2015a; E. Li et al., 1993; López-Otín et al., 2013; Romanov & Vanyushin, 1981; V. L. Wilson & Jones, 1983).

In young cells, the majority of CpGs within the genome have cytosine methylation. CpG methylation within promoters leads to transcriptional repression, through the formation of compact chromatin structures, such as heterochromatin. Conversely, promoters of genes that are highly expressed are devoid of DNA methylation, hence their name—CpG islands. DNA methylation is particularly important during development, when it is used to silence genes in tissues in which their expression is never going to be needed, or at developmental stages in which they no longer need to be expressed. As identical twins age, the pattern of DNA methylation becomes more and more divergent because of epigenetic drift caused by environmental factors or spontaneous stochastic errors in the process of transmission of DNA methylation. **Epigenetic drift** leads to unpredictable differences in the methylome among aging individuals. Caloric restriction delays age-related methylation drift (Gensous et al., 2019; Maegawa et al., 2017; Mendelsohn & Larrick, 2017). However, some of the methylation changes that occur with age are directional and involve specific regions of the genome.

This fact indicates that at least part of the DNA methylation changes during aging are not stochastic but could be associated with biological mechanisms involved in the aging process. DNA methylation, as well as life span, also differs between organisms of identical genetic makeup, such as queen and worker honeybees, when subjected to different environmental stimuli, such as diet, which results in an altered gene expression pattern. Strikingly, similar methylome profiles were experimentally found following RNAi-mediated silencing of a DNA methyltransferase enzyme, Dnmt3, in laboratory-bred bees (Kucharski et al., 2008; Shi et al., 2011). Although it is yet to be determined whether the diet-induced DNA methylation changes are indeed responsible for causing the transcriptional shift observed in these cases, it provides great promise for unravelling a key concept of epigenetic reprogramming.

With a few exceptions, mammalian aging is more commonly associated with CpG hypomethylation, especially at repetitive DNA sequences. This is likely to be at least partly responsible for the loss of heterochromatin during aging. Loss of CpG methylation at repetitive sequences will heighten the risk of retrotransposition events and, hence, genomic instability during aging, given that the retrotransposons comprise much of the repetitive DNA. The global decrease in DNA methylation upon aging may be attributed to the progressive decline in levels of the DNA methyltransferase DNMT1 (Jung & Pfeifer, 2015b) . In addition to the general DNA hypomethylation that occurs during aging, progressive age-dependent loss of DNA methylation also occurs at specific gene promoters, (Lu et al., 2002; Z. Zhang et al., 2002). Simultaneous with the general and localized DNA

hypomethylation during aging, hypermethylation occurs at specific CpG sites of the genome, presumably to repress expression of specific genes (Bekaert et al., 2015; Cedar & Bergman, 2012; Eipel et al., 2016; Fraga et al., 2007; Horvath, 2013; Jones et al., 2015; Jung & Pfeifer, 2015b; Q. Lin et al., 2016; Monk, 1995; Reik et al., 2001; Vanyushin et al., 1973; Weidner et al., 2014a) (137, 142, 148, 149).

A recent analysis suggested the possibility that transcription factor binding may hinder the ability of **DNA methyltransferases** to eventually recognize those sites, resulting in aberrant gene expression in aged cells (Avrahami et al., 2015; Helman et al., 2016) . The hypothesis seems promising but is yet to be addressed during aging in different cells, tissues, or organisms. (Beerman et al., 2013, 2013; Maegawa et al., 2010; Teschendorff et al., 2010; T. Yuan et al., 2015)

The development of novel next-generation sequencing technologies for genome-wide assessment of DNA methylation levels has permitted the confirmation and extension of earlier studies. A comparison between the methylome of CD4+ T cells from new-born and centenarian individuals further solidifies the notion of global decreases in DNA methylation with aging, accompanied by heterogeneous DNA methylation in the centenarian genome. However, the reproducibility of DNA methylation changes during aging at some sites is such that age prediction can be made fairly accurately by analysing DNA methylation patterns in only three specific CpG sites, at least for blood DNA (Garinis et al., 2008; Weidner et al., 2014b).

During **replicative senescence**, there are alterations in DNA methylation patterns globally and at specific sites, as observed during organismal aging. Notably, cell passage numbers and the population doublings can be accurately predicted from the methylation pattern at specific CpG sites (Koch et al., 2012; Koch & Wagner, 2013). This altered DNA methylation pattern during replicative senescence correlated with the change of expression of SIRT1 (Ions et al., 2013; Wakeling et al., 2009, 2015b). This might result from the effect of SIRT1 on DNA methylation of PcG target genes, although the mechanistic details of this phenomenon are unclear. One of the remaining challenges in the analyses of DNA methylation during aging is to identify the causal pathways that contribute to the functional decline of the DNA methylome during aging. (Wakeling et al., 2015a).

The age-dependent **changes in the DNA methylome** are reminiscent of those occurring in cancer. Global hypomethylation at the repetitive regions and site-specific hypermethylation at certain promoters have also been reported during cancer, suggesting a potential connection between age-dependent DNA methylation changes and increased cancer risk observed in the elderly population (Gautrey et al., 2014; Hengstler et al., 2009)

Notably, local hypermethylation events during aging sometimes occur at the promoters of tumor-suppressor genes, potentially preceding cell transformation events. The analogy between cancer and aging also extends to the PcG target genes because both aging and cancer are accompanied by DNA hypermethylation of these genes. Hence, a better understanding of the reasons behind the changes in the DNA methylome during aging may help us to also understand the causes of cancer.

The epigenetic clock

It is well established that as individuals age, there is a raft of molecular changes that occur within the cells and tissues. Changes in DNA methylation patterns have been shown to occur with aging and thus may be a fundamental mechanism that drives human aging. Epigenetic biomarkers of aging,

otherwise known as the epigenetic clock, have been developed using DNA methylation measurements. Referred to specifically as 'DNA methylation age' (DNAmAge), they provide an accurate estimate of age across a range of tissues, and at different stages of life, and are some of the most promising **biomarkers of aging**. DNAmAge has also permitted the identification of individuals who show substantial deviations from their actual chronological age, and this 'accelerated biological aging' has been associated with unhealthy behaviours, frailty, cancer, diabetes, cardiovascular diseases (CVD), dementia , and mortality risk. (Adam Woodcox, 2018)

In the last few years, two meta-analyses of 13 studies (*n* = 13,089) and 4 studies (*n* = 4658), respectively, have been undertaken to investigate the extent to which DNAmAge in blood predicts mortality risk. Both reported a significant association between increased DNAmAge and mortality risk. However, neither was undertaken as part of a systematic review, raising the possibility that the findings were not representative of all research that has been undertaken in the field. To date, there has also been no systematic review that has investigated whether DNAmAge biomarkers are predictors of age-related diseases or longevity.(Adam Woodcox, 2018)

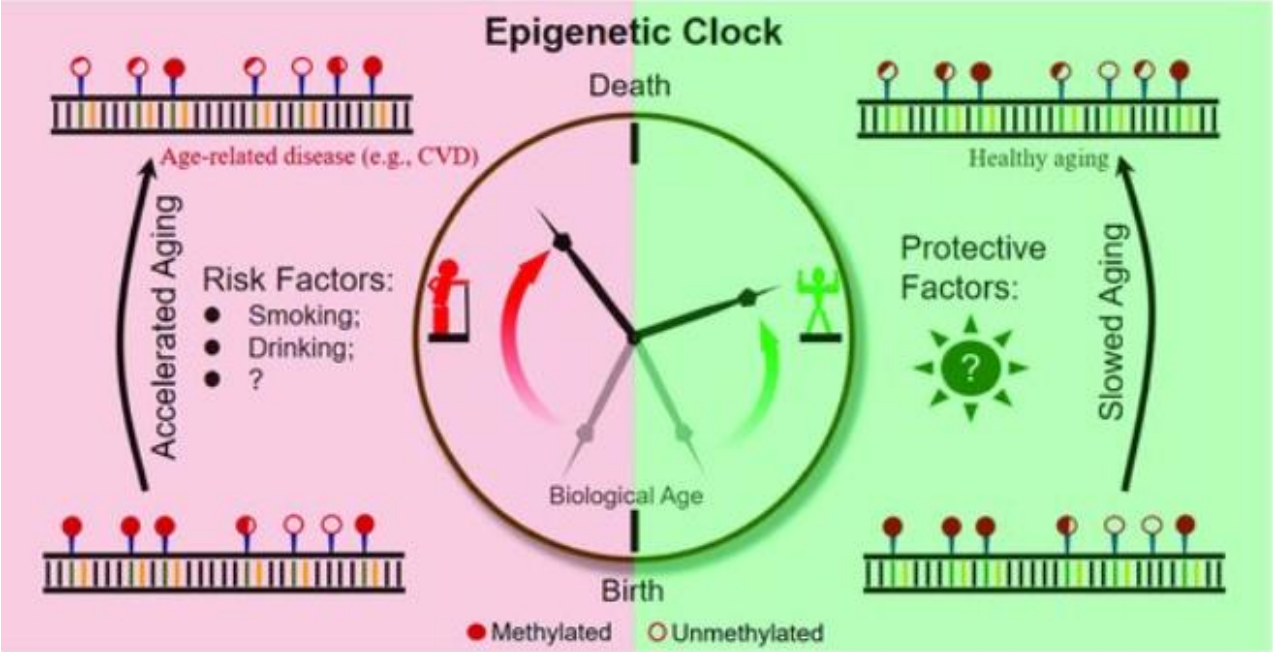

Fig Epigenetic clock (Pal & Tyler, 2016a), (Xiao et al., 2019)

Histones and healthy aging

Growing evidence in recent years also clearly implicates chromatin structure, which carries much of the epigenetic information, as a major player during the aging process. The basic unit of chromatin structure is the nucleosome, which consists of 147 base pairs of DNA wrapped around a histone octamer that comprises two copies of each core histone protein, H2A, H2B, H3, and H4.The addition of linker histones, such as histone H1, and other nonhistone proteins, such as heterochromatin protein 1 (HP1), facilitates the formation of higher-order repressive chromatin structures, such as

heterochromatin. Packaging of the genomic DNA into the highly organized chromatin structure regulates all genomic processes in the nucleus, including DNA replication, transcription, recombination, and DNA repair, by controlling access to the DNA. Studies on the aging of mammals are rather limited by the long life span of the commonly used model organisms. (Dinant & Luijsterburg, 2009; Kaller et al., 2006; Kornberg, 1974; Kornberg & Thomas, 1974; Luger et al., 1997; Maison & Almouzni, 2004; Rhodes, 1997).

One of the earlier proposed models of aging was the "**heterochromatin loss model of agin**g" (Bernadotte et al., 2016; Haithcock et al., 2005; K. L. Wilson, 2005). This model suggests that the loss of heterochromatin that accompanies aging leads to changes in global nuclear architecture and the expression of genes residing in those regions, directly or indirectly causing aging and cellular senescence. As with any other model of aging, the heterochromatin loss model is supported by experimental data, but there are also confounding observations. Loss of transcriptional silencing due to decay of the heterochromatin occurs during aging in all eukaryotes examined from yeast to humans, and there is evidence that accelerating or reversing this process can either shorten or lengthen life span, respectively. Gene silencing requires the absence of histone acetylation within heterochromatin regions. Accordingly, treatment with histone deacetylase (HDAC) inhibitors or deletion of genes encoding HDACs, such as yeast SIR2 or its sirtuin counterparts in metazoan species, shortens life span, whereas chemical activation or overexpression of SIR2 or **sirtuins extends life span** (Anastasiou & Krek, 2006; Dillin & Kelly, 2007; Finkel et al., 2009; L Guarente & Guarente, 2007; Leonard Guarente, 2011b; Haigis & Sinclair, 2010; Hall et al., 2013, 2013; V. D. Longo & Kennedy, 2006; B. J. Morris, 2013).

Recently, the aging research field has experienced a leap from the earlier paradigm of the heterochromatin loss model. Not only is the heterochromatin reorganized during aging, but a global loss of core histone proteins from the genome during aging has also been observed in multiple scenarios, and this has been shown to be a cause of aging in yeast. (Pal & Tyler, 2016a)

Not surprisingly, given their functional interdependence, DNA methylation and histone modifications are intertwined to exert the changes observed during aging. Numerous studies have reported age induced DNA hypermethylation of specific loci that contain known Polycomb group protein (PcG) target genes in both mice and humans (Bird, 2002; Bonasio et al., 2010; Horvath, 2013; Yuanyuan Li et al., 2018; Ng & Surani, 2011; Xie et al., 2013; Young, 2011)

PcGs repress gene expression by H3 K27 trimethylation in a dynamic manner that is reversible by H3 K4 methylation, as needed, during development. Whereas hypermethylation of DNA upon aging is enriched at the genomic regions carrying bivalent histone marks (that is, both H3 K4me3 and H3 K27me3) at poised promoters, DNA hypomethylation co-occurs with the histone modification marks H3 K9Ac, H3 K27Ac, H3 K4me1, H3 K4me2, and H3 K4me3 that are found mostly at enhancer regions (Bell et al., 2012; Calo & Wysocka, 2013; Kananen et al., 2016; Serfling et al., 1985; Xie et al., 2013; T. Zhang et al., 2020).

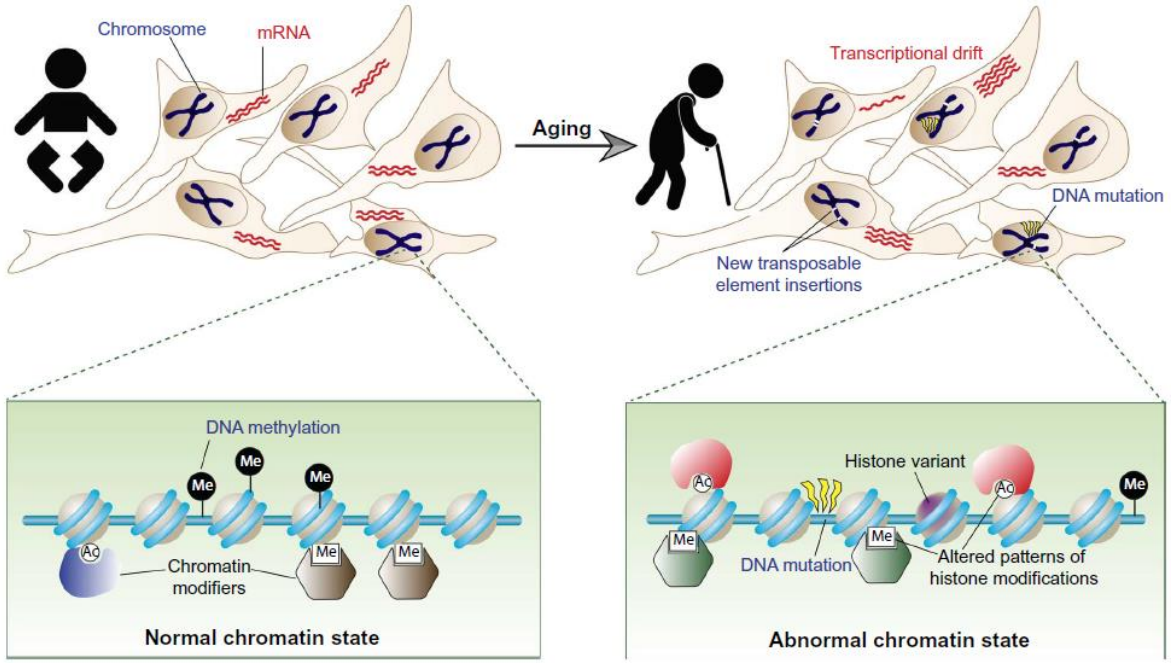

Fig. 1. Overview of epigenetic changes during aging. In young individuals, the cells within each cell type have a similar pattern of gene expression, determined in large part by each cell having similar epigenetic information. During aging, the epigenetic information changes sporadically in response to exogenous and endogenous factors. The resulting abnormal chromatin state is characterized by different histone variants being incorporated, altered DNA methylation patterns, and altered histone modification patterns, resulting in the recruitment of different chromatin modifiers. The abnormal chromatin state in old cells includes altered transcription patterns and transcriptional drift within the population. The abnormal chromatin state in old cells also leads to new transposable elements being inserted into the genome and genomic instability, including DNA mutations.

Fig epigenetic changes during aging (Pal & Tyler, 2016a)

Noncoding RNAs (ncRNAs) and aging

Various ncRNAs are the most recent players in the epigenetics field, influencing seemingly all biological processes in virtually all organisms. It is now widely accepted that approximately 60 to 90% of the human genome is transcribed, giving rise to an enormous array of ncRNA. Until recently, most of the studies focused on the **short ncRNAs**, but the functional importance of **long ncRNAs (lncRNAs)** is now becoming apparent. Although it is likely that the majority of these functions are epigenetic, with the ncRNAs having a significant influence on modulating gene expression and chromatin packaging, the complete array of biological functions of ncRNAs is yet to be understood. Disruption of ncRNA function has been implicated in numerous disease conditions, such as cancer, neurodegenerative disorders, cardiovascular disorders, and aging (Huarte, 2015; Yongzhen Li et al., 2019; Ren et al., 2018; Toomey et al., 2016) (Soriano-Tárraga et al., 2019). miRNAs are sncRNAs that negatively control their target gene expression post-transcriptionally and have been implicated in aging. Although miRNAs do not alter the chromatin structure, they are considered mediators of epigenetics because they lead to heritable changes in gene expression that do not involve a change in the DNA sequence. The best-characterized examples of roles of miRNAs during aging come from studies in C. elegans. Several miRNAs are involved in modulating life span and in controlling tissue aging. One of the most prominent examples includes the regulation of aging by the miRNA lin-4 and its pro-aging target miRNA lin-14. Loss of function of lin-4 shortens life span, whereas overexpression of lin-4 extends life span. In contrast, knocking down lin-14, even in adult animals, extends life span. Studies have demonstrated that the longevity-modulating miRNAs lin-4 and lin-14 function in the same pathway as DAF-2 and DAF-16, modulating life span through the insulin/ insulin-like growth factor 1 (IGF-1) signaling pathway. Indeed, the majority of miRNAs are down-regulated with age

(Goodall et al., 2019; Huan et al., 2018; Kinser & Pincus, 2020; McGregor & Seo, 2016; Soriano-Tárraga et al., 2019). The evolutionarily conserved nature of some of these miRNAs indicates that their role in life span regulation likely extends beyond this organism to larger eukaryotes, including humans. Clearly, changes in miRNA levels during aging are a means to regulate target gene expression, in addition to chromatin changes. (Abdelmohsen et al., 2013a; Szafranski et al., 2015)

lncRNAs also serve as important regulators of transcription through their interaction with chromatin or chromatin-associated factors, modulating aging and senescence directly or indirectly. One such example includes a specific lncRNA, Gas5, which is highly expressed in aged mice brain and has been associated with impaired learning. Another example is H19 lncRNA, a differentially spliced product from the H19 gene located at the IGF2/H19 imprinted locus, which interacts with methyl-CpG–binding domain protein 1 to form a complex to repress expression of an imprinted gene network in mice. Loss of imprinting from the IGF2/H19 locus has been shown during aging in both mice and human prostate associated with re-expression of certain inactive genes and loss of binding of chromatin-associated protein CTCF. This may serve as a potential reason for increased prostate cancer occurrence in aging men, further supporting the connection between aging and cancer development. Other lncRNAs that are implicated in **major senescence-associated pathways**, such as p53/p21 pathways, are differentially expressed in proliferating early passage compared to senescent late-passage fibroblast cells Similarly, differential expression patterns of various lncRNAs have been implicated in the pathophysiology of another age-onset neurological disorder, Huntington's disease, wherein some of these lncRNAs have been postulated to modulate **chromatin architecture and/or transcription**. Other ncRNAs, mostly products of the RNAi pathway, are involved in heterochromatin assembly in repetitive DNA elements in diverse organisms. (Abdelmohsen et al., 2013b; Castel & Martienssen, 2013; Guang et al., 2010; Kour & Rath, 2016).

In **the absence of Dicer** (which cleaves double-stranded RNA and pre-microRNA into short double-stranded RNA fragments called small interfering RNA and microRNA) in mice, there are signs of early senescence, signifying its role in longevity. A decrease in Dicer levels has also been observed in adipocytes collected from elderly humans, suggesting the possibility of a conserved mechanism of sncRNA dysregulation during aging in mammals (R. M. Anderson, 2012).

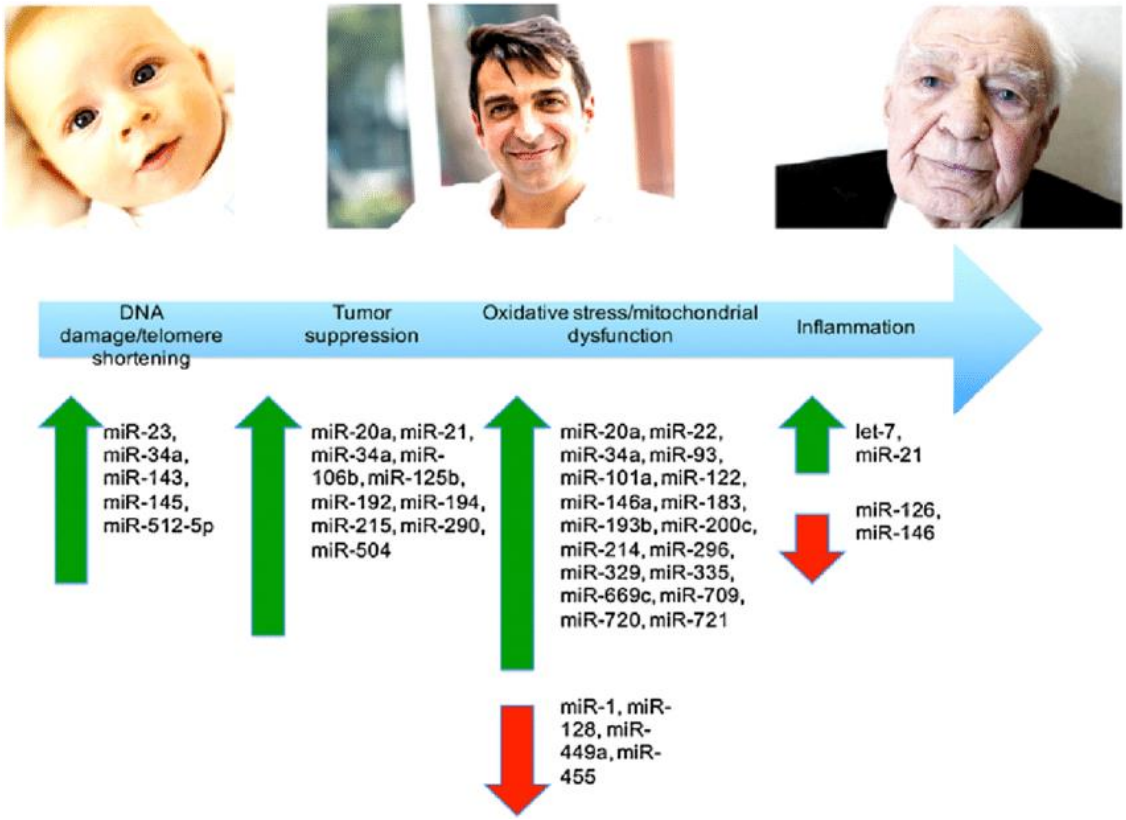

Fig miRNAS, aging, senescence (Williams et al., 2017)

Aging of the Immune system (I.S.) and epigenetics

Old age is associated with a reduced immune function that ultimately leads to elderly individuals becoming less responsive to vaccination and more susceptible to a range of infections. Changes in both the innate and adaptive arms of the immune system have been documented in aging. The intrinsic functional reduction in immune competence is associated with low-grade chronic inflammation, termed "**inflammaging,"** characterized by high levels of circulating cytokines and latent viral infections. This inflammatory state then further perpetuates the age-related immune dysfunction. Stem cell diversity also appears to reduce with age with an exponential increase in the occurrence of clonal hematopoiesis, where one mutant hematopoietic stem cell (HSC) produces an overwhelming proportion of mature blood cells, especially after the age of 45–60 in humans. The **thymus**, where T cells develop, begins to involute at puberty in both mice and humans due to age-related changes that affect both T-cell progenitors and the thymic microenvironment. Similarly, decreased hematopoietic activity in the bone marrow of mice and humans means that B-cell lymphopoiesis also decreases with age. **DNA damage** both promotes cellular senescence in order to allow **DNA repair** mechanisms and causes activation of the innate immune system to clear damaged cells. The reduced capacity of the aged immune system to clear these cells, therefore, results in an accumulation of genomically damaged and senescent cells within all tissues of the body, including within the immune system itself. DNA methylation levels globally reduce in HSCs and mature leukocytes (and other tissues) as mice or human age. Age-associated changes in DNA methylation have been reported in a number of human immune cell types including monocytes and $CD4^+$ and $CD8^+$ T cells and many of these changes appear to be cell type-specific. While DNA methylation at a

global level is reduced with age, discrete sites are hypermethylated. (Allis & Jenuwein, 2016; Briceño et al., 2016; Busslinger & Tarakhovsky, 2014; Cambier, 2005; Chambers et al., 2007; Keenan & Allan, 2019)

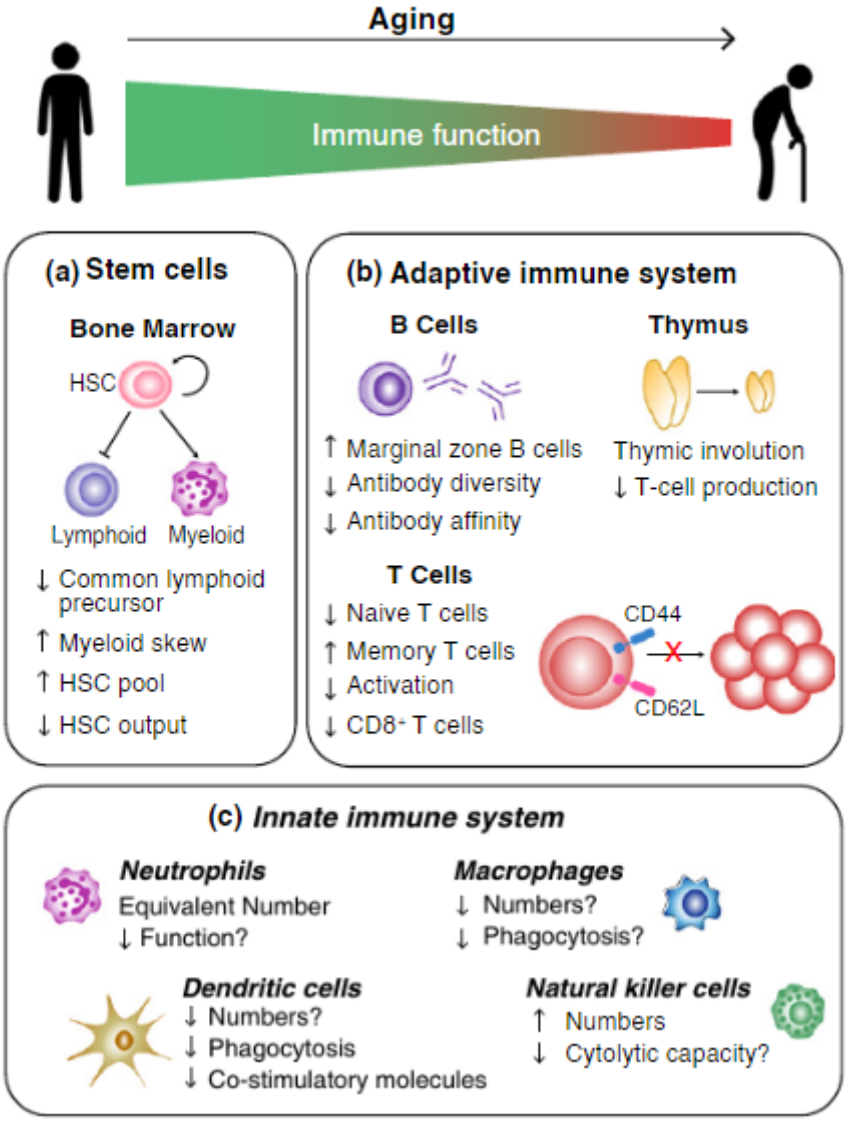

Fig Dysfunctions of the aged I.S. (Keenan & Allan, 2019)

Neurodegenerative diseases, aging and epigenetics

One of the most notable features of human aging is neurodegeneration and reduced brain function. Premature death of neurons is considered to be a major feature of neurodegenerative diseases. Studies of neurodegenerative processes implicate ncRNAs. Whereas some of the miRNAs correlate with neuroprotection, others clearly contribute toward neurodegenerative diseases and/or aging. For example, the mir-34 family appears to be an important determinant for brain aging in flies and maybe also in worms. There are higher levels of mir-34 in Alzheimer's disease mouse model brains and samples collected **from Alzheimer's disease** patients. The pro-survival factor BCL2 and the

antiaging deacetylase SIRT1 both serve as targets of mir-34, and the expression of the latter correlates inversely with mir-34 expression, revealing a potential mechanism for mir-34 function in the aged brain. Similarly, another miRNA, mir-144, seems to be enriched in aged brains and may also contribute to age-associated neurodegeneration through down-regulation of key protective factors.

Transgenerational epigenetic changes and aging

According to biological dogma, genetics governs all the inherited traits across generations, and epigenetic modifications are reset upon passage through the germ line. However, over the years, this notion was challenged when evidence of **epigenetic inheritance** through meiosis became acknowledged in certain processes, such as flower symmetry and colour in plants, or coat colour and size in mice. Recently, longevity mediated by histone methylation was shown to be epigenetically inherited for several generations , implicating transgenerational epigenetic inheritance for the first time in the regulation of life span. (Berger, 2012; Emerson et al., 2020; Greer et al., 2011; Mango, 2011; Muers, 2011).

Deficiencies in either of the three components of H3 K4me3 methylase complex (ASH-2, WDR-5, or SET-2), in only the parental generation, resulted in life span extension in C. elegans in the three subsequent generations, in the absence of methylase deficiency in these offspring's.

Microbiota and healthy aging

The accumulation of bacteria, archaea and eukaryota that colonizes the gastrointestinal tract is referred to as the "gut microbiota" and has developed over thousands of years with the host into a complicated and mutually beneficial relationship. The human gastrointestinal tract (GIT) is one of the largest interfaces (250-400 m2) between the host, environmental factors, and antigens in the human body. In an average lifetime, around 60 tons of food pass through the human digestive tract, together with an abundance of environmental microorganisms that pose a major threat to intestinal integrity. It has been estimated that more than 10^{14} microorganisms live in the GIT, which is about ten times the number of bacterial cells and more than one hundred times the genome content (microbiome) of the human genome. However, a recently revised estimate has shown that the ratio of human to bacterial cells is actually closer to 1: 1 (Thursby & Juge, 2017).

The typical **adult microbiota** contains about 1000-1150 types of bacteria. However, some experts estimate that only 160 of these species constitute the core microbiota that is found in almost all humans, while the occurrence of other species can vary widely (Qin, Li, Raes, Arumugam, Burgdorf, & Manichanh, p. 2010).

Human gut microbiome is composed of several different phyla, including **Bacteroidetes, Firmicutes and Actinobacteria.** However, it could be shown that> 90% of the bacterial species are members of Bacteroidetes or Firmicutes (Eckburg, M. Bik, Bernstein, Purdom, Dethlefsen, & Sargent, 2005).

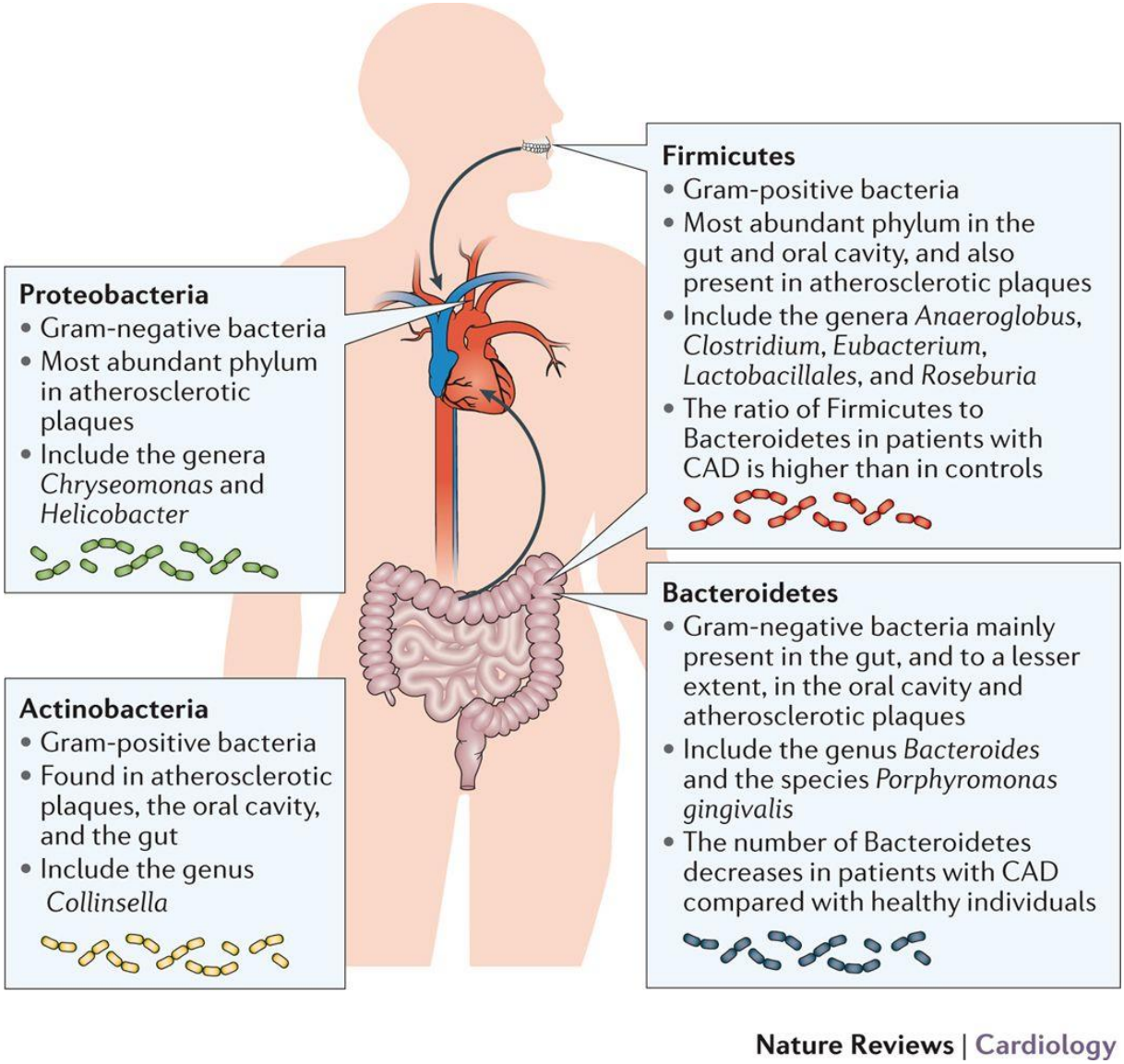

Fig Human microbiota (Jonsson & Bäckhed, 2017)

The gut microbiome plays a central role in many physiological and immunological processes, e.g. in the defense against pathogens and in the development of immune and intestinal barrier functions. (West, Renz, Jenmalm, Kozyrskyi, Allen, & Vuillermin, The gut microbiota, and inflammatory noncommunicable diseases: associations and potentials for gut microbiota therapies., 2015).

It is involved in many aspects of **metabolism**, including the production of bile acids, lipids, vitamins, choline, and polyamine (Nicholson, Holmes, Kinross, Burcelin, Gibson, & Jia, 2012).

The intestinal flora is also involved in the breakdown of indigestible polysaccharides (fermentation of resistant starch, oligosaccharides and inulin), whereby energy for the host is obtained from foods that are absorbed but not digested by the host (Bäckhed, Ding, Wang, Hooper, Koh, & Nagy, 2004).

According to the **16S ribosomal DNA sequencing** data of faecal samples, individual gut microbiota show distinct profiles, and this inter-individual variation is greater in older adults. Longitudinally, however, gut microbiotas of healthy adults are relatively stable even for decades. These phylogenetic

data are supported by metagenomic analysis of whole shotgun sequencing data: SNP variation patterns show stability over time. Thus, once established early in life (even within three years after birth), the gut microbiota seems to be rather stably maintained. Nevertheless, it is responsive to the host's dietary and health conditions, much as the host's epigenome is to various environmental cues. In fact, the **gut microbiota interfaces the gut environment with the epigenome**, but its communication with the host systems involves various signaling networks and their mediators. For instance, the "gut-brain axis" connects the gut microbiome with the central nervous system via neurons, hormones, or cytokines. Despite the marked inter-individual variation in the gut microbiota profile, an array of bacterial genes exists that individual hosts share, as shown by functional metagenomics]. This "functional core microbiome" is collectively provided by different microbial taxa, indicating that different microbial species can functionally replace one another. The presence of such a **core microbiome** makes sense if the core functions concern housekeeping or other important biochemical or physiological pathways(Cani & Delzenne, 2009).

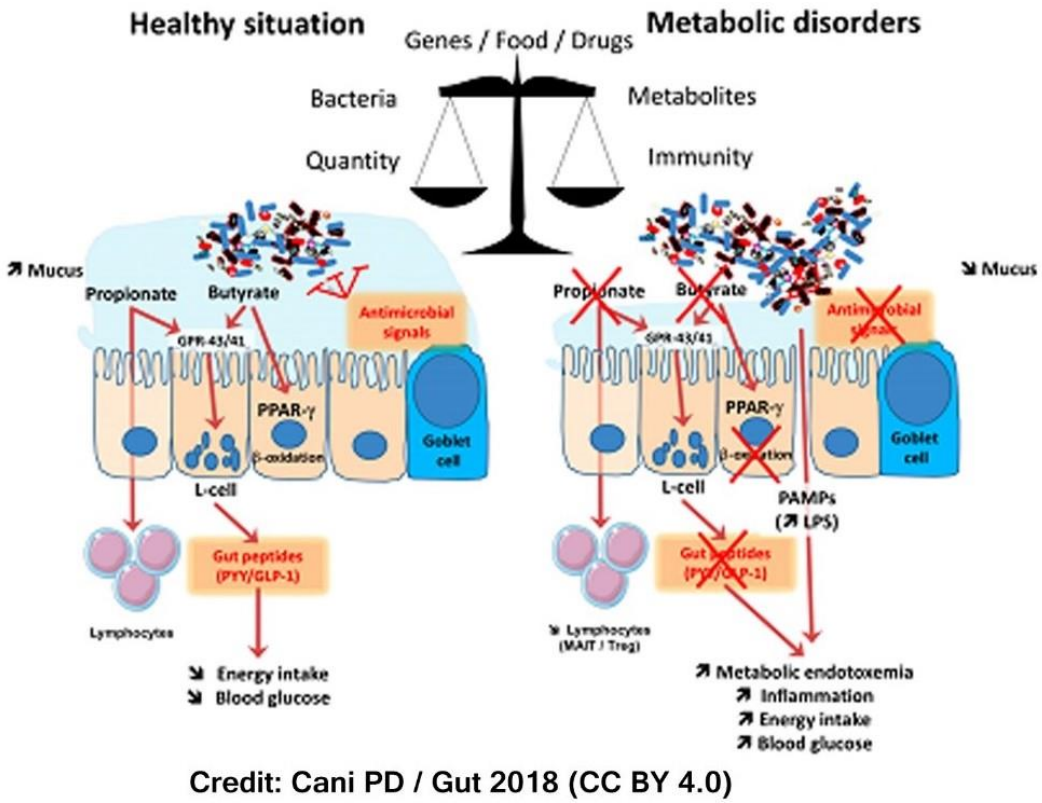

Fig Disbalanced microbiota and metabolic disorders (Cani, 2018)

Development of the gut microbiota: It was generally believed that the development of the microbiota begins from birth. Recently it became clear that the community structure of the intestinal microbiota and metabolism of the mother shifts during pregnancy (Koren et al., 2012) and that microbes colonize the amniotic fluid, the umbilical blood cord, and the placenta, indicating maternal **microbial colonization of the fetus** in utero (D'argenio, 2018) with important consequences for healthy further development.

After birth, the gastrointestinal tract is quickly populated, and life events such as illness, antibiotic treatment, and dietary changes lead to chaotic changes in the microbiota. The type of birth also

influences the composition of the microbiota, since, in small children given vaginally, the microbiota contains a high proportion of lactobacilli in the first few days, which is due to the high contamination of the vaginal flora with lactobacilli. In contrast, the microbiota of **Caesarean section** infants is exhausted and delayed in colonizing the genus Bacteroides but is colonized by facultative anaerobes such as Clostridium species. While the faecal microbiome of 72% of the vaginally born infants is similar to their mother's faecal microbiome, this percentage is reduced to only 41% in cesarean babies. In the early stages of development, the microbiota is generally low in diversity and is dominated by two main phyla, Actinobacteria and Proteobacteria. (Chong et al., 2018; Kundu et al., 2017; Milani et al., 2017; Round & Mazmanian, 2009; M. Tanaka & Nakayama, 2017)

During the first year of life, **microbial diversity** increases and the composition of the microbiota converges towards a pronounced, adult-like microbial profile with time patterns that are unique to each child. At around 2.5 years of age, the composition, diversity and functionality of the microbiota in infants are similar to those of the microbiota in adults. Although the composition of the gut microbiota is relatively stable in adulthood, it is still subject to disturbances from life events (Thursby & Juge, 2017).

Function of the microbiota: The **fermentation** of complex carbohydrates creates **short-chain fatty acids (SCFAs)** that are involved in many cellular processes and metabolic pathways, strengthen the intestinal barrier function and regulate the immune system and inflammatory reactions:

One potentially productive approach to the roles of the gut microbiota in human aging is to compile age-related changes in the gut microbiota and examine whether these changes have any biological relevance. Cross-sectional studies of fecal samples from individuals in different age groups suggest age-related changes in the gut microbiota composition and diversity, which concurs with longitudinal study results. In general, the gut microbiota of the elderly becomes more diverse and variable with advancing age. For instance, the three bacterial families in the core microbiota become less abundant in older age groups, while certain health-associated species become more abundant in older age groups including centenarians and semi-supercentenarians (aged 105-109). These changes in composition and diversity are also reflected in age-dependent reshaping of co-abundance networks. Certain changes in composition and diversity are associated with biological or functional age, independent of chronological age. Various measures of frailty have been used as indicators of biological age, and gut microbiota composition is associated with biological age. Also, gut microbial diversity inversely correlates with biological age, but not with chronological age. Furthermore, a co-abundance module consisting of Ruminococcus, Coprobacillus, and Eggerthella genera becomes abundant with an increase in biological age, independent of chronological age (Maffei 2017). The first two genera of this module belong to the Firmicutes phylum and the last one to the Actinobacteria. An interpretation of these results is that as biological age increases, overall gut microbiota richness decreases, while some microbial taxa associated with unhealthy aging emerge. Thus, what happens in the gut microbiota with advancing biological age can be very different from what happens with chronological age, which illustrates the importance of using a biological or functional measure in aging studies.

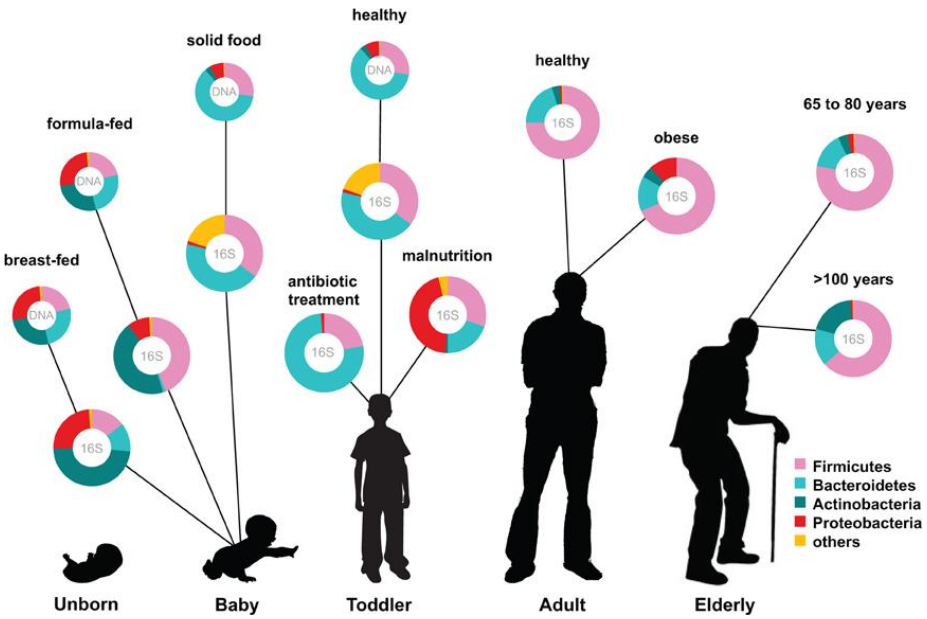

Fig Functions and metabolites of microbiota in aging (Nagpal et al., 2018)

Short-chain fatty acids are products of the breakdown of dietary fibres by the anaerobic gut microbiota. They can easily enter the circulation from the gut and have beneficial roles in energy metabolism. Acetate can reduce serum cholesterol and triglyceride levels, propionate can lower glucose levels, and butyrate can increase insulin sensitivity in mice. Age-related changes in the metagenomics of short-chain fatty acid production have been observed. For instance, frequencies of genes encoding short-chain fatty acid production and those involved in carbohydrate breakdown decrease, while those genes involved in protein breakdown increase. The reduced frequency of genes for short-chain fatty acid production is also associated with frailty. Thus, the short-chain fatty acids have the potential to modulate healthy aging. (Den Besten et al., 2013)

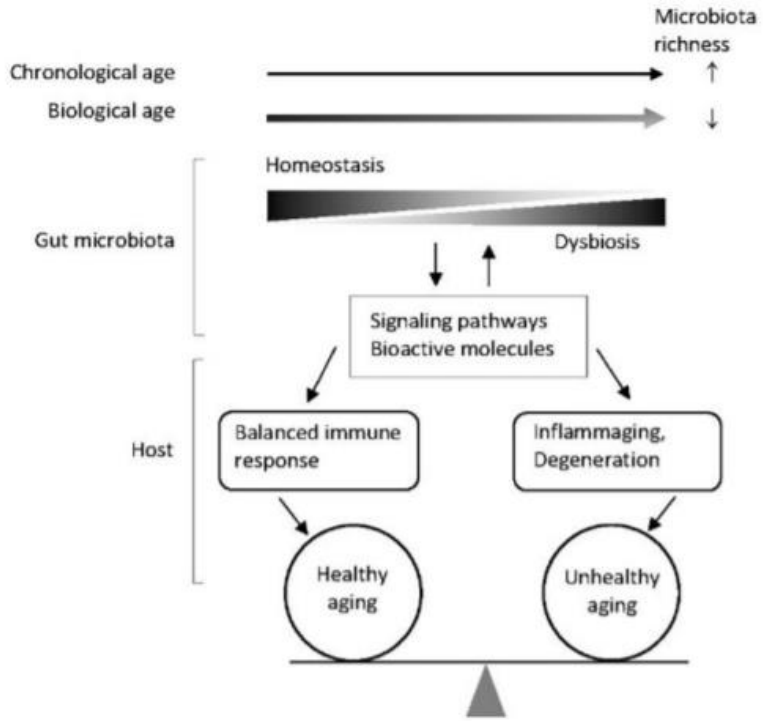

Fig. 1.
Biological age-dependent gut dysbiosis and unhealthy aging. Biology of aging is better approached using a functional measure of age. An increase in chronological age (in the direction of the arrow) is associated with an increase in phylogenetic richness of the gut microbiota, but an increase in biological age shows an inverse association. As biological age increases, homeostasis of the gut microbiota with the host decreases, while dysbiosis increases. The dysbiotic changes are communicated to the host through various signaling pathways and bioactive molecules, which either delays or promotes proinflammatory immune responses and age-related degenerative pathologies.

Fig Functions and metabolites of microbiota in aging (Nagpal et al., 2018)

Lifestyle and healthy aging

A healthy lifestyle has been defined as 'a way of living that lowers the risk of being seriously ill or dying early'; the emphasis is that 'health is not just about avoiding disease. It is also about physical, mental and social wellbeing' (World Health Organization 1999). The term 'lifestyle' refers to personal choices that might influence health, such as diet, **physical activity**, smoking and alcohol consumption. In an independent report, each of these four lifestyle factors are prioritised for the effective delivery of health and wellbeing in England (Bernstein et al 2010). The integration of lifestyle advice into the care given to all patients is an important step towards promoting healthy aging. According to Bowling and Dieppe (2005), healthy or successful aging is most commonly described by the general population in terms of self-perceived health.

Evidence for the shared benefits of a number of healthy lifestyle practices is more established than evidence pertaining to diet alone. Together, a **Mediterranean-type diet** along with non-smoking, moderate alcohol consumption and physical activity have been found to be associated with a more

than **50% lower risk of all-cause mortality** and mortality from coronary heart disease, cardiovascular disease and cancer (Knoops et al 2004). However, physical activity tends to decline as people age. In England, only 19% of people aged 65-74 years and 7% of people aged over 75 years do the recommended levels of exercise (NHS Information Centre 2008), which is currently 30 minutes of moderate physical activity at least five times per week (Department of Health 2004). Reduced physical activity can occur for a number of reasons, including lower level functioning, fear of falling, disability and low health literacy in terms of the benefits of physical activity. Yet there is strong evidence for the benefits of remaining active throughout a person's lifespan. Indeed, there is evidence that rather than being confronted with declining physical fitness, older people can take steps to improve physical function. One systematic review demonstrated that older adults with different levels of abilities could improve their functional performance by regular exercise training (Chin et al 2008). In the **Vienna active aging study** six months of elastic band resistance training lead to improvements in antioxidant defense, DNA stability and oxidative damage in institutionalized elderly (Franzke et al., 2018).

Nutrition and healthy aging

Nutritional sciences have determined the levels of calories, macronutrients, and micronutrients necessary to stave off starvation and deficiency diseases. These amounts are the basis for dietary recommendations. For the most part, these recommendations do not account for age, and the nutritional requirements of older adults are rarely specifically investigated. **Protein reference value** was recently raised for older adults (1mg/kg BW/d) compared to younger adults (0.8mg/kg BW/d) (DGE et al. 2019). The extent of muscle wasting differs greatly between individuals due to differences in the aging process *per se* as well as physical activity levels. Interventions for sarcopenia include exercise and nutrition because both have a positive impact on protein anabolism but also enhance other aspects that contribute to well-being in sarcopenic older adults, such as physical function, quality of life, and anti-inflammatory state. (KHN, 2018a, 2018b; Strasser et al., 2018) Age-associated physiologic changes also put older adults at risk of deficiency—the loss of gastric acidity can contribute to **vitamin B_{12}** deficiency. The integrative strategy combines foods into patterns of consumption. The patterns can be derived a priori based on hypothesized benefits of certain patterns (e.g., vegetarian pattern), or derived from the populations to reflect patterns of foods observed to be consumed together (e.g., Mediterranean diet pattern). A pattern-based approach may have advantages in communicating prevention messages since persons do not eat nutrients they eat foods, and these foods are selected not in isolation but as a part of a broad pattern of choices that make up the totality of diet. Primary among the practical challenges in conducting human nutrition research is the difficulty people have in recalling/tracking what they eat and drink. With the exception of controlled feeding studies, accuracy is elusive. Persons tend to systematically underestimate intake, and this underreporting can be correlated with other variables that also predict outcomes such as body mass index or education. (Stephen B. Kritchevsky, 2016)

Sandoval-Insausti and co-workers' explore relative macronutrient intake as it relates to incident **frailty** in 1,822 community-dwelling older Spanish adults (Sandoval-Insausti et al., 2016). Higher protein intake and higher monounsaturated fatty acid intake are both inversely associated with frailty risk. This is consistent with earlier data showing higher protein intake to be associated with the retention of lean mass in men and women and lower frailty risk in women (Houston et al., 2008; Stephen B. Kritchevsky, 2016; León-Muñoz et al., 2014; Rahi et al., 2016; Sandoval-Insausti et al.,

2016). **Mediterranean diets** are notable for being rich in monounsaturated fatty acids and have been linked to lower rates of frailty (León-Muñoz et al., 2014; Rahi et al., 2018; Talegawkar et al., 2012). Gopinath and co-worker's use data from the Australian Blue Mountain Eye Study to examine the relationship between dietary glycaemic index, glycaemic load, and fibre intake, and healthy aging over 10 years (Gopinath et al., 2016; Sieri et al., 2015). The study population was 49 years of age at baseline. Healthy aging was defined as the absence of disability, depressive symptoms, cognitive impairment, respiratory symptoms, and chronic diseases. Participants with energy-adjusted cereal/grain fibre intakes above the median were nearly twice as likely to age healthfully as those with intakes below the median. The total mortality rate was also lower in the high-fibre group. Several studies in this issue examine nutritional biomarkers.

Ma and co-workers randomized 180 Chinese adults with mild **cognitive impairment** to receive a folic acid supplement (400 mcg/d) or a placebo for 6 months. The supplementation improved both folic acid status and a number of other compounds involved in the one carbon metabolism. Remarkably, the intervention group showed statistically significant improvements in IQ, and digit span and block design performance. These findings reconfirm that nutrition plays an important role in how we age, and that there are choices to be made that can beneficially affect trajectories of physical and cognitive function (An et al., 2019; Ma et al., 2019; Moore et al., 2018). To advance, there needs to be greater investment in randomized trials of dietary interventions that are of sufficient size and duration to understand the functional benefits of potential nutritional choices. These trials should include mechanistic components focused on the hallmarks of biologic aging to link biologic changes to any functional benefits that might be observed. The linking of function and biologic read-outs would support the use of these read-outs in smaller shorter term to increase the pace of establishing health promoting nutritional approaches for older adults.

Omics approaches for the understanding of healthy aging

Recent advances in the development of high-throughput sequencing and different ‘‘omics’’ technologies now allow quantitative analyses of biological molecules at multiple levels, and how they change with aging, with high specificity and sensitivity. Recent advances in the development of high-throughput sequencing and different ‘‘omics’’ technologies now allow quantitative analyses of biological molecules at multiple levels, and how they change with aging, with high specificity and sensitivity. Despite this progress, integration of high throughput data remains a challenging problem. Due to variability of conditions, comparison and integration of omics data collected by different labs remains a challenge. Therefore, care should be taken when interpreting and integrating the data, considering experimental variables. Also, due to high technical variation, high-throughput approaches require proper data normalization and data quality control. Despite these challenges, the unbiased genome-wide analysis and monitoring how complex biological networks change with age are critical for understanding the mechanisms that underlie aging. Specifically, the aging field will benefit from applying systems biology approaches to efficiently integrate the dynamic transcriptional, translational, and metabolic changes with aging and to reliably infer regulatory factors from multi-layered data. Moreover, understanding of the aging process is not possible without taking into account molecular interactions (or **‘‘interactome’’**) occurring among different components of the biological system (proteins, lipids, nucleic acids, etc.) as well as how they affect physiological parameters associated with aging (i.e., ‘‘physiome’’). In fact, aging is indicated as one of

the goals of the ‘‘Human Physiome project’’, which is focused on precisely defining interactions that occur in the human body as a whole by using quantitative computer modeling (Lorusso et al., 2018).

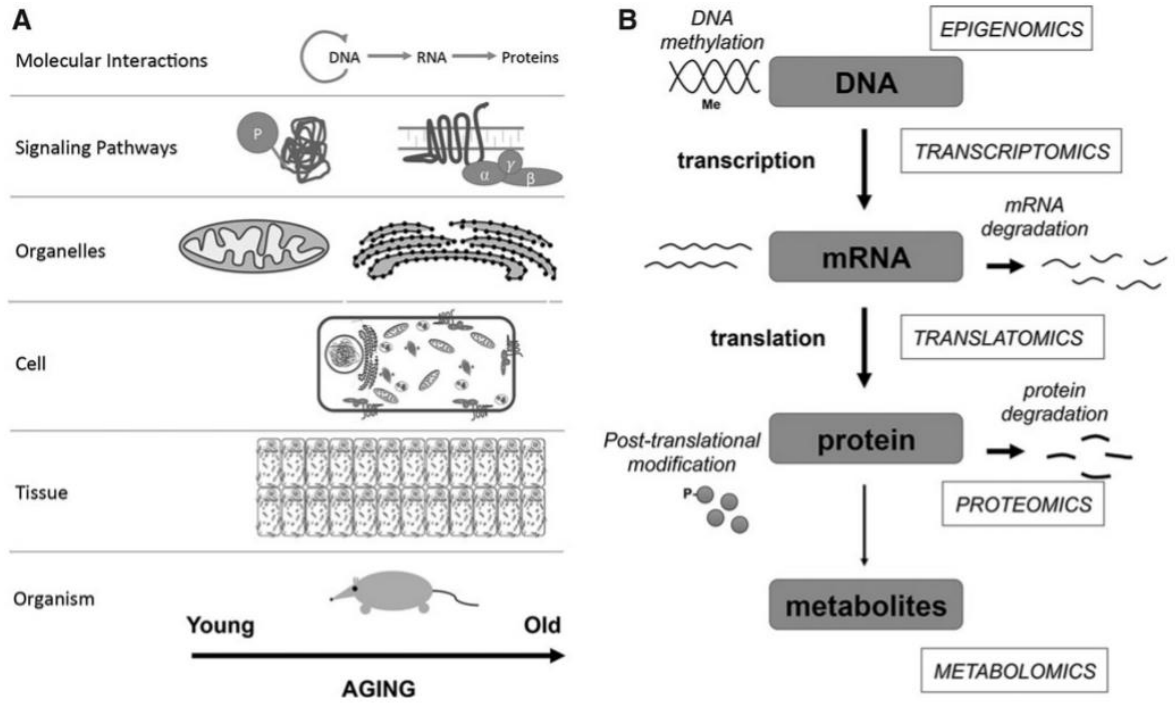

FIG. 1. Systems biology approaches in aging research. (A) Understanding the molecular mechanisms that underlie aging requires integration of multi-layered data about age-dependent changes that occur at the molecular, cellular, tissue, and organismal levels. **(B)** A scheme showing the Central Dogma of Molecular Biology and different ‘‘omics’’ approaches that are currently available for the high-throughput quantitative analysis of molecular changes associated with aging.

Fig System biology (Lorusso et al., 2018)

Genomics: Genomics is the study of whole genomes of organisms and incorporates elements from genetics. Genomics uses a combination of recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyse the structure and function of genomes. It differs from 'classical genetics' in that it considers an organism’s full complement of hereditary material, rather than one gene or one gene product at a time. Moreover, genomics focuses on interactions between loci and alleles within the genome and other interactions such as epistasis, pleiotropy, and heterosis. Genomics harnesses the availability of complete DNA sequences for entire organisms and was made possible by both the pioneering work of Fred Sanger and the more recent next-generation sequencing technology. https://www.ebi.ac.uk/training/online/course/genomics-introduction-ebi-resources

Transcriptomics: Aging is controlled by a combination of genetic, environmental, and stochastic factors. A number of diverse genes have been shown to modulate aging. Studies in invertebrate organisms have led to the identification of hundreds of genes, whose deletion or knockdown can dramatically increase lifespan (Adams, 2008). Moe recently, data indicate that Life span has little to do with genes, analysis of large ancestry database shows. Furthermore, Longevity gene' such as SIRT 6 might be responsible for more efficient DNA repair. (T. E. Johnson, 2002; Keyes et al., 2015; *Life*

span has little to do with genes, analysis of ancestry database shows - STAT, n.d.; *"Longevity gene" responsible for more efficient DNA repair - ScienceDaily*, n.d.; Passarino et al., 2016).

Transcriptional regulation: Expression of many genes is altered with aging, and these changes may have a causative effect on aging and the development of age-associated pathologies. A number of research groups used microarrays and RNA sequencing (RNA-Seq) to analyze age-dependent changes in gene expression in different model organisms as well as in humans. In addition, multiple transcriptome analyses have been performed to identify genome-wide transcriptional changes associated with increased longevity (Fleischer et al., 2018, 2018; Holly et al., 2013; Peters et al., 2015; Tarkhov et al., 2019; Zhuang et al., 2019).

These studies revealed profound changes in the transcriptome with age, which reflect age-associated degenerative processes, but might also result from transcriptional response or adaptation to aging. Moreover, aging is accompanied by increased cell-to-cell and increased inter-individual variation in gene expression, which was attributed to stochastic variation or transcriptional noise. To identify changes in mRNA transcript levels with aging, several bioinformatics tools and statistical methods have been developed for multiple comparison analysis and functional enrichment analysis of RNA-Seq data. In addition, gene expression clustering and dimension reduction approaches, for example, principal component analysis, are commonly used to identify genes with similar expression patterns, which could help identify potential regulators. However, it is hard to establish which of the differentially expressed genes are the regulatory factors and which are the consequences of regulatory changes based only on expression patterns. To identify upstream regulatory factors, one of the commonly used methods is to search for the enrichment of binding sites in the promoter regions of genes with a similar transcription pattern. This approach relies on information about the promoter sequence binding sites identified by chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) analysis. (Anaı¨s et al., 2013; Jaini et al., 2014; Peng et al., 2016; Triska et al., 2017)

Translatomics: Despite the importance of transcriptional regulation in the control of gene expression during aging, many of the genes are also regulated at the level of translation. However, the mechanistic details by which translational control affects the aging process remain poorly understood. Recent technological advances in proteomics and next-generation sequencing now enabled researchers to quantitatively measure the rates of protein translation at the genome-wide level that could facilitate investigation of translational control of gene expression during aging. Recently, a novel approach based on next-generation sequencing, named ribosome profiling or Ribo-Seq, has emerged as a powerful technology to study mRNA translation and mechanisms of translational regulation at the genome-wide level (K. Huang et al., 2019; Neuhaus et al., 2016; J. Zhao et al., 2019).

Proteomics: Although the rate of mRNA synthesis determines the bulk of the specific protein's concentration in the cell, and the regulation at the level of transcription for the most part governs protein expression, regulation at the level of protein translation and turnover appears to fine-tune protein concentration to the cellular needs 197 proteins were recently found to be positively associated, and 20 proteins were negatively associated with age. Growth differentiation factor 15 (GDF15) had the strongest, positive association with age (T. Tanaka et al., 2018; Ubaida-Mohien et al., 2019). Current techniques for high-throughput analysis of protein concentration include liquid chromatography coupled with mass spectrometry (LC-MS/MS), antibody based immune-histochemistry, protein arrays, and fluorescence-based imaging using fluorescent reporters (e.g., GFP-tagged proteins). However, one of the common limitations for the proteomics measurements is sensitivity of detection. Currently, only a small fraction of the proteome can be quantitatively

measured in a single experiment. Also, compared with transcriptomics that can detect different transcript isoforms produced as a result of alternative splicing, proteomics analysis is restricted to a limited number of protein variants.

Tissue-specific aging: Biological age of an organ and the way different organs age depend on its unique physiological function and specific cellular properties. Moreover, one tissue or organ can affect aging of other tissues and even the whole organism. It is important to emphasize the need to survey multiple organs and cell types and to integrate different omics data. Integration of multiple omics approaches such as transcriptomics, proteomics, metabolomics, as well as global analysis of pre-mRNA splicing, and methylation could reveal an in-depth picture of how different components deteriorate with aging, contribution of specific organs to aging, and a better understanding of aging at the level of the whole organism. (Chatsirisupachai et al., 2019; H. Choi et al., 2019; Tu, n.d.)

Post-translational modifications: Among other factors, the activity of the protein is determined by its expression, rate of protein synthesis and protein turnover, as well as protein localization within cells and regulatory . In turn, PTMs can affect the biochemical properties of the protein by changing its structure, enzyme active site environment, and protein- protein interactions. Protein posttranslational modifications, either spontaneous or physiological/pathological, are emerging as important markers of aging and aging-related diseases, though clear causality has not yet been firmly established (Santos & Lindner, 2017).

Epigenomics: Epigenomic changes include DNA methylation and multiple histone modifications that can influence the transcription of genes through inhibiting the binding of transcription factors, heterochromatin formation, and affecting genome stability. The environmental and experimental perturbations of the epigenome and chromatin remodellers have been shown to directly affect lifespan in a variety of organisms. Further, epigenetic modifications have been proposed to be useful biomarkers of aging (Muñoz-Najar & Sedivy, 2011)(Soriano-Tárraga et al., 2019).

Metabolomics: Gradual accumulation of molecular damage, which is produced by numerous metabolic processes, has been proposed to be the principal driver of aging. (Frisard et al., 2007; C. Y. Liu et al., 2016; Redman et al., 2018). Such cumulative damage is not limited to oxidative damage and may include by-products of enzymatic reactions, alterations of gene expression, errors in RNA and protein synthesis, and accumulation of DNA mutations, among others. Moreover, the diversity of these damage forms and by-products of various metabolic processes is expected to increase with advanced age Therefore, analysis of metabolic signatures associated with the old age (Krumsiek et al., 2015) could provide a better understanding of the mechanisms of aging. Two commonly used methods to quantitatively measure low-molecular-weight molecules, or metabolites, include the LC-MS/MS and nuclear magnetic resonance (NMR) spectroscopy. Currently, the Human Metabolome Database (HMDB; https://hmdb.ca/) contains more than 52,000 entries, including both water-soluble and lipid-soluble small molecule metabolites. These studies revealed significant changes in metabolic profiles with age across different species and organs. Most metabolites demonstrate organ-specific patterns, whereas some of the small-molecule metabolites correlate with aging across different organs. In addition to organ-specific changes, significant differences in metabolite levels were observed between men and women in humans. (Krumsiek et al., 2015) However, a set of small-molecule metabolites consistently correlates with age independently of gender, suggesting that metabolite signatures could be used for the development of reliable biomarkers to predict biological age. Importantly, by using non-targeted metabolomics, researchers found that the diversity of metabolites (total number of distinct metabolites) was increased with aging. Untargeted plasma metabolomics quantifying 770 metabolites on a cross-sectional cohort of 268 healthy individuals including 125 twin pairs covering human lifespan enabled a random forest (RF) analysis which was

successful to predict age in adult subjects (≥16 years) using 52 metabolites (R^2 = .97). (Bunning et al., 2020). The appearance of new small-molecule metabolites with aging implies that some of them might represent by-products of metabolism or other damage forms that accumulate in old organisms. Further advances in sensitivity of metabolomics techniques and structural identification of non-targeted hits that accumulate with aging may provide insights into causes of aging.

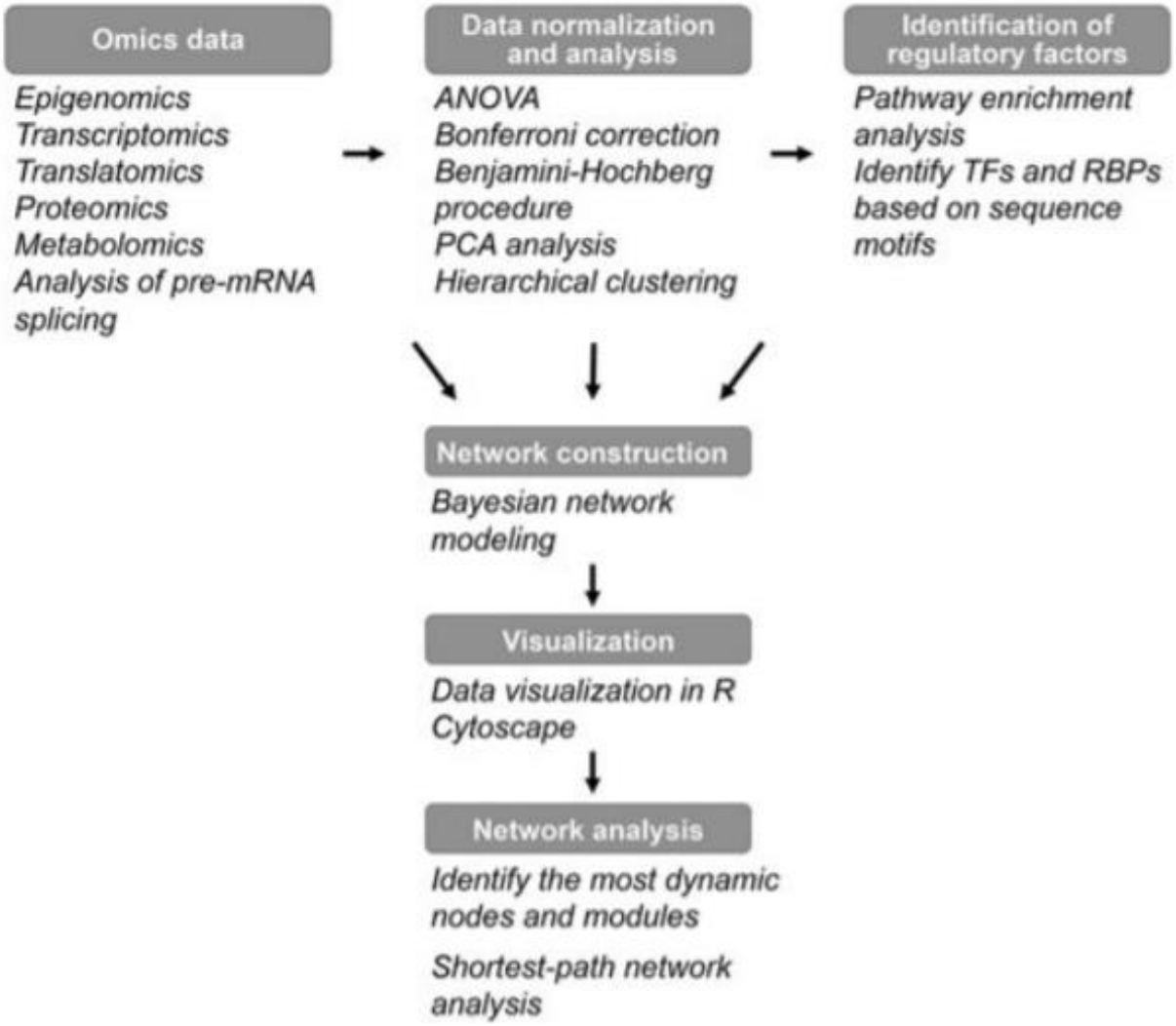

Fig Work flow for data integration (Lorusso et al., n.d.)

Pre- and intervention against premature aging and development of complex diseases

Avoidance of premature aging and the development of complex diseases addresses multiple aspects such as the avoidance of hazards from the environment e.g. toxins or stress, a healthy lifestyle including safe forms of exercise or working balance as well as nutritional aspects WHO concludes: http://www.emro.who.int/about-who/public-health-functions/health-promotion-disease-prevention.html

Disease prevention: Disease prevention, understood as specific, population-based, and individual-based interventions for primary and secondary (early detection) prevention, aiming to minimize the burden of diseases and associated risk factors.

Primary prevention refers to actions aimed at avoiding the manifestation of a disease (this may include actions to improve health through changing the impact of social and economic determinants on health; the provision of information on behavioral and medical health risks, alongside consultation and measures to decrease them at the personal and community level; nutritional and food supplementation; oral and dental hygiene education; and clinical preventive services such as immunization and vaccination of children, adults and the elderly, as well as vaccination or post-exposure prophylaxis for people exposed to a communicable disease.

Secondary prevention deals with early detection when this improves the chances for positive health outcomes (this comprises activities such as evidence-based screening programs for early detection of diseases or prevention of congenital malformations; and preventive drug therapies of proven effectiveness when administered at an early stage of the disease).
It should be noted that while primary prevention activities may be implemented independently of capacity-building in other health care services, this is not the case for secondary prevention. Screening and early detection are of limited value (and may even be detrimental to the patient) if abnormalities cannot be promptly corrected or treated through services from other parts of the health care system. Moreover, a good system of primary health care with a registered population facilitates the optimal organization and delivery of accessible population-based screening programs and should be vigorously promoted.
Health promotion: Health promotion is the process of empowering people to increase control over their health and its determinants through health literacy efforts and multisectoral action to increase healthy behaviours. This process includes activities for the community-at-large or for populations at increased risk of negative health outcomes. Health promotion usually addresses behavioural risk factors such as tobacco use, obesity, diet, and physical inactivity, as well as the areas of mental health, injury prevention, drug abuse control, alcohol control, health behaviour related to HIV, and sexual health.

Disease prevention and health promotion share many goals, and there is considerable overlap between functions. On a conceptual level, it is useful to characterize disease prevention services as those primarily concentrated within the health care sector, and health promotion services as those that depend on intersectoral actions and/or are concerned with the social determinants of health.

The preservation of health and prevention of disease is also one of the most important guiding principles of traditional **Chinese medicine**. Health preservation is a branch of Chinese medicine that teaches practical ways of preventing illness and maintaining good health throughout one's life. This pursuit of health and longevity has especially influenced the lifestyles of traditional Chinese and Asian cultures for over 2,000 years (Q. Wang et al., 2018).

Health-related quality of life: (HRQoL) Health-related quality of life (HRQOL) is an individual's or a group's perceived physical and mental health over time. https://www.cdc.gov/hrqol/index.htm
However, HRQOL is a subjective appreciation of how personal characteristics and health influence well-being but studies aimed to quantitatively measure the influence of dietary, lifestyle, and demographic factors on HRQoL (Pano et al., 2020).

WHO, health and lifestyle: To ensure a healthy lifestyle, WHO recommends eating lots of fruits and vegetables, reducing fat, sugar and salt intake and exercising.12 steps to healthy eating as well as aspects of physical activity are given https://www.euro.who.int/en/health-topics/disease-prevention/nutrition/a-healthy-lifestyle.

Healthy aging and biotech: Many diverse concepts to " fight aging" are under development by biotechnology https://www.fightaging.org/. Diets, functional foods (Wichansawakun & Buttar, 2018), drugs (Justice et al., 2019) or vaccines (Yoshida et al., 2020) were discussed to reduce senescent cells in tissues. Recently senolytic CAR-T cells targeting senescent cells were shown to reverse senescence-associated pathologies (Amor et al., 2020) and blood plasma fractions or modifications were shown to rejuvenate tissue in mice (Mehdipour et al., 2020).

The following chapters focus on aspects of nutrition, foods, food ingredients and their specific functions

Fasting/ caloric restriction

To date, caloric restriction (i.e., a reduction in caloric intake without malnutrition) is the only non-genetic intervention that has consistently been found to extend both mean and maximal life span across a variety of species. Key early studies in rodents revealed that mice fed 55–65% caloric restricted diets through their life exhibited a 35–65% greater mean and maximal lifespan than mice eating a non-purified ad libitum diet (Weindruch, 1996). Although attenuated, these effects remain present even when moderate caloric restriction (20–40%) is implemented in middle-aged mice (Weindruch et al., 2001). Importantly, prolonged caloric restriction has also been found to delay the onset of age-associated disease conditions such as cancer and diabetes in rodents (Weindruch et al., 2001) and nonhuman primates (Colman et al., 2009). Thus, findings from animal studies, including recent primate studies, suggest prolonged caloric restriction has the potential to extend health-span and thereby increase the quality of life. In recent studies conducted in overweight humans, caloric restriction has been shown to improve a number of health outcomes including reducing several cardiac risk factors (Fontana et al., 2004, 2007; Lefevre et al., 2009), improving **insulin-sensitivity** (Larson-Meyer et al., 2006), and enhancing mitochondrial function (Civitarese et al., 2007). Additionally, prolonged caloric restriction has also been found to reduce oxidative damage to both DNA (Heilbronn and Ravussin, 2003; Heilbronn et al., 2006; Hofer et al., 2008) and RNA, as assessed through white blood cells (Hofer et al., 2008). Thus, findings of initial human clinical trials appear to support the promise of caloric restriction demonstrated in animal studies, at least in overweight adults. Several different biological mechanisms may account for the increase in healthspan and longevity observed in response to caloric restriction in preclinical models. For example, aging is characterized by an exponential increase of oxidatively damaged proteins, and caloric restriction has been found to downregulate the expression of genes involved in oxidative stress and ameliorate oxidative damage in several different tissues (Hofer et al., 2009; Kayo et al., 2001; Lee et al., 1999; Marzetti et al., 2009; Opalach et al., 2010; Phillips and Leeuwenburgh, 2005). Additional biological changes associated with the caloric restriction that may contribute to the observed increases in health span and longevity include enhanced cellular quality control through autophagy ("self-eating" of damaged organelles), improved function of the ubiquitin-proteosome system, and the maintenance of a healthy population of mitochondria through biogenesis (generation of new mitochondria) (Aris et al., 2013; Dutta et al., 2012; Kayo et al., 2001; Lee et al., 1999; Rangaraju et al., 2009; Wohlgemuth et al., 2007, 2010).

Despite this health-promoting biological changes, most individuals have difficulty engaging in caloric restriction over the long-term (Scheen, 2008). Due to poor long-term compliance, an important area of study is whether compliance can be improved through the use of natural and/or pharmaceutical compounds that enhance satiety and/or whether similar biological effects may be achieved through alternative behavioural approaches.

One alternative dietary approach that may produce similar biological changes as caloric restriction that has received increasing interest from the scientific community is **Intermittent Fasting**. In contrast to traditional caloric restriction paradigms, food is not consumed during designated fasting periods but is typically not restricted during designated feeding periods. The length of the fasting period can also vary but is frequently several continuous hours. Evidence that this approach may have beneficial effects on longevity first appeared several decades ago (Carlson and Hoelzel, 1946). Since this time, a growing body of literature suggests that fasting periods and intermittent fasting regimens can trigger similar biological pathways as caloric restriction (i.e., increased autophagy and mitochondrial respiratory efficiency), which can result in series of beneficial biological effects including increased circulation and cardiovascular disease protection, and modulation of reactive oxygen species and inflammatory cytokines, periods have also been shown to have antimutagenic, antibacterial, and anticarcinogenic effects (Lee and Longo, 2011). (Anton & Leeuwenburgh, 2013) (A. Ahmed et al., 2018; Patterson & Sears, 2017).

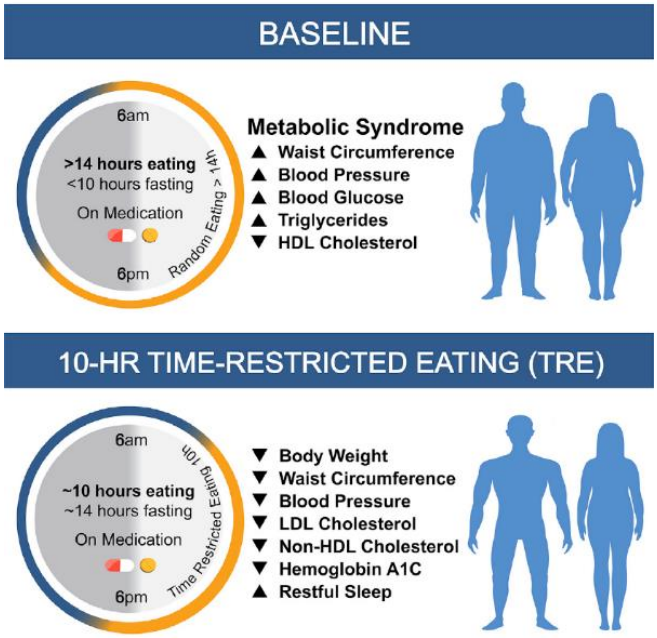

Fig time-restricted fasting (M.J. Wilkinson et al 2020)

Wilkinson and Manoogian et al. studied the impact of time-restricted eating in metabolic syndrome by reducing participant's daily eating window from R14 h to a self-selected 10 h window for 12 weeks. Time-restricted eating led to weight loss, healthier body composition, lower blood pressure, and decreased levels of cardiovascular disease-promoting lipids. (Wilkinson et al., 2020) Therefore, time-restricted eating is a potentially powerful lifestyle intervention to improve metabolic syndrome. (M.J. Wilkinson et al 2020).

According to Weindruch and Sohal in 1997 reducing food availability over a lifetime (caloric restriction) has remarkable effects on aging and the life span in animals. The authors proposed that the health benefits of caloric restriction result from a massive reduction in the production of damaging oxygen-free radicals. At the time, it was not generally recognized that because rodents on caloric restriction typically consume their entire daily food allotment within a few hours after its provision, they have a daily fasting period of up to 20 hours, during which ketogenesis occurs. Since then, hundreds of studies in animals and scores of clinical studies of controlled intermittent fasting regimens have been conducted in which metabolic switching from liver-derived glucose to adipose cell-derived ketones occurs daily or several days each week. Although the magnitude of the effect of intermittent fasting on life-span extension is variable (influenced by sex, diet, and genetic factors), studies in mice and nonhuman primates show consistent effects of caloric restriction on the healthspan (D. L. Longo et al., 2019).

Studies in animals and humans have shown that many of the health benefits of **intermittent fasting** are not simply the result of reduced free-radical production or weight loss. Instead, intermittent fasting elicits evolutionarily conserved, adaptive cellular responses that are integrated between and within organs in a manner that improves glucose regulation, increases stress resistance and suppresses inflammation. During fasting, cells activate pathways that enhance intrinsic defences against oxidative and metabolic stress and those that remove or repair damaged molecules. During the feeding period, cells engage in tissue specific processes of growth and plasticity. However, most people consume three meals a day plus snacks, so intermittent fasting does not occur. Preclinical studies consistently show the robust disease-modifying efficacy of intermittent fasting in animal models on a wide range of chronic disorders, including obesity, diabetes, cardiovascular disease, cancers, and neurodegenerative brain diseases (D. L. Longo et al., 2019).

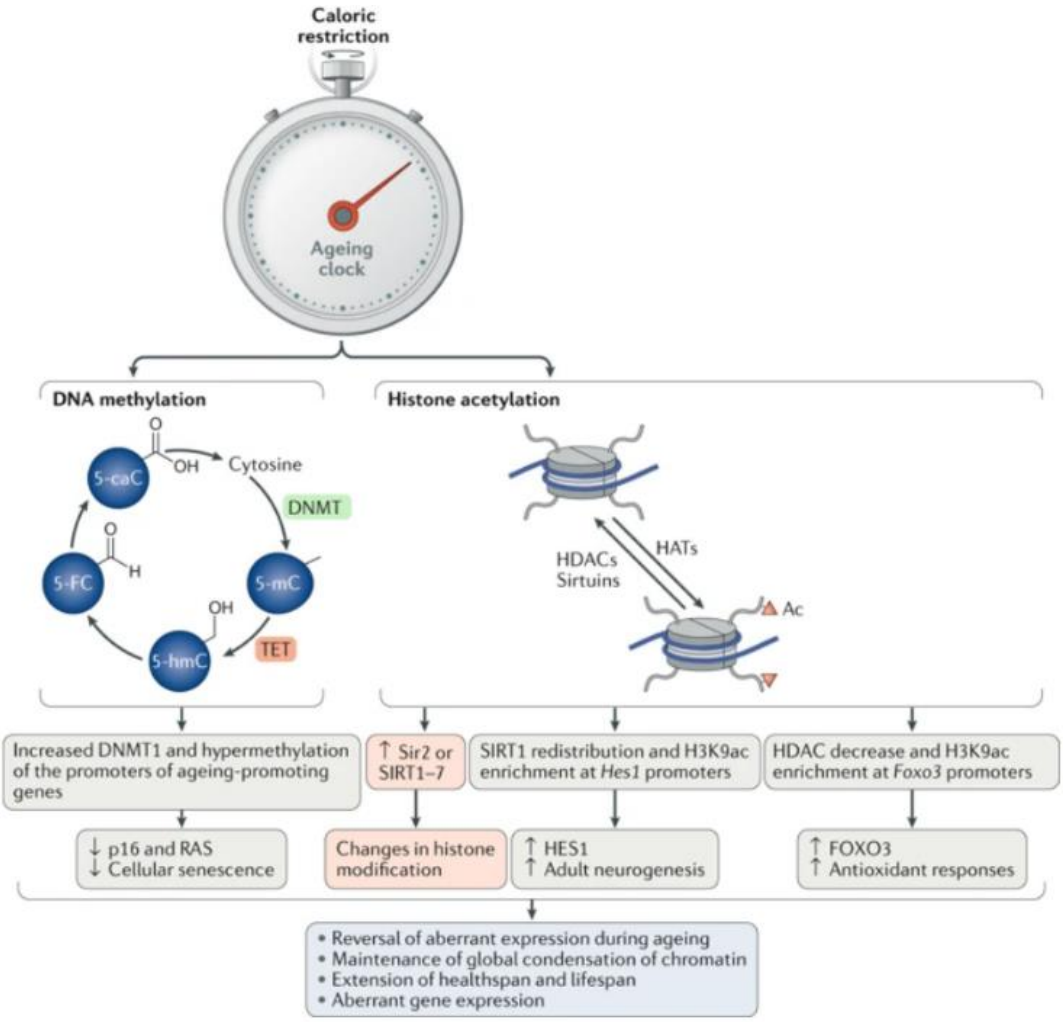

Fig Diets, caloric restriction and the epigenetic clock clock, (Y. Zhang & Kutateladze, 2018) Ac, histone acetylation; 5-caC, 5-carboxycytosine; 5-FC, 5-formylcytosine; HATs, histone acetyltransferases; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine

Caloric restriction (CR) is a powerful rejuvenation strategy to turn back the clock of aging. CR influences epigenetic processes via two representative mechanisms: DNA methylation and histone modification. For example, CR activates DNA methyltransferases (DNMTs) to hypermethylate the promoter regions of aging-promoting genes (such as those encoding p16 and RAS, the activation of which induces cellular senescence), ceasing the expression of these genes. CR also modulates ten–eleven translocations (TETs) to change the global DNA methylation landscape. At the histone level, CR is widely reported to be able to activate Sir2 or the seven homologs of SIR2 in mammals (SIRT1–SIRT7). On the other hand, CR causes a redistribution of SIRT1 on chromatin, causes enrichment of Lys9-acetylated histone H3 (H3K9ac) at the promoters of Hes1(a basic helix–loop–helix transcriptional regulator) and promotes adult neurogenesis. Other histone deacetylases (HDACs) may be inhibited by CR, leading to higher levels of H3K9ac at the promoter of the transcription factor Foxo3, and induce antioxidant responses. Together, CR-induced epigenetic changes result in the maintenance of global condensation of chromatin, a reversal of aberrant gene expression and eventual extension of healthspan and lifespan. (Weiqi Zhang et al., 2020) (Zhang et al. 2020).

Periodic flipping of the metabolic switch not only provides the ketones that are necessary to fuel cells during the fasting period but also elicits highly orchestrated systemic and cellular responses that carry over into the fed state to bolster mental and physical performance, as well as disease resistance. patients with metabolic disorders (obesity, insulin resistance, hypertension, or a combination of these disorders).

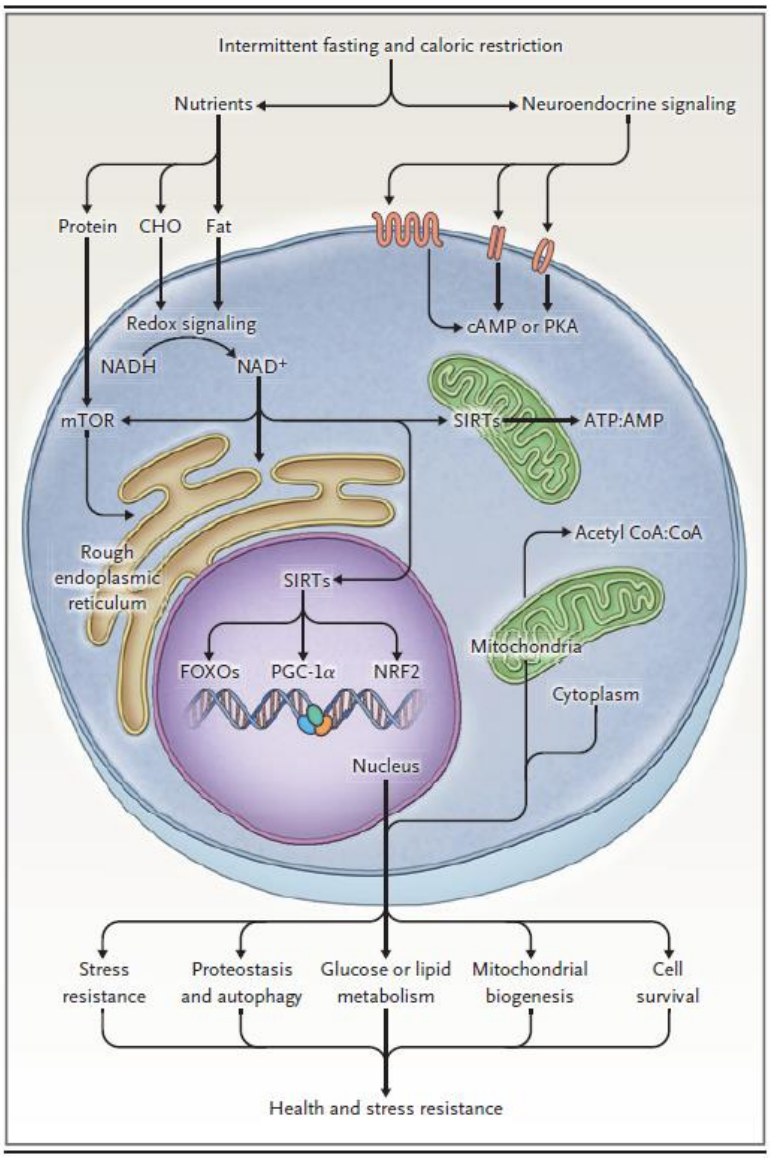

Fig Cellular Responses to Energy Restriction That Integrate Cycles of Feeding and Fasting with Metabolism. Total energy intake, diet composition, and length of fasting between meals contribute to oscillations in the ratios of the levels of the bioenergetic sensors nicotinamide adenine dinucleotide (NAD+) to NADH, ATP to AMP, and acetyl CoA to CoA. These intermediate energy carriers activate downstream proteins that regulate cell function and stress resistance, including transcription factors such as forkhead box Os (FOXOs), peroxisome proliferator–activated receptor γ coactivator 1α (PGC-1α), and nuclear factor erythroid 2–related factor 2 (NRF2); kinases such as AMP kinase (AMPK); and deacetylases such as sirtuins (SIRTs). Intermittent fasting triggers neuroendocrine responses and adaptations characterized by low levels of amino acids, glucose, and insulin. Down-regulation of the insulin–insulin-like growth factor 1 (IGF-1) signaling pathway and reduction of circulating amino acids repress the activity of mammalian target of rapamycin (mTOR), resulting in inhibition of protein synthesis and stimulation of autophagy. During fasting, the ratio of AMP to ATP

is increased and AMPK is activated, triggering repair and inhibition of anabolic processes. Acetyl coenzyme A (CoA) and NAD+ serve as cofactors for epigenetic modifiers such as SIRTs. SIRTs deacetylate FOXOs and PGC-1α, resulting in the expression of genes involved in stress resistance and mitochondrial biogenesis. Collectively, the organism responds to intermittent fasting by minimizing anabolic processes (synthesis, growth, and reproduction), favouring maintenance and repair systems, enhancing stress resistance, recycling damaged molecules, stimulating mitochondrial biogenesis, and promoting cell survival, all of which support improvements in health and disease resistance. The abbreviation cAMP denotes cyclic AMP, CHO carbohydrate, PKA protein kinase A, and redoxreduction–oxidation (De Cabo & Mattson, 2019).

Glucose and fatty acids are the main sources of energy for cells. After meals, glucose is used for energy, and fat is stored in adipose tissue as triglycerides. During periods of fasting, triglycerides are broken down into fatty acids and glycerol, which are used for energy supply. The liver converts fatty acids to ketone bodies, which provide a major source of energy for many tissues, especially the brain, during fasting. In the fed state, blood levels of ketone bodies are low, and in humans, they rise within 8 to 12 hours after the onset of fasting, reaching levels as high as 2 to 5 mM by 24 hours (A. Ahmed et al., 2018; D. L. Longo et al., 2019; Patterson & Sears, 2017).

The metabolic switch from the use of glucose as a fuel source to the use of fatty acids and ketone bodies results in a reduced respiratory-exchange ratio (the ratio of carbon dioxide produced to oxygen consumed), indicating the greater metabolic flexibility and efficiency of energy production from fatty acids and ketone bodies. Ketone bodies are not just fuel used during periods of fasting; they are potent signaling molecules with major effects on cell and organ functions. Ketone bodies regulate the expression and activity of many proteins and molecules that are known to influence health and aging (De Cabo & Mattson, 2019).

These include peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), fibroblast growth factor 21,22,23 nicotinamide adenine dinucleotide (NAD+), sirtuins, poly(adenosine diphosphate [ADP]–ribose) polymerase 1 (PARP1), and ADP ribosyl cyclase (CD38). By influencing these major cellular pathways, ketone bodies produced during fasting have profound effects on systemic metabolism. Moreover, ketone bodies stimulate the expression of the gene for brain-derived neurotrophic factor with implications for brain health and psychiatric and neurodegenerative disorders (De Cabo & Mattson, 2019).

Repeated exposure to fasting periods results in lasting adaptive responses that confer resistance to subsequent challenges. Cells respond to intermittent fasting by engaging in a coordinated adaptive stress response that leads to increased expression of antioxidant defenses, DNA repair, protein quality control, mitochondrial biogenesis and autophagy, and down-regulation of inflammation (Fig. 3). These adaptive responses to fasting and feeding are conserved across taxa. Cells throughout the bodies and brains of animals maintained on intermittent fasting regimens show improved function and robust resistance to a broad range of potentially damaging insults, including those involving metabolic, oxidative, ionic, traumatic, and proteotoxic stress. Intermittent fasting stimulates autophagy and mitophagy while inhibiting the mTOR (mammalian target of rapamycin) protein-synthesis pathway. These responses enable cells to remove oxidatively damaged proteins and mitochondria and recycle undamaged molecular constituents while temporarily reducing global protein synthesis to conserve energy and molecular resources (De Cabo & Mattson, 2019.)

In conclusion, preclinical studies and clinical trials have shown that intermittent fasting has broad-spectrum benefits for many health conditions, such as obesity, diabetes mellitus, cardiovascular disease, cancers, and neurologic disorders.

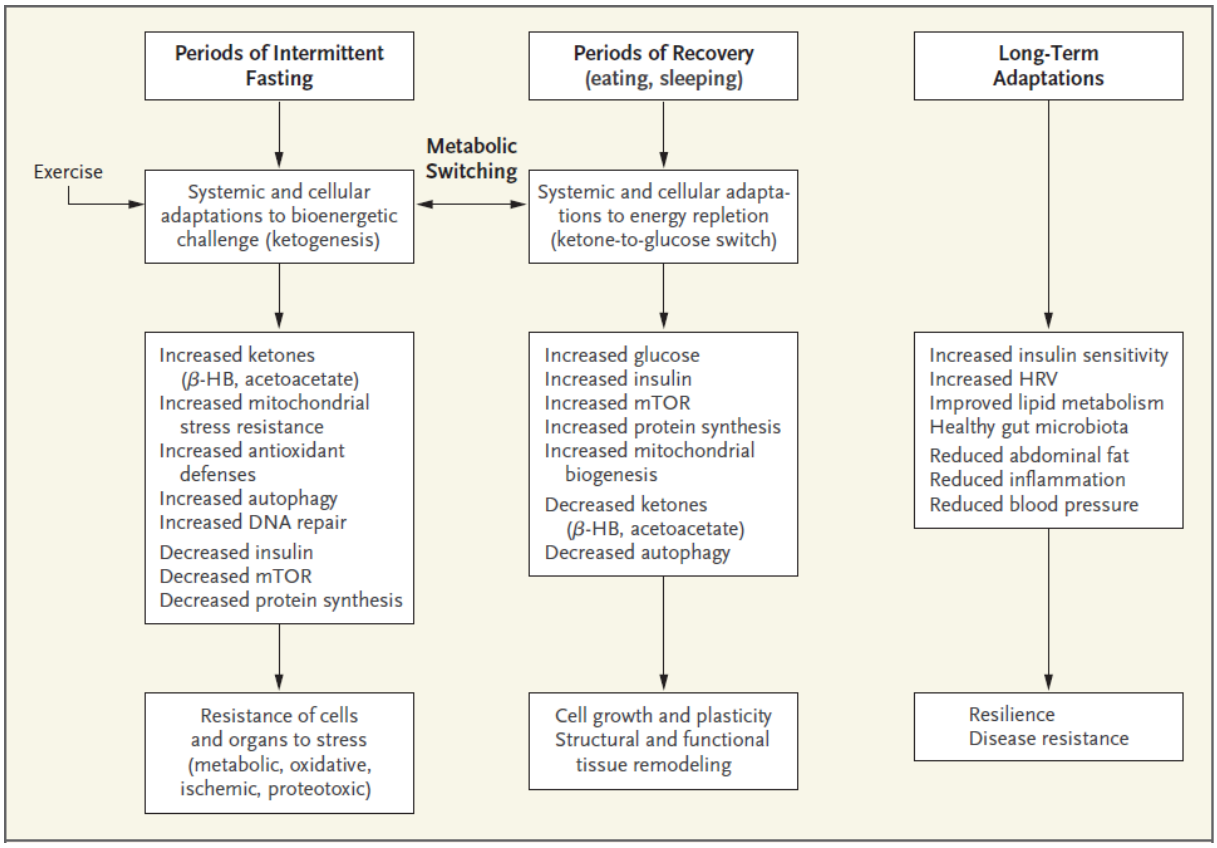

Fig Cellular and Molecular Mechanisms Underlying Improved Organ Function and Resistance to Stress and Disease with Intermittent Metabolic Switching. Periods of dietary energy restriction sufficient to cause depletion of liver glycogen stores trigger a metabolic switch toward use of fatty acids and ketones. Cells and organ systems adapt to this bioenergetic challenge by activating signaling pathways that bolster mitochondrial function, stress resistance, and antioxidant defences while up-regulating autophagy to remove damaged molecules and recycle their components. During the period of energy restriction, cells adopt a stress-resistance mode through reduction in insulin signaling and overall protein synthesis. Exercise enhances these effects of fasting. On recovery from fasting (eating and sleeping), glucose levels increase, ketone levels plummet, and cells increase protein synthesis, undergoing growth and repair. Maintenance of an intermittent-fasting regimen, particularly when combined with regular exercise, results in many long-term adaptations that improve mental and physical performance and increase disease resistance. HRV denotes heart-rate variability. (De Cabo & Mattson, 2019).

Nutrition and the way to a personalised diet

The experience of the second world war changed our whole view of public health and food policy. Well-fed children and healthy adults were dependent on a good diet. Despite all this effort, deficiency diseases remained a huge problem, with millions of children dying or suffering from

malnutrition, anaemia, and vitamin A deficiency. While the developing world was worrying about infant malnutrition, the problem of **obesity** was emerging in America and Europe.

As societies became wealthier eating was for pleasure and the idea of a balanced diet was emerging. The 1970s saw a change in ideas about public health in the West. The disease pattern had changed, and heart disease and cancers of the lung, breast, and large bowel were now leading causes of death. More recently there has been a huge increase in nutritional studies, all trying to explain why cancer rates differ so widely around the world; dietary factors such as salty smoked foods have been identified as cancer promoters. Experts developed complex theories on why diet produces chronic diseases that develop over several decades. Lately WHO has now drawn together the dietary evidence linked both to the chronic diseases of adulthood and to malnutrition. New nutritional goals for a country have been established. These suggest that, ideally, a country's food supplies should contain only modest amounts of saturated fatty acids, sugar, and salt, with the majority of the energy coming from starchy foods such as cereals and tubers. A substantial intake of vegetables and fruit is also important. This amounts to a completely different concept of the balanced diet, in which protein and energy are no longer the priorities while excessive intake of fat, saturated fatty acids, sugar, and salt are a cause for concern. (James W.O.T. 1991, New concepts of a balanced die*t*)

The United States had its first new **food pyramid** 1992. Named "MyPyramid," it was designed in accordance with the US Dietary Guidelines for Americans and jointly published in April 2005 by the US Department of Health and Human Services (HHS) and the US Department of Agriculture (USDA). These guidelines are "the cornerstone of federal nutrition policy and education" and are based on "what experts have determined to be the best scientific knowledge about diet, physical activity and other issues related to what [Americans] should eat and how much physical activity [they] need. Following an extensive public campaign USDA announced that, "MyPyramid is about the ability of Americans to personalize their approach when choosing a healthier lifestyle that balances nutrition and exercise". Central to the campaign was a web-based tool, now modified available at https://www.choosemyplate.gov/ When a person entered his age, sex, and activity level into the online form a personalised MyPyramid Plan appeared. This plan listed the recommended number of daily servings for 5 food categories—grains, vegetables, fruits, milk, and meat and beans—along with recommendations for oils and "discretionary calories." It then further delineated the amounts of whole grains and vegetables that should be in the diet, including weekly servings of dark green, orange, and starchy vegetables. (John Neustadt Integrative Medicine, 2005)

The food pyramid has been replaced by MyPlate and elderly get support like "What's On Your Plate? Smart Food Choices for Healthy Aging" (https://www.nia.nih.gov/health/healthy-eating).

Around 2010 **the first 1,000 days** of life theory developed. This time spanning roughly between conception and one's second birthday has been established to be a unique period of opportunity when the foundations of optimum health, growth, and neurodevelopment across the lifespan are established. Imprinting of the immune system, the epigenetic system and microbiota occurs in this time. Maternal nutrition and maternal microbiota have a central influence on these developments (Al Nabhani & Eberl, 2020).

Within the EU-funded **Food4Me project**, a multi-centre study was performed, as a proof of principle that a fully internet delivered personalised nutrition advice could make a difference in people's lifestyle. The project envisaged personalised nutrition advice at three levels:

- The person's diet only

- The diet combined with knowledge of the person's phenotype (measurable traits, such as physical and biochemical measurements, e.g. height, weight, or cholesterol level),
- The diet, phenotype and genotype (that determine a person's heritable gene identity, e.g. a gene variant associated with weight gain).

Food4Me developed a novel, internet delivered, food frequency questionnaire for personalised dietary analysis. It allowed for feedback based on the subject's prevailing food choices. The new method that included a digital photographic atlas was used to quantify food intake, took approximately 20 minutes to complete, and was validated in 2 peer-reviewed studies. https://www.eufic.org/en/healthy-living/article/personalised-nutrition-food4me-project / https://cordis.europa.eu/project/id/265494/reporting

The results of these analyses imply that attitudes towards personalised nutrition appear to be primarily driven by perceptions of benefit and how achievable it is to access or adopt.

The use of nutrition in the prevention of diseases still needs case by case evaluation. Some experts feel that "Preventive dietetics has largely lost its importance in conventional medicine. Hyperuricemias, hypercholesterolemia or hypertension may be mentioned as a few examples in which, in view of the powerful pharmacotherapeutic possibilities of dietetics in the field of clinical medicine, practically no importance is attached" (Prof W. Marktl 2020, p. comm).

The development of functional foods

The primary function (nutritional value) of food is to provide us with food energy and the necessary macro and micronutrients. The realization that our food can (must) do more than just provide us with energy and macro and micronutrients has been known to mankind for millennia. They also contribute to our well-being and can influence health status and prevent illnesses (health value). Food that fulfills this function to an extent or in a special way is referred to as "functional food". The awareness of this has always been well established in Asian cultures, but also not unknown in our culture, as the statement of the ancient Greek doctor Hippocrates (around 460 to 370 BC) proves: "Your food should be your medicine and your medicine your food." (Berghofer et al., 2015) https://broschuerenservice.sozialministerium.at/Home/Download?publicationId=541

It has been known for a long time that a clear relationship exists between the food we eat and our health. The current concept of functional foods has resulted from the gradual recognition that healthy diets result from eating nutritious foods and from the identification of the mechanisms by which foods modulate metabolism and health. When we eat food, our first and most basic aim is to obtain nutrients for our body and to satisfy our metabolic needs. However, some groups of foods, in addition to their nutritional properties, present other additional properties for health. These types of foods are called functional foods and may be defined as any food that has a positive impact on an individual's health, physical performance, or state of mind, in addition to its nutritious value. https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/functional-foods

The concept of functional food was first introduced in 1984 in Japan to improve the consumer's health through the diet fortified with functional ingredients. In the 1980s, researchers in Japan began to look intensively at the health benefits of food. The so-called FOSHU concept (FOSHU = foods for specified health use) was established, which was fixed in 1991 by Japanese law. Food manufacturers

who want to label their food with a special FOSHU seal must undergo a corresponding approval process. In 2002, 293 foods were already approved in this area in Japan (Shimizu, 2002, 2003).

In the USA, the functional foods concept was initiated and coined by Stephen DeFelice and Steve McNamara in 1989. Actually, they suggested the term “nutraceuticals” based on the 1983 Orphan drug act that was established to permit the distribution of drugs destined to treat rare medical conditions that affect a small number of people in the United States. Nutraceuticals are substances that are a food or part of a food that provides medical and/or health benefits, including the prevention and treatment (these are “drug” terms) of disease. (*Functional Food - an overview (pdf) | ScienceDirect Topics*, n.d.)

The European Food Safety Authority (EFSA) defines functional foods as: “A food, which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. A functional food can be a natural food or a food to which a component has been added or removed by technological or biotechnological means, and it must demonstrate their effects in amounts that can normally be expected to be consumed in the diet” (EC 1924/2006 on nutrition and health claims made on foods).

Experts belonging to the Functional Food Center, USA (FFC) currently define functional foods as “natural or processed foods that contain known or unknown biologically active compounds, which, in defined, effective, and non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers for the prevention, management, or treatment of chronic disease or its symptoms”. In this context, bioactive compounds, which are considered as a backbone of the functional foods, are understood as “primary and secondary metabolites of nutritive and non-nutritive natural components generating health benefits by preventing or are Functional Foods essential for “ Sustainable Health”? In the USA, the definition of Nutraceuticals includes “functional foods” – i.e. foods that provide a specific health benefit based on their ingredients. Nutraceuticals are natural, bioactive chemical compounds that have health-promoting, disease-preventing, or general medicinal properties (María Dolores del Castillo, Amaia Iriondo-DeHond, 2018).

Functional foods are similar in appearance to conventional foods; the former being consumed as part of the normal diet. In contrast to conventional foods, functional foods, however, have demonstrated physiological benefits and can reduce the risk of chronic disease beyond basic nutritional functions, including maintenance of gut health. When food is being cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called "functional food". Thus, functional food provides the body with the required amount of vitamins, fats, proteins, carbohydrates, *etc*., needed for its healthy survival (Food and Agriculture Organization of the United Nations (FAO), authors Report on Functional Foods, Food Quality and Standards Service (AGNS).

Other additional considerations have been proposed to define a functional food, including three additional conditions in particular: 1. it is a food (not a capsule, tablet, or powder) derived from natural ingredients; 2. it can and should be consumed as part of the daily diet; 3. it has a particular function when eaten, serving to regulate a particular body process, such as:

- enhancement of biological defense mechanisms
- prevention of specific diseases
- recovery from specific diseases
- control of physical and mental disorders
- slowing of the aging process.

Research supporting the beneficial properties of functional foods to combat cancers, coronary heart disease (CHD), diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic disorders, ulcers, etc. is based on chemical mechanisms using in vitro and cell-culture systems, various disease states in animals, and epidemiology of humans.

Also, the term “sustainable health” was introduced as an objective of functional foods as: “a healthy and active aging avoiding the risk of diseases”. Healthy foods and particularly functional foods are needed to achieve this goal. Sustainable health may be accomplished by delivering high-quality care and improved public health without exhausting natural resources or causing severe ecological damage. Sustainable health can also be achieved by protecting and improving health now and for future generations using different strategies such as healthy nutrition that may be based on functional foods. Plans for minimizing the environmental impact on health and nutrition are also needed (María Dolores del Castillo, Amaia Iriondo-DeHond, 2018).

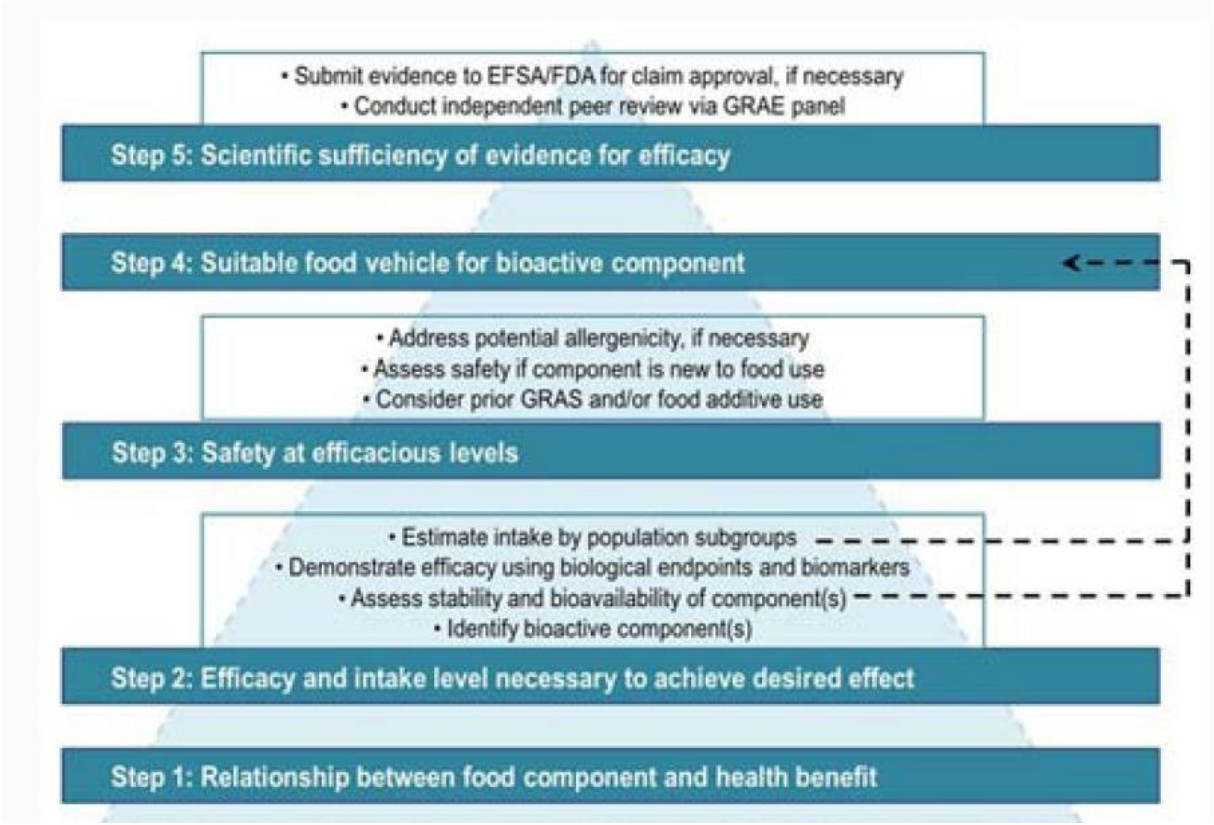

Figure summarizes the steps needed for collecting enough scientific evidence for the validation of functional foods (María Dolores del Castillo, Amaia Iriondo-DeHond, 2018)

The European Union acknowledges that “Over the last years a new, potential role of food has emerged, that of fulfilling a specific "physiological" function. While food that fulfills this role are called "functional" food” consumed as part of the usual diet. It provides health effects that go beyond traditional nutritional effects. As such it is closely related to but different from concepts such as food supplements or nutraceuticals. The markets for this new type of food are more developed in the USA.

So far, a coherent regulatory framework for functional food had been lacking within the EU, but in December 2006 the European Parliament and the Council adopted a new Regulation (EC 1924/2006) on nutrition and health claims made on foods". (see also chapter regulations)

The global market for functional foods (foods with specific health claims, energy drinks not included) was an impressive $ 43.27 billion in 2013. This was a 26.7% increase in value since 2009. Since then, the growth has been somewhat slower due to the global economic crisis and the stricter regulation in the EU (Leatherhead Food Research, 2014). Due to the high market potential, it is understandable that even small manufacturers (SMEs) want to be part of it. Since it is difficult for SMEs to obtain their own claims, they can only use generally permitted, nutrition-related statements, which, however, do not result in a unique selling proposition and no differentiation from the competition. For these reasons, the search for alternative or alternative options will increasingly occur in the future. (Berghofer et al., 2015)

Gruenwald (2015) reports that due to what he considers to be a very strict regulation of the "Health Claims" Regulation, it is attractive for many manufacturers to "switch to foods for special medical purposes". This food category (FSMP - foods for medical purposes) is a sub-category of "foods intended for a particular diet" (PARNUTS - foods intended for particular nutritional uses). They were only recently reorganized in REGULATION (EU) No. 609/2013. It enables strong claims to be advertised without the restrictions of the Health Claims Regulation. An FSMP must differ significantly from a normal food in terms of the manufacturing process or the composition, but it can also be available to other consumers via the intended group of consumers. Beauty claims have been officially rejected by EFSA and the EU Commission as health claims (Berghofer et al., 2015)

Typically, functional foods include:

- Probiotics, Prebiotics, Synbiotics and postbiotics.
- Dietary fibre
- Omega 3 fatty acids, oleic acids and phytosterols
- Phytoestrogens
- Phenolic compounds

Pro-, Pre-, Syn- and Postbiotics

Probiotics

Probiotics: Based on a 2001 Food and Agriculture Organization of the United Nations – World Health Organization (FAO-WHO) expert group consensus statement, probiotics, can be defined as 'live microorganisms, which when administered in adequate amounts, confer a health benefit on the host'. The majority of the probiotic products contain a defined and a limited list of microbial taxa, which mostly include lactic acid bacteria (LAB) such as Lactobacillus spp. and Bifidobacterium spp., which have the status of being generally regarded as safe (GRAS). Notably, health effects of probiotics are very strain and disease-specific, as reviewed elsewhere. As such, evidence on each of them might be different and there is a tremendous amount of data on specific probiotic strains accompanied by a large diversity in study outcomes.

Ideally, probiotics should be physiologically and genetically characterized and should be able to arrive in a viable state in the gut after product processing, storage conditions and gastric passage. Additionally, the health effects should be demonstrated in human studies.

It has been suggested that probiotics can influence the gut microbiota through the suppression and inhibition of pathogens as well as preventing adhesion and establishment of these pathogens in the gut. Furthermore, probiotics may have a role in immune system development, synthesis of important nutritional elements such as vitamins, and the reinforcement of the intestinal barrier integrity through the upregulation of genes involved in tight junction signaling.

For the probiotic members of the genera *Lactobacillus, Bifidobacterium and Streptococcus*, immunomodulatory properties have been shown, with beneficial effects on cell-mediated immunity and inflammation. Probiotic modulation of immune development represents a promising application, particularly in young infants where the most pronounced immune-modulating effects have been documented. As such, probiotics in infant and young child nutrition have shown promising results for the management and treatment of allergies, gut and respiratory infections, irritable bowel syndrome (IBS), ulcerative colitis (UC) and infant colic.

Clinical efficacy and prophylactic effects of probiotics has also been suggested in adults for several conditions, including antibiotic-associated diarrhea, acute gastroenteritis, IBS, UC, and acute respiratory infections. Moreover, probiotic use has been associated with a variety of immunomodulatory effects.

Changes in composition and functioning of the gut microbiota as a result of probiotic use is less clear, although some studies report that probiotics induce alterations in gut microbiota composition that co-occur with health-promoting effects. However, due to a lack of experimentally demonstrated causal relationships, it is difficult to claim that such microbiome alterations are indeed beneficial.

(Arumugam et al., 2016; Bermudez-Brito et al., 2012; Bertelsen et al., 2016; Burks et al., 2015; Cencic & Chingwaru, 2010; Ciampa et al., 2017; Collado et al., 2009; de Almada et al., 2016; Deshpande et al., 2018; Gibson et al., 2017; Gosálbez & Ramón, 2015; Gurry, 2017; Hemarajata & Versalovic, 2013; Kataria et al., 2009; Lahtinen, 2012; Macfarlane et al., 2006; Markowiak & Ślizewska, 2017; Miller et al., 2017; Mohammadi & Golchin, 2020; Nikbakht et al., 2018; O'Toole et al., 2017; Ouwehand et al., 2000; Sánchez et al., 2017; Sanders et al., 2019; Suez et al., 2019; Taverniti & Guglielmetti, 2011; C. M. Thomas & Versalovic, 2010; D. W. Thomas et al., 2010; Van Der Aa et al., 2010, 2011; Vandenplas et al., 2015; Y. Wang et al., 2013).

Prebiotics

Prebiotics: The International Scientific Association of Probiotics and Prebiotics recently reviewed the definition and scope of prebiotics and produced a consensus statement on the definition of prebiotics: 'a substrate that is selectively utilized by host microorganisms conferring a health benefit'. Prebiotics can change the microbiota composition by stimulating the growth of certain species, thereby promoting health benefits in the host. Numerous carbohydrates which are fermentable for microorganisms only have been reported to convey such prebiotic effects, including human milk oligosaccharides (HMOs), several dietary fibre types, phenolics and phytochemicals, conjugated linoleic acid and polyunsaturated fatty acids, resistant starch and a wide range of oligosaccharides, with wide ranges of health effects (Flint et al., 2012). The well-studied and most frequently used prebiotic oligosaccharides include short-chain galactooligosaccharides (scGOS) and long-chain

fructooligosaccharides (lcFOS). The main effects of many of these prebiotics are based on the enhancement of the growth and activity of specific *Bifidobacterium* spp., where relative abundances are characteristic for breastfed infants and young children. Via this microbiota modulating effect, prebiotic oligosaccharides in infant formula are known to induce changes in gut metabolic activity and bring stool consistency and frequency closer to that of breast-fed infants. Besides effects in babies, prebiotics is associated with physiological and pathophysiological properties throughout life, including toddlers, adolescents, adults, and the elderly. These effects include, amongst others, improvement of gastrointestinal functioning and barrier function, increase in mineral absorption, modulation of energy metabolism and satiety, and reduction of the risk of intestinal infections.

(Bertelsen et al., 2016; Gibson et al., 2017; Giovannini et al., 2014; Macfarlane et al., 2006; Markowiak & Ślizewska, 2017; Roberfroid et al., 2010; Sanders et al., 2019; Sierra et al., 2014; Slavin, 2013; D. W. Thomas et al., 2010; Umu et al., 2017; Vandenplas et al., 2015; Wegh et al., 2017)

Synbiotics

Synbiotics are often defined as 'synergistic mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and colonization of live beneficial microorganisms in the gastrointestinal tract of the host'. Synbiotics can modulate the gut microbiota composition and modulate microbial metabolite production. Infant formula with added synbiotics have shown to support normal growth in infants with cow's milk allergy, modulate the intestinal microbiota and prevent asthma-like symptoms in infants with atopic dermatitis. On top of this, it was shown that infant formulas supplemented with synbiotics containing scGOS/lcFOS and *Bifidobacterium breve* M-16V compensate the delayed Bifidobacterium colonization documented for C-section born infants. In C-section born infants these synbiotics modulate the production of acetate and the acidification of the gut. These observed physiological conditions, described as indicators of gut health, emulate the ones observed in vaginally born infants. With regards to adults, several meta-analyses suggest positive effects of synbiotics in constipation, on lowering of high fasting blood glucose levels and on the risk of developing postoperative sepsis after gastrointestinal surgery.

(Arumugam et al., 2016; Burks et al., 2015; Chua et al., 2017; Gurry, 2017; Markowiak & Ślizewska, 2017; Van Der Aa et al., 2010, 2011).

Postbiotics

Postbiotics are functional bioactive compounds, generated in a matrix during fermentation, which may be used to promote health. The term postbiotics can be regarded as an umbrella term for all synonyms and related terms of these microbial fermentation components. Therefore, postbiotics can include many different constituents including metabolites, short-chain fatty acids (SCFAs), microbial cell fractions, functional proteins, extracellular polysaccharides (EPS), cell lysates, teichoic acid, peptidoglycan-derived muropeptides, and pili-type structures. Postbiotics is also a rather new term in the '-biotics' field. Where consensus exists for the definitions of pre- and probiotics, this is not yet the case for postbiotics (Wegh et al., 2019).

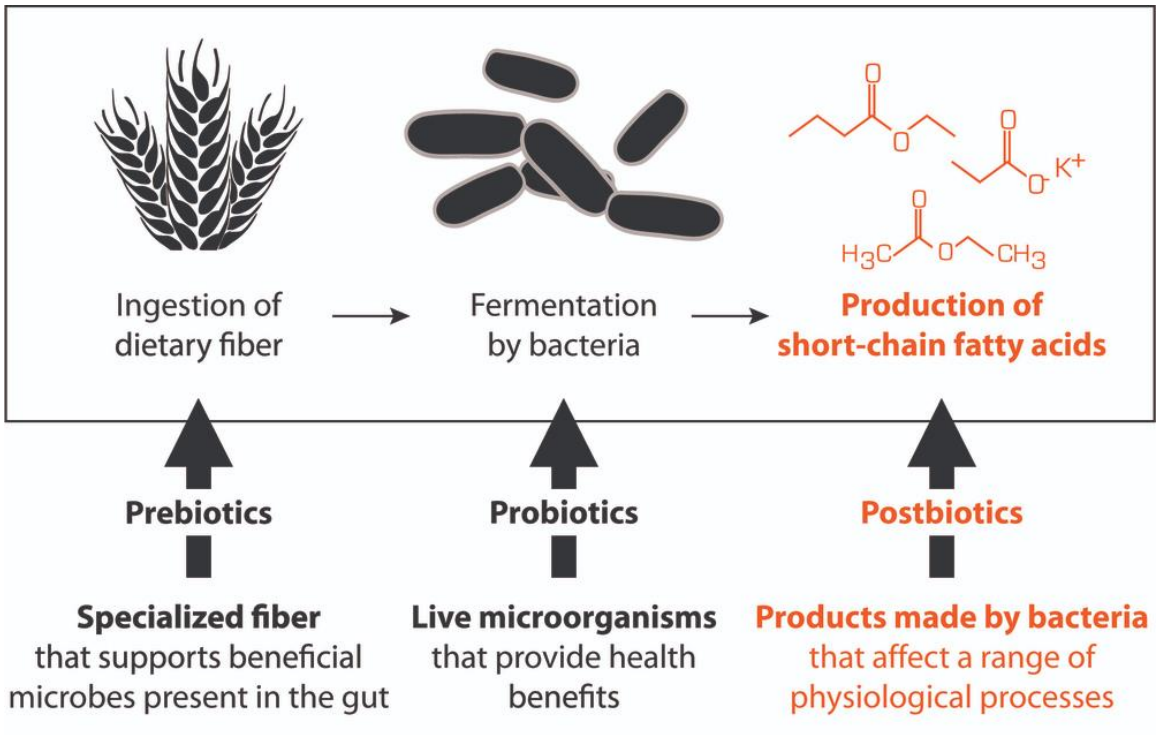

Fig Postbiotic, https://www.thinkbiome.com/postbiotics

Novel food, food supplements, neutraceuticals, phytoceuticals, medicinal foods

"There are widespread inconsistencies and contradictions in the many published definitions of 'nutraceuticals' and 'functional foods', demonstrating uncertainty about what they actually are. There are no internationally agreed definitions of 'nutraceuticals' and 'functional foods', or of similar terms, such as 'health foods', or of terms related to herbal products, which are sometimes referred to as 'nutraceuticals'. The term 'dietary supplement' is widely used to designate formulations that are also called 'nutraceuticals', 'Fortified foods', sometimes called 'designer foods', are foods to which compounds of proven therapeutic or preventive efficacy (e.g. folic acid) have been added. (Aronson, 2017) (Santini et al., 2018).

Some definitions for food-derived products

Food supplement (United States Government Office, 1994)	A product (other than tobacco) in the form of a capsule, powder, softgel or gelcap intended to supplement the diet to enhance health that bears or contains one or more of the following dietary ingredients: a vitamin, mineral, amino acid, or other botanical or dietary substance.	United States Food and Drug Administration (FDA). Dietary Supplement Health and Education Act (DSHEA). U.S. Department of Health and Human Services. 1994. United States. Public Law 103–417, available at FDA Website: http://www.fda.gov. [42]
Food supplement (EU, 2002)	Food product whose purpose is to supplement the normal diet and which consists of a concentrated source of nutrients or other substances with nutritional effects or physiological, single or in combination, marketed in dosed formulations, such as capsules, tablets, tablets or pills, designed to be taken in small individual quantities measured.	EU Directive 2002/46/EC [17]
Phytochemical (Bloch and Thomson, 1995)	Substances found in edible fruit and vegetables that can be ingested daily (in quantities of grams) by humans and that exhibit a potential to favourably modulate human metabolism to prevent cancer and other diseases (isoflavones, resveratrol, garlic allyl sulphides, tomato lycopene, onion quercetin etc.).	Bloch A, Thomson CA. Position of The American Dietetic Association (phytochemicals and functional foods). J Am Diet Assoc 1995; 95: 493–496. [74]
Nutraceuticals (De Felice, 1995)	Food or part of food that provides medical or health benefits, including the prevention and/or treatment of a disease.	DeFelice SL. The nutraceutical revolution: its impact on food industry R&D. Trends Food Sci Technol 1995; 6: 59–61. [2]
Nutraceuticals (Zeisel, 1999; DSHEA, 1994)	A diet supplement that delivers a concentrated form of a biologically active component of food in a nonfood matrix to enhance health.	Zeisel SH. Regulation of "Nutraceuticals". Science. 1999: 285; 1853–5. Food and Drug Administration, FDA, Dietary Supplement Health and Education Act of 1994 (DSHEA), United States. [75]
Nutraceuticals (Brower, 1998)	Any substance that is a food or a part of a food and is able to induce medical and health benefits, including the prevention and treatment of disease.	Brower V. Nat Biotechnol 1998; 16: 728. [76]
Nutraceuticals (Merriam Webster Dictionary, 2015)	A foodstuff (as a fortified food or dietary supplement) that provides health benefits in addition to its basic nutritional.	Merriam-Webster Online Dictionary. 2015. Merriam-Webster Inc., P.O. Box 281, Springfield, MA 01102, United States. [77]
Nutraceuticals (ENA, 2016)	Nutritional products that provide health and medical benefits, including the prevention and treatment of disease.	European Nutraceutical Association (ENA). 2016. Science behind Nutraceuticals. In E. N. Association (Ed.), (Vol. 2016). 594 Basel, Switzerland. [78]
Functional food (Zeisel, 1999)	Nutrient consumed as part of a normal diet but delivering one or more active ingredients (that have physiological effects and may enhance health) within the food matrix.	Zeisel SH. Regulation of "Nutraceuticals". Science 1999: 285; 1853–5. Food and Drug Administration, FDA, Dietary Supplement Health and Education Act of 1994

Fig definitions functional foods, nutraceuticals, supplements (Santini et al., 2018)

In the US, recent legislation regarding food safety includes the Food Safety Modernization Act, signed in 2011 by Barack Obama. FSMA has given the Food and Drug Administration (FDA) new authorities to regulate the way foods are grown, harvested, and processed. The law grants the FDA a number of new powers, including mandatory recall authority, which the agency has sought for many years. The FSMA requires the FDA to undertake more than a dozen rulemakings and issue at least 10 guidance documents, as well as a host of reports, plans, strategies, standards, notices, and other tasks. FDA guidance documents cover e.g. Food facility registration, Current Good Manufacturing Practices (CGMPs), Hazard Analysis & Critical Control Points (HACCP), Retail Food Protection, Imports & Exports. https://www.fda.gov/food/guidance-regulation-food-and-dietary-supplements

The European Union has implemented a regulatory system for marketing and labellingof foods a new regulation came into force on 1st of January 2018 replacing previous regulations. https://ec.europa.eu/food/safety/novel_food_en (Also see chapter regulation)

Allocations of products to specific legal categories is often difficult.

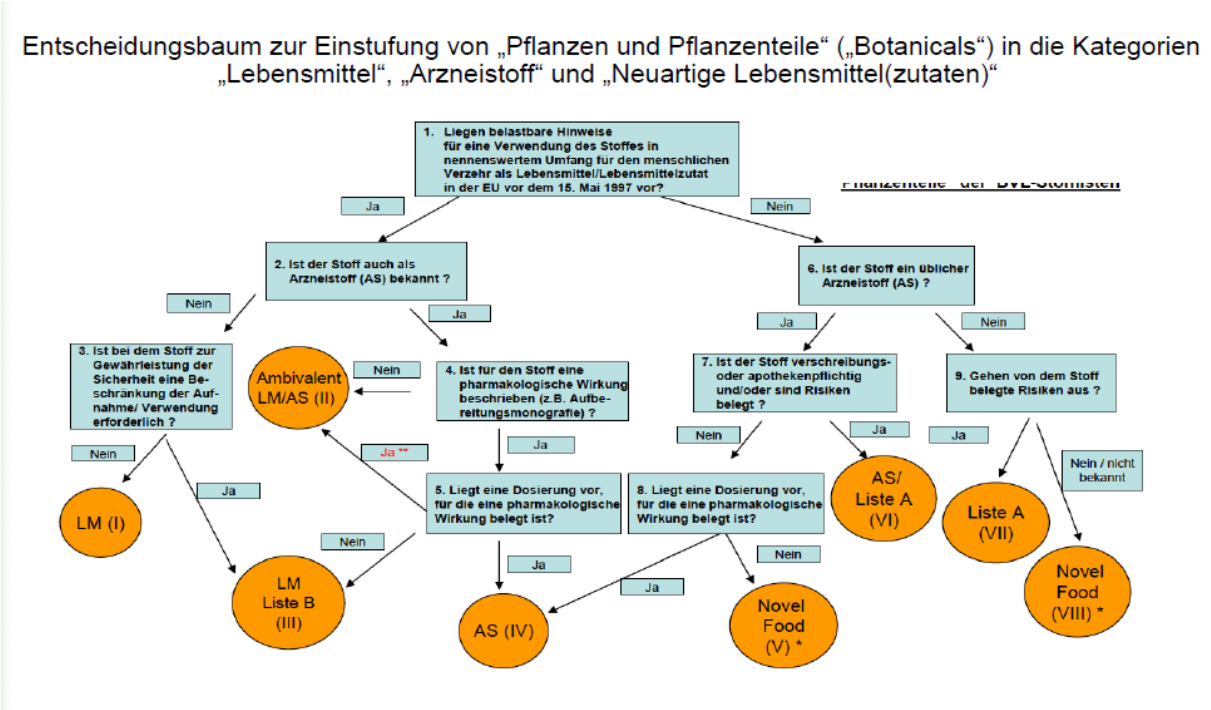

Fig The complexity of allocation of products into the categories of pharmaceuticals, medicinal foods, botanicals, functional foods, novel foods or foods (EU, nd)

Supplements, Dietary supplements

Botanical dietary supplements are generally available as whole plants, plant parts, powdered plant material, or plant extracts. These supplements are marketed in various forms, including as powders, tablets, capsules, gummies, teas, tinctures, and essential oils. A variety of botanical dietary supplements are used in complementary and integrative health practices (https://nccih.nih.gov/health/integrative-health). Although there is overlap in the botanical species used in dietary supplements and other forms of complementary medicine, such as Ayurveda and Traditional Chinese Medicine, the applications can vary widely, and safety considerations associated with these practices are beyond the scope of the current work.

Many different stakeholders recognize the importance of ensuring the quality and safety of botanical dietary supplements. This endeavors is multi-dimensional and involves consideration of the chemical properties and toxicological profiles of the raw botanical ingredient(s), excipients present in the finished product, and reagents involved in the processing or manufacturing of the finished product, as well as possible sources of contamination at any step along the process from the harvest of the raw ingredients to storage of the finished product. A complicating factor in the evaluation of botanical quality and safety is their inherent complexity. Botanical dietary supplements are typically complex mixtures and can display a high degree of variability (both natural and introduced). Sources of variability in finished products can range from compositional differences between batches of raw materials to differences in processing and manufacturing of the source biomass. The constituents of individual batches may differ based on factors such as a geographical location where the plant material is grown (e.g., altitude, climate, and time and growth stage at harvest. Furthermore, the processes and practices that individual manufacturers use are often unique to the company and

proprietary, so while batch-to-batch variation within a company may be minimal, variability between products that are nominally the same from different companies may be considerable. Due to this widespread variability, chemical evaluation of composition has joined botanical morphology as an important tool in the manufacture, study and regulation of these products. Standardization, a process that measures *and adjusts* the amount of and ratio between key constituents has been adopted as a means of controlling batch-to-batch variability. Due to the complexity and variation in botanical dietary supplement composition, there are significant issues with comparing test articles across studies and, therefore, reproducibility in botanicals research is a challenge. Protocols for dietary supplement research must consider many different factors, including populations (generalizability), responders vs. non-responders, timing and duration of exposure, endpoints of concern, dose levels, and earlier phase studies. Data from exploratory clinical trials and studies of natural products have been highly inconsistent, and many problems are replicating the effects of botanicals that have been reported in the literature. (Shipkowski et al., 2018)

Due to many inconsistencies in study design and knowledge about product composition, there is a low level of confidence in published data on potential biological targets of botanical dietary supplements. (Landis et al., 2012; R. Morris, 2012)

As part of efforts to standardize botanical quality, there are now many publications that set out minimal quality standards for botanical raw materials and very simple finished products. These include the European Pharmacopoeia, the United States Pharmacopeia (USP), Pharmacopoeia of the People's Republic of China, the Hong Kong Materia Medica Standards, and others. These science-based, quality monographs for botanicals contain specifications on the identity, content and composition, purity, and performance of individual botanicals. These publications set out specifications and tests for use in Good Manufacturing Practice (GMP) settings, but are not mandatory for dietary supplement products in the U.S. (www.usp.org).

Use patterns and regulatory guidance for botanical dietary supplements that differ around the world. According to the US Dietary Supplement Health and Education Act (DSHEA) of 1994, the term "dietary supplement" can be defined using several criteria; namely (a) a product (other than tobacco) that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract, or combinations of these ingredients , (b) a product intended for ingestion in pill, capsule, tablet, or liquid form, (c) a product not represented for use as a conventional food or as the sole item of a meal or diet, (d) anything labelled as a "dietary supplement", and (e) products such as a newly approved drug, certified antibiotic, or licensed biologic that was marketed as a dietary supplement or food before approval, certification, or license (unless if this provision is waived by an authority such as Secretary of Health and Human Services, as in USA) .

The European Union defines: As an addition to a normal diet, food business operators market food supplements, which are concentrated sources of nutrients (or other substances) with a nutritional or physiological effect. Such food supplements can be marketed in "dose" form, such as pills, tablets, capsules, liquids in measured doses, etc. The objective of the harmonised rules on those products in Directive 2002/46/EC is to protect consumers against potential health risks from those products and to ensure that they are not provided with misleading information (Cencic & Chingwaru, 2010).

Case study curcumin as supplements, opinion of EU- EFSA

The Panel on Food Additives and Nutrient Sources added to Food provides a scientific opinion re-evaluating the safety of curcumin (E 100). Curcumin has been previously evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the EU Scientific Committee on Food (SCF). In 2004 JECFA allocated an ADI of 0–3 mg/kg bw/day. The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that became available since then and the data available following a public call for data. The Panel considered that the indications provided by the positive results for curcumin in several *in vitro* and *in vivo* tests for genotoxicity, especially those detecting chromosomal aberrations and DNA adducts should not be disregarded and that the available *in vivo* genotoxicity studies were insufficient to eliminate the concerns regarding genotoxicity. The Panel noted that all statistically significant effects noted by NTP in a long-term carcinogenicity study in rats and mice refer to benign neoplastic lesions (adenomas) and that the incidences for malignant neoplastic lesions (carcinomas) did not reach statistical significance. The Panel also noted that the effects observed were not dose-dependent, were in line with historical control values, and not consistent across sexes and/or species. The Panel agreed with JECFA that curcumin is not carcinogenic. The Panel also concluded that this eliminates the concerns over genotoxicity. The Panel concluded that the present database supports an ADI of 3 mg/kg bw/day based on the NOAEL of 250–320 mg/kg bw/day from the reproductive toxicity study for a decreased body weight gain in the F2 generation observed at the highest dose level and an uncertainty factor of 100. The Panel concluded that at the maximum levels of use, intake estimates for 1- to 10-year old children at the mean and the high percentile (95^{th}) are above the ADI in some European countries. The Panel noted that intake of curcumin from the normal diet amounts to less than 7% of the ADI of 3 mg/kg bw/day.

https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1679

Nutraceuticals

The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice, MD, founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford, NJ (Kalra, 2003). DeFelice proceeded to define nutraceutical as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease". When functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) other than anaemia, it is called a nutraceutical. It should be noted that the term nutraceutical, as commonly used in marketing, has no regulatory definition. Thus, nutraceuticals differ from dietary supplements in the following aspects: (1) nutraceuticals must not only supplement the diet but should also aid in the prevention and/or treatment of disease and/or disorder; and (2) nutraceuticals are used as conventional foods or as sole items of a meal or diet. Dietary components play beneficial roles beyond basic nutrition, leading to the development of the functional food concept and nutraceuticals. A functional food for one consumer can act as a nutraceutical for another consumer. Examples of nutraceuticals include fortified dairy products (e.g., milk) and citrus fruits (e.g., orange juice).

Several naturally derived food substances have been studied in cancer therapies. Vitamin E, selenium, vitamin D, green tea, soy, and lycopene are examples of nutraceuticals widely studied in human health. While many of these 'natural' compounds have been found to have high therapeutic potential; future studies should include well-designed clinical trials assessing combinations of these compounds to realize possible synergies they bring into human health.

Polyunsaturated fatty acids (PUFAs) (which include the omega-3 and omega-6 fatty acids) and phytochemicals also play an important role as healthy dietary bioactive compounds (Brower V., 1998)(Zeisel S.H., 1999)(Kalra E.K., 2003).

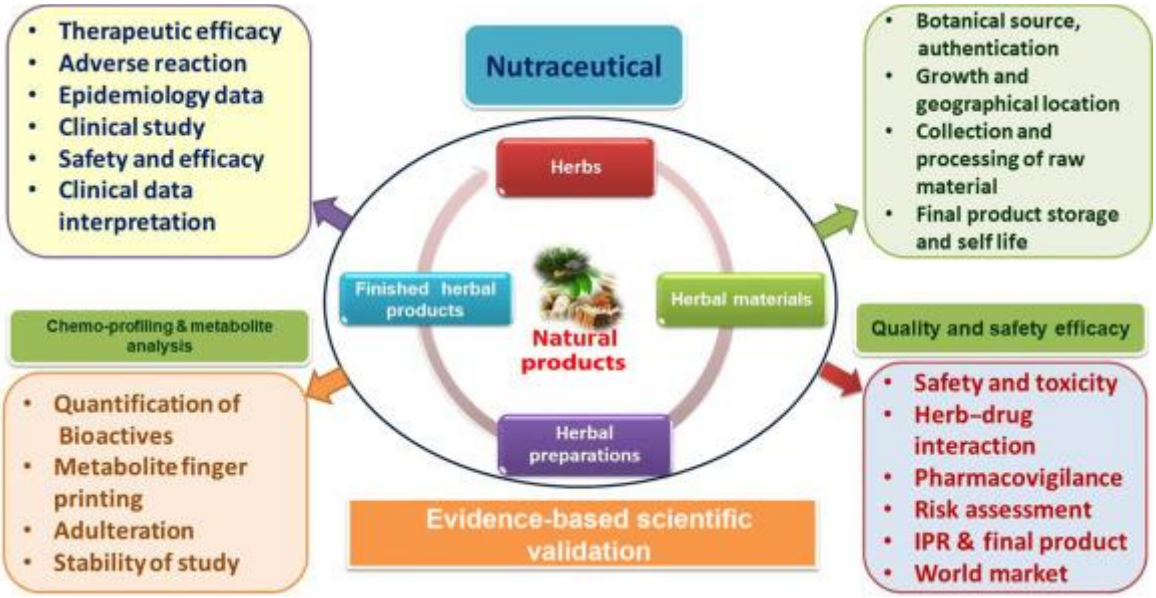

Fig Parameters for the evaluation of a nutraceutical (Mukherjee, 2019)

Medical foods

Medical foods are considered to be administered to a “patient receiving active and ongoing medical supervision (e.g., in a health care facility or as an outpatient) by a physician who has determined that the medical food is necessary to the patient’s overall medical care.” Furthermore, medical foods cannot be used for a condition that can be managed with a simple adjustment of the normal diet, such as diabetes or vitamin and mineral deficiencies. Medical foods include nutritionally complete formulas; nutritionally incomplete formulas containing proteins, carbohydrates, or fats; formulas for metabolic disorders in patients over 12 months of age; and oral rehydration formulas. These foods differ from dietary supplements and FDA-approved drugs in a number of ways. The main difference between medical foods and dietary supplements is that medical foods are used to manage a chronic disease or condition under medical or physician supervision, whereas supplements are intended for healthy individuals and can be obtained over-the-counter (OTC). Ciampa et al., 2017). The use of medical foods for specific diseases must also be supported by recognized science.

US- FDA regulates food for special medical purposes. The term medical food, as defined in section 5(b) of the Orphan Drug Act (21 U.S.C. 360ee (b) (3)) is "a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation. Medical foods are not those simply recommended by a physician as part of an overall diet to manage the symptoms or reduce the risk of a disease or condition. Not all foods fed to patients with a disease, including diseases that require dietary management, are medical foods. Instead, medical foods are foods that are specially formulated and processed (as opposed to a naturally occurring foodstuff used in a natural state) for a patient who requires the use of the product as a major component of a disease or condition's specific dietary management. https://www.fda.gov/food/guidance-documents-regulatory-information-topic-food-and-dietary-supplements/medical-foods-guidance-documents-regulatory-information

European community provides rules for the composition and labelling of foods intended for the dietary management (under medical supervision) of individuals who suffer from certain **diseases, disorders or medical conditions** have been laid down by the Commission Directive 1999/21/EC, adopted under the old legislative framework of Directive 2009/39/EC.
https://ec.europa.eu/food/safety/labeling_nutrition/special_groups_food/medical_en

These foods are intended for the exclusive or partial feeding of **people whose nutritional requirements cannot be met by normal foods**. The Directive 1999/21/EC lays down essential requirements on their composition and gives guidance for the minimum and maximum levels of vitamins and minerals.

Nutritional substances that may be used in the manufacture of foods for special medical purposes are laid down in Commission Regulation (EC) No 953/2009. European EFSA gives guidance for the preparation of dossiers for foods for special medicinal purposes:
https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2015.4300

Case study EU EFSA opinion on N-acetyl-L-cysteine as a medical food

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to evaluate N-acetyl-L-cysteine (NAC) for use in foods for particular nutritional uses and in food for special medical purposes.

The panel noted that NAC is deacetylated in animal tissues to L-cysteine. However, the extent to this deacetylation occurs in humans after oral ingestion has not yet been completely clarified. Therefore, the bioavailability of L-cysteine from oral NAC cannot finally be evaluated. A direct comparison of plasma levels of L-cysteine from orally administered NAC and L-cysteine should be undertaken. The Panel also noted that data on the bioavailability of L-cysteine from NAC in the presence of other N-acetyl amino acid derivatives is not available. When combining different N-acetyl derivatives as sources for the respective amino acids, the efficiency of their deacetylation may be affected. The bioavailability of amino acids from a product containing more than one acetylated nutrient source would have to be assessed as a part of the product development. Normally such information would be available as a part of a file supporting the intended use of the product.
The proposed uses in foods for all age groups except for healthy infants and young children might result in intakes of up to 2.9 g NAC/day. Such intakes would exceed the therapeutic doses of 0.4 - 0.6 g NAC/day, which has mucolytic effects. In addition, gastrointestinal disturbances, such as

vomiting and diarrhea, cannot be excluded in sensitive persons at these intakes. Furthermore, there is some concern because of a potential prooxidative effect of NAC, which should be clarified by appropriate studies. For these reasons, the requested general use of NAC for replacement of L-cysteine in foods for particular nutritional uses by NAC is not acceptable.

The Panel is aware of the use of NAC in foods for special medical purposes (FSMPs). The Panel noted that under the current Community legislation FSMPs should be used under medical supervision. The supervising physician will be in a position to weigh up any risks and benefits to the patient. Given these considerations, the Panel concluded that the inclusion of NAC in the list of substances permitted for FSMP's is acceptable.

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2004.21

Science-based categorisation of bioactive plant ingredients

Generally, medicines contain just one active substance, synthetically, whereas medicinal plants are practically a mixture of over dozens or even hundreds of chemicals that act synergistically. Moreover, medicinal plants contain a large number of vitamins and minerals, easily assimilated by human body. Many recent studies demonstrate that vitamins and minerals obtained through chemical synthesis have not the same beneficial effect as similar natural products. It may be due to the fact that in natural products there is a synergistic and complementary action between vitamins, minerals, and enzymes, while synthetic compounds (vitamins or minerals) are isolated and even obtained as a different enantiomeric form. The importance of botanic products for humanity is due mainly to their phytocompounds, active principles with therapeutic properties. Several studies have investigated these plant-derived compounds. Depending on the role they hold in living organisms, natural substances are divided in the next major categories: (i) primary metabolites, molecules common to all biological systems (proteins, fats, sugars) and (ii) secondary metabolites, compounds that could be specific for different species as a direct result of the evolution process of a particular phylogenetic group. Bioactive molecules are basically those secondary metabolites exhibiting therapeutic, preventing, toxicological, and immune-stimulating activity (Segneanu et al., 2017).

Compound type	Pharmacological properties
Terpenoid	Antimicrobial, antiviral, antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory [15, 34]
Phenolics acids	Anticarcinogenic and antimutagenic, anti-inflammation and anti-allergic [16, 20, 25, 31–35]
Alkaloids	Antispasmodic, antimalarial, analgesic, diuretic activities, local anesthetic, antihypertensive, antiasthma, antimalarials, diuretic, bactericidal [14–16, 20, 21]
Flavonoids	Antioxidant activity, cardiovascular protective, anti-inflammatory, hepatoprotective, antiviral, antibacterial [20, 22–24, 34]
Saponins	Antitumor, antiviral, antifungal, anti-inflammatory, immunostimulant, antihypoglycemic, antihepatotoxic and hepatoprotective, anticoagulant, neuroprotective, antioxidant [16, 20, 24–27, 34]
Tannins	Antioxidant, anti-carcinogenic, diuretics, hemostatic, anti-mutagenic, metal ion-chelators, antiseptic, [14, 16 , 20, 25, 28–32]

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Fig Properties of compounds (Segneanu et al., 2017)

The term ‘secondary plant substances’ covers substances of very different structures. From the roughly 100,000 known secondary plant substances, 5,000 to 10,000 occur in human food (Watzl 2008). As the name suggests, secondary plant substances are formed in small quantities in the secondary plant metabolism. They are a multitude of chemically heterogeneous compounds, which generally have pharmacological effects. Due to their chemical structure and functional properties, secondary plant compounds are divided into different groups, namely: polyphenols, carotenoids, phytoestrogens, glucosinolates, sulphides, monoterpenes, saponins, protease inhibitors, phytosterols and lectins. Although secondary plant compounds are not considered essential human nutrients, they do have an influence on a number of metabolic processes and various health-promoting effects

are attributed to them. They are said to protect against various types of cancer and mediate the effects of cardiovascular diseases, such as the dilatation of blood vessels and a reduction in blood pressure. Secondary plant compounds also have neurological, anti-inflammatory, and antibacterial effects (DGE, 2012).

Knowledge on the importance of secondary plant compounds and their positive health effects has increased considerably. This can largely be explained by the growing focus on large prospective observational studies, called cohort studies, and, above all, intervention studies with isolated secondary plant compounds. Ultimately, only intervention studies provide the necessary causal evidence which demonstrates the relationship between the intake of secondary plant compounds and the resultant disease-preventing effects. Since the 2008 Nutrition Report by the German Nutritional Biochemist, Bernhard Watzl (WATZL, 2008), numerous epidemiological studies confirm the effects of secondary plant compounds on human health and their attribution to decreasing the risk of getting a disease (WATZL, 2008). Based on the current scientific data, it is possible to evaluate the preventive effect of secondary plant substances. However, recommendations on the daily or average individual intake cannot be concluded at this stage (DGE, 2012).

Polyphenols

Dietary polyphenols are one of the most important groups of natural antioxidants and chemopreventive agents found in human diets including fruits, vegetables, grains, tea, essential oils, and their derived foods and beverages. Epidemiological, clinical, and nutritional studies strongly support the evidence that dietary phenolic compounds enhance human health by lowering risk and preventing the onset of degenerative diseases including cancers, cardiovascular diseases, and metabolic disorders (H. Zhang & Tsao, 2016).

Modulation of oxidative stress mediated inflammation by phenolic compounds

Phenolic compounds	Anti-inflammation	Biomarkers	Mechanisms	Cell/animal models	Ref.
Phenolic acids containing propolis extract	Inhibition of inflammasome mediated inflammatory responses	IL-1β, casp-1↓	Interfering with inflammasome pathway	Ex vivo LPS-stimulated macrophage from C57BL/6 and Caspase-$1^{-/-}$ mice	[26]
Apigenin	Inhibition of LPS-induced inflammation	casp-1, IL-1β↓	Inhibition of NLRP3 inflammasome activation	THP-1 cells	[28]
Homoplantaginin	Inhibition of palmitic acid-induced Inflammation	IL-1β, ICAM-1, and MCP-1, casp-1, IL-1β↓	Suppressing ROS-sensitive thioredoxin-interacting protein	Endothelial Cells	[50]
Quercetin and allopurinol	Amelioration of kidney injury	IL-1β and IL-18↓	Inhibition of NLRP3 inflammasome activation and increasing PPAR expression	STZ-induced rat model	[51]
Luteoloside	Supression of metastasis and proliferation of HCC	ROS, NLPR3, casp-1, IL-1β↓	Inhibition of NLRP3 inflammasome activation	HCC	[52]
Rutin	Modulates ASC expression	casp-1, IL-1β, ASC-NLRP3, IL-18, TNF-α↓	Inhibition of NLRP3 inflammasome activation	Cerulein-induced rat model	[53]
Catechin	Migration of gouty inflammation	IL-1β↓	Modulation of NLRP3 inflammasome activation	Monosodium Urate-Induced C57BL/6, human macrophage THP-1 cells	[54]
Ellagic acid	Prevention of inflammasome assocaited PAH	IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ, MIP-1, MDA, NLPR3, casp;↓ SOD↑	Inhibition of NLRP3 inflammasome activation	MCT-induced rats	[55]
EGCG	Suppression of melanoma growth	NLRP1, IL-1β, NFκB↓	Inhibition of NLRP3 activation	Human metastatic melanoma cells	[56]
Resveratrol	Inducing autophagy and ameliorating hepatic inflammation	IL-1β, NLPR3;↓ IL-1β, TNF-α, IL6;↓ liver TG↑	Inhibition of NLRP3 activation	Murine macrophage and Prg-IgAN mouse model; mouse model of HF diet-induced obesity	[29•,30]

Cas-1: caspase-1; LPS: lipopolysaccharide; MCT: monocrotaline; PAH: pulmonary artery hypertension; MDA: malondialdehyde; MIP-1: macrophage inflammatory protein-1; HCC: hepatocellular carcinoma cells; ICAM-1: intercellular adhesion molecule-1; STZ: streptozotocin; ASC: apoptosis associated speck-like CARD containing protein; EGCG: epigallocatechin-3-gallate; HF: high fat, TG: triglyceride.

Fig Modulation of oxidative stress mediated inflammation by polyphenolic compounds (H. Zhang & Tsao, 2016).

While a large body of evidence exists for the in vitro antioxidant and anti-inflammatory effects of polyphenols, these effects are not always validated in vivo, in fact, many conflicting results have been reported. The inconsistency between observations in the in vitro and in vivo studies raise new questions and challenges, particularly those related to the roles of polyphenols and their metabolites in inflammation-related health issues such as colonic degenerative diseases. While free or simple conjugates of polyphenols are absorbed in the upper gastrointestinal (GI) tract, their bioavailability is very low compared to the vitamin antioxidants. Phenolic compounds are also metabolized after intestinal uptake before being delivered to different tissues or organs by blood circulation to exert their various effects. The unabsorbed phenolics and those bound to cell wall materials can be further metabolized or released by the gut microbiota in the colon. These metabolites or bound phenolics can therefore positively affect local inflammatory status, or indirectly act as prebiotics to promote the growth of probiotics, leading to improved gut health (H. Zhang & Tsao, 2016).

Dietary phenolics are powerful antioxidants in vitro, being able to neutralize free radicals by donating an electron or hydrogen atom to a wide range of reactive oxygen, nitrogen, and chlorine species.

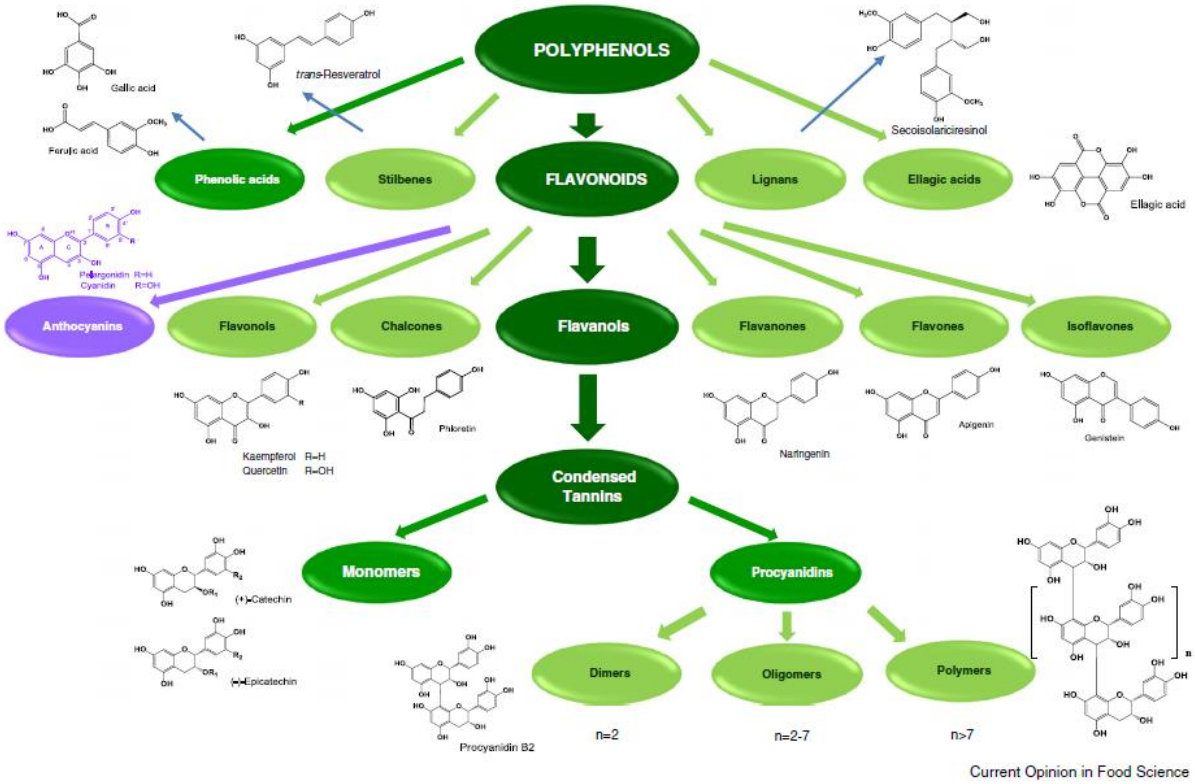


Fig Polyphenols (H. Zhang & Tsao, 2016)

In addition to reducing oxidative stress-mediated inflammation, phenolic compounds can also attenuate the pro-inflammatory cytokine-induced activation of NFkB via different molecular mechanisms. However, the key molecule from the upstream of the NFkB pathway targeted by polyphenols has only been identified in very recent years (H. Zhang & Tsao, 2016).

Polyphenols in foods

	Source (serving size)	Polyphenol content	
		By wt or vol	By serving
		mg/kg fresh wt (or mg/L)	*mg/serving*
Hydroxybenzoic acids (2, 6)	Blackberry (100 g)	80–270	8–27
Protocatechuic acid	Raspberry (100 g)	60–100	6–10
Gallic acid	Black currant (100 g)	40–130	4–13
p-Hydroxybenzoic acid	Strawberry (200 g)	20–90	4–18
Hydroxycinnamic acids (2, 5–7)	Blueberry (100 g)	2000–2200	200–220
Caffeic acid	Kiwi (100 g)	600–1000	60–100
Chlorogenic acid	Cherry (200 g)	180–1150	36–230
Coumaric acid	Plum (200 g)	140–1150	28–230
Ferulic acid	Aubergine (200 g)	600–660	120–132
Sinapic acid	Apple (200 g)	50–600	10–120
	Pear (200 g)	15–600	3–120
	Chicory (200 g)	200–500	40–100
	Artichoke (100 g)	450	45
	Potato (200 g)	100–190	20–38
	Corn flour (75 g)	310	23
	Flour: wheat, rice, oat (75 g)	70–90	5–7
	Cider (200 mL)	10–500	2–100
	Coffee (200 mL)	350–1750	70–350
Anthocyanins (8–10)	Aubergine (200 g)	7500	1500
Cyanidin	Blackberry (100 g)	1000–4000	100–400
Pelargonidin	Black currant (100 g)	1300–4000	130–400
Peonidin	Blueberry (100 g)	250–5000	25–500
Delphinidin	Black grape (200 g)	300–7500	60–1500
Malvidin	Cherry (200 g)	350–4500	70–900
	Rhubarb (100 g)	2000	200
	Strawberry (200 g)	150–750	30–150
	Red wine (100 mL)	200–350	20–35
	Plum (200 g)	20–250	4–50
	Red cabbage (200 g)	250	50
Flavonols (11–18)	Yellow onion (100 g)	350–1200	35–120
Quercetin	Curly kale (200 g)	300–600	60–120
Kaempferol	Leek (200 g)	30–225	6–45
Myricetin	Cherry tomato (200 g)	15–200	3–40
	Broccoli (200 g)	40–100	8–20
	Blueberry (100 g)	30–160	3–16
	Black currant (100 g)	30–70	3–7
	Apricot (200 g)	25–50	5–10
	Apple (200 g)	20–40	4–8
	Beans, green or white (200 g)	10–50	2–10
	Black grape (200 g)	15–40	3–8
	Tomato (200 g)	2–15	0.4–3.0
	Black tea infusion (200 mL)	30–45	6–9
	Green tea infusion (200 mL)	20–35	4–7
	Red wine (100 mL)	2–30	0.2–3
Flavones (11–12, 14, 18)	Parsley (5 g)	240–1850	1.2–9.2
Apigenin	Celery (200 g)	20–140	4–28
Luteolin	Capsicum pepper (100 g)	5–10	0.5–1
Flavanones (19–21)	Orange juice (200 mL)	215–685	40–140
Hesperetin	Grapefruit juice (200 mL)	100–650	20–130
Naringenin	Lemon juice (200 mL)	50–300	10–60
Eriodictyol			
Isoflavones (22–25)	Soy flour (75 g)	800–1800	60–135
Daidzein	Soybeans, boiled (200 g)	200–900	40–180
Genistein	Miso (100 g)	250–900	25–90
Glycitein	Tofu (100 g)	80–700	8–70
	Tempeh (100 g)	430–530	43–53
	Soy milk (200 mL)	30–175	6–35
Monomeric flavanols (6, 17, 26, 27)	Chocolate (50 g)	460–610	23–30
Catechin	Beans (200 g)	350–550	70–110
Epicatechin	Apricot (200 g)	100–250	20–50
	Cherry (200 g)	50–220	10–44
	Grape (200 g)	30–175	6–35
	Peach (200 g)	50–140	10–28
	Blackberry (100 g)	130	13
	Apple (200 g)	20–120	4–24
	Green tea (200 mL)	100–800	20–160
	Black tea (200 mL)	60–500	12–100
	Red wine (100 mL)	80–300	8–30
	Cider (200 mL)	40	8

Fig Polyphenols in food (H. Zhang & Tsao, 2016)

Besides fasting, numerous studies strongly support the protective effects of polyphenols on glucose homeostasis. Two of the most common flavanols, EGCG and resveratrol, have been demonstrated to affect numerous processes, including insulin secretory function, glucose uptake and tolerance, insulin resistance, oxidative stress, inflammation and mitochondrial function. Modification of the glucose metabolism especially caloric restriction using the Warburg effect is under discussion for an combinational therapy fighting cancers including cytokine therapies, targeted antibodies, adoptive cell transfers, genetically engineered chimeric antigen receptor (CAR) T cells, cancer vaccines, genetically engineered oncolytic viruses, and immune checkpoint blockade (ICB). (Turbitt et al., 2019)

Epigenetic active foods

Over the past decade, remarkable breakthroughs in our understanding of epigenetic biology have coincided with an increased public interest in the impact of diet and lifestyle choices on health. It is well established that a balanced diet enhances life expectancy and helps to prevent or treat certain

diseases, such as obesity, diabetes, cancer, and mental disorders. However, the biological mechanisms underlying these effects are not yet well understood. One possibility is through directly affecting catalytic activities of the enzymes responsible for 'writing' or 'erasing' the epigenetic modifications. Wang et al. identified two phytochemicals, dihydrocaffeic acid (DHCA) and malvidin-3'-O-glucoside (Mal-gluc), metabolic intermediates derived from Concord grape juice, grape seed extract, and trans-resveratrol—that attenuate depression-like behaviour's in mice (Jun Wang et al., 2018). Using a mouse model, Wang et al. showed that treating mice with DHCA and Mal-gluc, which were added to drinking water, increased their resilience to stress, and reduced depression-like behaviours. Specifically, the authors found that DHCA reduced expression of "methyl-DNA writer"—the DNA methyltransferase 1 (DNMT1). DNMT1 methylate's intronic sequences of interleukin 6 (*IL-6*) genes, reducing the level of this pro-inflammatory cytokine which has previously been implicated in the development of depressive disorders. Mal-gluc, on the other hand, reduced the expression of an "acetyllysine eraser", histone deacetylase 2 (HDAC2), which led to a significant increase in histone H3 acetylation on promotor of the RAS-related botulinum toxin substrate 1 (*Rac1)* gene, whose expression level was found to be downregulated under chronic stress.

Malnutrition in early life has directly and indirectly been linked to a number of disorders in adulthood, giving rise to the hypothesis of epigenetic memory. In their study, Yuan et al. showed that lactation in mice causes epigenetic changes that influence the likelihood of the development of obesity later in life (Jun Wang et al., 2018). Milk lipids are known to activate the nuclear receptor, PPARα (peroxisome proliferator-activated receptor), an important transcriptional regulator of hepatic liver metabolism. Using a genome-wide analysis of DNA methylation, Yuan et al. identified several PPARα target genes, including the fibroblast growth factor-21 (*Fgf21*), that undergo PPARα-dependent DNA demethylation upon pharmacological activation of PPARα in mice. Fgf21 is a liver hormone essential in a number of metabolic functions, including bodyweight maintenance and regulation of energy homeostasis. Yuan et al. found that once established in early life, the DNA (de)methylation status of Fgf21 remains unchanged in the adulthood, and that the reduced DNA methylation of *Fgf21* correlates with a reduction in diet-induced obesity in older animals. The authors further demonstrated that *Fgf21* demethylation is stimulated during the lactation period of new-born mice. The results of this study are striking and may have a profound impact on our understanding of the mechanisms contributing to obesity. These data suggest a link between breastfeeding and weight gain suppression and also elegantly illustrate how specific epigenetic modifications built in early life produce a long-lasting effect.

Many other factors have been shown to markedly contribute to changes in epigenetic programs and responses. For example, Krautkramer et al. reported that gut microbes and their metabolites affect host chromatin state, increasing histone poly-acetylation and producing short-chain fatty acids (SCFAs) (Krautkramer et al., 2016; Mischke & Plösch, 2016). A "Western-type" diet rich in processed foods and high sugar drinks was found to limit microbial SCFAs production, prevent many of the microbiota-dependent events to occur, and lead to alterations in hepatic gene expression. Another example is the effect of a low-carb ketogenic diet that was shown to rescue hippocampal memory defects in a mouse model of Kabuki syndrome, characterized by loss of site-specific histone methylation and deficiency in chromatin opening. This diet promotes formation of β-hydroxybutyrate, an HDAC inhibitor, and leads to changes in H3ac and H3K4me3 in the hippocampus and rescue of the neurogenesis and memory phenotypes of these mice models. Ketogenic diet, butyrate and β-hydroxybutyrate are discussed for an epigenetic intervention of many neuropsychiatric disorders, e.g. depression (Bourassa et al., 2016; Włodarek, 2019).

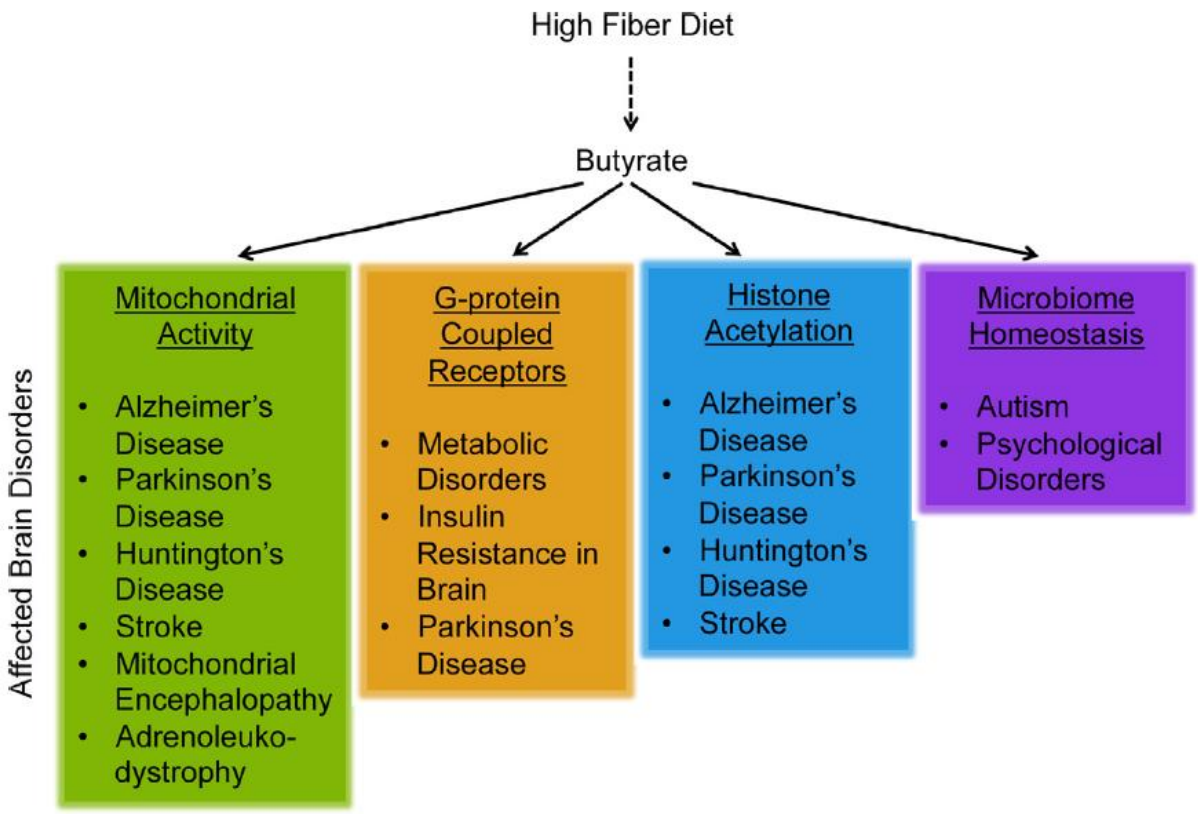


Fig Neuroprotective effects of butyrate and the diseases which may benefit from butyrate treatment or a high fibre diet. (Bourassa et al., 2016)

The term **'nutriepigenomics'** describes interactions of nutrients and their effects on human health through epigenetic modifications defined as stable heritable patterns of gene expression occurring without changes in the DNA sequence. These epigenetic patterns include interacting components at the transcriptional level, DNA methylation, and histone modifications, and at the posttranscriptional level, RNA interference. In addition, to the nutrition of an individual, we have to consider also transgenerational, prenatal, and postnatal effects as epigenetic alterations at critical time points during development can result in stable changes and predispose individuals to disease later in life. Numerous nonmendelian features of metabolic syndrome, cancer, or central nervous system disorders, clinical differences between men and women or monozygotic twins are associated with epigenetic effects of fetal and/ or lifelong nutrition. One famous example of nutriepigenomics is the development of a honeybee into a queen or worker with identical genomes following different feeding with royal jelly or a diet of pollen and nectar, respectively.

Nutrients affecting one of the two metabolites of the 1-carbon metabolism, S-Adenosylmethionin, a ubiquitous methyl donor, or S-adenosylhomocysteine, an inhibitor of methyltransferases, potentially alter the methylation of DNA and histones. Methylated promoter and other regulatory regions of a gene are usually associated with gene repression, whereas DNA demethylation within these regions leads to gene activation. Polyphenols, including curcumin, genistein, epigallocatechin gallate (EGCG), resveratrol, and equol, are well known for their beneficial effects via modulation of nuclear factor kappa B (NFkB) expression, chromatin remodeling through regulation of histone deacetylases (HDACs), and DNA methyltransferases activities. Components such as gut microbial-derived butyrate, sulforaphane, and curcumin affect histone acetyl-transferase (HATs) and/or HDACs activities leading

to changes in chromatin structure. Vitamins like, biotin, niacin, and panthothenic acid, influence histone modifications, for example, biotin influences histone biotinylation AQ5 and niacin histone ADP-ribosylation. Resveratrol, butyrate, sulforaphane, and diallyl sulfide inhibit HDACs, whereas curcumin inhibits HATs .Although the action of many bioactive substances is specific to enzymes and proteins involved in the regulation of different components of the epigenome, interaction with other nutrients and lifestyle factors in physiological and pathological conditions must be taken into account as well. In addition, epigenetic components exert effect over each other. It adds an additional layer of complexity to the action of epigenetically active nutrients. Studies demonstrate that DNA methylation and histone modifications that act together to establish chromatin structure are involved in miRNA regulation and vice versa. Thus, deeper knowledge of bioactive nutrients/diets for characterization of their effects on the epigenome modifying enzymatic activities (acetylation, methylation, phosphorylation, ribosylation, oxidation, ubiquitination, and sumoylation) influencing drug absorption, distribution, metabolism, and excretion is needed (Marlene Remely et al., 2015)**.**

Bioactive food components may play a role in the prevention of various diseases, but researchers still face challenges which inhibit the implementation of such compounds into clinical practice. A major factor is the lack of knowledge underlying the mechanisms and effectiveness of these metabolites and whether in vitro results are replicable in vivo in humans. Another concern is the poor **bioavailability** of the nutrients, which may be increased by combining certain bioactive compounds with other nutrients such as curcumin with peperino from black pepper. However, pipeline has been discussed for prevention abut also progression of cancerogenesis. (Manayi et al., 2017; Rather & Bhagat, 2018; Zadorozhna et al., 2019)Furthermore, dose-dependent effects have been seen with some metabolites indicating a higher disease risk with elevated nutrient doses. (Stefanska et al., 2012).

Nutriepigenetic research mostly looks at cancer, possibly because of the strong link between epigenetic modifications and cancer development and progression. Possible future research could be geared towards different kinds of diseases that are affected by epigenetic mechanisms. As emphasized by multiple studies mentioned above there is an urgent need for more in vivo and clinical trials exploring the possible beneficial effects of bioactive food compounds.

The use of bioactive food components for novel disease treatments may be promising as at an adequate dose these compounds show no toxic effects and appear safe for human use, making them ideal for disease treatment. Due to the low bioavailability of certain compounds, foods or diets may not be the ideal treatment option. This does not make the current findings irrelevant. The mechanism by which a certain compound acts upon a disease could be used to find alternative synthetic chemicals, such as CDF-diflourinated-curcumisynthetic instead of curcumin, which offer the same beneficial effects and higher bioavailability. The use of higher dose supplements or combination of certain foods may increase bioavailability. Caution is advised as some synthetic analogs have shown negative side effects (e.g. synthetic HDAC inhibitors) and therefore plant-derived alternatives (e.g. Sulforaphane) could be an alternative here. In general, safety relevant dose effects are often poorly understood for nutraceuticals. (Ronis et al., 2018) Even though bioactive compounds may not yet be used in clinical practice, they still provide important mechanistic information of possible disease treatment possibilities. Combined therapy (standard therapy combined with bioactive compounds) is proposed as a promising alternative to standard treatments by almost all papers mentioned above.

Examples of bioactive food components and epigenome interactions

Polyphenols (bioflavonoids and catechins) such as quercetin, fisetin, myricetin, catechin, epicatechin and epigallochatechin-3-gallate (EGCG) have been shown to inhibit DNA methylation by effecting DNMT activity. Polyphenols are found amongst others in a variety of fruits and vegetables, black and green tea, chocolate, and coffee.

An in vitro study performed on prokaryotic and human cells proposed two mechanisms of DNA methylation inhibition by polyphenols:

Polyphenol structures all contain a catechin group which is the main component allowing the inhibition of DNMT activity. Dietary catechol's can be methylated by the enzyme catechol-O-methyltransferase (COMT) using the same methyl donator SAM as in DNA methylation. This specific methylation mechanism is called O-methylation. During O-methylation of various polyphenols S-Adenosylmethionine (SAM) is turned into S- Adenosylhomocystein (SAH), which is a feedback inhibitor of DNMTs. It is thought that higher SAH concentrations through O-methylation may lead to the inhibition of DNMTs and thus indirectly inhibit DNA methylation. The second mechanism is the direct inhibition of DNMTs through EGCG, which is independent of the O-methylation pathway. EGCG is a noncompetitive inhibitor of DNMTs and its effect is enhanced by the presence of Mg2+ (Won et al., 2005).

Cancer development and progression are often affected by epigenetic mechanisms that silence tumor suppressor genes which are crucial defense mechanisms for the cell. Silencing of genes has been linked to hypermethylation of CpG islands in promotor sequences.

EGCG, a catechin found in green tea, has been shown to reduce global DNA methylation by downregulating DNMT activity and to reactivate silenced tumor suppressor genes in human skin cancer cells. Although the study was in vitro and lasted only 6 days the results are promising to consider polyphenols for potential chemoprevention. Moreover, EGCG has been linked to antioxidant, anti-inflammatory and DNA repairing effects which also may play an important role in cancer prevention. Furthermore, there have been no negative side effects reported in adequate green tea consumption, while too high doses of EGCG caused cell apoptosis (Nandakumar et al., 2011).

A systematic review examining green tea for ovarian cancer prevention confirmed the promising properties of green tea and its components. Green tea was associated with decreasing expression of cancer-related proteins, decreased risk of ovarian cancer and increased survival for patients with ovarian cancer. The authors emphasized a need for further studies, especially in vivo models, analyzing the exact mechanisms of EGCG on cancer prevention. Furthermore, optimal doses, intake method and timing of green tea need to be evaluated. The study concludes that although the effects of green tea sound promising more research is needed to introduce green tea or its components into medical field (Trudel et al., 2012).

One can conclude that in vitro human models do confirm the positive effects of polyphenols, the question remaining is whether these findings can be extrapolated in vivo and at what doses.

Sulforaphane's impact on cancer and osteoporosis: Sulforaphane is an isothiocyanate found in cruciferous vegetables such as broccoli or broccoli sprouts. It has been shown to be an HDAC inhibitor which may play a role in cancer and neurogenerative diseases. Some synthetic HDAC inhibitors are already being used in the clinical field, but these have led to dose-dependent toxicities.

Researchers have therefore turned their attention to plant-derived chemicals such as sulforaphane which shows no toxicity towards non-cancer cells. A review confirmed the potential of sulforaphane as an HDAC inhibitor in prostate cancer both in vivo and in vitro models. It was suggested that the cytotoxic effect of the broccoli-derived molecule may be even greater when combined with "traditional" cancer therapies (combined therapy). This may be increasingly relevant for patients who develop resistance to cancer therapy. (Ganai, 2016)

Osteoporosis and other bone-related diseases may be due to changes in bone homeostasis caused by age. Thaler and colleagues found that sulforaphane could lead to higher bone density in mice by promoting osteoblast differentiation through DNA demethylation and by decreasing osteoclast activation by inhibiting the expression of osteoclast activators. Sulforaphane was also shown to induce bone matrix mineralization in vitro by enhancing the expression of osteoblast-specific transcription factors through DNA demethylation. The authors noted that the mice used were very young (8 weeks old) and for a better understanding experiments with older mice should be conducted. Even though the study was conducted on mice (in vivo) and in cell cultures (in vitro) it opens up opportunities for continued research on the potential benefits of sulforaphane and may allow new therapeutic strategies to be developed (Thaler et al., 2016).

Curcumins role in epigenetic modifications: Curcumin is a compound present in turmeric which has been linked to all three major epigenetic modifications (DNA methylation, histone modification and miRNA regulation). A systematic review analyzing the effects of phytochemicals on miRNAs indicated that curcumin showed upregulation and downregulation in pancreatic cancer cells (in vitro) and downregulation of oncogenic miRNA in gastric cancers. It was noted that such findings have not been effectively replicated in in-vivo studies. Low bioavailability in vivo has lead researchers to propose CDF-diflourinated- curcumisynthetic, a synthetic analog of curcumin, as a potential chemo-preventative compound in pancreatic cancer (Parasramka et al., 2012).

Antioxidant and anti-inflammatory effects have been linked to curcumin, but the exact mechanisms are unclear. An in vitro study conducted on human retinal pigment epithelium (RPE) cells evaluated the effect of curcumin on H_2O_2-induced miRNA expression. H_2O_2 (hydrogen peroxide), a reactive oxygen species caused by oxidative stress, is responsible for age-related macular degeneration by attacking the RPE. The authors showed that curcumin could protect RPE cells from oxidative damage by regulating miRNA expression of proinflammatory and antioxidant genes. Cells pretreated with curcumin that were exposed to H_2O_2 showed reduced H_2O_2-induced miRNA expression. For example, curcumin downregulated miR-302, a miRNA that inhibits epigenetic regulators such as DNMT1 and causes DNA demethylation. This study does not only provide possible evidence for novel treatments for oxidative-stress mediated diseases though curcumin, but it may help to further understand the antioxidative and anti-inflammatory effects of the compound (Howell et al., 2013).

Curcumin has also been found to be an HDAC inhibitor and to modulate the expression of HDACs in mice and cell lines in different cancer types. While in vitro and in vivo data confirm the benefits of curcumin in cancer cells, Soflaei et. al. points out a lack of clinical evidence and proposals of a combined treatment using curcumin and standard cancer therapies. The authors emphasize the safety of curcumin use for humans and it's anti-cancer properties, propagating a promising cancer treatment. (Soflaei et al., 2018)

Link et. al. supported the hypothesis that curcumin may regulate DNA methylation in colorectal cancer cells. An in vitro analysis of human cancer cells showed that long term curcumin treatment induced methylation of specific genes associated with colorectal cancer. The authors suggested that

curcumin does not influence DNA methylation by modulating DNMTs but rather through regulation of gene expression (Link et al., 2013).

Natural compound	Natural sources	Pharmacological effects	Epigenetic mechanisms of action
Folate, cobalamin, riboflavin, pyridoxine, methionine	Folate and riboflavin: spinach, asparagus, beans, peas, lentils, sunflower seeds, almonds Cobalamin: fish, shellfish, poultry, milk, eggs Pyridoxine and methionine: grains, nuts, dragon fruit, sesame seeds	Anti-cancer, anti-proliferative, chemoprevention of malignant transformation	Regulation of one-carbon metabolism, SAM/SAH ratio, DNMT and MBD expression; regulation of miRNAs (tumour suppressor miR-122, miR-34a, miR-127, and oncogenic miR-21, miR-222)
Retinoic acid	Vietnamese gac, crude palm oil, yellow and orange fruits (mango, papaya), orange root vegetables (carrots), spinach, sweet potatoes	Anti-cancer, anti-proliferative, differentiating, pro-apoptotic	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN and ERs; regulation of miRNAs targeting DNMTs; regulation of tumour suppressor miRNAs (miR-15, miR-16, let-7a, let-7c, miR-34a, miR-342) and oncogenic miRNAs (miR-10a); GNMT regulation; histone acetylation
Vitamin D3	Sun exposure, fish, fish liver oils	Anti-cancer, anti-proliferative, differentiating, pro-apoptotic	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1, PTEN and ERs; regulation of histone acetylation; regulation of oncogenic miRNAs (miR-181a, miR-181b)
Resveratrol	Roots of hellebore, grapes, mulberries, apricots, pineapples, peanuts	Anti-cancer, antioxidant, anti-proliferative, anti-angiogenesis, anti-inflammatory, pro-apoptotic, cardioprotective	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1 and PTEN; activation of deacetylase SIRT1 and p300 HAT; down-regulation of UHRF1; regulation of miRNAs
Genistein and daidzein	Soybeans, lupin, kudzu, psoralea, fava beans, coffee	Anti-cancer, antioxidant, antihelminthic, anti-metastatic, cancer protective	Regulation of DNMTs expression and enzyme activity by affecting p21, AP-1 and PTEN; increase in HAT activity; regulation of miRNAs (tumour suppressor miR-1296, miR-16, and oncogenic miR-27a)
EGCG	Green tea	Anti-cancer, antioxidant, anti-proliferative, anti-angiogenesis, anti-inflammatory, pro-apoptotic, cancer protective	Regulation of SAM/SAH ratio by COMT-mediated reactions; direct inhibition of DNMTs by binding to catalytic domain of the enzyme; regulation of tumour suppressor miRNAs (miR-16)
Curcumin	Spice turmeric	Anti-cancer, antioxidant, protects against heart failure	Direct inhibition of DNMTs by binding to catalytic domain of the enzyme; inhibition of HDACs and p300 HAT; regulation of miRNAs (tumour suppressor miR-22, miR-15a, miR-16, and oncogenic miR-21, miR-199a)

Fig Epigenetic active compounds and activities (Stefanska et al., 2012)

Sirtuin activation by “SIRT foods”

Sirtuins, commonly also referred to as silent mating type information regulation 2 homologous (SIRT), are a class of proteins which can be found in all living organisms, from bacteria and archaebacteria to mammals, and were first discovered in the 1990s in an effort to find yeast mutants with longer life durations. (SINCLAIR, 2006) SIRT1 is mainly located in the cell nucleus, but it can also be found in the cytosol. SIRT2 is also located in the cytosol, where it has its main site. SIRT3, SIRT4

and SIRT5 are mitochondrial proteins, but SIRT3 can also be located in the cell nucleus and cytosol under different cellular conditions. SIRT6 and SIRT7 are located in the cell nucleus and nucleolus respectively (Alhazzazi, 2011, p. 80-88).

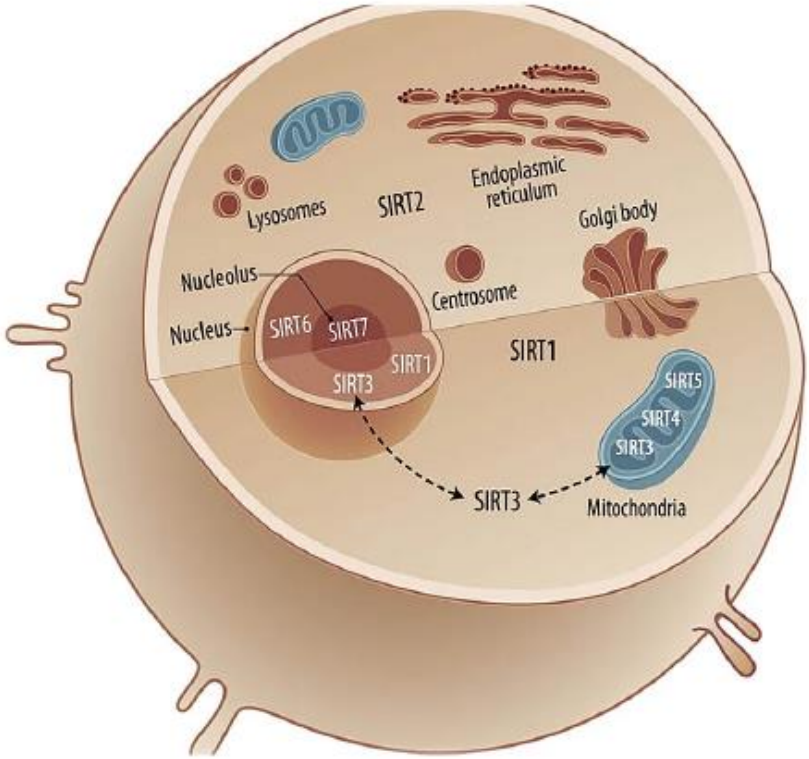


Figure 1: Subcellular localization of sirtuins

Fig localisation sirtuins, (Alhazzazi, 2011)

Humans possess a total of seven sirtuins (SIRT1-SIRT7). Just like in yeast, they act as energy sensors in our cells and are activated when there is a lack of energy. Thanks to their properties, sirtuins are therefore multifunctional and regulate many metabolic processes as well as the aging process (RAUH, 2013). In fact, increased activity of a yeast's sirtuin, silent information regulator two (Sir2), can extend its life. It ensures the silencing of certain chromatin regions by deacetylating histones. This attenuation of chromatin activities, such as during replication, recombination, and transcription, seems to be essential for prolonging the life of Sirt2. Interestingly, an increase in sirtuin activity also prolongs the lifespan of more complex organisms, such as the worm Caenorhabditis elegans or the fruit fly Drosophila melanogaster. Recent studies suggest that sirtuins also plays an important role in the regulation of the life span of mammals. In more complex species, sirtuins can deacetylate a number of cellular regulatory proteins, in addition to histones, and can influence their activity in a positive or negative manner. However, the life-enhancing effect of increased sirtuin on mice has not yet been clearly documented.

Strong evidence supports a role for SIRT1 mediating an oxidative stress response by directly deacetylating several transcription factors that regulate antioxidant genes. Notably, SIRT1 activates several members of the FOXO family of transcription factors which promote the expression of stress

response genes including SOD2. For example, SIRT1 functions in an autoregulatory loop along with the early growth response protein ERG1 to regulate SOD2 to protect contracting muscle cells from oxidative stress. SIRT1 also promotes mitochondrial biogenesis by activating peroxisome proliferator-activated receptor co-activator 1-α (PGC-1α). PGC-1α increases mitochondrial mass and upregulates the expression of oxidative stress genes including glutathione peroxidase (GPx1), catalase, and manganese SOD (MnSOD). Finally, SIRT1 inactivates the p65 subunit of NF-κB through direct deacetylation. NF-κB inhibition suppresses the inducible nitric oxide synthase (iNOS) and nitrous oxide production and thus may lower the cellular ROS load. Given its role in the antioxidant response, whether SIRT1 activation contributes to CR mediated lifespan extension has been extensively studied. CR fails to increase the lifespan of SIRT1 knock-out mice, and these mice do not increase their physical activity, a phenotype typically associated with calorically restricted mice. Similarly, SIRT1 overexpression mimics a caloric restriction phenotype. Precisely how SIRT1 functions during CR remains an open question, but emerging evidence suggests that p53 plays an important role in modulating SIRT1 during CR. Mitochondria account for the majority of cellular ROS production. Mitochondrial SIRT3 deacetylates and activates several enzymes that are critical in maintaining cellular ROS levels. SIRT3 deacetylates SOD2 at two important lysine residues to boost its catalytic activity and the catalytic activity of SOD2 is diminished when SIRT3 is deleted. SIRT3 knock-out mice fail to reduce their levels of lipid peroxidation and protein carbonylation that are typically observed during caloric restriction indicating that SIRT3 is necessary for caloric restriction to mitigate oxidative stress. Additionally, SIRT3 stimulates the activity of mitochondrial isocitrate dehydrogenase, IDH2, during caloric restriction through direct deacetylation. IDH2 promotes the conversation of NADP+ to NADPH which in turn provides the reducing equivalents for conversion of oxidized to reduced glutathione. In support of this biochemical data, SIRT3 is required to protect calorically restricted mice from age-associated hearing loss. Another link between SIRT3 and oxidative stress comes from the field of oncology. Since ROS can severely damage nucleic acids, it is not surprising that oxidative stress can promote tumorigenesis. SIRT3 knock-out mouse embryonic fibroblasts (MEFs) exhibit higher ROS levels, greater genomic instability, and increased sensitivity to oncogenic transformation compared to wild-type fibroblasts (Kim et al, 2010). Intriguingly, overexpression of SOD2 suppresses oncogenic transformation in SIRT3 knock-out MEFs suggesting that SIRT3 may protect against tumorigenesis through an oxidative stress mechanism. In support of the above in vitro data, mice deficient for SIRT3 are more susceptible to cancer and many human tumors display reduced SIRT3 levels compared to healthy tissues. In addition to suppressing the formation of cancer, SIRT3 can also combat established tumors (Leonard Guarente, 2011a; Merksamer et al., 2013),

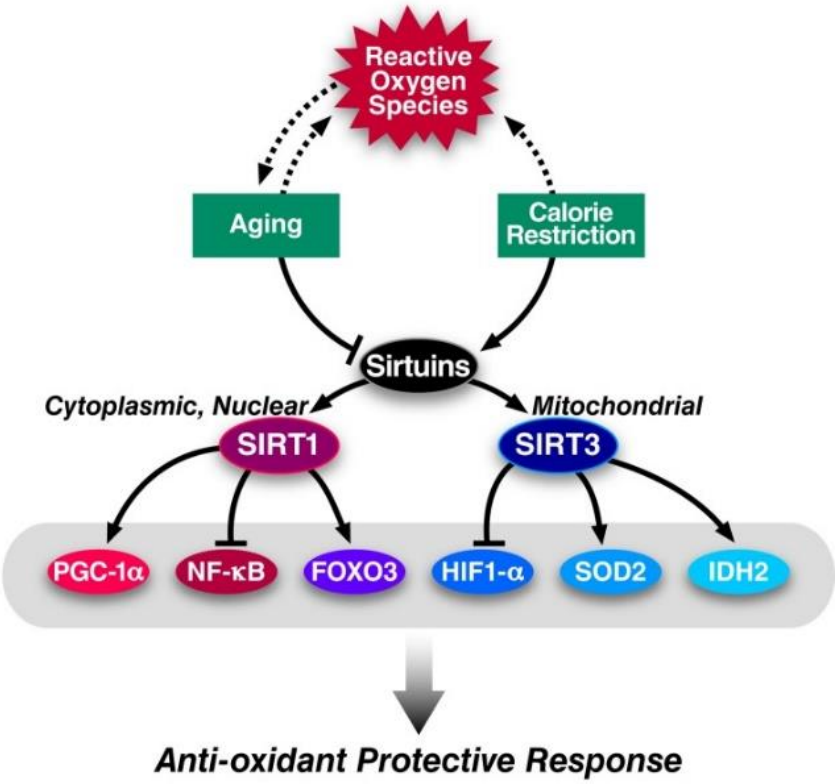


Fig Role of SIRTS, Merksamer et al., 2013

The close link between the sirtuin function and cellular metabolism plays a central role in regulating the lifespan. For example, sirtuins are necessary to activate the life-prolonging effect which occurs during the restriction of calories. Studies have shown that in animals, including mammals, a reduced calorie intake leads to a general increase in fitness and prolonged life span. For example, in mice, Sirt1 activity is increased when calorie restrictions are in place (Bober 2007). Recently, research has focused on ways how to activate these sirtuins without fasting and foods which stimulate sirt enzymes came into broader interest. Sirt-Foods are plant-based foods composed of polyphenols – secondary plant substances. These substances act on the sirtuins and imitate the 'lack of energy' signal which normally results from fasting, dieting and/or exercise. In other words, SirtFoods can activate sirtuins without fasting taking place. In countries such as Japan and India, varying sirtuins are part of the daily diet. Also known as 'blue zones', these countries have the lowest incidences of lifestyle-related diseases, including hypertension, obesity, diabetes, fatty liver, and cancer, due to higher sirtuin activating diets. In addition, the polyphenols contained in SirtFoods have been closely linked to positive effects on epigenetic mechanisms and many other genes known for promoting a long and, above all, healthy life (Piwpong, 2018, p. 98- 112). The sirtuin genes, and the resulting sirtuin enzymes, are activated during low energy cycles. Certain natural plant compounds such as Red wine, Strawberries, Onions, Soy, Parsley, Extra virgin olive oil, Dark chocolate (85% cocoa), Green tea, Buckwheat, Turmeric or Walnuts may be able to increase the level of these proteins in the body, and foods containing them have been dubbed "sirtfoods." SIRT foods have attracted a broad interest in public media: https://www.healthline.com/nutrition/sirtfood-diet#section1
https://www.bbcgoodfood.com/howto/guide/what-sirtfood-diet

Senolytic foods

Accumulating evidence suggests that targeting some of the aging hallmarks, for example, cellular senescence, can significantly improve human health and extend health span. **Cellular senescence** is a phenomenon where normal cells stop dividing. Senescent cells (SCs) accumulate in various tissues during the aging process. On one hand, cellular senescence blocks the propagation of damaged cells in order to maintain tissue homeostasis. On the other hand, it plays a causative role in irreparable, deleterious cellular damage and loss of tissue homeostasis, which relates to aging and aging-associated diseases. Accumulating evidence demonstrates that elimination of SCs can reduce age-dependent deterioration in tissues and organs, which is useful in improving the treatment of age-associated diseases and alleviating the side effects of therapy-induced. Small molecules that can selectively kill SCs, called **senolytics,** have the potential to both prevent and treat age-related diseases, thereby extending health span. Until now, several classes of senolytic agents, including natural compounds such as quercetin, fisetin , piperlongumine , and curcumin analog EF24, and targeted therapeutics, which are mainly senolytic target inhibitors, have been identified. Compared to the targeted senolytics, natural senolytic compounds are less potent, but have low toxicity. They may also have a better chance of being translated into the clinical setting to treat age-related diseases or used as a lead for the development of more specific and potent senolytic agents (W. Li et al., 2019)(Bielak-Zmijewska et al., 2019).

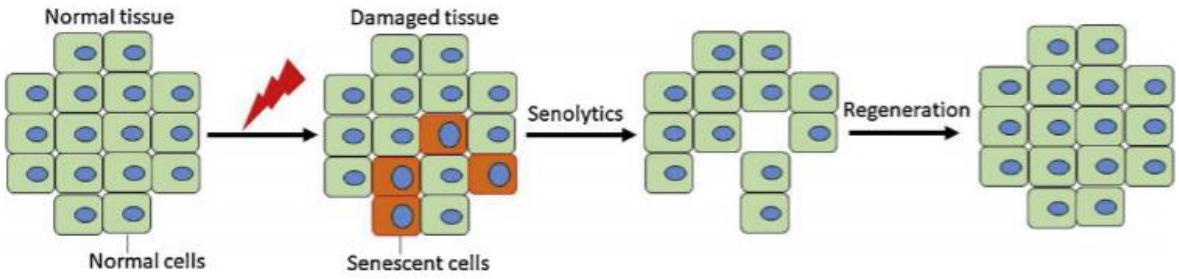


Fig cellular senescence (W. Li et al., 2019)

Fasting mimetics

As the world population ages, chronic diseases such as diabetes, cardiovascular disease, cancer, and neurodegeneration become ever more prevalent. Interventions that favour healthy aging would constitute powerful strategies with which to limit human diseases that have a broad socioeconomic impact. Fasting regimens such as intermittent fasting or dietary adaptations such as caloric restriction is among the few regimens that extend life and beneficially affect health in all tested model organisms, including rodents and nonhuman primates. However, few people seem capable of changing their dietary routines for extended periods. Thus, supplementation with caloric restriction mimetics (CRMs), which would pharmacologically mimic the beneficial effects of caloric restriction or fasting, has gained attention as an attractive and potentially feasible strategy (Madeo et al., n.d.).

Weight loss or gain, metabolic rate but also nutrition-related disorders, like obesity or diabetes mellitus 2 (DM2), result from several factors, such as genetics, epigenetics, environment and lifestyle, and the composition of gut microbiota. Individual responses to nutrition and diet have been extensively investigated as a consequence of genetic disposition, especially single nucleotide polymorphism. Genetic variants can only explain a minor part of inheritance. Recently, it became obvious that epigenetic characteristics are also involved into personal responses to diet.

Caloric restriction (CR) and fasting are becoming more popular and are known for their beneficial effect on energy metabolism and furthermore on lifespan, increasing longevity. Although the mechanisms are not yet fully understood, one mechanism may involve the upregulation of SIRT1, which is located in the cell nucleus, requires NAD+ as a cofactor and is negatively regulated by NADH. CR decreases the cellular ATP concentration and increases the NAD+/NADH ratio. SIRT1 but also SIRT6 can interfere with the NF-kB pathway through deacetylation of Polymerase II p65 subunit, leading to inhibition of NF-kB signaling. NF-kB is involved in the etiology of insulin resistance induced by the altered pattern of inflammatory cytokines in the adipocytes. This might be one possible mechanism where HDACs interact with insulin sensitivity. More recently there is growing evidence that caloric restriction, fasting, and fasting mimetics may trigger senolysis (Shetty et al., 2018).

SIRT1 has been identified as a potential locus at which changes in cellular energy metabolism could modify clock function by causing deacetylation of core clock components. SIRT1 is present and functional in adipose tissue. SIRT1 gene expression increases several-fold during the early phase of adipogenesis but is also functional in mature differentiated adipocyte, promoting triglyceride mobilization with prolonged food limitation by repressing the Peroxisome proliferator-activated receptor gamma (PPAR-g). Thus, SIRT1 could cause daily frequency in adipogenetic potential by effects on PPAR-g and core clock components.

PPARs regulate the expression of several genes involved in metabolic processes that are potentially linked to the development of some diseases such as hyperlipidemia, diabetes, and obesity. Serum PPARG levels in obese subjects were significantly decreased as compared to healthy controls. PPARα is involved in ensuring energy availability and plays a prominent role in fasting and its function is to both stimulate lipolysis of white adipose tissue to supply fatty acids to non-adipose tissue organs and to control ketogenesis in the liver to obtain energy from fatty acids. Ketone body formation, ketogenesis, is elevated when blood glucose levels drop. Moderately elevated blood ketone bodies occur normally during fasting and prolonged exercise. For instance, β-Hydroxybutyrate is a ketone body primarily synthesized in the liver from the oxidation of fatty acids.

Various polyphenols found in plants are able to activate biochemical pathways in mammalian cells so as to confer health benefits. These molecules include resveratrol, catechins, quercetin and genistein. There is considerable overlap between these pathways and those activated by caloric restriction. The chemicals have therefore been termed “calorie restriction mimetics”. In both cases, the response induced steels the cell to resist adverse or stressful conditions. As a result of their effects cells become more resistant to oxidative stress, inflammatory pathways are suppressed, and changes are seen in the cell cycle. Resveratrol has been shown to extend lifespan in various organisms and to extinguish disease processes in obese mice, so that instead of dying early, the mice have a normal lifespan. The changes induced seem in large part to be mediated by their activation of sirtuin enzymes. Sirtuins deacetylate and thereby activate transcription factors that alter the expression of various genes involved in stress-resistance (B. J. Morris, 2010).

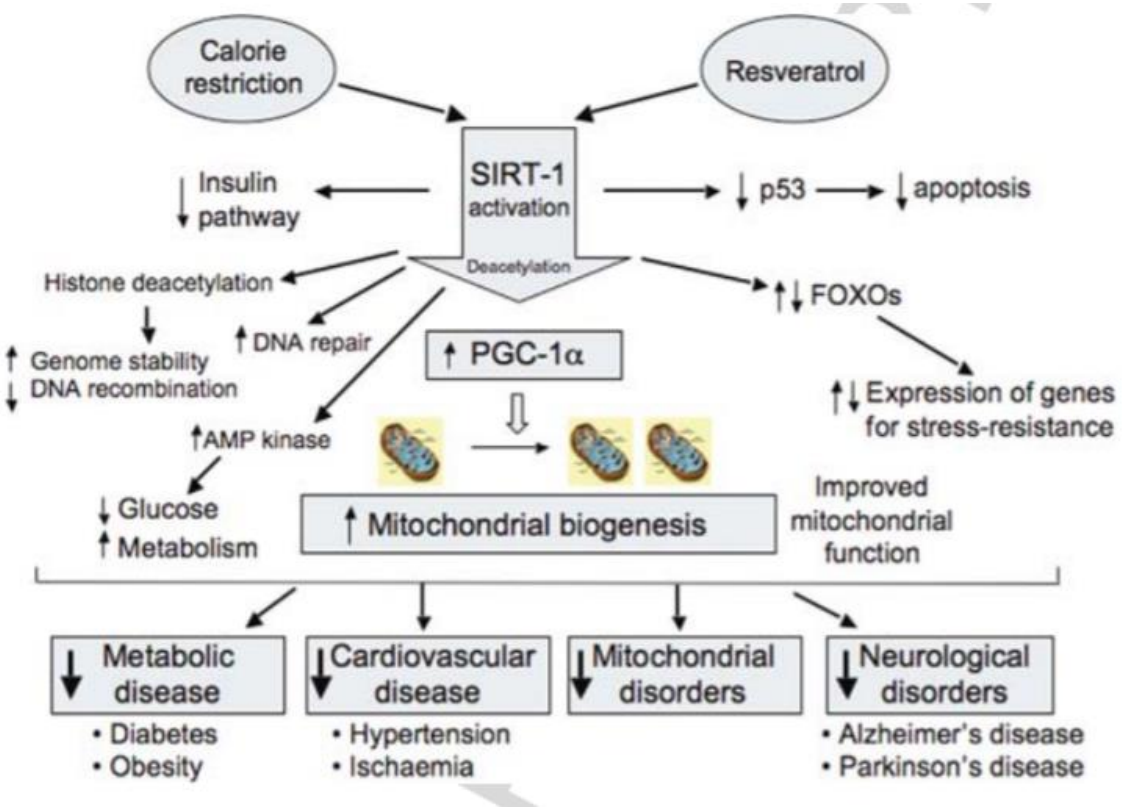


Fig General pathways and processes activated by calorie restriction and resveratrol (B. J. Morris, 2010)

In a recent clinical trial the decrease of senescent cells by senolytics Dasatinib plus Quercetin was shown in individuals with diabetic kidney disease (Hickson et al., 2019).

Synthetic sirtuin activators and inhibitors have potential as pharmaceuticals in the treatment of cancer. Nicotinamide is a well-known inhibitor of sirtuins. Sirtinol, various indoles and their derivatives, as well as the calchone represent additional sirtuin inhibitors of possible clinical use. Activators of Sirt1 have been discovered by screening of chemicals (Milne et al. 2007) were some were much more potent than resveratrol, which itself is the most potent natural Sirt1-activator. (Everitt et al., 2010).

Case study Resveratrol, EGCG, Spermidine

Resveratrol: Resveratrol is a natural polyphenolic compound synthesized by various plant species, grapes, peanuts, and berries. This secondary plant ingredient is said to have properties with a positive effect on the human organism, such as antioxidant, anti-inflammatory, anti-aging, cardioprotective and neuroprotective effects. For example, Dohyun Park's study from 2016 of mice demonstrated that, similar to fasting, resveratrol can stop inflammatory cells in vitro and prolong life, but without the need for calorie restrictions. In addition, resveratrol has preventive properties against chronic diseases, including diabetes and various metabolic disorders, and can be used as a treatment option. Resveratrol was first discovered by the Japanese researcher, Michio Takaoka, in

1939. Takaoka isolated the substance from the leaves of the medicinal plant Veratrum grandiflorum, a white lily species. The highest content of resveratrol was found in Japanese knotgrass (Polygonum cuspidatum or Ko-jo-kon or Darakchasava) in 1963 and, until today, it remains the main source of resveratrol extraction (TAKAOKA, 1939, p. 1090-1100). It was not until 1976 that resveratrol was first detected in red grapes. While the substance is mainly found in the grape skin, small concentrations of resveratrol can also be found in the seeds, stems, vines, and roots. Unsurprising, the highest concentration is found in vines whose immune system is under severe strains due to external influences. Conversely, grape vines growing in moderate or stable conditions tend to produce less resveratrol. Therefore, untreated, natural, or organic grapes contain significantly more resveratrol than chemically treated ones. Resveratrol can also be found in, among others, peanuts, blueberries, cranberries, raspberries, mulberries, plums. Resveratrol is sensitive to light and oxygen and survives both the fermentation process and long storage periods. For example, the more time the wine has for natural maceration, the higher the content of resveratrol (STERVBO, 2007,). Several studies have demonstrated the anti-inflammatory and growth-inhibiting properties of resveratrol in cancer and primary cells and animal models. The anti-aging effects of resveratrol are due to the activation of SIRT1, the NAD-dependent histone deacetylase. Resveratrol is considered the most powerful SIRT1 activator and there are generally two ways in which resveratrol acts in relation to SIRT1, namely: either by binding directly to SIRT1, and thereby promoting the deacetylation of a number of SIRT1 substrate proteins, or by increasing the NAD+ level (DEWALD, 2019). In another study conducted by Joseph Baur in 2006, resveratrol improves health and survival of mice on a high-calorie diet and a life-enhancing effect of resveratrol was shown in a mammalian organism fed by a hypercaloric and high-fat diet. Despite the fact that it has been shown, for the first time, that a substance classified as a 'functional food ingredient' prolongs the life span of a mammal and, at the same time, prevents the manifestation of the metabolic syndrome resulting from a high-fat super nutritional diet, these results should be interpreted with caution. The doses of resveratrol administered are in the pharmacological range. Currently, it is still relatively unclear whether and, if so, what type of secondary health risks could occur when consuming more than 1 g of resveratrol as a supplement on a daily basis (corresponding to the amount in approximately 110 L of red wine) (ABRAHAM, 2009, p. 445-453.) EGCG: EGCG ((−)-Epigallocatechin-3-gallate), the main catechin of green tea (50–75%), has been shown to support many potential health effects, including antioxidant, anticarcinogenic, hypocholesterolemic, and cardioprotective epigenetic activities. It was found to increase energy expenditure and weight loss, reduce fat mass, and facilitate weight maintenance after weight loss. Anti-obesity effects might be mediated through antioxidative and singlet oxygen quencher properties. Inhibition of destructive effects of ROS might act through selective inhibition of specific enzyme activities such as (Dnmts) DNA methyltransferases, repair of chromosomal aberrations, and suppressing inflammation in the development of obesity. EGCG supplementation (0.1%) in obese and diabetic C57BL/KsJ-db/db mice decreased the levels of insulin, IGF-1 (insulin-like growth factors), IGF-2, free fatty acid, and expression of TNF-α, interleukin- (IL-) 6, IL-1β, and IL-18 mRNAs in hepatic tissue.

The green tea polyphenol epigallocatechin-3-gallate (EGCG) inhibits Dnmt activity resulting in a decreased 5- methylcytosine concentration; 20 μmol/L of EGCG already inhibited Dnmt activity in oesophageal (KYSE-150), colon (HT-29), prostate (PC-3), and breast (MCF7 and MDAMB- 231) cancer cells, although no effects of EGCG on Dnmt activity (2–50 μmol/L) are also shown in cancer cells. Another Dnmt inhibitory pathway of flavan-3-ols results from an increase of S-adenosyl-l-homocysteine (SAH).A reduced Dnmt activity reactivates methylation-silenced genes in a dose (5–50 μmM of EGCG) and time dependent (12– 144 h) manner. (Xiong et al., 2018) EGCG is discussed for its

activity to decrease telomere length in tumorcells but elongation in untransformed cells. (Pointner et al., 2017a; Udroiu et al., 2019)

EGCG is widely consumed as a dietary supplement. Its potential properties include slowing aging and extending lifespan, although how exactly this is achieved remains unclear. EGCG promoted healthy lifespan in Caenorhabditis elegans. The life-extending mechanism was stimulated by EGCG-induced production of reactive oxygen species (ROS). Additionally, EGCG triggered mitochondrial biogenesis to restore mitochondrial function. The EGCG-induced increase in lifespan depends on known energy sensors such as AMPK/AAK-2, as well as SIRT1/SIR-2.1 and FOXO/DAF-16. Interestingly, aging decreased the response to EGCG and progressively neutralized its beneficial effects on longevity. Collectively, our findings link EGCG to the process of mitohormesis and suggest an inducible, AMPK/SIRT1/FOXO-dependent redox signaling module that could be invoked in different contexts to extend healthy lifespan. (Xiong et al., 2018)

Spermidine: Spermidine, also referred to as monoaminopropylputrescine, is a secondary plant substance and natural polyamine, and is formed as a by-product during the formation of spermine from putrescine and decarboxylated S-adenosylmethionine. It is found in all-natural organisms, from bacteria to humans, and its highest concentration is most frequently found in the seminal fluid of mammals. Scientists suspect that this phenomenon has an evolutionary background and that its purpose is to protect the genetic material in the sperm. In other words, it helps to preserve the health of the offspring. It is also closely related to cell growth. However, the exact physiological function of spermidine in the growing cell – in other words in the production of nucleic acids and proteins or membrane stabilisation – has yet to be studied in its entirety (Steinmüller, 2015). Spermidine has several qualities, one of which is autophaghy. Both in unicellular organisms, as well as multicellular organisms such as humans, spermidine activates autophagy. Only under the microscope this little wonder of nature becomes visible. When placing a spermidine on a cell the two immediately interact. Countless small 'garbage bags' are formed, in which the cellular waste is collected and digested in a type of 'cellular stomach'. It activates the same process that is initiated when person has been fasting over multiple days.

Case study Antiviral nutraceuticals

Biological opportunities for intervention with viral infections, epigenetics, (A. Haslberger et al., 2020)

Work identifying SARS-CoV-2-human protein-protein interactions indicated interactions between SARS-CoV-2 proteins and epigenetic regulators of gene expression such as histone deacetylase 2 (HDAC2). (Gordon et al., 2020) Epigenetic reprogramming is discussed as a major possibility to control viral gene expression or latency. Reactivating HCMV from the latent phase or repressing the virus lytic and reactivation phases by epigenetic-targeted therapy represent encouraging options to overcome latency and viral shedding or otherwise replication and infectivity, which could lead eventually to control the infection and its complications. This concept is similarly studied in the context of hepatitis B and C virus, herpes simplex virus, and Epstein-Barr virus. SIRT3, a class III HDAC, restricted HBV cccDNA transcription. Treatment with the small molecule C646 that specifically inhibits p300/CBP, the histone acetyltransferases (HAT) for H3K27ac, and H3K122ac reduced HBV transcription in a dose-dependent manner (Egger et al., 2004; Nehme et al., 2019). Like other ssRNA viruses, when SARS-Cov-2 infects host cells, the virus requires continuous cellular transcription for viral mRNA synthesis. This mechanism implies a functional association with the host's genome

expression so that it adapts the infected cell for the host-to-pathogen confrontation at each replication. A key role is that RNA viruses can recruit host DNA methyltransferases (DNMTs) to methylate and decrease gene function of specific genes including those for shaping innate and adaptive immune responses (Boehm & Ott, 2017; Geiman & Robertson, 2002; Marcos-Villar et al., 2018). Inhibition of DNMTs will re-activate the immunity-related gene function responsible for combatting viral infection. RNA methyltransferases include enzymes such as METTL3/14 that may facilitate RdRP stability and mRNA cap guanine-N7-methyltransferase that is responsible for protecting the virus's ability to replicate (Byszewska et al., 2014; A. W. Li, n.d.).
Furthermore, SARS-CoV-2 infection could be shown to limit autophagy by interfering with AMP-protein activated kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1). Targeting of these pathways by exogenous administration of spermidine, 33 AKT inhibitor MK-2206, and the Beclin-1 stabilizing, antihelminthic drug niclosamide inhibited SARS34 CoV-2 propagation by 85, 88, and >99%, respectively in vitro.

Quercetin: Quercetin, a flavonoid found in fruits and vegetables, has unique biological properties that may improve mental/physical performance and reduce infection risk. These properties form the basis for potential benefits to overall health and disease resistance, including anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, and psychostimulant activities, as well as the ability to inhibit lipid peroxidation, platelet aggregation, and capillary permeability, and to stimulate mitochondrial biogenesis. Flavonoid molecules are widely distributed in plants. They are found in a variety of foods including apples, berries, Brassica vegetables, capers, grapes, onions, shallots, tea, and tomatoes, as well as many seeds, nuts, flowers, barks, and leaves. Quercetin is also found in medicinal botanicals, including *Ginkgo biloba*, *Hypericum perforatum*, and *Sambucus canadensis. Q*uercetin intake was reported to be 4.37 mg/day, where the main food sources of flavonol were apples (7.4%). The highest concentration is 234 mg/100 g of edible portion in capers.

Dietary intake of quercetin ranges from 50 to 800 mg/day. Evidence indicates that quercetin glucosides are far better absorbed than its rutinosides (the major quercetin glycoside in tea). Quercetin and derivatives are transformed into various metabolites (phenolic acid) by enteric bacteria and enzymes in intestinal mucosal epithelial cells. Quercetin was reported as a long-lasting anti-inflammatory substance that possesses strong anti-inflammatory capacities. Several studies in vitro using different cell lines have shown that quercetin inhibits tumor necrosis factor (TNF-) and IL-8 production in cells. Protective effects of quercetin against inflammation in human umbilical vein endothelial cells (HUVECs) indicated that the effect was mediated via the downregulation of vascular cell adhesion molecule 1 (VCAM-1) and CD80 expression. Quercetin was found to decrease stress-induced senescent cells and to suppress the senescence-associated pro-inflammatory response. (Coppé et al., 2010) Quercetin significantly induces the gene expression, as well as the production of Th-1, derived interferon- (IFN-) and down-regulates Th-2 derived interleukin 4 (IL-4) by normal peripheral blood mononuclear cells. Quercetin inhibited influenza infection with a wide spectrum of strains, with half-maximal inhibitory concentration (IC50) between 7.756 and 1.931 g/mL. Mechanism studies identified that quercetin showed interaction with the HA2 subunit. Moreover, quercetin could inhibit the entry of the H5N1 virus. This study indicates that quercetin showing inhibitory activity in the early stage of influenza infection provides a future therapeutic option to develop effective, safe, and affordable natural products for the treatment and prophylaxis of IAV infections. (Chirumbolo, 2010; Ganesan et al., 2012; Gansukh et al., 2016; Hewlings & Kalman, 2017; Yao Li et al., 2016; S. C. Lin et al., 2019; W. Wu et al., 2015) Quercetin, like some other flavonoids, inhibits Angiotensin-Converting Enzyme known as a docking station of SARS. IC_{50}values for luteolin, quercetin, rutin, kaempferol, rhoifolin, and apigenin K were 23, 43, 64, 178, 183, and 196 µM, respectively (Guerrero et al., 2012) (Häckl et al., 2002).

Curcumin: The polyphenol curcumin aids in the management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia. Ingesting curcumin by itself does not lead to the associated health benefits due to its poor bioavailability, which appears to be primarily due to poor absorption, rapid metabolism, and rapid elimination. Several components can increase bioavailability. For example, piperine is the major active component of black pepper and, when combined in a complex with curcumin, has been shown to increase bioavailability by 2000%. Curcumin combined with enhancing agents provides multiple health benefits. Curcumin's effect on free radicals is carried out by several different mechanisms. It can scavenge different forms of free radicals, such as reactive oxygen and nitrogen species; it can modulate the activity of GSH, catalase, and SOD enzymes active in the neutralization of free radicals; also, it can inhibit ROS-generating enzymes such as lipoxygenase/cyclooxygenase and xanthine hydrogenase/oxidase. Curcumin has been shown to block NF-B activation increased by several different inflammatory stimuli such as markers of inflammation (soluble CD40 ligand, sCD40L), interleukin 1 beta (IL-1), interleukin 6 (IL-6), soluble vascular cell adhesion molecule 1 (sVCAM-1).

Curcumin shows antiviral and antibacterial activity against the influenza virus, hepatitis C virus, HIV, and strains of *Staphylococcus*, *Streptococcus*, and *Pseudomonas*. Antiviral activity was observed against several different viruses including hepatitis viruses, influenza viruses, and emerging arboviruses like the Zika virus (ZIKV) or chikungunya virus (CHIKV). Interestingly, it has also been reported that the molecule inhibits human immunodeficiency virus (HIV), herpes simplex virus 2 (HSV-2) and human papillomavirus (HPV). Also, a modest inhibition of the HIV-1 and HIV-2 proteases was shown. (Ali & Banerjea, 2016; Anggakusuma et al., 2014; Barthelemy et al., 1998; Basu et al., 2013; Praditya et al., 2019) *Compounds contained in Curcuma sp., Citrus sp., Alpinia galanga, and Caesalpinia sappan* were evaluated as anti-SARS-CoV-2 inhibitor through its binding to 3 protein receptors. The selected protein targets are RBD-S (PDB ID:6LXT), PD-ACE2 (PDB ID: 6VW1), and SARS-CoV-2 protease (PDB ID:6LU7). The affinities of bonds formed are represented as a docking score. The results show that hesperidin, one of the compounds in Citrus sp., has the lowest docking score for all three protein receptors representing the highest affinity to bind the receptors. Moreover, all of the citrus flavonoids possess a good affinity to the respected receptors as well as curcumin, brazilin, and galangin, indicating that those compounds perform inhibitory potential for the viral infection and replication. In general, docking studies indicate that *Citrus sp*. exhibits the best potential as an inhibitor to the development of the SARS-CoV-2, followed by galangal, sappan wood, and *Curcuma sp*. that can be consumed in daily life as prophylaxis of COVID-19 (Utomo et al., 2020).

Epigallocatechin gallate (EGCG): A plethora of beneficial activities for human health was shown for the green tea EGCG in areas such as inflammation, metabolic disease, premature aging, and neurological diseases. Mechanisms include antioxidative and anti-inflammatory activities, modulation of epigenetic methylation, histone modification, and miRNAs, DNA stability, and DNA repair. (Chacko et al., 2010; *Immunonutrient Supplementation - Google Books*, n.d.; Pointner et al., 2017b; Marlene Remely et al., 2017; Singh et al., 2011; Tomás-Barberán & Andrés-Lacueva, 2012; Yiannakopoulou, 2015a). Also senescence, an import mechanism for a viral infection is addressed by EGCG (S. Ahmed, 2010; Blagosklonny, 2007; Stephanie Lilja, Julia Oldenburg, Berit Hippe, n.d.) EGCG has been reported to possess a broad spectrum of antiviral activities against DNA viruses such as herpes simplex virus (HSV; *Herpesviridae*), adenovirus (*Adenoviridae*), human papillomavirus (HPV; *Papovaviridae*), and hepatitis B virus (HBV; *Hepadnaviridae*), and against (+)-RNA viruses such as hepatitis C virus (HCV; *Flaviviridae*), Zika virus (ZIKV; *Flaviviridae*), dengue virus (DENV; *Flaviviridae*), West Nile viruses (WNV; *Flaviviridae*), Chikungunya virus (CHIKV; *Togaviridae*) and (−)-RNA viruses such as human immunodeficiency virus (HIV; *Retroviridae*), Ebola virus (EBOV; *Filoviridae*) and influenza virus (*Orthomyxoviridae*). (Bhat et al., 2014; Calland et al., 2015;

Carneiro et al., 2016; C. W. Chang et al., 1994; Ciesek et al., 2011; Green, 1949; Hartjen et al., 2012; L. He et al., 2013; W. He et al., 2011; Isaacs et al., 2008, 2011; Kaihatsu et al., 2018; Kawai et al., 2003; Kuzuhara et al., 2009; S. Li et al., 2011; Ling et al., 2012; S. Liu et al., 2005; Nakayama et al., 1993; Nance et al., 2009; Patrick Reid et al., 2014; Pradhan & Nguyen, 2018; Sharma et al., 2017; Song et al., 2005; Tillekeratne et al., 2001; Vázquez-Calvo et al., 2017; Weber et al., 2003; Williamson et al., 2006; C. Zhao et al., 2014; Zu et al., 2012).

EGCG-fatty acid derivatives are to be expected to increase viral and cellular membrane permeability. EGCG-fatty acid monoesters showed improved antiviral activities against different types of viruses, probably due to their increased affinity for viruses and cellular membranes. (Kaihatsu et al., 2018).

Antiviral mechanisms might come from interactions of EGCG with DNMTs, ACE-2, and helicase. (A. W. Li, n.d.) Tea polyphenols [catechin, epicatechin, and (-)-epigallocatechin-3-O-gallate (EGCG)] and bioflavonoids (quercetin, fisetin, and myricetin) inhibited SssI DNMT- and DNMT1-mediated DNA methylation in a concentration-dependent manner. (Coppé et al., 2010) The IC(50) values for catechin, epicatechin, and various flavonoids ranged from 1.0 to 8.4 microM, but EGCG was a more potent inhibitor, with IC(50) values ranging from 0.21 to 0.47 microM. (Won et al., 2005; Yiannakopoulou, 2015b).

Phloretin: Phloretin is one of the best known and abundant dihydrochalcone characterized by the presence of the 2,6-dihydroxyacetophenone pharmacophore. It is a versatile molecule with anticancer, anti-osteoclastogenic, antifungal, antiviral, anti-inflammatory, antibacterial, and estrogenic activities and able to increase the fluidity of biological membranes. The main biological action of phloretin is the inhibition of glucose cotransporter 1. Phloretin furthermore, possesses antioxidative properties and effects the synthesis of proinflammatory molecules PGE2, IL-8, IL-6, MCP-1, and ICAM-1. Phloretin prevented TNF-α-stimulated upregulation of VCAM-1, ICAM-1, and E-selectin expression in a concentration-dependent manner. To the same extent as for TNF-α, phloretin also inhibited IL-1β-induced upregulation in the expression of all 3 adhesion molecules. Inhibition of cytokine-induced adhesion molecule expression for VCAM-1, ICAM-1, and E-selectin was detected already at the level of mRNA. (Stangl et al., 2005a, 2005b; Zielinska et al., 2019). Phloretin significantly decreased infectious titers of two ZIKV strains. The 50% effective concentration (EC 50) of phloretin against MR766 and PRVABC59 was 22.85 µM and 9.31 µM, respectively. Further analyses demonstrated that decreased viral production was due to host-targeted inhibition, including decreased apoptotic caspase-3 and -7 activities and reduced phosphorylation of Akt/mTOR pathways. Also, upon disruption of cellular glucose availability within host cells using 2-deoxy- d -glucose, ZIKV propagation was inhibited. (Behzad et al., 2017; Cseh et al., 2000; Stangl et al., 2005a, 2005b; Zielinska et al., 2019).

Berberine: In Chinese medicine, berberine has a strong importance in the clinical therapy of influenza virus infections. Berberine is an isoquinoline derivative alkaloid isolated from many medicinal herbs, such as Rhizoma coptidis and Cortex phellodendri (Y. Wu et al., 2011). The activity of berberine as an antiviral substance was also shown against a variety of strains of the chikungunya virus (CHIKV). CHIKV infection specifically activated the major mitogen-activated protein kinase (MAPK) signaling pathways extracellular signal-related kinase (ERK), p38, and c-Jun NH_2-terminal kinase (JNK). Upon treatment with berberine, this virus-induced MAPK activation was markedly reduced. Subsequent analyses with specific inhibitors of these kinases indicated that the ERK and JNK signaling cascades are important for the generation of progeny virions. In contrast to specific MAPK inhibitors, berberine lowered virus-induced activation of all major MAPK pathways and resulted in a stronger reduction in viral titers. Furthermore, a significant reduction of CHIKV-induced inflammatory disease was seen with berberine in a mouse model (Varghese et al., 2016).

The MEK-ERK signaling pathway and autophagy play also an important role in the pathophysiology of enterovirus71 (EV7l1) replication. Inhibition of the MEK-ERK signaling pathway and autophagy (H. Wang et al., 2017; H. Q. Wang et al., 2013)was shown with the isoquinoline alkaloid isolated from *Berberis vulgaris L.* (H. Q. Wang et al., 2013).

Berberine derivatives were evaluated for their activity for suppression of tumor necrosis factor (TNF)-α-induced nuclear factor (NF)-κB activation. (Y. X. Wang et al., 2017). A human phosphokinase array revealed that CHIKV infection specifically activated the major mitogen-activated protein kinase (MAPK) signaling pathways extracellular signal-related kinase (ERK), p38, and c-Jun NH_2-terminal kinase (JNK). Upon treatment with berberine, this virus-induced MAPK activation was markedly reduced. Subsequent analyses with specific inhibitors of these kinases indicated that the ERK and JNK signaling cascades are important for the generation of progeny virions. In contrast to specific MAPK inhibitors, berberine lowered virus-induced activation of all major MAPK pathways and resulted in a stronger reduction in viral titers. Further, we assessed the *in vivo* efficacy of berberine in a mouse model and measured a significant reduction of CHIKV-induced inflammatory disease

Sulforaphane: Originally defined to support bone health (Thaler et al., 2016), the naturally occurring isothiocyanate sulforaphane (SFN) has also an antiviral activity. It has been shown that the osteoblast supporting transcription factor Runx2 is required for the long-term persistence of antiviral $CD8^+$ memory T cells (Olesin et al., 2018). Supplementation of an SFN-rich broccoli homogenate further increased live attenuated influenza virus (LAIV)- induced granzyme B production in NK cells and granzyme B levels appeared to be negatively associated with influenza RNA levels in nasal lavage fluid cells. The authors concluded that nasal influenza infection may induce complex changes in peripheral blood NK cell activation and that SFN increases virus-induced peripheral blood NK cell granzyme B production, an effect that may be important for enhanced antiviral defense responses. (Müller et al., 2016) These results show that SFN induces active DNA demethylation via the up-regulation of the *Tet* genes *in vitro*. This epigenetic reprogramming of the chromatin leads to apoptosis of preosteoclasts but only to a lower extent of preosteoblasts. A recent study shows that significantly higher mRNA levels of Notch2, Jagged1, RANKL, and IL-1β were observed in EBV positive compared to EBV negative periodontitis lesions suggesting that RANKL, Notch2, and IL-1β play a role in viral defense (A Jakovljevic, Andric, et al., 2018; A Jakovljevic, Knezevic, et al., 2018; Aleksandar Jakovljevic et al., 2020; Nikolic et al., 2019).

Nigella sativa, Thymoquinone: Nigella sativa (N. Sativa) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of traditional medicine. N. Sativa has been traditionally used for the treatment of a variety of disorders, diseases, and conditions pertaining to the respiratory system, digestive tract, kidney and liver function, cardiovascular system and immune system support, as well as for general well-being. A recent publication showed that an extract containing Ns oil supported the immune response and reduced the pathogenicity of the H9N2 avian influenza virus which is structurally related to SARS-CoV-2 in chicken. Many active compounds have been isolated, identified, and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%). The most important compound of N. Sativa is Thymoquinone (TQ) which is known for epigenetic action. (Ahmad et al., 2013; El–Dakhakhny, 1963; Khan et al., 2019; Mohammadi & Golchin, 2020) (Mohammadshahi et al., 2018).

Thymoquinone is known in DNA methylation/demethylation e.g. upregulation of ubiquitin-like-containing plant homodomain (PHD). Ring finger domain 1 (UHRF1) influences cancer cells to repress

tumor suppressor genes through their promoter hypermethylation during cell proliferation. Thymoquinone can suppress UHRF1 and, thus, might be able to repair epigenetic aberration in cancer cells through a DNA demethylating process, probably involved in the downregulation of DNA methyltransferase 1 (DNMT 1).

Salvia officinalis: In addition to antibacterial action, S. officinalis has been reported to induce antifungal, antiviral, and antimalarial effects. The antiviral activity of S. officinalis is most probably is mediated by safficinolide and sage one, two diterpenoids that are found in its aerial parts (El–Dakhakhny, 1963; Ghorbani & Esmaeilizadeh, 2017).

The discussed interactions between the mechanism of bioactive plant ingredients and viral, especially SARS related infections give some hope of regimens of plant-derived pre- or interventions or combinations with pharmaceutically derived medication. A better understanding of the interactions of molecules involved in the epigenetic regulation of viral infection as well as in senescence should also booster the development of functional foods for immune- senescence and healthy aging.

Bioavailability, stability, formulation, safety

Bioavailability, stability, formulation, mixability in case of composite products of functional foods or nutraceuticals is of major importance for health effects and safety. All these characteristics depend on the source of the product and need to be evaluated case by case. For example for polyphenols it is important to realize that the polyphenols that are the most common in the human diet are not necessarily the most active within the body, either because they have a lower intrinsic activity or because they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated (Shahidi & Alasalvar, 2016).

Plasma concentrations reached after polyphenol consumption vary highly according to the nature of the polyphenol and the food source. They are on the order of 0.3–0.75 _mol/L after consumption of 80–100mg quercetin equivalent administered in the form of apples, onions, or meals rich in plant products (90, 92, 107). When ingested in the form of green tea (0.1–0.7_mol/L for an intake of 90–150 mg), cocoa (0.25–0.7 _mol/L for an intake of 70–165 mg) (210–213), or red wine (0.09 _mol/L for an intake of 35 mg) (137), catechin and epicatechin are as effectively absorbed as is quercetin

Metabolites that are found in blood and target organs and that result from digestive or hepatic activity may differ from the native substances in terms of biological activity. Extensive knowledge of the bioavailability of polyphenols is thus essential if their health effects are to be understood. Much about the intestinal mechanisms of the gastrointestinal absorption of polyphenols remains unknown. Most polyphenols are probably too hydrophilic to penetrate the gut wall by passive diffusion, but the membrane carriers that could be involved in polyphenol absorption might be of interest.

In foods, all flavonoids except flavanols are found in glycosylated forms, and glycosylation influences absorption. However, hydrolysis of glucoside is known in fermentation e.g. for tempeh (Y. C. Huang et al., 2018). Glycosylation does not influence the nature of the circulating metabolites. Intact glycosides of quercetin, daidzein, and genistein were not recovered in plasma or urine after ingestion as pure compounds or from complex food.

Dietary fibre is generally associated with polyphenols in plant foods and stimulates intestinal fermentation, which could influence the production of particular microbial metabolites.

Administration of polyphenols without a food matrix could markedly affect their bioavailability. (Manach et al., 2004)

Specific active metabolites are produced by the colonic microflora. Equol produced from soya daidzein appears to have **phytoestrogenic** properties equivalent to or even greater than those of the original isoflavone. There is great interindividual variability in the capacity to produce equol. Only 30-40% of the occidental people excrete significant quantities of equol after consumption of isoflavones, and these persons are called**" equol producers**". The corresponding percentage among Asian populations is unknown, but a recent study suggested that the percentage in Japanese men could be as high as 60% .The ability or inability of persons to produce equol seems to remain the same for at least several years. The composition of the intestinal flora plays a major role. Inoculation of germ-free rats with human flora from equol producers confers on these rats the capacity to produce this metabolite, whereas colonization with flora from non–equol producers leaves the rats in capable of producing equol. Equol is not recovered in plasma from infants who are fed soy-based formulas, which suggests that the bacteria responsible for its production are not developed in the first months of life. (Morton et al., 2002); (Arai et al., 2000)(Nielsen & Williamson, 2007)(Setchell et al., 1998)(Atkinson et al., 2005)(J.-P. Yuan et al., 2007).

Also, the **stability** of bioactive food ingredients needs to be evaluated case by case. For example, the thermal stability (60◦ C, 80◦ C, 100◦ C), antioxidant activity, and ultraviolet C light (UV-C) stability of standard polyphenols solutions (catechin, gallic acid, and vanillic acid) and of vegetal extracts from spruce bark and grape seeds were investigated. Exposure of the standard solutions and vegetal extracts to high temperatures revealed that phenolic compounds were also relatively stable (degradations ranged from 15 % to 30 % after 4 h of exposure). The highest antioxidant activity was obtained for ascorbic acid and gallic acid followed by catechin and caffeic acid and the grape seeds. The results show that, after 3 h of UV-C exposure, approximately 40 % of vanillic acid, 50 % of gallic acid, and 83 % of catechin were removed. Similar degradation rates were observed for vegetal extracts, with the exception of the degradation of catechin (40 %) from grape seeds. In addition, the photo-oxidation of polyphenols in the presence of food constituents such as citric acid, ascorbic acid, sodium chloride, and sodium nitrate was assessed. (Volf et al., 2014)

Unfortunately, the bioactivity of many polyphenols for nutraceutical applications shows a lack in **long-term stability**, making these natural compounds very sensitive to light and heat. Moreover, polyphenols often present a poor bio-disponibility mainly due to low water solubility. Lastly, many of these molecules possess a very astringent and bitter taste, which limits their use in food or in oral medications. To circumvent these drawbacks, delivery systems have been developed, and among them, encapsulation would appear to be a promising approach. Many encapsulation methods are described in the literature, among which some have been successfully applied to plant polyphenols. the plethora of health benefits reported in the scientific literature also results from the capacity of polyphenols to interact with proteins (enzymes, membrane receptors, tissue proteins) in a specific way, thus allowing them to protect or modulate their activity. Polyphenols act as potent inhibitors of ROS-generating enzymes by complexing the protein. The process of polyphenol complexation is directly influenced by the protein characteristics and polyphenol characteristics. The main types of interactions involved in the complexation mechanism are non-covalent bond formation and hydrophobic interactions. Therefore, the administration of phenolic compounds requires the formulation of a finished protecting product able to maintain the structural integrity of the polyphenol until the consumption or the administration, mask its taste, increase its water solubility and bioavailability, and convey it precisely towards a physiological target.

Among the existing **stabilization** methods, encapsulation is an interesting means. The use of encapsulated polyphenols instead of free compounds is the source of numerous works. The particles obtained are called microcapsules or microspheres according to the internal structure, core-shell-like, or matrix, respectively. Microparticles may contain a solid, liquid, or gaseous active substance, with a size range between about 1 micron and 1 millimeter. Particles with a smaller size, < 100 nm are called nanoparticles, and nanocapsules and nanospheres can also be distinguished according to their internal structure. The coating materials include polymers of natural or synthetic origin or lipids. (Munin & Edwards-Lévy, 2011).

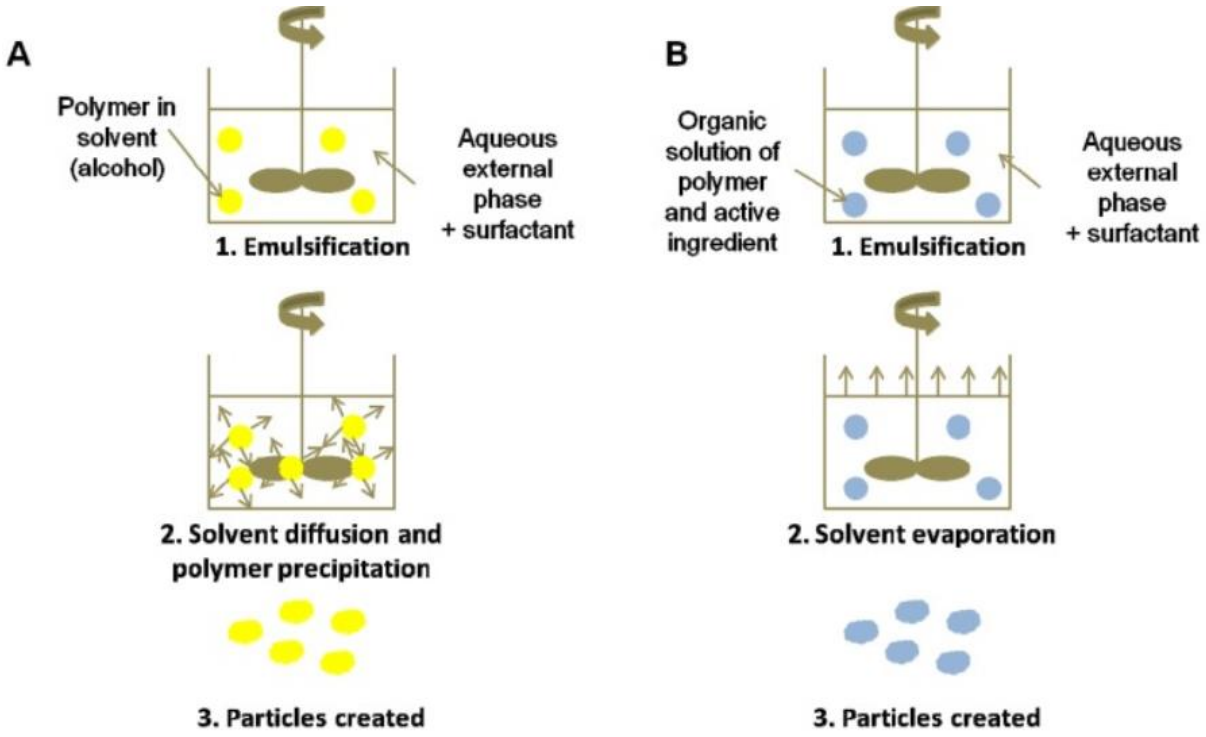


Fig Stabilisation, encapsulation, (Munin & Edwards-Lévy, 2011)

The wide array of new health products - nutraceuticals, nutritional supplements, functional foods, dietary supplements, foods for special medical purposes and foods for special dietary uses – as well as different national regulations make a harmonised understanding of safety evaluation difficult and is often confusing to consumers and industry.

Health foods are defined rather ambiguously and partially defined by the health claims that they can contribute. The health foods have to be produced using Good Manufacturing Practices (GMP) and will be reviewed with reference to safety, efficacy, and quality control. They are monitored by sampling and testing, post-market monitoring and re-evaluation of health effects.

The **labelling** of such products is strictly regulated. In health foods some substances may be used that are not permitted in conventional foods. Such foods shall not claim the function to prevent or treat any diseases. They shall have accurate label of active/marker ingredients and their contents. The functions and ingredients must be consistent with those on the label and instructions.

Production aspects of nutraceuticals

Nutraceutical, a hybrid term from 'nutrition' and 'pharmaceutical' coined by Stephen L. DeFelice in 1989, was defined as 'any substance that is a food or a part of food and provides medical or health benefits, including the prevention and treatment of disease'. (DeFelice, 1995). Later on, the concept of nutraceutical was modified as 'a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food'. (Sabharwal, n.d.) Nutraceuticals are now more specific to structurally and functionally diverse bioactive compounds that exert long-term medicinal or physiological benefits other than purely nutritional or direct pharmaceutical effects, which distinguishes them from functional foods and drugs. They can be derived from plants (e.g. phytochemicals, vitamins), from animals (e.g. polysaccharides), from microorganisms (e.g. poly amino acids) and from marine sources (e.g. glucosamine and chitosan). Phytochemicals are a broad spectrum of plant-derived bioactive secondary metabolites that are commonly found in fruits, vegetables, beans and grains. They are involved in plant defences against biotic or abiotic stresses, and also exhibit health-protecting or disease-preventing effects on humans. Phytochemicals is a vast and very important repertoire for nutraceuticals, which include polyphenolic compounds (flavonoids, isoflavonoids, stilbenoids and curcuminoids), alkaloids, terpenoids (monoterpenes, diterpenes, tetraterpenes, polyterpenes, steroid saponins, lycopene and carotenoids) and their derived compounds.

Extraction from plants In order to extract different phenolic compounds from plants with a high degree of accuracy, various solvents of differing polarities must be used.

Multiple solvents have been commonly used to extract phytochemicals, and scientists usually employed a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time.

Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest; Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water. Also, microwave-assisted extraction (MAE) and ultrasonic-assisted extraction are used. Purification of bioactive molecules includes thin-layer and column chromatographic methods. Silica, alumina, cellulose, and polyamide exhibit the most value for separating the phytochemicals. Determination of the structure of desired molecules uses mostly a wide range of spectroscopic techniques such as UV-visible, Infrared (IR), Nuclear Magnetic Resonance (NMR), and mass spectroscopy (Altemimi et al., 2017).

Microbial production of nutraceuticals *E. coli* and *S. cerevisiae* have generally been developed as platform organisms for *de novo* or semi-*de novo* production of almost all kinds of polyphenolic compounds. Some of the biggest advantages of *E. coli* include its fast growth and ease of genetic manipulation; for *S. cerevisiae* its Generally Regarded As Safe (GRAS) status and its ability to functionally express plant metabolic enzymes. In all cases, in order to achieve polyphenolic compound production in microbes, plant-originated enzymes are firstly overexpressed via either codon optimization or construction of enzyme chimeras that facilitate bacterial expression. The first strategy is feeding precursors to the heterologous hosts to produce corresponding polyphenolic compounds. The second approach is *de novo* biosynthesis of phenolic compounds from simple carbon sources like glucose and glycerol, which is more appealing for industrial applications (Jian Wang et al., 2016).

Safety and regulatory aspects of foods, supplements, functional foods

Like in medical fields (Cuervo & Clarke, 2003) the analysis of the balance of benefits and harm of food additives or functional foods are a delicate undertaking. Intervention in the often highly complex regulatory pathways of metabolisms may result in a significant improvement of pathophysiological pathways. But often unnoticed side effects on unrelated molecular pathways may cause unintentional harmful side effects. The identification of unintended side effects, risk assessment, benefit risk assessment, risk management and risk communication is an often complicated task. (Jain & Mathur, 2015). EU EMEA worked on a benefit risk assessment model: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/report-chmp-working-group-benefit-risk-assessment-models-methods_en.pdf. Furthermore, risk benefit assessment has to consider personalised health aspects as well as the personal values of clients. (Baldt, 2019).

Case study. Benefits and possible risks of L. carnitine

Carnitine is an essential nutrient for fat metabolism, and new research suggests that it also plays a role in blood glucose homeostasis. Carnitine has received attention over the years as a therapy for hyperlipidemia, muscle weakness, and symptoms associated with mitochondrial disorders. This review provides an overview of the role of carnitine in metabolism, evaluation of deficiency states, and the decision-making process for initiation of carnitine supplementation. Role of Carnitine in Metabolism Carnitine is required for the metabolism of long-chain fatty acids for energy. Beta-oxidation of long-chain fatty acids, an essential component of energy metabolism, occurs in the matrix of the mitochondria of cells. Carnitine is the carrier molecule that is required to transport the fatty acid from the cytosol across the outer and inner membranes of the mitochondrion and into the matrix for metabolism and creation of energy. Carnitine is required for the metabolism of long-chain fatty acids for energy. Beta-oxidation of long-chain fatty acids, an essential component of energy metabolism, occurs in the matrix of the mitochondria of cells. Carnitine is the carrier molecule that is required to transport the fatty acid from the cytosol across the outer and inner membranes of the mitochondrion and into the matrix for metabolism and creation of energy (Mogensen & Pfister, 2013).

Carnitine occurs naturally in the diet, and 75% of carnitine in the body comes from food. Sources of dietary carnitine are red meat, fish, and dairy products Carnitine occurs naturally in the diet, and 75% of carnitine in the body comes from food. Sources of dietary carnitine are red meat, fish, and dairy products. Carnitine needs to be supplemented in deficiencies and studies in athletes have shown that carnitine supplementation fosters exercise performance. As reported in the majority of studies, an increase in maximal oxygen consumption and a lowering of the respiratory quotient indicate that dietary carnitine has the potential to stimulate lipid metabolism. Treatment with L-carnitine also has been shown to induce a significant post-exercise decrease in plasma lactate, which is formed and used continuously under fully aerobic conditions. Data from preliminary studies have indicated that L-carnitine supplementation can attenuate the deleterious effects of hypoxic training and speed up recovery from exercise stress. Recent data have indicated that L-carnitine plays a decisive role in the prevention of cellular damage and favourably affects recovery from exercise stress. Uptake of L-

carnitine by blood cells may induce at least three mechanisms: 1) stimulation of hematopoiesis, 2) a dose-dependent inhibition of collagen-induced platelet aggregation, and 3) the prevention of programmed cell death in immune cells. Carnitine has direct effects in regulation of gene expression (i.e., carnitine-acyltransferases) and exert effects via modulating intracellular fatty acid concentration. (Karlic & Lohninger, 2004) It is recommended that Carnitin supplementation should include analysis of markers of beta-oxidation, and nutritional and functional parameters. However also interactions of carnitine with cancer cells and potentially cancer pathogenesis is discussed: Cancer cells must maintain metabolic homeostasis in a wide range of conditions, including harsh microenvironments in which cancer cells must continue to meet the high bio-energetic demand in order to undergo replication. These cells achieve metabolic homeostasis by regulating the dynamics of nutrients present in the microenvironment, and the ability of cancer cells to utilize them to produce energy and to synthesize macromolecules. Assessing the response to carnitine supplementation involves monitoring carnitine and biochemical blood values, markers of beta-oxidation, and nutritional and functional parameters Recent findings have suggested that carnitine system (CS) could be considered as a gridlock to finely trigger the metabolic flexibility of cancer cells. Indeed, the components of this system are involved in the bi-directional transport of acyl moieties from cytosol to mitochondria and vice versa, thus playing a fundamental role in tuning the switch between the glucose and fatty acid metabolism. Therefore, the CS regulation, at both enzymatic and epigenetic levels, plays a pivotal role in tumors (Melone et al., 2018).

In the US recent legislation regarding food safety includes the Food Safety Modernization Act (FSMA), signed in 2011 by Barack Obama. FSMA has given the Food and Drug Administration (FDA) new authorities to regulate the way foods are grown, harvested, and processed. The law grants the FDA a number of new powers, including mandatory recall authority, which the agency has sought for many years. The FSMA requires the FDA to undertake more than a dozen rulemakings and issue at least 10 guidance documents, as well as a host of reports, plans, strategies, standards, notices, and other tasks. FDA guidance documents cover e.g. Food facility registration, Current Good Manufacturing Practices (CGMPs), Hazard Analysis & Critical Control Points (HACCP), Retail Food Protection, Imports & Exports. https://www.fda.gov/food/guidance-regulation-food-and-dietary-supplements

SAFETY AND REGULATORY ASPECTS OF SUPPLEMENTS IN US: Under Dietary Supplement Health and Education Act of 1994 (DSHEA), the manufacturer is responsible for the safety of dietary supplements before marketing; it is not required to demonstrate its safety if the ingredient is in use before 1994. The manufacturer need not go for registration or approval by US Food and Drug Administration(FDA). Under DSHEA, the dietary supplements are exempted from Food Additive Delaney Clause that is the burden of proof of safety of the products lies with FDA. DSHEA allows information from books, articles, or scientific abstracts to be used along with the sale of dietary supplements; however, the burden of proof to prove the information is false lies with FDA. Dietary supplement ingredients not used before 15th October 1994, are subject to additional safety review and must be notified to FDA ahead of the marketing. Manufacturer must report to FDA about the new ingredient at least 75 days before to be introduced. Under DSHEA, the manufactures may make structure-function claims or claims on general well-being along with a statement that “not been evaluated by FDA”. Claims may be made without pre-approval of FDA; Manufacturers making claims must have substantiation that the claim is truthful and not misleading; the health claims to be

informed to FDA before 30 days of marketing; The product may include a disclaimer that the product is not a drug and do not receive FDA premarket approval; drug claims of diagnosis, mitigate, cure or prevention of disease cannot be made (Sattigere et al., 2018).

US- FDA regulates food for special medical purposes. The term medical food, as defined in section 5(b) of the Orphan Drug Act (21 U.S.C. 360ee (b) (3)) is "a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation. Medical foods are not those simply recommended by a physician as part of an overall diet to manage the symptoms or reduce the risk of a disease or condition. Not all foods fed to patients with a disease, including diseases that require dietary management, are medical foods. Instead, medical foods are foods that are specially formulated and processed (as opposed to a naturally occurring foodstuff used in a natural state) for a patient who requires use of the product as a major component of a disease or condition's specific dietary management. https://www.fda.gov/food/guidance-documents-regulatory-information-topic-food-and-dietary-supplements/medical-foods-guidance-documents-regulatory-information

Novel food regulation (NFR), EU: The European Union has implemented a regulatory system for marketing and labellingof foods a new regulation came into force on 1st of January 2018 replacing previous regulations. This guideline was developed to help food business operators understand the scope of the new NFR and the principles to apply when verifying the status of their products.

A new food is only a novel food if it was not used on the EU market to a significant degree before 15 May 1997 for human consumption and falls into one of the 10 categories. Both conditions must be met. - Some foods are now explicitly included (e.g. insects, foods from tissue cultures, engineered nanomaterial, etc). Such foods, if lawfully marketed in the EU after 15 May 1997 can remain on the market if an application for authorisation is submitted before 1 January 2019 and the food is subsequently authorised. - Although additives, food enzymes and flavourings are excluded from the scope of the NFR, when such substances are used as ingredients, but not for a technological function, they may still come under the scope of the NFR. - Also, foods that do not meet the specifications or conditions of use specified in the Union List of authorised novel foods, require a new novel foods authorisation. Foods that have only been used in food supplements before 15 May 1997 and are now intended to be used in regular foods, require authorisation as novel food. Examples of Novel Food include new sources of vitamin K (menaquinone) or extracts from existing food (Antarctic Krill oil rich in phospholipids from *Euphausia superba*), agricultural products from third countries (chia seeds, noni fruit juice), or food derived from new production processes (UV-treated food (milk, bread, mushrooms and yeast). Pre-market authorisation of Novel Foods on the basis of an evaluation in line with the above principles is necessary. https://ec.europa.eu/food/safety/novel_food_en

SAFETY AND REGULATORY ASPECTS OF SUPPLEMENTS IN the EU: The European Union Food Safety Authority (EFSA) is the keystone body in European Union (EU) for Risk Assessment regarding food and feed safety on existing and emerging risks with food chain. As risk assessor, EFSA produces scientific opinion and gives advice on food safety and quality and provides a platform for European policies and legislation to support the European Council, European parliament, and EU member states. EFSA establishes consultation with national food authorities and other stakeholders. It has scientific committees and panels that help deliver scientific advice; it has developed a comprehensive

body of good risk assessment plan to guide its scientific panel and committee experts to help ensure EFSA opinions respect the highest scientific standards. Directive 2002/46/EC on food supplements envisages the setting of maximum and minimum amounts of vitamins and minerals in supplements. Since Jan 2018 the new Regulation (EU) 2015/2283 is applicable for novel foods. EFSA guidance on safety evaluation of sources of nutrients and bioavailability of nutrient is given in https://www.efsa.europa.eu/en/efsajournal/pub/5294. Rules on nutrition and health claims have been established by Regulation (EC) No 1924/2006. The Regulation started to apply on 1 July 2007. Definition of claims and permitted claims are given in https://ec.europa.eu/food/safety/labeling_nutrition/claims/health_claims_en.

Botanicals: The EU does not have a centralised authorisation procedure for the use of botanicals and derived preparations in food. Nonetheless, the use of botanicals and derived preparations in food has to comply with the general requirements set out in Regulation (EC) No 178/2002, which lays down the general principles and requirements of food law in the EU. This assigns primary legal responsibility for the safety of the products placed on the market to business operators. Some botanicals are considered as traditional herbal medicinal plants and are used both in medicinal products and in food supplements. The European Medicines Agency (EMA) is responsible for assessing both the safety and efficacy of herbal preparations when used as medicines. It is not the role of EFSA or EMA to determine the classification of a botanical as a medicine or a food supplement. http://www.efsa.europa.eu/en/topics/topic/botanicals

A regulation for “Food For Specific Groups”, especially for infants and young children, food for special medical purposes, and total diet replacement for weight control ('Food for Specific Groups'), Regulation (EU) No 609/2013 was adopted in 2013 and applied from July 2016. It aims to protect specific vulnerable groups of consumers (infants and young children, people with specific medical conditions and people undertaking energy-restricted diets to lose weight). The regulation sets general compositional and labellingrules and require the Commission to adopt, through delegated acts, specific compositional and labellingrules for Infant and follow-on formula, processed-cereal based food and other baby food, Food for special medical purposes, Total diet replacement for weight control

The regulation simplifies the regulatory framework, by eliminating those rules that are unnecessary and contradictory and by replacing them with a new framework that takes into account the developments on the market and in EU food law. In particular, the new Regulation abolishes the obsolete concept of "dietetic food" by repealing Directive 2009/39/EC, which previously laid down general rules for these products categories (specific rules on the different product categories adopted in the past under Directive 2009/39/EC remain applicable until the new specific rules have replaced them – see dedicated pages for more info)

The regulation also establishes a single Union list of substances that can be added to these foods including minerals and vitamins, empowers the Commission to adopt interpretation decisions clarifying whether a given food falls within the scope of the Regulation and under what specific food category, in order to ensure uniform implementation of the rules, requires the Commission to transfer rules on gluten-free foods and very low gluten under Regulation (EU) No 1169/2011 on food information to consumers in order to ensure clarity and consistency and establishes that meal replacement products for weight control should be regulated solely under Regulation (EC) No 1924/2006 on nutrition and health claims in order to ensure legal certainty. https://ec.europa.eu/food/safety/labeling_nutrition/special_groups_food_en

Food for special medical purposes must be used under medical supervision. It is dedicated to patients with impaired absorption of food, abnormal absorption and metabolization of food and incorrect excretion of certain nutrients or metabolites, or with other medically-determined nutrient requirements, whose dietary management cannot be achieved by modification of normal diet alone. (*Fundamental regulations concerning novel food products and food for special medical purposes with plant ingredients in: Herba Polonica Band 65 Heft 3 (2019)*, n.d.)

Health claim regulation in the EU: In December 2006, the European Parliament and the Council adopted a new Regulation (EC 1924/2006) on nutrition and health claims made on foods". The regulation defines health claims as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health". The two principal categories of health claims are:

- reduction of disease risk claims and claims referring to children's development and health (Article 14).
- claims other than those referring to the reduction of disease risk and children's development and health (Article 13).

Three different types of Article 13 claims are defined, i.e. health claims describing or referring to (a) the role of a nutrient or other substance in growth, development and the functions of the body, (b) psychological and behavioural functions, and (c) slimming or weight control or reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet. https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/functional-food-european-union

Case study EFSA health claim Glucomannan

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to konjac mannan (glucomannan) and reduction of body weight, reduction of post-prandial glycaemic responses, maintenance of normal blood glucose concentrations, maintenance of normal (fasting) blood concentrations of triglycerides, maintenance of normal blood cholesterol concentrations, maintenance of normal bowel function and decreasing potentially pathogenic gastro-intestinal microorganisms. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders. The food constituent that is the subject of the health claims is konjac mannan (glucomannan). The Panel considers that konjac mannan (glucomannan) is sufficiently characterised. Reduction of body weight the claimed effects are "weight management" and "contributes to weight management". The target population is assumed to be overweight individuals. In the context of the proposed wordings, the Panel assumes that the claimed effects refer to the reduction of body weight. The Panel considers that the reduction of body weight is a beneficial physiological effect for overweight individuals. In weighing the evidence, the Panel took into account that most of the intervention studies, which were of adequate sample size and duration, found a statistically significant effect of glucomannan on body weight loss in the context of a hypocaloric diet when administered as a pre-load before meals, and that the mechanism by which glucomannan could exert the claimed effect is established. On the basis of the data presented, the Panel concludes that a cause and effect relationship has been established between the consumption

of glucomannan and the reduction of body weight in the context of an energy-restricted diet. The Panel considers that in order to obtain the claimed effect, at least 3 g of glucomannan should be consumed daily in three doses of at least 1 g each, together with 1-2 glasses of water before meals, in the context of an energy-restricted diet. The target population is overweight adults. Reduction of post-prandial glycaemic responses. The claimed effect is “reduction of glycaemic response”. The target population is assumed to be individuals willing to reduce their post-prandial glycaemic responses. In the context of the proposed wordings, the Panel assumes that the claimed effect relates to the reduction of post-prandial glycaemic responses. The Panel considers that reduction of post-prandial glycaemic responses may be a beneficial physiological effect. No references were provided from which conclusions could be drawn for the scientific substantiation of the claimed effect. On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of glucomannan and the reduction of post-prandial glycaemic responses. Maintenance of normal blood glucose concentrations. The claimed effects are “glycaemic control” and “contributes to maintain a healthy blood sugar level”. The target population is assumed to be the general population. In the context of the proposed Glucomannan related health claims 3 EFSA Journal 2010;8(10):1798 wordings, the Panel assumes that the claimed effect refers to the long-term maintenance or achievement of normal blood glucose concentrations. The Panel considers that long-term maintenance of normal blood glucose concentrations is a beneficial physiological effect. In weighing the evidence, the Panel took into account that only one small intervention study of short duration on a highly selected population sub-group was presented for the substantiation of the claimed effect, and that no evidence on the sustainability of the effect was provided. On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of glucomannan and the maintenance of normal blood glucose concentrations. Maintenance of normal (fasting) blood concentrations of triglycerides. The claimed effect is “helps to maintain physiological lipid levels in the blood”. The target population is assumed to be the general population. In the context of the proposed wordings, the Panel assumes that the claimed effect relates to the maintenance of normal (fasting) blood concentrations of triglycerides. The Panel considers that maintenance of normal (fasting) blood concentrations of triglycerides may be a beneficial physiological effect. In weighing the evidence, the Panel took into account that only one out of seven studies presented reported a significant decrease in plasma concentrations of triglycerides following consumption of glucomannan. On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of glucomannan and the maintenance of normal blood concentrations of triglycerides. Maintenance of normal blood cholesterol concentrations. The claimed effects are “helps to maintain physiological lipid levels in the blood” and “heart health”. The target population is assumed to be the general population. In the context of the proposed wordings, the Panel assumes that the claimed effect relates to the maintenance of normal blood cholesterol concentrations. A claim on glucomannan and maintenance of normal blood cholesterol concentrations has already been assessed with a favourable outcome. Maintenance of normal bowel function. The claimed effects are “bowel functions”, “intestinal health/bowel function” and “bowel function/colonic function”. The target population is assumed to be the general population. In the context of the proposed wordings, the Panel assumes that the claimed effects refer to the maintenance of normal bowel function by promoting intestinal regularity and reducing intestinal transit time. The Panel considers that maintenance of normal bowel function in the context of a reduction in intestinal transit time, and an increase in the frequency of bowel movements within the normal range might be a beneficial physiological effect. No studies were provided from which conclusions could be drawn for the scientific substantiation of the claimed effect. On the basis of the data presented, the Panel concludes that a cause and effect relationship

has not been established between the consumption of glucomannan and the maintenance of normal bowel function. Decreasing potentially pathogenic gastro-intestinal microorganisms. The claimed effect is “prebiotic action/bifidogenic action”. The target population is assumed to be the general population. In the context of the proposed wording, the Panel assumes that the claimed effect refers to the capacity of food(s)/food constituent(s) to increase the numbers of bacteria considered to be beneficial. The Panel considers that the evidence provided does not establish that increasing numbers of gastro-intestinal microorganisms is a beneficial physiological effect. The Panel considers that decreasing potentially pathogenic gastro-intestinal microorganisms might be a beneficial physiological effect. No human studies were provided from which conclusions could be drawn for the scientific substantiation of the claimed effect. The Panel concluded that a cause and effect relationship has not been established between the consumption of glucomannan and decreasing potentially pathogenic gastro-intestinal microorganisms.

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.1798

Whereas most stakeholders agree in the need of scientifically sound and clear information for consumers the European Health Claim Regulation has been in the spotlight of critical discussion in many area and occasions such as probiotics (Legrand, n.d.; Rijkers et al., 2011). More recently discussions centered around claims in the area of ant viral and immune supporting claims for COVID 19 infections.

Case study Discussions about health claims

EU health claims and botanicals: Back in the spotlight amid COVID-19 crisis
https://www.foodnavigator.com/Article/2020/05/08/EU-health-claims-and-botanicals-Back-in-the-spotlight-amid-COVID-19-crisis

While consumers frightened by the COVID-19 pandemic stocked-pilled food (and toilet paper), it was tempting for some companies to overpromote their products with regards to their ability to ‘treat’ or ‘cure’ the disease. This was especially the case for products that may sound ‘natural’, like plant derived products. Recently several national authorities recalled the basic principle that claims must not be false, ambiguous or misleading for the consumer nor attribute a property of treating or curing a human disease. This was for instance the case in Belgium where the FASFC seized a tea from China that was labelled as "product for the treatment of flu, to fight the coronavirus ” and encouraged the consumer to be extra-vigilant regarding this kind of so-called ‘miracle’ product. This is not the first time that a product derived from a plant has drawn attention with nonauthorized claims. While not always that extreme, claims surrounding botanical products are often problematic in the EU. There is no clear legal definition of “botanicals”, but this term often designates “a substance, used either as a food or a medicine, derived from plants, fungi, algae or lichen ”. Botanicals are subject to a wide variety of claims that promote their health benefits. When made on foods to be delivered as such to the final consumer, such claims should be made in accordance with Regulation (EC) n°1924/2006, which applies to nutrition and health claims made in commercial communications, whether in the labeling, presentation or advertising (the NHC Regulation). If the NHC Regulation offers some flexibility in the use of statements which refer to general benefits and in the choice of wording, often

claims associated with botanicals are flexed to the point that it may trigger the reclassification of the product. The boarder between botanical food products and medicinal products, in particular traditional herbal medicinal products, is very thin. The choice of claims that accompany such products is consequently of particular importance. Nowadays, there are only few authorized health claims for botanicals such as “Monacolin K from red yeast rice contributes to the maintenance of normal blood cholesterol levels ”, while hundreds have been rejected and the assessment of more than 1,500 has been put “on hold” by the European Commission. The NHC Regulation provides that health claims must be authorized following a strict scientific assessment by the European Food Safety Agency (EFSA). But following the adoption of the NHC Regulation the Commission received thousands of applications that have been consolidated in a list of 4637 health claims to be examined by EFSA. When EFSA started to carry out its scientific assessment, the first 500 opinions issued were negative, as most of the claims failed to demonstrate the cause and effect relationship between the consumption of the botanical substance and the alleged health benefit. This resulted in the Commission decision to request the EFSA to suspend temporarily its assessment of the health claims relating to botanical substances. In 2012, the European Commission published a list of 2000 claims for which the scientific assessment has been put “on hold” and which could therefore continue to be used in accordance with the transitional scheme provided for in Article 28(5) and (6) of the NHC Regulation. To this date, despite several challenges before the Court of Justice regarding the legality of such an extended transition period, most of the claims referring to botanicals remain on hold and thus can be used only if they comply with the other provisions of the NHC Regulation and possible national legislations. As the assessment of health claims on plants and their preparations are currently on hold, and that part of the NHC Regulation has not been fully implemented, the European Commission launched in 2015 its REFIT evaluation focusing on nutrient profiles and health claims on plants and their preparations added to foods. As part of the REFIT, a study was commissioned. One aspect of this study was whether the scientific assessment of health claims related to botanicals could recognize the notion of “traditional use” as an adequate element for their substantiation. A key element for the REFIT evaluation was to take into consideration the broader regulatory framework that applies to plants and their preparations when used in food and in particular the possible overlap between the NHC Regulation and the THMP Directive 2004/24/EC. Interestingly, the REFIT Platform recommended in its opinion to wait for the results of the REFIT of the NHCR before deciding on whether there is a need to collect additional evidence on the performance of the THMP Directive 2004/24/EC and whether on this basis, an amendment of the latter is needed. To date the outcome of the REFIT evaluation for the NHC Regulation has not been made public. Based on the work program of the European Commission for 2020, there is still hope to finally see the long-awaited report finally published. Nevertheless, given the current Coronavirus crisis, the publication might be postponed along with the presentation of the “farm to fork strategy” Strategy which should put the spotlight on consumer information.

The false promises of dietary supplements in the corona crisis

https://www.bccourier.com/the-false-promises-of-dietary-supplements-in-the-corona-crisis/

Vegetable, natural – and mostly unregulated. Dietary supplements with herbal ingredients are a billion-dollar market. It is a thorn in the side of consumer advocates and the pharmaceutical industry because it has been regulated and controlled too laxly for years. The problem becomes particularly clear in the corona pandemic. “For example, it is currently being claimed on social media about all possible substances that they would be able to prevent infection with the corona virus,” wrote the health spokeswoman for the SPD faction, Bärbel Bas. “It can be dangerous for all of us if effective

protective measures are subsequently dispensed with." How deceptive such information can be is shown in a case in which the Hessen Consumer Advice Center advertised as classified inadmissible: A health food store advertised herbal dietary supplements with cistus herb as "immune-boosting". This statement is not permitted with regard to herbal ingredients, only the added vitamins and minerals may be advertised with the label "maintains the immune system". The Federal Ministry of Food and Agriculture (BMEL) even issued a warning against claims of effectiveness such as "Protects against viruses". "Healthy people are promised something immune-boosting," says Wiebke Franz from the Hessen Consumer Center. "That's not okay."

Botanicals in unregulated gray area

The case points to a much bigger problem: dietary supplements Plant ingredients, so-called botanicals, exist in a largely unregulated gray area. The reasons for this lie in Brussels, Berlin, in the state capitals, at the municipal level and with the consumer himself. In the easiest case, buyers and patients lose money and time, in the worse they risk interactions with other Medications or not take a more effective treatment because they believe in the healing effects of the dietary supplements. In the worst case, contaminated or even poisoned preparations end up on the market and in the shopping basket. The origin of the problem lies with the EU Commission: It made it in the Health Claims Regulation of 2006 to check the health claims of vitamins, minerals and also herbal ingredients. In the case of vitamins and minerals, this succeeded: There is now a binding list from the EU Food Safety Authority EFSA about which claims can be used for which ingredients. The tone of the approved claims is extremely conservative. Calcium is "needed to maintain normal bones".

Pharmaceutical industry is upset

The problem with botanicals: A review of their claims has been suspended for ten years. Claims previously used may continue to be used. Actions for failure to act by manufacturers of herbal medicinal products that have to be approved in a complex manner have so far been dismissed. That annoys the pharmaceutical industry. "It is incomprehensible the position of the EU Commission that a regulation is simply not implemented despite clear guidelines," says Nicole Armbrüster, business area manager for biological and herbal medicines at the Federation of the Pharmaceutical Industry. "In my view, this is a neglect of consumer protection." There is another regulatory gap with the maximum amount of ingredients. A market check by the consumer advice center for isoflavone products shows that 14 from 22 products (64 percent) had such a high consumption recommendation that the EFSA / BfR orientation values for the daily intake of soy or red clover isoflavones were exceeded. There are no uniform EU-wide maximum values even for vitamins and minerals. Last week, however, the BMEL announced that it would ask the EU Commission to set maximum values.

Federalism as a third problem

The third problem is federalism. "The manufacturers of nutritional supplements can quickly bring innovative products onto the market, which is also due to the fact that there are lower hurdles for market launch in Germany," says Armbrüster. Food supplements are considered food and only have to register with the Federal Office for Consumer Protection and Food Safety (BVL). The authority then forwards the registration for review to the control authorities of the federal states in which the preparation is to be distributed in the future. On request, the BVL announced that "mainly coordinative tasks", "such as the central receipt of the food supplement advertisements" were carried out. The manufacturers are responsible for the safety of food and this includes food supplements. Two SWR journalists researched that the control by the state authorities does not work: a preparation with thorn apple extract that is toxic to humans could be registered unhindered

and was not checked for months. “I expect this authority to also check compliance with the legal provisions,” Bärbel Bas writes. “It is the responsibility of the Federal Minister of Food and Agriculture to ensure that harmful substances in food supplements are not put into circulation.”

Food supplements on the shelf next to medication

The BPI and the consumer advice center are calling for central approval instead of registration and stricter control mechanisms. Because botanicals are now traded in large quantities on the Internet, which makes regional control all the more ineffective. In the end, the problem lies in retail and with consumers. In drugstores and pharmacies, food supplements are often used alongside herbal medicines. “A pack of a ginkgo leaf can contain both: food supplements and medicine,” says Nicole Armbrüster. “But both fall under completely different laws.” Medicines were used to cure, alleviate or prevent illnesses, while dietary supplements supplement the diet – without claiming to be cured. Armbrüster and Franz would therefore like better advice especially in pharmacies. Ultimately, supplements should only be taken if they are indicated by the doctor. German pharmacies spend 560 million euros a year on vitamins and minerals alone. 2018 225 million packs of food supplements were sold.

Clinical trials for functional foods

Many different types of clinical trials are designed by manufacturing companies and others to explore specific product features impacting human health. In addition, many government organizations regulate clinical trials and claims about trial findings globally, including the United States (US) Food and Drug Administration (FDA) and Federal Trade Commission (FTC) as well as the European Medicines Agency (EMA), European Food Safety Authority (EFSA) and other governments and agencies. The general term clinical trial, may include two basic types of studies which may be “observational” (without any planned interventional or placebo treatment; treatment is outside the control of the investigator) or “interventional” (the specific treatment is carefully planned and controlled by the investigator). Observational studies may include large-scale epidemiological studies intended to assess disease associations within populations and across lifetimes. Interventional studies include RCTs to test a particular investigational product.
Clinical trials may be pragmatic (designed to assess treatment effectiveness in routine clinical practice conditions) or explanatory (designed to evaluate the efficacy of a treatment in a highly controlled setting, the archetypical drug trial). Clinical trials can use a placebo control (which compares an active product to the same product without the active component), a “historical” control (which compares previously reported data with a new group), or may have no comparator group at all (a single treatment is applied to a group without any comparator group). All clinical trials should be conducted using Good Clinical Practices (GCP) with appropriate Human Subject Protections (HSPs) and all products used in human testing should be produced under Good Manufacturing Practices (GMP) using a well-established Quality Management System (QMS).

Both observational studies and experimental trials can provide useful data for identifying diet-disease relationships. Two experimental designs are conventionally used: parallel and crossover. Each of these designs possesses advantages and disadvantages. (AbuMweis et al., 2010) For certain functional ingredients, selection of an appropriate control arm is straightforward, while for others it is challenging. Studies should be short enough to optimize subject compliance, be cost-effective, and avoid high subject dropout rates, while being lengthy enough to ensure biological efficacy. The dose,

frequency, and diurnal timing of intake of the active food ingredient all need to be chosen carefully. Food trials are often designed to evaluate specific marketing claims needing scientific substantiation while drug trials document the safety and efficacy of a specific drug for a specific intended use (e.g., to treat, mitigate or cure a human disease). Food trials tend to be more pragmatic and exploratory as they document human experiences with specific foods in the context of the human diet while drug trials tend to be more explanatory as they document specific drug doses and schedules and specific disease responses. Food trials typically enrol healthy individuals while drug trials enroll patients with a specific disease type potentially needing the research treatment. Foods are complex mixtures of ingredients (e.g., plant parts, meats, eggs, chemicals, beverages, whole meals, etc.) designed to be palatable and which may have the general health effect under investigation while drugs are highly purified and designed to have a specific effect on a disease. Food trials do not typically require a safety review in the US by the FDA before the trial can commence (JL, 2017).

Food trials are less expensive than drug trials. In the US, per subject costs for food trials are roughly $3,000-$5,000 (typically as part of small, less than $500,000 marketing budget for an individual food product) while per subject costs for drug trials is roughly $30,000-$50,000 (typically as part of large, $1.3B dollar R&D budget for an individual drug product). Food trials were estimated to cost $25,000-$250,000 and one publication reported drug trials to cost $1M-50M based on data from 2004-2012 In the EU, food trials were estimated to cost €2,000 per subject while drug trials were estimated to cost €169,613 per subject (Sertkaya et al., 2016).

According to a *JAMA* study, the average clinical trial needed to push a cardiovascular drug through FDA approval costs $19 million. Factors that contribute most heavily to trial costs are the number of patients researchers need to recruit to document a drug effect, how many sites are needed around the world and the length of the trial itself. Drugs are more expensive to test when they have smaller effects that require observing more patients for longer periods of time.
https://www.cardiovascularbusiness.com/topics/healthcare-economics/why-its-so-expensive-develop-new-cv-drugs

Case study. Discussion on costs, prices, and monopolies

Following high, fast-growing costs for developments, including clinical trials, for pharmaceuticals as well as high-quality functional foods triggered a discussion about the costs and prices of these products.

The core of drug pricing problem is one fundamental fact: Drug companies enjoy government-sanctioned and -enforced monopolies over the supply of many drugs. Monopolies are established by high costs for clinical studies which can only be afforded by big industries. Monopolies result then from patents awarded for 20 years from the time the patent is filed. Once new drugs are approved the monopolies assured by patents enable pharmaceutical companies to charge any price they choose. They generally pick prices that not only cover their development costs but also generate profits that exceed those of most other industries: for example, the average profit margin for the 25 largest software companies (which are cited as having the same high R&D investment and low production and distribution costs as pharmaceutical companies) was 13.4 percent in 2015, while the average profit margin for the 25 largest drug companies was 20.1 percent in 2015. Drugmakers are also free to raise prices whenever they want at rates they alone determine.

Drug companies say their monopoly earnings are necessary to sustain the research and development that produce new drugs. In effect, they are saying that they need to be able to charge the very high prices we now see for patented drugs so they can innovate. This raises the questions of how much money society should allocate toward pharmaceutical innovation and who should decide.

Only a countervailing nonmarket force of equal strength can bring those prices down. Western industrial countries, recognizing this, authorize their governments to step in and moderate drug prices for the benefit of their citizenry. Some set prices by fiat, while others negotiate with drug companies. In the latter case, the negotiations are sometimes guided by comparative effectiveness analysis that estimates the value of new drugs to patients.

https://www.commonwealthfund.org/blog/2018/its-monopolies-stupid

Consumers expectations and functional foods

Many consumers worldwide are rather critical about industrial food production, sceptic about arguments for functional foods and prefer holistic ideas of foods where a variety of social, cultural, and physical-environmental factors strongly influence eating practices and behaviours, such as food manufacturing, production and retailing, the built environment, and food-related sociocultural issues (Traverso-Yepez & Hunter, 2016). In contrast, a rapidly growing number of citizens with mostly above average amounts of often internet and social media derived information demands for ways of fortifying their health and nutrition.

A global survey polling over 21,100 (Sääksjärvi et al., 2009) showed significant differences in consumer attitudes to food, nutrition and health between fifteen pan-European countries. The consumer's characteristics, such as demographic origin and personal motivation to engage in the care for health, play an important role in the acceptance of functional food . Socio-demographic characteristics of the consumer, such as gender, education, and age, are the most important factors of the acceptance of functional food. Gender Studies addressing consumer attitudes and acceptance of functional foods have often found gender differences. In general, women tend to be more open and interested in functional foods than men are (Childs and Poryzees 1998), and also seem to be more health conscious . (Ares & Gámbaro, 2007; Childs & Poryzees, 1998).

An important motive for the consumption of functional food is the maintenance of good health condition and the extent to which functional food contributes to this. An additional factor in the success of functional products is confidence in the information and the knowledge of the effects of a particular product on health condition. (Urala, 2000) However, human knowledge of the distinctive attributes of functional food is not sufficient to stimulate consumption. The probability of the purchase of a functional product increases when the consumer associates the functional characteristics (e.g. dietary fibre) with the consequences of their consumption (e.g. reduces the risk of diseases of the stomach and intestines). In addition to healthful properties, the carrier (base product) contributes to the positive perception of a functional product. Moreover, the functional component affects the perception and acceptance of functional food by consumer. What is more, the source of information about the origin of functional components plays an important role in making the decision to purchase the product. Organoleptic attributes, in particular taste, are one of the most important factors determining the success of functional products (Verbeke, 2005)(Moreau, P.C., D.R. Lehmann, 2001) (Kraus, 2015).

Obtaining the consumer's input in the development process can assist in better understanding the consumer's needs in such a way that product developers and research and development departments can then overcome the aspects confusing them. (Kraus, 2015). Safe science-based holistic uses of opportunities for a personalised health prevention following an improved balanced information of citizens need to enable am informed decision of consumers for such opportunities. Consumers organisations are often seen as reliable sources for valid information.

Case study. Consumers recommendation fishoil, Omega 3

The benefits of Omega 3 fish oil in foods, fish oil or medication for health, especially cardiovascular protection has been discussed intensively for a long time. Depending on objectives, methods, products, their quality and study design clinical studies came to different results and conclusions. A specific problem of Omega 3 PUFA and studies with them could come from the quality of the products especially the oxidative stability of encapsulated fish oil dietary supplements (Kolanowski, 2010)

A recent systematic review found that higher omega-3 PUFA levels in diets or blood were associated with a 26% reduction in the risk of metabolic syndrome (MetS), odds ratio (OR)/relative risk (RR) 0.74, 95% confidence interval (CI) 0.62-0.89). This inverse association was evident among studies with Asian populations but not among those with American/European populations, authors concluded that higher intakes of omega-3 PUFAs, but not omega-6 PUFAs, was associated with lower MetS risk. (Jang & Park, 2020) Another meta-analysis found an efficacy of omega-3 PUFAs in depression (Liao et al., 2020).

A multicenter, randomized, double-blind, placebo-controlled trial involving patients with established cardiovascular disease or with diabetes and other risk factors, who had been receiving statin therapy and who had high fasting triglyceride level and a low-density lipoprotein cholesterol level found a cardiovascular risk reduction for hypertriglyceridemia using Icosapent ethyl, a highly purified eicosapentaenoic acid ethyl ester.

In a new guideline, the American Heart Association (AHA) recommends drugs with omega-3 fatty acids for the treatment of hypertriglyceridemia (Skulas-Ray et al., 2019)

In Mai 2020 the German organisation Stiftung Warentest, which gives regular advice to consumers, concluded that benefits of 20 food supplements from pharmacies, drugstores and supermarkets, health food stores and online shops have not been adequately proven , neither for the heart nor for other health aspects. The outcome for three drugs analyzed is also modest. The article concluded that the tested food supplements to protect against cardiovascular diseases are superfluous. This applies to both healthy and high-risk patients. https://www.test.de/medikamente/wirkstoff/fischoel-omega-3-fettsaeuren-w846/ The conclusions were mainly drawn from the Cochran analysis (Kho & AbdelhamidAS, 2018). Test done by the organisation included fatty acid spectrum and the content of omega-3 fatty acids in dietary supplements, fatty acid composition, total fat, free fatty acids, vitamins D. "Other supplier-related or test-related materials cannot be made available externally".

The recommendation concluded that there is no reason to take such additives. "Fatty sea fish such as herring or salmon contains a large amount of OmegA-3 FATTY acids. The need can be met even without fish. Healthy vegetable oil or walnuts are sufficient. Whereas the recommendation for a healthy lifestyle and nutrition, carefully including PUFA sources, was broadly accepted various

comments of readers of the article addressed problems of possible individually different needs as well as access and possibilities to achieve requirements for a healthy lifestyle and nutrition, such as working routines.

According to the German Society for Nutrition, people in this country are adequately supplied with plant-based omegA-3 FATTY ACID " Prof Juergen Koenig, Dep for nutrition, Univ of Vienna pointed to aspects of balances in the consumption of omega 3-fatty acids and of omega 6-fatty acids. According to the German Society of Nutrition, these to fatty acid classes should be consumed at a ratio of 1:5 (omega 3/omega 6). Typically, Western diets provide a higher amount of omega 6-fatty acids together with an overall high amount of total fat. In addition, studies on effects of single fatty acid classes should be critically evaluated on compensation effects (e.g., replacing saturated fatty acids by unsaturated fatty acids or replacing saturated fatty acids by carbohydrates, etc., p. comm)

Towards a preventive personalised healthcare

The centuries starting after the 1970s may be seen as the time of the rise of modern biology and biotechnology. Soon technology-excited individuals started to explore information from modern biology for their use to enhance their personal quality of life.

Biohacking between science, do it yourself try and error.

Without understanding what it is, biohacking has gone viral. Internet followers rub their eyes in disbelief when they read that Twitter CEO Jack Dorsey experienced the benefits of fasting intermittently and drinking "salt juice" each morning. Or that former NASA employee Josiah Zayner injecting himself with DNA using the gene-editing technology CRISPR. Maybe some of us read that Bay Area folks engage in "dopamine fasting." These are all types of biohacking, a broad term for a lifestyle that is growing increasingly popular, and not just in Silicon Valley, where it really took off.

Biohacking — also known as DIY biology — is an extremely broad and amorphous term that can cover a huge range of activities, from performing science experiments on yeast or other organisms to tracking your own sleep and diet to changing your own biology by pumping a younger person's blood into your veins in the hope that it'll fight aging.

The type of biohackers currently gaining the most notoriety are the ones who experiment — outside of traditional lab spaces and institutions — on their own bodies with the hope of boosting their physical and cognitive performance. They form one branch of transhumanism, a movement that holds that human beings can and should use technology to augment and evolve our species.

Some biohackers have science PhDs; others are complete amateurs. And their ways of trying to "hack" biology are as diverse as they are. It can be tricky to understand the different types of hacks, what differentiates them from traditional medicine, and how safe — or legal — they are.
https://www.vox.com/future-perfect/2019/6/25/18682583/biohacking-transhumanism-human-augmentation-genetic-engineering-crispr

The term “biohacking” is used and understood in a wide variety of contexts. There is no uniform definition of what biohacking exactly is. A widely used general definition says that “Biohacking is do-it-yourself biology, a biotechnical, social movement in which individuals and organizations make use of the achievements in biology and environmental science for their own purposes.” In this context the term hacking means taking control of something. Biohacking is also described as the art and science of optimizing and maximizing well-being and performance with the latest biological and technological tools, addressing the identification of sufficient disease risk predictors to enable their early detection and the generation of tailored therapeutic options.

In a biohackers world knowing and optimizing as many biological parameters as possible is a key feature to design and run their own precision health program to reach the best version of themselves. Hot topics of the biohacking community are the optimization of lifestyle habits. Modulation of daily routines and habits, like prioritization, nutrition, and metabolism, fasting, ketogenic diets, cognitive performance, sleep, brain health, nootropics, nutraceutical, etc. in combination with quantifying yourself by the usage of biosensing wearables, genetic analysis, and lab Basis of Information. Biohacking is certainly a tool of a generation that is used to dig for information in the web and in social networks, often neglecting scientific sources of information. Hypes and often superficial science-based knowledge is mixed together in different groups with more or less scientific background. Biohacking or “do it yourself” biology has been on the rise in recent years – it now even has various organised conferences. Following a recent VICE news documentary about a start-up company called Ascendance Biomedical – who are self-testing drugs – biohacking has had further exposure outside of its circle of devout followers.

Biohacking is an open innovation and social movement that seeks to further enhance the ability of the human body. This includes humans trying to get cyborg like features, achieve hyper human senses, and also seek out new medicines and cures for disease via the promotion of self-experimentation.

According to their website, Ascendence Biomedical are currently exploring HIV/AIDS and herpes elimination, and “muscular optimisation”. It sounds futuristic and appealing, but those critical of the approach say a major concern is that the methods of the biohacking community are housed outside of the relevant scientific processes – as governmental, academic, charitable and pharmaceutical institutions that operate with high safety standards for medical research are held to. This means that the biohacking pathway is anything but safe, as it is not regulated testing.
http://theconversation.com/the-dangers-of-biohacking-experiments-and-how-it-could-harm-your-health-100542

OPTIMIZATION BY PERSONALIZATION. Self-tracking is a well-known powerful tool to identify and impact several unhealthy habits, but also provides the option to monitor efforts and effectiveness of changing lifestyle habits. Several tracking devices for nutrition, mind, sleep, training and recovery and stress response are already available on the strongly growing healthcare market. All of these biohacks are then used to provide a personalised healthcare management system, as a holistic approach that enables better prevention or therapy results, thus improving the quality of care and life.

Biohacking, OMICS sciences, and monitoring biohacking regimes. Every individual has its unique molecular fingerprint while dealing with the environmental inputs, that influence the chemical composition of a biological system. This individual signature is created by our genetic makeup, which determines the body’s potential to deal with environmental inputs. Our cells have the possibility to

adapt to various environmental influences via genomic and epigenetic driven gene regulation mechanisms by dynamic changes in the accessibility of the DNA to transcription machinery binding but without changes in the nucleotide sequence.

The monitoring of such molecular interactions at multiple functional levels is combined in the research area called omics sciences. The intensive omics research will be able to explain and predict a phenotype by assessing a multi-omics signature, derived from genome, methylome, transcriptome, proteome, and metabolome as well as metagenomic data collection. Studying each omics level in isolation often ends up with the issues of failing in reproduce former findings, due to the lack of information. But large omics datasets also are a real challenge interpreting all that huge data sets properly.

Biohacking picks up the hot topic of personalised healthcare driven by omics-based data collection and propagates the usage mainly in personalised natural or complementary medicine based therapeutic approaches. But a crucial realistic view on strong scientific evidence and the quality of selected studies used for selling biohacking regimes, kits and testing online are widely missed.

Especially the supply of epigenomics, proteomics and metabolomics-based analysis panels had increased on the market in the last years.

In future scenarios omics-based science approaches will strongly facilitate the transition of studying healthcare interventions from population-based assessments to targeted individual-based assessment and management. Today we are only at the beginning of studying the “Omics” cascade to develop new biomarkers, drug targets and personalised therapies. Thus, biohacking on the basis of those biomarkers without critical validation is more than questionable.

Case study Biohacking changes the idea of nutrition

Biohacking changes the idea of nutrition to a technology for optimisation. The best example to understand this is the story of one of the founders of the biohacking idea, Dave Asprey's a multimillionaire who had everything. But he weighed 134 kg.

He tried various diets — everything from low-calorie to high protein; Zone to Atkins, but apparently nothing worked. So Asprey set about trying to “hack” his own biology; to map out a system “in an attempt to find one little hole he could exploit in order to take over.”

By doing this Asprey says he discovered which foods (and drinks) were good for him - both physically and mentally. The result is a diet high in saturated fat; one which holds that red meat is good, brown rice is bad, cuts out fruit, and that resulted in a publication called The Bulletproof Diet.

In 2004 Asprey’s learned in the Himalaya yak butter tea and the health benefits of the Tibetans’. That moment, he says, transformed his life. When he got home, he tried blending regular butter in tea but it tasted terrible, so he blended grass-fed butter — which most appropriated yak butter - with coffee instead. Bulletproof Coffee was born. According to his slick-looking Bulletproof website, Asprey read “countless thousands of research papers online, [spent] more than 10 years working with some of the world’s top health and nutrition researchers, [and read] over 150 nutrition books” to come up with his diet which he says will “not only make you stronger and leaner” but will also "upgrade" your brain and “reduce your risks of cancer, heart disease, stroke, diabetes, and Alzheimer’s.”

Many of the ideas of Bulletproof and other nutrition hacking sites make nutritional scientist crying. But we must admit that in recent years nutritional sciences experienced a vivid debate about carbohydrates, proteins, and fat.

https://www.thelancet.com/journals/lanpub/article/PIIS2468-2667(18)30135-X/fulltext?fbclid=IwAR1hx9T9kVuoa2kxQSELVSMqc-5QvYEMUCJGCkndTen5o7SYX0Cxaq-DqYw

https://annals.org/aim/fullarticle/2752328/unprocessed-red-meat-processed-meat-consumption-dietary-guideline-recommendations-from?fbclid=IwAR1o3pKBNqwGAPgK09BBjMv_ZkLaeKaYT4xrJ-saYHueFAYb6D6ZoknZgkw

https://www.heart.org/-/media/files/healthy-living/company-collaboration/inap/fats-white-paper-ucm_475005.pdf

A central idea of Asprey and the biohacking community is certainly that everyone has to hack, to find out the best way, the best technology for himself. Whereas this try and error method looks frightening to scientist, also solid science is looking for individual variances in the metabolic make-up of each individual and individualised diets based on solid sciences and analysis are developing quickly. The overall aim of biohacking is to promote health and longevity by the modulation of lifestyle habits. Addressing energy levels, stabilized mood and focus, which in turn should create happiness, confidence, willpower, and intelligence, but the hypothesis that the desire for the feeling of having control of the body and mind can create a feeling of security by keeping your habits in check by self-measurement regimes. Security is of great importance in an insecure, increasingly complex world, where global crises are no longer manageable, but what is manageable? The body and it is environment. Starting to take responsibility for own health has a great potential for the prevention or therapy of lifestyle-associated diseases, like metabolic syndrome and its comorbidities like diabetes, coronary heart disease, or cancer.

Self-tracking maybe applies a level of mindfulness to your overall health. This can positively affect the transformation of habits and daily routines in a healthier way and supports the maintenance of the new habits, which indeed is the greatest challenge.

But the emerging biohacking trend has to be controlled, reviewed, and critically discussed by the scientific community and healthcare professionals. Getting false or misleading results by dubious analytical service providers or overdose supplements are possible risks of uncontrolled not standardized biohacking methods. Insufficient scientific evidence by lacking the validation of biomarkers used, and principle ethical issues concerning DNA sequence manipulation, data security also have kept in mind when reading about new achievements in this growing field of biological research.

At the biohackers summit 2019 in Finland participants agreed that Biohacking is defined as better living through science, technology, and nature with biological and technological tools. Biohacking is the application of genetics, epigenetics, physiology, nutrition, and bioinformatics to take your physical & mental performance, health and wellbeing to a new level. Especially bioinformatics is another popular target for do-it-yourself biology research. Whereas science strongly aims for data confidentiality and struggles with owners' rights of biobanks, the biohacker community strongly believe that the tools and knowledge of science belong to everyone and is ready to use hackers

possibilities of utilizing nutrigenetic, nutriepigenetic and biometric data. A near clash of cultures seems likely.

Basis of Individualisation, the human genome project

The main goals of the Human Genome Project were first articulated in 1988 by a special committee of the U.S. National Academy of Sciences, and later adopted through a detailed series of five-year plans jointly written by the National Institutes of Health and the Department of Energy.

James Watson was appointed to lead the NIH component, which was dubbed the Office of Human Genome Research. The following year, the Office of Human Genome Research evolved into the National Center for Human Genome Research.

In 1990, the initial planning stage was completed with the publication of a joint research plan, "Understanding Our Genetic Inheritance: The Human Genome Project, The First Five Years, FY 1991-1995." This initial research plan set out specific goals for the first five years of what was then projected to be a 15-year research effort.

HGP researchers deciphered the human genome in three major ways: determining the order, or "sequence," of all the bases in our genome's DNA; making maps that show the locations of genes for major sections of all our chromosomes; and producing what is called linkage maps, through which inherited traits (such as those for genetic disease) can be tracked over generations.

The HGP has revealed that there are probably about 20,500 human genes. This ultimate product of the HGP has given the world a resource of detailed information about the structure, organization, and function of the complete set of human genes. This information can be thought of as the basic set of inheritable "instructions" for the development and function of a human being.

The International Human Genome Sequencing Consortium published the first draft of the human genome in the journal *Nature* in February 2001 with the sequence of the entire genome's three billion base pairs some 90 percent complete. More than 2,800 researchers who took part in the consortium shared authorship. A startling finding of this first draft was that the number of human genes appeared to be significantly fewer than previous estimates, which ranged from 50,000 genes to as many as 140,000. The full sequence was completed and published in April 2003.

Upon publication of the majority of the genome in February 2001, Francis Collins, then director of the National Human Genome Research Institute, noted that the genome could be thought of in terms of a book with multiple uses: "It's a history book - a narrative of the journey of our species through time. It is a shop manual, with an incredibly detailed blueprint for building every human cell. And it is a transformative textbook of medicine, with insights that will give health care providers immense new powers to treat, prevent, and cure disease."

The tools created through the HGP also continue to inform efforts to characterize the entire genomes of several other organisms used extensively in biological research, such as mice, fruit flies, and flatworms. These efforts support each other because most organisms have many similar, or "homologous," genes with similar functions. Therefore, the identification of the sequence or function of a gene in a model organism, for example, the roundworm *C. elegans,* has the potential to explain a homologous gene in human beings, or in one of the other model organisms.
https://www.genome.gov/human-genome-project

Whereas the sequencing of the first genome took 13 years to accomplish; a decade later, next generation sequencing (NGS) technologies – including whole genome or exome sequencing (WGS/WES) becoming increasingly available in many hospital laboratories an enabled the sequencing of hundreds of genomes with different objectives.

The 1000 Genomes Project was an international research consortium that was set up in 2007 with the aim of sequencing the genomes of at least 1,000 volunteers from multiple populations worldwide in order to improve our understanding of the genetic contribution to human health and disease. The project goal was to produce a catalogue of human variation down to variants that occur at 1% frequency or less over the genome, in order to facilitate genetic studies on common human disease.

A major paper, published in the October 1, 2015, issue of *Nature*, marks the completion of the final phase of the colossal project: a comprehensive, open-access database of genetic variation from 2,504 individuals from 26 populations across the globe .The genotypes were obtained using a combination of whole-genome sequencing, deep exome sequencing and high-density single nucleotide polymorphisms (SNPs) microarrays. The characterization of the variants was based on a set of 24 sequence analysis tools. Overall, the project discovered and characterized more than 88 million variants, including 84.7 million SNPs, 2.6 million short insertions/deletions, and 60,000 structural variants, that were integrated into a high-quality haplotype scaffold (Altshuler et al., 2010; Craddock et al., 2010; Devuyst, 2015; J. Hardy & Singleton, 2009; Manolio et al., 2009; Sachidanandam et al., 2001; B. J. Wilson & Nicholls, 2015).

As compared to the reference human genome, a typical genome differs at ~ 4 to 5 million sites, 99.9% of these variants being SNPs and short indels. The number of variant sites is greatest in individuals from African ancestry, as expected from the out-of-Africa model of human expansion. Analyses of the variants most likely to affect gene function revealed that a typical genome contained ~150 sites with protein-truncating variants, ~10,000 sites with peptide-sequence altering variants and ~ 500,000 variant sites overlapping regulatory regions such as promoters, enhancers, or transcription factor binding sites. Importantly, ~2,000 variants per genome were associated with complex traits through genome-wide association studies (GWAS) and 24 – 30 variants per genome implicated in rare diseases through ClinVar (a database of the relationships among human variations and phenotypes). Other analyses provided information about population history, the demography of ancestor populations, and resolution of genetic association studies. (Auton et al., 2015)

The results of the 1000 Genomes Project, which attest to the benefits of “consortium-based science,” complete a set of genomic information that has already been in use for several years. Such information is particularly useful for the design of genotyping arrays, population genetics (e.g. genotype imputation in GWAS, defining variants in regions of interest, filtering of likely neutral variants), and investigations on natural selection, population structure, and admixture. The major advantages of the 1000 Genomes Project data set include the broad representation of human genetic variation (with a much improved coverage of South Asian and African populations); the use of multiple analysis strategies, increasing the quality of filtering and mapping and allowing the capture of more diverse types of genetic variants (Devuyst, 2015)

Missing heritability

Genome-wide association studies have identified hundreds of genetic variants associated with complex human diseases and traits and have provided valuable insights into their genetic

architecture. Most variants identified so far confer relatively small increments in risk, and explain only a small proportion of familial clustering, leading many to question how the remaining, 'missing' heritability can be explained (Manolio et al., 2009). Possibilities to analyse the potential sources of missing heritability and propose research strategies, including and extending beyond current genome-wide association approaches have since then been discussed to illuminate the genetics of complex diseases and enhance its potential to enable effective disease prevention or treatment.

The problem of missing heritability, that is to say, the gap between heritability estimates from genotype data and heritability estimates from twin data, has been a source of debate for about a decade. Human traits, especially social and behavioural traits, remains controversial. The dawn of the genome-wide association study (GWAS) era, around the year 2007, brought with it the question: can we identify specific genetic variations that explain the heritability estimated from twin studies? The small sample sizes of early GWAS meant they had power to identify only common genetic variants with relatively strong effects, and the amount of trait variation that these variants explained was typically only a small fraction of the heritability estimated by twin studies. For height, by 2010 around 40 variants had been identified that collectively explained around 5% of the variation in height, compared to a twin heritability of around 80%. This gap became labelled ’the problem of missing heritability’ and has stimulated heated debate ever since. Many different explanations for the ’missing heritability’ have been proposed. 1) that complex traits are highly polygenic and affected by many rare variants; 2) that twin studies have overestimated heritability. (Youngid, 2019). Epigenetic programs may account for a significant fraction of the “missing heritability.”

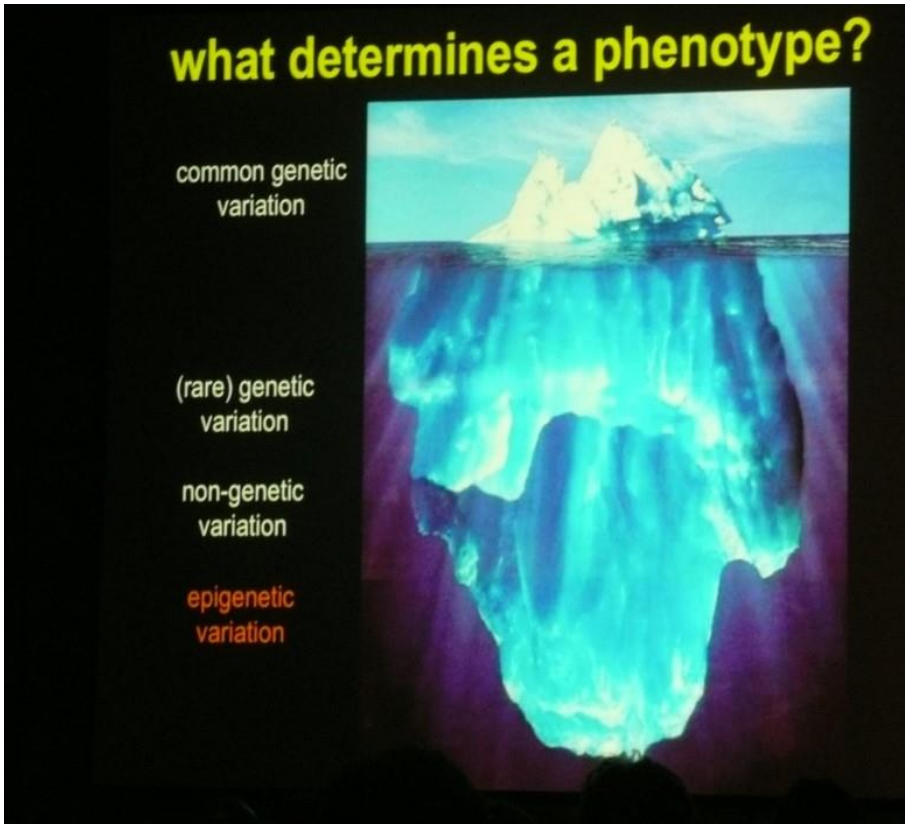


Fig Gene Environment interactions, Phenotype, anonymus

Consistent components of complex traits, such as those linked to human stature/height, fertility, and food metabolism or to hereditary defects, have been shown to respond to environmental or nutritional condition and to be epigenetically inherited. The knowledge acquired from epigenetic genome reprogramming during development, stem cell differentiation/de-differentiation, and model organisms is today shedding light on the mechanisms of (a) mitotic inheritance of epigenetic traits from cell to cell, (b) meiotic epigenetic inheritance from generation to generation, and (c) true transgenerational inheritance. Such mechanisms have been shown to include incomplete erasure of DNA methylation, parental effects, transmission of distinct RNA types (mRNA, non-coding RNA, miRNA, siRNA, piRNA), and persistence of subsets of histone marks (Trerotola et al., 2012)**.**

From clinical genetics to personalised medicine

The focus of traditional clinical genetics has been on identifying monogenic disorders, often pre-specified on the basis of a person's family history, ethnicity, or medical history. These variants – mutations – are usually of high penetrance, i.e. carrying the mutation is associated with a high likelihood of developing the disorder in question. The family history may point to dominant, recessive, X-linked, or some other form of a single gene (monogenic), Mendelian inheritance. Patients are usually referred on the basis of unusual family history, birth of a child with a serious congenital anomaly, or diagnosis of a suspected genetic condition (Becker et al., 2011).

New NGS methods enabled then the strategy of genetic profiling to offer individual risk information for multifactorial disorders (e.g., cardiovascular disease, cancers, and type 2 diabetes, where disease risk results from the interaction between several genes (polygenic) as well as non-genomic factors. Genetic profiling often involves measuring single nucleotide polymorphisms (SNPs): variations in the smallest building blocks of DNA. While SNPs may lead to mutations that cause monogenic disorders (which are generally rare, usually sufficient in themselves to cause disease, and are more readily identifiable as causative), SNPs associated with more common, complex diseases, generally convey only minor excess risk making them more difficult to identify when embedded within a background of widespread non-pathogenic variation across the genome.

Genetic profiling may be narrowly targeted, using defined panels of SNPs designed to provide risk information for a specific health condition (e.g., colorectal cancer), or may

be less targeted, with SNP-based genome-wide profiling addressing multiple disorders. Least targeted are those approaches that use NGS technologies to provide "complete" genomic information on an individual. Thus, the scope of "personalised medicine" may range from targeted testing of one or several mutations associated with rare monogenic, high penetrance disorders at one extreme to, at the other, sequencing a patient's exome or genome without targeting specific variants. (B. J. Wilson & Nicholls, 2015).

Ultimately, the service model underpinning the delivery of personalised medicine is that of "genomics in medicine" – the integration of genomic testing with other clinical investigations, delivered by health care professionals who are not medical geneticists, and where a positive test result does not imply a serious genetic diagnosis, rather is interpreted alongside the entirety of a patient's health and medical dat (B. J. Wilson & Nicholls, 2015)

Population screening

Population screening involves a test being offered to all individuals in an eligible group, usually defined by age, as part of an organised program. The group is eligible because there is strong scientific evidence that they are at most risk and will get the most health benefit from screening. No screening test is 100 per cent accurate, which is why screening is offered at recommended intervals. For a screening program to succeed there must be evidence that early diagnosis and treatment increases the chance of successfully treating or managing the disease. Population screening therefore offers a test or investigation to apparently healthy individuals in order to detect unrecognized disease or its precursors, with the intention of improving the outcome for individuals identified in this way. Screening tests will often modify the risk that an individual has the disorder in question, shifting the group with positive screening results from low(standard or population) risk to a high (increased) risk, without this amounting to a firm, positive diagnosis. In such screening programmes, further investigations may be required to confirm or exclude the suspected diagnosis. Population screening programmes are made available to the whole population or to large subgroups (e.g. all new-born infants, all pregnant women) where there is no specific reason to think that the individuals screened are likely to be affected. Where there is reason to single out an individual as already being at risk of the condition, then diagnostic testing may be appropriate without the screening. The screening is ordered to individuals who are healthy and asymptomatic; screening is not a response to pre-existing concerns from those who think they might be ill, or those who do have symptoms of disease which resulted in a number of ethical concerns. Concerns of this nature led Wilson and Jungner, on behalf of the World Health Organization, to draw up criteria to be met before screening (Auton et al., 2015). https://apps.who.int/iris/handle/10665/37650

Personalised genetic screening

Individual genetic variants generally confer only a small increase in individual disease risk, and even panels with multiple variants are poor at discriminating disease risk in individuals. However, when combined with age, genetic panels may offer more accurate risk stratification and indicate more tailored approaches to the timing or intensity of screening tests (so-called “personalised screening”). For example, for individuals in a highest risk stratum, surveillance might begin at a younger age or screening frequency shortened, while individuals in lower risk strata might benefit from a reduction in screening intensity.

Pashayan et al model this approach using the UK National Breast Screening Programme as a case study. They use as an example a panel of 67 common SNPs that explain approximately 14% of the genetic component of breast cancer risk. They calculate that profiling using this panel plus age would lead to some women as young as 35, and some as old as 79, being offered screening because they reach the threshold of 2.5% 10 year absolute risk; conversely, 24% fewer women aged 47–73 would be reclassified below the risk threshold and therefore not offered screening. Overall, 3% fewer cases would be detected (lower sensitivity), but there would be a lower rate of false positive screens (higher specificity). If this group’s analyses are valid, the addition of targeted genetic profiling would potentially lead to some missed cancers, a reduction in unnecessary diagnostic investigations, a higher detection rate of (predicted to be more aggressive) cancers in younger individuals, and reduction in over-diagnosis of indolent or latent cancers (Pashayan et al., 2013). Similar arguments

have been proposed for screening for colorectal cancer and prostate cancer. Genetic profiling for stratification of population-based screening must be formally evaluated. Predictions about risk reclassification, disease detection, and changing eligibility for screening need to be confirmed in empirical studies in representative populations, and randomized controlled trials comparing personalised screening with current approaches would allow quantification of benefits, harms, and costs in actual practice. While it is anticipated that personalised screening would reduce negative psychosocial impacts of screening, there are multiple issues which would need to be taken into account (discussed below) before genetic profiling could be considered for widespread implementation (B. J. Wilson & Nicholls, 2015).

Genetic screening interventions

Screening intervention	Target population	Example conditions
Currently available		
Pre-conceptional screening	Individuals planning pregnancy	Recessive conditions, eg, cystic fibrosis
Antenatal screening	Pregnant individuals	Major chromosomal anomalies, eg, Down syndrome
Newborn screening	Neonates	Inborn errors of metabolism, eg, phenylketonuria
Cascade screening	First and second degree relatives of individual with genetic disorder	Recessive conditions, eg, familial hypercholesterolemia
Population carrier screening	Defined population subgroups	Genetic conditions with high prevalence in subgroup, eg, hemoglobinopathies
Direct-to-consumer tests	Individuals willing to purchase	Common disease susceptibility, eg, cardiovascular disease
Potential/in development		
Disease-based case finding	Patients with common serious conditions	Common conditions with genetic subtypes, eg, some cancers
Personalized/stratified population screening	Target population for standard (non-genetic) screening	Conditions screened for at population level, eg, colorectal cancer
Case finding in whole genome/exome sequencing	Patients undergoing whole genome/exome sequencing for clinical diagnostic investigation	Rare "actionable" genetic mutations, eg, retinoblastoma

Fig Genetic screening concepts, (B. J. Wilson & Nicholls, 2015)

Problems of direct to consumer (DTC) genetic testing

Although DTC genetic testing may provide an opportunity for consumers to recognize the importance of genetics in diverse phenotypes, including diseases DTC genetic testing has important drawbacks.

Most genetic tests performed by DTC companies are limited to few major genetic variants related to the phenotypes of interest, which leads to poor discriminatory power. In addition to genetic factors, disease incidence is influenced by environmental and lifestyle factors including age, sex, race, nutrition, exercise, and stress, often modulating epigenetics or microbiota. Thus, DTC genetic testing itself does not guarantee that a consumer with a high genetic risk score will suffer from a certain disease. Instead, it only indicates that one has a genetic propensity for that disease. For example, 23andMe conducts an *APOE*genetic test in relation to Alzheimer disease. The average likelihood of developing Alzheimer disease in carriers of the relevant allele is more than twice as high than in people who do not carry it. However, not everyone with the *APOE* e4 gene will develop Alzheimer disease, and having the *APOE* e2 gene, which confers resistance to Alzheimer disease, is likewise not a guarantee that one will never get Alzheimer disease (Oh, 2019).

Conflicting evidence with direct to consumer nutrigenetic tests

In recent years, several companies have started selling DNA testing kits via the internet directly to consumers (DTC) (Crow, 2019). Such "consumer genetics" companies communicate genetic test results to the customer without medical supervision and offer a wide range of predictions about the personal risk for developing common diseases including cancer, autoimmune or cardiovascular diseases. Overall, "direct-to-consumer genetic testing" services, or DTC-GT, are supposed to guide the user toward more informed decisions about lifestyle choices. Usually, the genetic test starts with an online order of a kit by a consumer, necessary for the collection and delivery of a saliva sample; the companies then extract the consumer's DNA and assess the presence or absence of specific genetic variants known to be associated with (for example) an increased disease risk, or with the regulation of a trait of interest and various health conditions. Interestingly, several companies have focused their service toward health-related outcomes such as fitness (e.g., performance and injury tests) (Webborn et al., 2015), pharmacogenetics (e.g., personalised treatment) (G. Zhang & Nebert, 2017)and nutrigenetics (e.g., weight control, and food intolerance and sensitivity).

The continuous growth of DTC-GT companies is fuelled by both the ongoing dramatic drops in DNA sequencing and genotyping costs, and by the availability of a wealth of human genetic variation data. Furthermore, the Human Genome in 2003 provided researchers with the full sequence of the human genome ("reference genome sequence"), which allows researchers to define genetic variants (differences in the sequence of DNA among individuals) and to study their functional consequences.

The most common class of genetic variants are termed single nucleotide polymorphisms (SNPs), So far, scientists have found more than 600 million SNPs in human populations around the world. The second most abundant class of genetic variants is insertions and deletions (INDELs), where a person's DNA sequence at a given position of the genome has more (insertions) or less (deletions) nucleotides with respect to the reference sequence.

SNPs and INDELs, both clinically relevant as well as neutral genetic variants, are registered in a free public archive, the Single Nucleotide Polymorphism Database (dbSNP) https://pubmed.ncbi.nlm.nih.gov/11125122/ . A specialized subset of dbSNP entries is collected in ClinVar (Landrum et al., n.d.), a freely available, public archive of human genetic variants with proven or suspected clinical relevance. A unique identification tag—the so-called "rs identifier" or "rs ID"—is assigned to each genetic variant stored in dbSNP. For example, the ID rs1801280 corresponds to a genetic variant that changes the aminoacidic sequence of the protein encoded by the gene NAT2 (N-Acetyltransferase 2, see **https://www.ncbi.nlm.nih.gov/snp/rs1801280**. Rs identifiers are

particularly useful when searching for information about a variant, because they are unambiguous, unique and stable; in contrast, descriptive names of genetic variants (based on their genetic position or on amino acid changes) can be more ambiguous, depending for example on the version of the human genome used as a reference.

The majority of common genetic variants have little or no effect on health or development. However, in certain special cases, they can influence an individual's response to certain drugs (Barbarino et al., 2018), or increase the risk of developing a complex disease such as type 1 diabetes (Nyaga et al., 2018) or Alzheimer's disease slightly (Cuyvers & Sleegers, 2016).

In addition, a wide number of genetic variants associated with clinical, biochemical, and anthropometric traits are available. In a recent paper, Zenin et al. (Zenin et al., 2019) identified 35 traits with significant and large genetic correlation to health-span, which can be classified into four clusters: (1) sociodemographic factors, lifespan, smoking and coronary artery disease; (2) high-density lipoprotein (HDL)-related traits; (3) obesity-related disease and body mass index (BMI); and 4) type 2 diabetes-related traits. Therefore, there is a genetical correlation between health-span, as a morbidity-free life period, personal history, and life-traits, which might be controlled by physiological processes such as nutrition.

When traits and diseases are highly heritable (i.e., monogenic traits and disorders determined by one or a few variants), genetic testing will be accurate and very predictive. In contrast, when traits and diseases are only partially influenced by genetic factors and the heritability is low, the predictive ability of tests that consider only a single genetic variant will never be very accurate (Tam et al., 2019). Simply put, in case of complex traits and diseases, having a genetic variant will not mean developing a certain phenotype.

The complexity of these concepts means that a generic user does not have the tools to understand the truthfulness and reliability of these tests unless direct-to-consumer genetic-testing (DTC-GT) companies declare in a transparent way the origin of the predictors used and the reliability of their advice. Reports provided by the companies are heterogeneous about content and representation of results. Most of the example documents (82%) are presented as personalised reports with name, surname, and sample ID. Twelve companies (55%) provide information about genes and SNPs. Only four companies cite GWAS and polygenic score risk. Overall, results are first represented in a summary of all traits studied and of the genetic results (73%) followed by trait-by-trait sections, where consumers are provided with information about traits analysed (77%), gene names and variants (82%) and a graphical representation of the genetic results (59%) indicating whether the genotype is favourable or not, along with the increased, low, moderate or typical risk. Genetic results are clearly explained (82%) with one or more sentences that explain the significance, followed by recommendations (86%). Only 10 companies provide a bibliography to support the reported information. Eleven companies state a disclaimer sentence, indicating that the information provided is not for diagnostic or clinical use, and suggest consulting with a healthcare professional before making any major changes to diet (Floris et al., 2020):

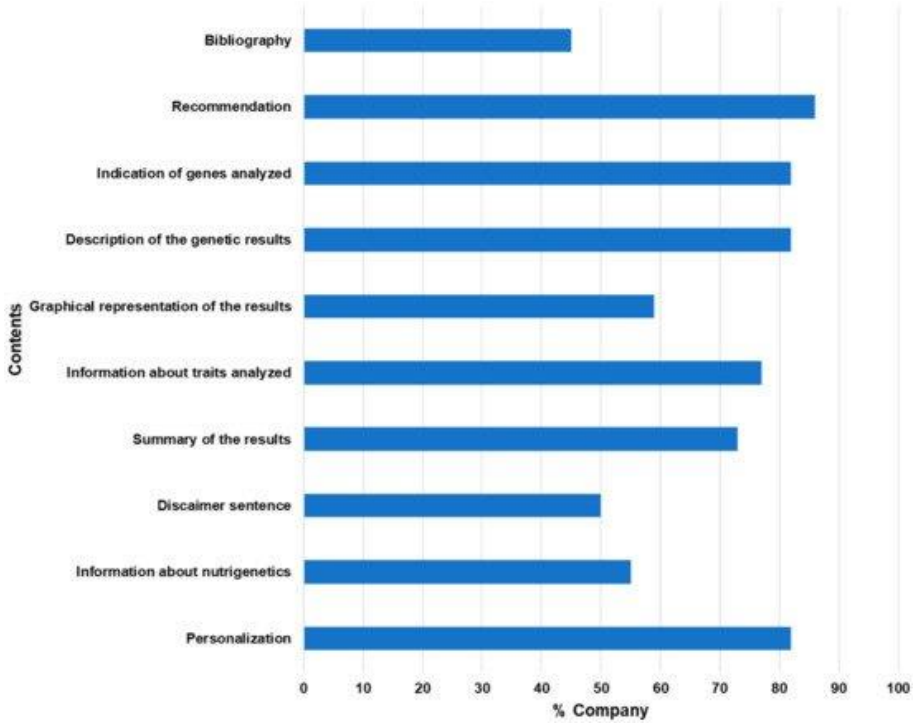


Fig Analysis of the contents of the report examples provided by the selected DTC-GT companies. In the graph, the percentages of companies including specific contents in their report examples are represented in the graph (Floris et al., 2020).

Despite the complexity of these arguments, according to a MIT Technology Review article published in February 2019, "more than 26 million consumers added their DNA to four leading commercial ancestry and health databases" https://www.technologyreview.com/2019/02/11/103446/more-than-26-million-people-have-taken-an-at-home-ancestry-test/ .

Ethical and legal issues: The inaccurate and scientifically unproven claims of some DTC-GT raise ethical issues, as costumer's health is at stake. There have been attempts to regulate genetic testing, but more needs to be done to achieve a reliable standard. In the United States the Clinical Laboratory Improvement Amendments (CLIA) is responsible for the validation of genetic tests, the standards to pass validation vary from state to state. In contrast, the EU protocol "Additional Protocol to the Convention on Human Rights and Biomedicine, Concerning Genetic Testing for Health Purposes" tries to unify genetic testing regulations. It emphasizes the need for medical supervision of genetic test with important health outcomes, as well as the need for adequate scientific validity and quality. The EU project "EuroGentest2" puts effort into harmonizing genetic testing in Europe and tries to ensure accurate and reliable results for patients (*EuroGentest: Home*, n.d.; San-Cristobal et al., 2013.) Regulation of genetic and nutrigenetic testing, especially for online consumer use, is essential to guarantee consumer safety. Furthermore, a standard for genetic testing would make identifying disease risk easier and more accurate.

A further concern is the marketing strategy of genetic testing companies. Websites of such companies often advertise the benefits of genetic testing without regarding the risks (lack of scientific evidence, misleading interpretations, health anxiety, genetic discrimination, confidentiality of test results etc.), omitting important information for the consumer. This lack of information may cause serious health consequences for customers who wrongly interpret their results as a medical diagnosis. Without proper genetic consulting or sufficient information provided by genetic testing

companies costumers do not understand the meaning of their disease risk and the impact the environment can have on genes. With poor predictive values DTC-GT risk to create needless health anxiety and false hopes for costumers, especially considering that those who are at a high risk for cancer or other diseases are more interested in talking genetic tests. Concerns also arise when genetic testing companies' market-specific drugs or nutritional supplements on their website. This questions the intention of the company in regards to their costumers health and wallet (Covolo et al., 2015).

An investigation done by the US GAO mentioned above noted deceptive marketing strategies by GT companies. The investigation made undercover calls to seek health advice based on the company's genetic tests results. Experts reviewing claims of test found many scientifically false. This shows how misinformation can easily reach consumers through uneducated company representatives. It is therefore crucial that DTC-GT are supervised by a health professional to inhibit misleading information and marketing strategies that may harm consumer health (*Misleading Test Results Are Further Complicated by Deceptive Marketing and Other Questionable Practices Statement of Gregory Kutz, Managing Director Forensic Audits and Special Investigations*, 2010).

A systematic review looked at potential biomarkers for breast cancer and obesity. The study concluded that there is still a lack of research to confirm the effectiveness of biomarkers in detecting breast cancer. The authors criticized that DNA methylation markers in serum detected cancer, but they could not differentiate between cancer types. Another misleading issue is that methylation occurs sporadically in an organism and its presence does not directly indicate tumor development. Researchers fear that this may lead to overdiagnosis. The authors emphasized the need for more research on the effectiveness of biomarkers in clinical trials, rather than focusing on new discoveries. It was predicted that a combination of genetic, epigenetic and noncoding RNA markers would result in better screening accuracy (Goettler et al., 2016).

Furthermore, SNPs may have different or no effects on different ethnic groups. The MTHFR polymorphisms e.g. showed conflicting outcomes in regard to CVD in the Chinese and American population. This may be due to the genetic information itself or to cultural or political factors (e.g. mandatory folate supplementation).

Genetic variability is dependent on sex as well as ethnical background. Many of the studies done on SNPs and disease risk have been conducted on white populations, the results can therefore not be translated to other ethnic groups with certainty. A database comparing the genetic background of different ethnic groups, such as the Genographic Project Beta 2.0 (Behar et al., 2007), could help interpret current data more accurately. It is clear that more scientific research on different ethnic groups (and larger populations) is needed to predict genetic risk more accurately (San-Cristobal et al., 2013). It is unethical to fmarket genetic test to consumers of certain ethnicities that will not benefit and could potentially be harmed by the inaccurate results. Before any genetic testing can be used in the medical field these issues must be confronted to assure all ethnicities receive correct disease predictions and to hinder one race to benefit more from the health care system than others.

Basis of personalisation: Gene environment interactions

An organism's complex trait composition is the product of genetic, epigenetic, and environmental inputs and their cumulative interactions through development. The genetic mechanisms that control complex traits are 'Mendelian 'in their transmission pattern. They and comprise DNA-sequence

differences and are responsible for the bulk of phenotypic variation across the human population. Epigenetic contributors, by contrast ,are 'non-Mendelian' and rely on mechanisms that bypass DNA sequence to impart phenotype. Non-Mendelian or epigenetically-driven phenotypic variation (EPV) can be thought of as variability that occurs in inbred populations, and can be classified according to distribution as either continuous (e.g. height) or discrete (e.g. .flower colour), or a combination (eusocial insect morphs). The latter examples are termed 'polyphenisms', scenarios in which the same genotype yields distinct phenotypes within a population, with no intermediates (e.g. worker and soldier ant). Both, continuous EPV and polyphenisms result predominantly from developmental responses to the environment, a broader phenomenon termed 'phenotypic plasticity. Studies suggest that phenotypic plasticity is triggered preferentially during critical windows of high sensitivity including embryonic development and growth phases. More recently, the pre-conceptual germlines of the parents, and even ancestral germlines have been highlighted as additionally relevant 'intergenerational' windows of sensitivity for triggering plasticity.

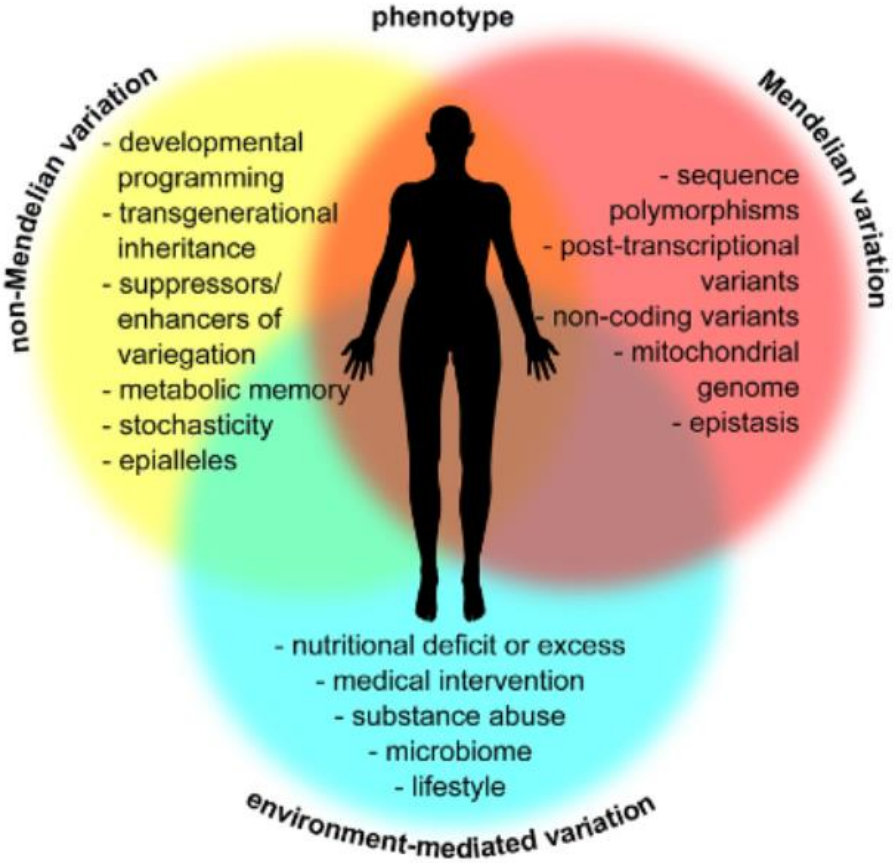


Fig Epigenetic control of variation and stochasticity in metabolic disease (Panzeri & Pospisilik, 2018)

Most disease traits, in turn, are composites of many complex traits. Further, at the genic level, dozens, if not hundreds, of relevant alleles can exist in the population. A recent survey of coding sequence variation across 60,706 individuals identified 7.9% of high-confidence regions as multiallelic, i.e. contained multiple distinct sequence variants. Because of the extreme interconnectivity of cell regulatory networks, even at the cellular level, predicting the impact of a sequence variant is difficult as the resultant variation acts in the context of all other variant sand

their potential additive, synergistic and antagonistic interactions. This phenomenon is known as epistasis. Gene expression is constantly modulated by external and internal cellular signaling, and thus by cellular or organismal 'environment. Environment can include factors such as stress, but also factors such as microbiome composition. In this case the influence is reciprocal: our nutrition and genetics shape the composition of microbiota, and thus our own environments.

In humans, for example the FTO locus not only associates extremely robustly with BMI but also it is a unique example of a locus that associates with phenotypic variation in BMI. Interestingly, physical activity attenuates (byw30%) the influence of the FTO locus on body mass index (BMI). This study demonstrates that interactions between genes and lifestyle exists in human and importantly that a given genetic susceptibility is modifiable by lifestyle. (Abe & Bonini, 2013; Anway et al., 2005; Baillie-Hamilton, 2002; Carone et al., 2010; Daxinger & Whitelaw, 2012; Dolinoy et al., 2010; Ebert & Sharp, 2012; Timothy M. Frayling et al., 2007a; Gapp et al., 2014; Herbert et al., 2006; Houle et al., 2010; Iafrate et al., 2004; Kappil et al., 2016; Manolio et al., 2009; Montgomery et al., 2013; Morgan et al., 1999; Panzeri & Pospisilik, 2018, 2018; Scuteri et al., 2007a).

Epigenetics explains gene-environment interactions

Until the 1950s, the word epigenetics was for regulated processes that, beginning with the genetic material, shape the final product (Waddington, 2014)

More modern definitions of epigenetics reflect the understanding that although the complement of DNA is essentially the same in all of an organism's somatic cells, patterns of gene expression differ greatly among different cell types, and these patterns can be clonally inherited. This has led to a working definition of epigenetics as "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence". (Riggs & Porter, 1996) Epigenetics, therefore is a bridge between genotype and phenotype and paved a way for a new understanding of the interaction between environment (such as nutrition or social environment) and genetics after the human genome projected focused the scientific interest on mostly genetic progress (A. Haslberger, 2009).

Environment has a critical role in the natural selection process for Darwinian evolution. The primary molecular component currently considered for neo-Darwinian evolution involves genetic alterations and random mutations that generate the phenotypic variation required for natural selection to act. The vast majority of environmental factors cannot directly alter DNA sequence. Epigenetic mechanisms directly regulate genetic processes and can be dramatically altered by environmental factors. Therefore, environmental epigenetics provides a molecular mechanism to directly alter phenotypic variation generationally. Lamarck proposed in 1802 the concept that environment can directly alter phenotype in a heritable manner. Environmental epigenetics and epigenetic transgenerational inheritance provide molecular mechanisms for this process. Therefore, environment can on a molecular level influence the phenotypic variation directly. The ability of environmental epigenetics to alter phenotypic and genotypic variation directly can significantly impact natural selection. Neo-Lamarckian concept can so facilitate neo-Darwinian evolution (A. Haslberger, 2009).

Epigenetic influences on traits may come for hereditary as well as environmental effect. Since the 1980s we have known that 'imprinted' loci can escape the erasure of DNA-methylation control that occurs during gametogenesis and early embryogenesis, enabling robust parent-of-origin patterns of

gene expression during development and across generations. More recently, ERVs and less-well characterized ‘ metastable epialleles’ have been demonstrated to escape reprogramming. These demonstrations provided molecular proof for the existence of at times robust, but non-Mendelian mechanisms that underpin gene expression effects and thus phenotype. Paternal diet can affect histone H3K27me3 status in sperm, demonstrating that sperm histone modifications may be sensitive to paternal nutritional cues. Maternal behaviour during the early suckling period can influence offspring robustness through to adult life, likely through sustained alteration in DNA methylation and histone acetylation at the glucocorticoid receptor (GR) promoter. (Carone et al., 2010),(Dolinoy et al., 2010)(Dolinoy et al., 2010)¸(Weaver et al., 2004). Maternal supplementation with antioxidants reduces this oxidative stress and prevents adiposity in rats fed with Western diet. Epigenetic modifications are clearly key mediators in these processes as essentially all known epigenetic modifiers rely on intermediary metabolites such as S-adenosyl-methionine (SAM), (Champagne, 2010) Acetyl-Coenzyme-A (CoA),a-ketoglutarate, and NADH to exert their function. Impaired glucose metabolism in women during pregnancy is associated with altered DNA methylation at the leptin genes in both maternal and feto-placental compartments, with increased risk of developing adult obesity and type 2 diabetes in the offspring. Changes in DNA methylation and histone acetylation in the promoter region ofPdx1, a transcription factor critical for b cell function and development, are associated with reduced pancreatic b cell mass and pre-diabetic state following intrauterine growth restriction (IUGR) in rats. (Bouchard et al., 2010; Henikoff & Matzke, 1997; Kaelin & McKnight, 2013).

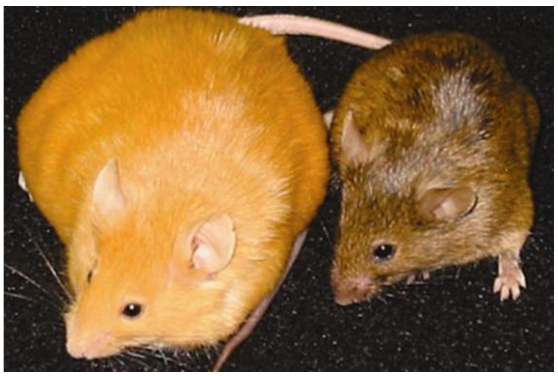
The epigenetic players are key factors in modulating the expression of genes in response to the environment. Position Effect Variegation (PEV) systems in yeast and Drosophila have proven to be elegant systems not only to screen for novel epigenetic regulators but also to highlight the molecular pathways that underpin interactions between environment and chromatin control. PEV was first described in Drosophila upon observation that an X-ray induced mutation. A large number of different PEV rearrangements have been described, generated by ethane methyl sulphonate (EMS), X-ray treatment (Elgin & Reuter, 2013).

Many toxins have since then be shown to trigger pathological epigenetic changes such as bisphenols (Alavian-Ghavanini et al., 2018). Epigenetics has also been shown to play a decisive role in the neuronal adaptations underlying learning and memory, the response to environmental challenges. Mechanisms implicated in stress-related epigenetic regulation were genes involved in the stress- or hypothalamus-pituitary-adrenal (HPA) axis due to its prominent role in the pathophysiology of stress related disorders (Klengel & Binder, 2015). Many nutraceuticals have been shown to exert their beneficial activity in prevention and intervention of complex diseases via epigenetic modifications (Carlos-Reyes et al., 2019)(Marlene Remely et al., 2015).

Case study: The Agouti mouse model and epigenetic influences of nutrition

Back in 2000, Randy Jirtle, a professor of radiation oncology at Duke University, and his postdoctoral student Robert Waterland designed a ground-breaking genetic experiment that was simplicity itself. They started with pairs of fat yellow mice known to scientists as agouti mice, so called because they carry a particular gene—the agouti gene—that in addition to making the rodents ravenous and yellow renders them prone to cancer and diabetes. Jirtle and Waterland set about to see if they

could change the unfortunate genetic legacy of these little creatures. Typically, when agouti mice breed, most of the offspring are identical to the parents: just as yellow, fat as pincushions, and susceptible to life-shortening disease. The parent mice in Jirtle and Waterland's experiment, however, produced a majority of offspring that looked altogether different. These young mice were slender and mousy brown. Moreover, they did not display their parents' susceptibility to cancer and diabetes and lived to a spry old age. The effects of the agouti gene had been virtually erased. Remarkably, the researchers effected this transformation without altering a single letter of the mouse's DNA. Their approach instead was radically straightforward—they changed the moms' diet. Starting just before conception, Jirtle and Waterland fed a test group of mother mice a diet rich in methyl donors, small chemical clusters that can attach to a gene and turn it off. These molecules are common in the environment and are found in many foods, including onions, garlic, beets, and in the food-supplements often given to pregnant women. After being consumed by the mothers, the methyl donors worked their way into the developing embryos' chromosomes and onto the critical agouti gene. The mothers passed along the agouti gene to their children intact, but thanks to their methyl-rich pregnancy diet, they had added to the gene a chemical switch that dimmed the gene's deleterious effects. "It was a little eerie and a little scary to see how something as subtle as a nutritional change in the pregnant mother rat could have such a dramatic impact on the gene expression of the baby," Jirtle says. "The results showed how important epigenetic changes could be." Our DNA—specifically the 25,000 genes identified by the Human Genome Project—is now widely regarded as the instruction book for the human body. But genes themselves need instructions for what to do, and where and when to do it (E., n.d.).



Recently, the *Agouty mouse* model was also utilized to evaluate the effects of maternal exposure to the endocrine active chemical bisphenol A (BPA) on the fetal epigenome. BPA is a high-production volume chemical used in the manufacture of polycarbonate plastic and epoxy resins, and it is present in many commonly used items including food and beverage containers, baby bottles, and dental sealants. To evaluate the effects of maternal BPA exposure on the fetal epigenome, female agouty mice received phytoestrogen-free AIN-93G diet or phytoestrogen-free AIN-93G combined with 50 mg BPA/kg diet 2 weeks prior to mating and throughout gestation and lactation. Maternal dietary BPA did not significantly influence litter size, litter survival, wean weight, genotypic ratio, or sex ratio (data not shown). In contrast, maternal dietary BPA exposure shifted the coat colour of A^{vy}/a offspring toward yellow and decreased methylation and nine CpG sites within the A^{vy} IAP. CpG methylation was also decreased at the $Cabp^{IAP}$ metastable locus, indicating that BPA-induced hypomethylation is not gene-locus specific, and may also impact as yet unidentified epigenetically labile genes in the mouse and, potentially, the human genome. Moreover, the BPA-induced hypomethylation of the fetal epigenome was abolished by maternal dietary nutritional supplementation with either methyl donors (folic acid, betaine, vitamin B_{12}, and choline) or the phytoestrogen genistein. These findings demonstrate that simple dietary changes can protect against the deleterious effects of environmental toxicants on the fetal epigenome (Dolinoy, 2008).

Markers enable a personalised pre- and intervention

Biomarkers are a central tool to assess biological changes due to age, disease or treatment in a patient and thus play a crucial role for personalised health prevention. Biomarkers started to be developed in areas like cancer and neurological diseases. Experiences in these areas were rapidly translated to other areas, especially metabolic diseases, and personalised nutrition

There are various biomarkers addressing the different levels of carcinogenesis: starting with genetic markers to altered gene expression, protein status and the metabolic level. Genetic mutation markers are mostly relevant in hereditary diseases whereas epigenetics has a fundamental function in both inherited and sporadic diseases. Compared to other, epigenetic biomarkers can be advantageous as it provides information on the patient's genetic and environmental background. Although the focus of personalised medicine is on genomic research, the exposome, all non-genetic internal and external influences that are determining a person's health, plays a crucial role in disease development. Epigenetic studies are one way to investigate both the genetic background and the environmental exposures (Goettler et al., 2016). The main benefits of biomarkers are that they are non-invasive (blood samples) and have the potential to offer early personalised screening for disease.

Molecular **genetic markers** represent one of the most powerful tools for the analysis of genomes and enable the association of heritable traits with underlying genomic variation. Availability of a wide array of molecular genetic markers offers tools for quick detection and characterization of genetic variation. Two forms of DNA sequence-based markers, single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs), predominate in modern genetic analysis. The most studied molecular genetic markers, SNPs, are distributed over the whole genome. The number of SNPs is estimated to range from 0.5 to 1 SNP per 100 base pairs (bp). A large number of genes associated with various cancer types, other complex diseases or metabolic regulation contain single nucleotide polymorphisms (SNPs). SNPs are located in gene promoters, exons, introns as well as 5'- and 3'-untranslated regions (UTRs) and affect gene expression by different mechanisms. These mechanisms depend on the role of the genetic elements in which the individual SNPs are located (Deng et al., 2017).

Besides SNPs, there are other important classes of genetic variants frequently used as molecular genetic markers, such as VNTRs (variable number of tandem repeats, a polymorphic sequence containing 20–50 copies of 6–100 bp repeats), STRs (short tandem repeats, also known as SSRs or microsatellites, a subclass of VNTR in which a repeat unit consists of only 2–7 nucleotides) and CNP (copy number polymorphisms, variation in the number of copies (CNV) of a DNA sequence in the >1 kb size range, which are common and widely distributed in the human genome). Platforms for DNA analyses include DNA microarrays, genotyping arrays, SNP arrays, Next-generation sequencing.

Epigenetic biomarkers reflect the mechanisms of epigenetics, CpG methylation, histone modification and non-coding RNAs.

DNA methylation mapping in cancer genomes shows that the vast majority of cancer types exist in hundreds of genes with high or low methylation and the highest CpG island hypermethylation frequency takes place in gastric cancer number of tumorsuppressor genes acting in cell cycle, apoptosis, cell adhesion, and invasion are inactivated by hypermethylation such as CDH1 (cadherin 1) and MLH1 (mutL homolog 1). In the same way changes of CpG methylation is seen in aging and aging related complex diseases or mechanisms. (see chapter molecular clock). Nowadays epigenetic

methylation is analysed using NGS sequencing following bisulfide conversion or immunoprecipitation (Barros-Silva et al., 2018).

Marker of **histone modification** are of important interest mostly in cancer analysis. Histone modifications can be detected by chromatin-immunoprecipitation (ChIP) using antibodies for histone methylations e.g. at sites H3K9me3 and H4K20me3 and subsequent real-time polymerase chain reaction.

The various forms of **non-coding RNAs**, especially miRNAs, as well as lncRNAs have been used as epigenetic biomarkers in the area of complex diseases, especially cancer and neurological diseases, or for metabolic or aging related mechanisms. Analysis of sets of miRNAs is usually done using NGS platforms.

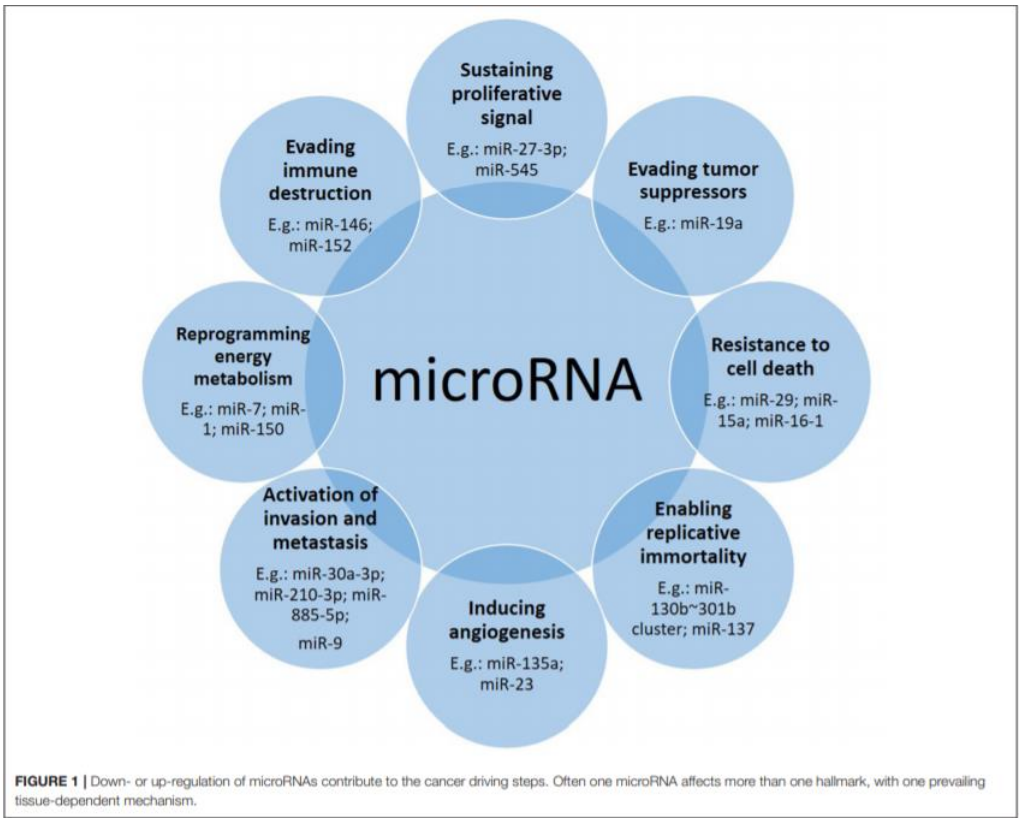


FIGURE 1 | Down- or up-regulation of microRNAs contribute to the cancer driving steps. Often one microRNA affects more than one hallmark, with one prevailing tissue-dependent mechanism.

Fig miRNAs in Cancer, (Detassis et al., 2017)

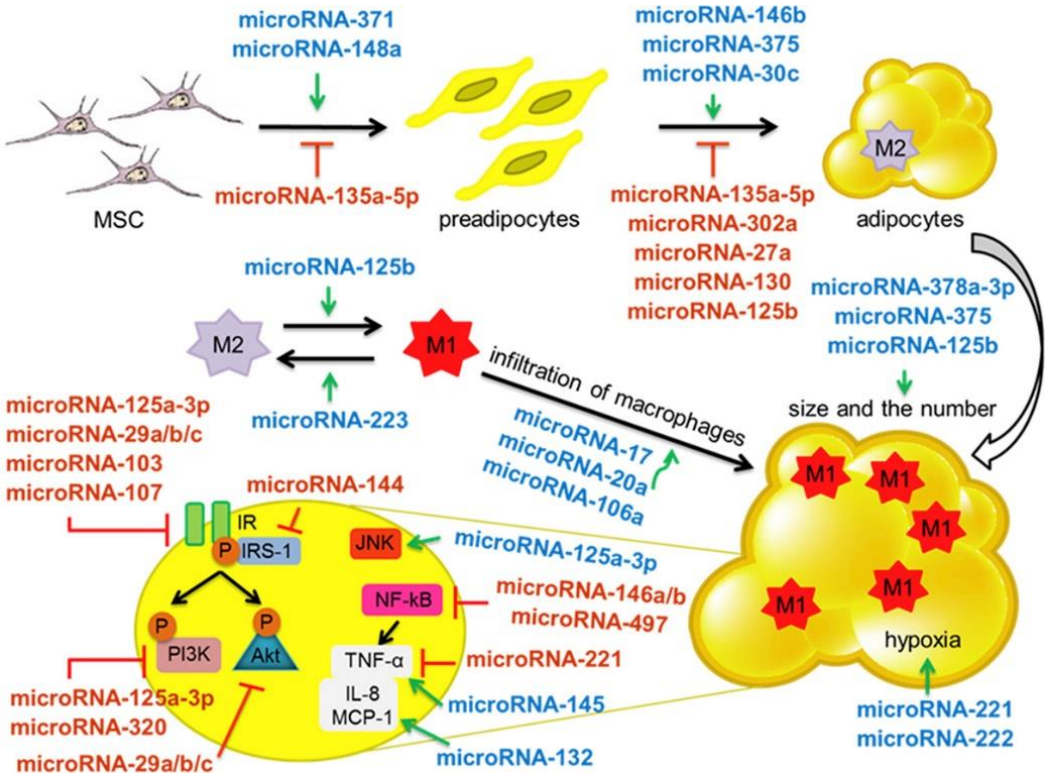
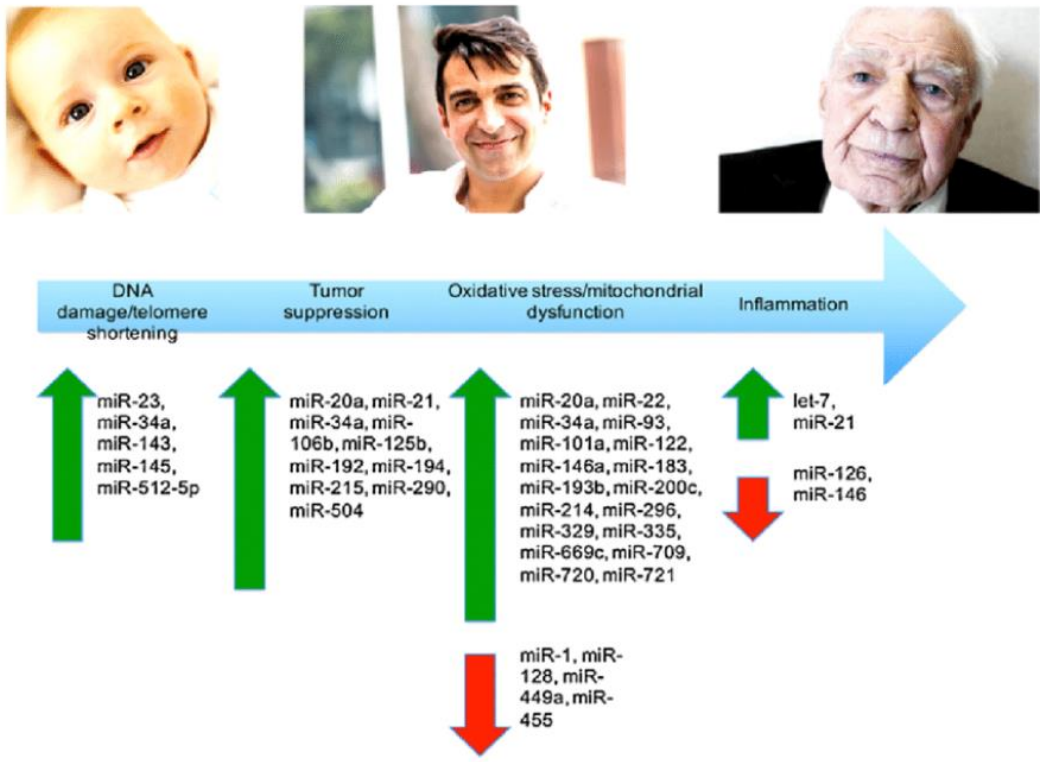


Fig MiRNAs in adipogenesis, chronic low-grade inflammation, and insulin resistance in obesity (Cruz et al., 2017)



The progression of aging at the cellular level can be seen as induction of a series of cellular events. As each event occurs, a corresponding miRNA profile can be seen. The above figure documents some of the miRNA signatures seen during each stage of senescence.

Fig: MiRNAs in aging, (Barros-Silva et al., 2018)

Recently **cell-free DNA** (cfDNA), often released after tissue destruction received a special importance as biomarkers in the area of early detection, staging and specification of cancer. (Cruz et al., 2017). But cfDNA is also developing as biomarker in areas of autoimmunity or metabolic pathogenesis. (Andreatta et al., 2018; Breitbach et al., 2012; Ferrandi et al., 2018; Haller et al., 2017; Vittori et al., 2019)

In the context of oncology, liquid biopsies consist of harvesting cancer biomarkers, such as circulating tumor cells (CTCs), tumor-derived cell-free DNA (ctDNA), and extracellular vesicles (EVs), from bodily fluids. These biomarkers provide a source of clinically actionable molecular information that can enable precision medicine. The use of liquid biopsies with CTCs, ctDNA, and EVs and the cargo they carry, as compared to solid-tumor biopsies, provides a minimally invasive method for early and longitudinal assessment of predictive and prognostic information related to the disease. Each biomarker offers different clinical opportunities, therefore potentially providing complementary information to allow for effective management of many oncological diseases. For example, analysis of ctDNA exclusively may not provide information on the heterogeneity of cancer cells; as mutation status may vary between cells in a tumor tissue, one may miss information as to what tumor cell population the ctDNA originated. Analysis of different phenotypes of CTCs garnered from the heterogeneous tumor microenvironment may provide more specific information related to mutation status originating from specific subpopulations, thereby informing proper chemotherapies including combination therapies (*i.e.*, precision medicine). (Campos et al., 2018)

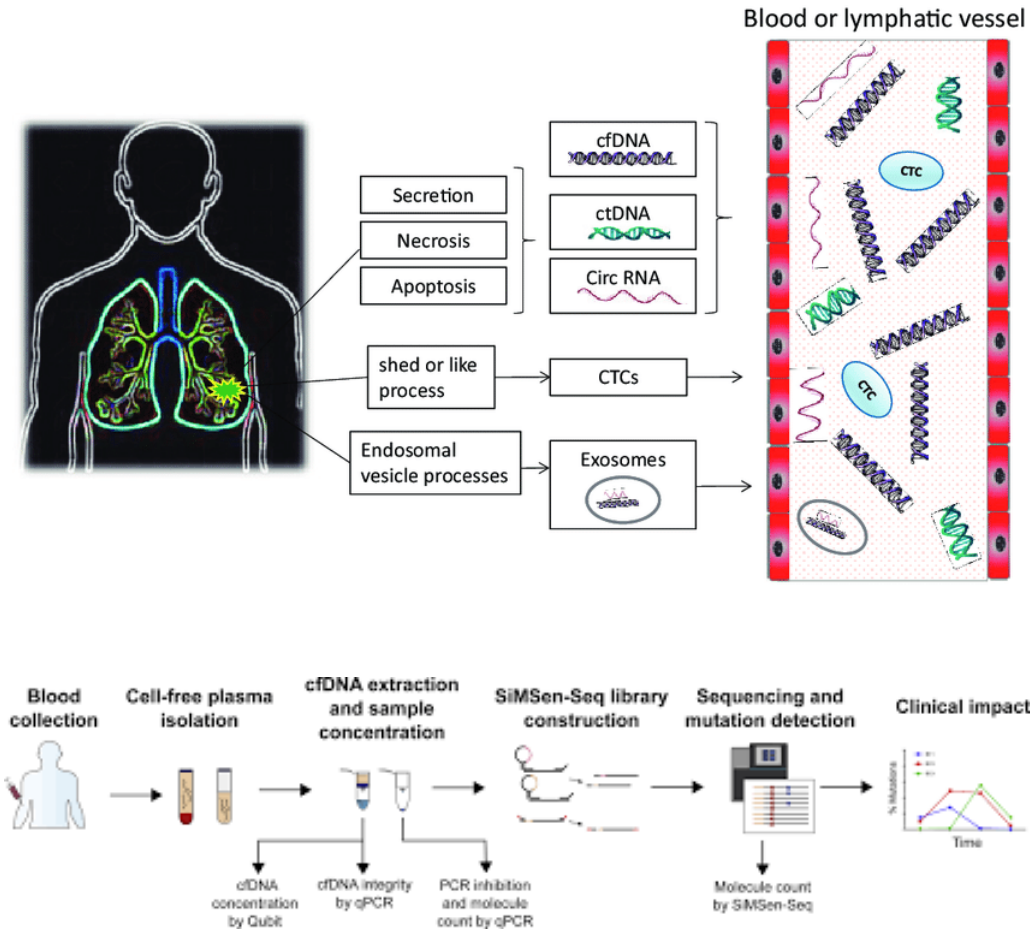


Fig The relevance of cell free DNA (cfDNA), (Johann et al., 2018; Johansson et al., 2019)

Metabolomic markers have gained a specific importance in the analysis of dietary patterns and cancer metabolism. Studies applied multivariate methods to identify panels of metabolites that discriminate between high and low scores within a pattern or between diet patterns. This is an important strategy because a panel of metabolites would be expected to best capture the multidimensionality of complex dietary patterns (McCullough et al., 2019).

In the cross-sectional KarMeN (Karlsruhe Metabolomics and Nutrition) study fasting blood and 24 h urine samples were analysed by mass spectrometry coupled to one- or comprehensive two-dimensional gas chromatography or liquid chromatography, and by nuclear magnetic resonance spectroscopy from 301 healthy men and women aged 18–80. This yielded in total more than 400 analytes in plasma and over 500 analytes in urine. Predictive modelling was applied on the metabolomics data set using different machine learning algorithms. Based on metabolite profiles from urine and plasma, it was possible to identify metabolite patterns which classify participants according to sex with > 90% accuracy. Plasma metabolites important for the correct classification included creatinine, branched-chain amino acids, and sarcosine. Prediction of age was also possible based on metabolite profiles for men and women, separately. Several metabolites important for this prediction could be identified including choline in plasma. The metabolite profile of human urine and plasma allows the prediction of sex and age with high accuracy, which means that sex and age are

associated with a discriminatory metabolite signature in healthy humans and therefore should always be considered in metabolomics studies (Rist et al., 2017).

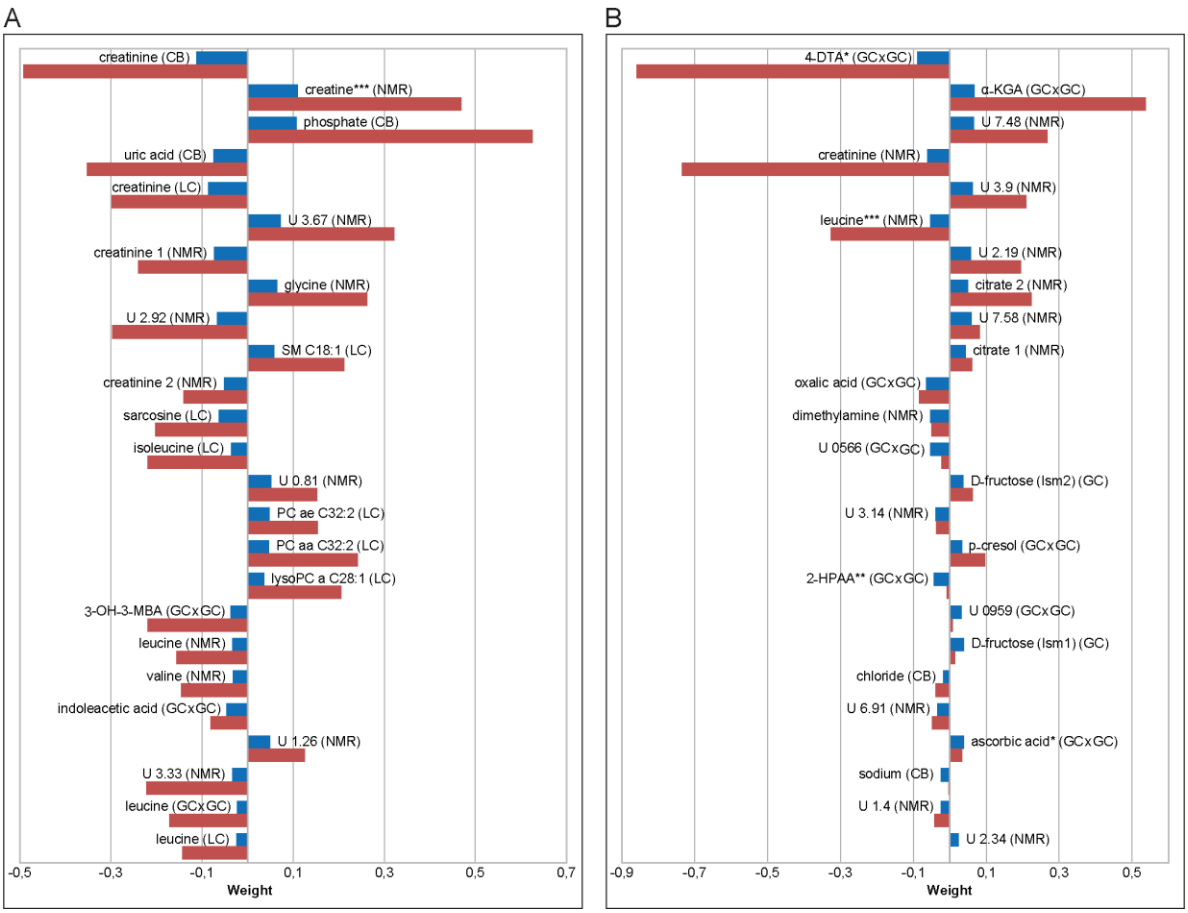


Fig: **Metabolite patterns for the prediction of sex.** Top 25 metabolites important for the correct prediction of sex of the KarMeN study participants in all algorithms applied on plasma (A) and 24 h urine (B) metabolite profiles (Rist et al., 2017).

Personalisation in drug development

The inevitable multiple phases of drug assessment from research and development (pre-human and clinical studies) to post-clinical evaluations have mandated the drug development process to be a slow-paced and over-priced procedure. The average capitalized cost for a new drug has been estimated at $2.5 billion in the R&D stage and a total of $2.8 billion post-approval. (DiMasi et al., 2016) Formulating new drugs for various disease categories such as cancer, orphan diseases and neurological disorders becomes even more challenging and time-consuming when difficulties such as drug resistance and incomplete understanding of disorder pathophysiology come into play. Reliability and predictability in the outcome of drug treatment following animal models, genetically modified animals or humanized mice are often poor.

Recently, organ-on-a-chip (OOC) technology has drawn attention to be used in drug development and clinical drug testing model by reflecting the genetic characteristics of cells in each patient. OOC is an engineered assembly of a controlled compartment to study, measure, and control cell behaviour

and response to various drug stimuli by replicating the cellular behaviour of the target tissue microenvironment. OOC has evolved from a combination of various engineering platforms such as microfluidic systems, engineered biomimetic tissues, and non-invasive monitoring system to address the difficulties of conventional drug testing models. Owing to recent advances in engineered biomaterials, it is now possible to design organoids with two-dimensional (2D) and three-dimensional (3D) scaffolds equipped with suitable extracellular matrix (ECM) to closely mimic human cell adhesion, migration, differentiation, and function *in vitro* system. Many types of human and animal stem cells have been used to generate organoids for the OOCs. Especially, using human-induced pluripotent stem cells (hiPSCs), which are obtained from patient's skin tissue or be directly harvested as pathogenic cells from patients, can be used to engineer personalised tissue constructs or disease models. Therefore, hiPSC-integrated OOCs provide a useful tool to establish personalised drug testing platforms that can mimic human physiology tuned for specific patient groups and individuals (Jodat et al., 2019).

Personalised Precision Medicine

Hippocrates already emphasized the importance of individualizing medical care, proclaiming ''It is more important to know what sort of person has a disease than to know what sort of disease a person has'. From this point of view, personalised medicine seems to have always been within the scope of medical practice and research (Abettan, 2016; Dörr et al., 2015; FISCHER, 2016; Fischer et al., 2015; Langanke et al., 2015; Meyer zu Schwabedissen, 2015; Michl, 2015b, 2015a; Ott & Fischer, 2015; Shara Yurkiewicz, 2010).

The idea of personalised medicine was first introduced by Roger William in the 1950s. It became more attainable only in the early 2000s when the human genome was mapped, and scientists could study the subtle individual genomic differences.

Recent genetic knowledges allow to anticipate drug response, enable more targeted therapeutic and can help us to choose more beneficial treatment in particular cases, whereas conventional medicine can only develop blockbusters. The hope is that prescriptions could be tailored to an individual's genotype and that this more accurate prescription will replace the current ''one-size-fits-all'' paradigm of drug development and usage. By early identifying those who will not benefit from particular treatments because of their genotype, it could enable to drastically reduce adverse drug reactions.

For example, Abacavir, a drug used by HIV patients, is relatively safe by nearly all patients but can cause severe and life-threatening allergic reactions by 6 percent of patients who are hypersensitive. In 2004, researchers correlated this toxic reaction with a single genetic variant. Currently, HIV patients have to be screened for this particular variant before the drug is prescribed. (Chaponda & Pirmohamed, 2011)

Molecular insight into disease mechanisms, the practice of health care is moving from reactive to preventive. Whereas traditional medicine most of the time can only offer a delayed intervention on an already existing disease, genetic testing would enable to predict the risk of developing some diseases, which can help the patient to prevent the occurrence of this illness by taking appropriate preventive actions. This shift from a reactive disease-treatment oriented medicine towards the proactive approach of preventive medicine would be radically new.

Of course, recent genomic successes entail a lot of changes, enabling new possibilities that raise new questions and new ethical challenges. First, by enabling to predict the risk of developing a particular disease, genetic testing lead to make recommendations about lifestyles allowing to counterbalance the genetically increased risk. This situation leads to increase the patient's own responsibility. For example, if a genetic test helps to identify an increased risk of developing a coronary heart disease, high risk people would probably be expected to adopt certain measures that are known to prevent the development of this disease, such as diet, exercising, etc. Knowing their susceptibility, if these people do not comply with the prevention recommendations, they are liable to be more considered as responsible for their disease. Thus, ''the discourse on personalizing medicine encourages the mentality of facilitating individual responsibility for health'' (Árnason, 2012).

Personalised medicine is also associated with an increased risk of discrimination. The possibility of dividing up the population into two groups (good vs. non responders) may lead to increase inequalities in access to health care. This would be particularly concerning in insurance-based healthcare systems and might result in bad or non-responders paying greater premiums. This could lead to call into question the principles of fairness, solidarity, and justice on which many healthcare systems are based. This issue becomes extremely worrying where stratification in probability of drug response coincides with ethnic categories. It could further increase inequalities already faced by some socially disadvantaged groups. (J. A. Johnson, 2008; Kalow, 2004; Leonard M. Fleck, 2010).

The extended possibility to acquire many disease- specific predictive information could lead to create a new class of individuals: the ''pre-patients'' or ''potential patients''. This would challenge the standard concept of disease, since health would not be opposed to illness, but to the risk of illness, which would be the new concern of medical practice. It could also transform the nature of the doctor-patient relationship: ''the gaze of the doctor [could] be directed more towards genetic factors than other factors relevant to the patient's condition'' (Árnason, 2012). Furthermore, increased power of prediction could lead to more directive kinds of relationship between doctor and patient in order to impose preventive strategies on the patient.

Personalised precision medicine, cancer

Variety of targets for therapy: Personalization in complex diseases such as cancer includes a 20years history of targeted therapies (Bedard et al., 2020). In recent years, a much deeper understanding of the mechanisms underlying evolution and progression of various neoplasms (cancer) has been achieved. As a result, novel targeted therapies that specifically interfere with these pro-oncogenic machineries have been developed. The underlying processes and molecular pathways, summarized by Hanahan and Weinberg, are now known as the hallmarks and major drivers of cancer evolution (Bauer et al., 2018). There are two main approaches for targeted cancer therapy: antibodies and small molecules. Antibodies are typically characterised by high selectivity; however, their targets are often restricted to the cell surface and they require intravenous or subcutaneous dosing because of their large molecular weight. By comparison, small molecule inhibitors vary in selectivity, and by virtue of their small size, can potentially bind a wider range of extracellular and intracellular targets. To date, there are 43 small molecule inhibitors approved by the US Food and Drug Administration (FDA) for oncology indications. Although most of these approvals were based on the prolonged survival of patients with advanced cancer that were refractory to conventional chemotherapy, many of these drugs show superiority over cytotoxic chemotherapy, with fewer side-effects as first-line therapy in the recurrent or metastatic setting. There are also examples of small

molecule inhibitors that are approved as treatment for minimal residual disease or as adjuvant therapy delivered with curative intent. Most approved small molecule inhibitors target intracellular kinases that regulate cell signalling through the transfer of phosphate groups to target proteins. A broad range of targets are currently being investigated, including those involved in protein–protein interactions, cancer metabolism, and immune modulation. However, not all selective small molecule inhibitors require individualised patient selection. The figure illustrates the complexity and multiplicity of targets for personalised therapy.

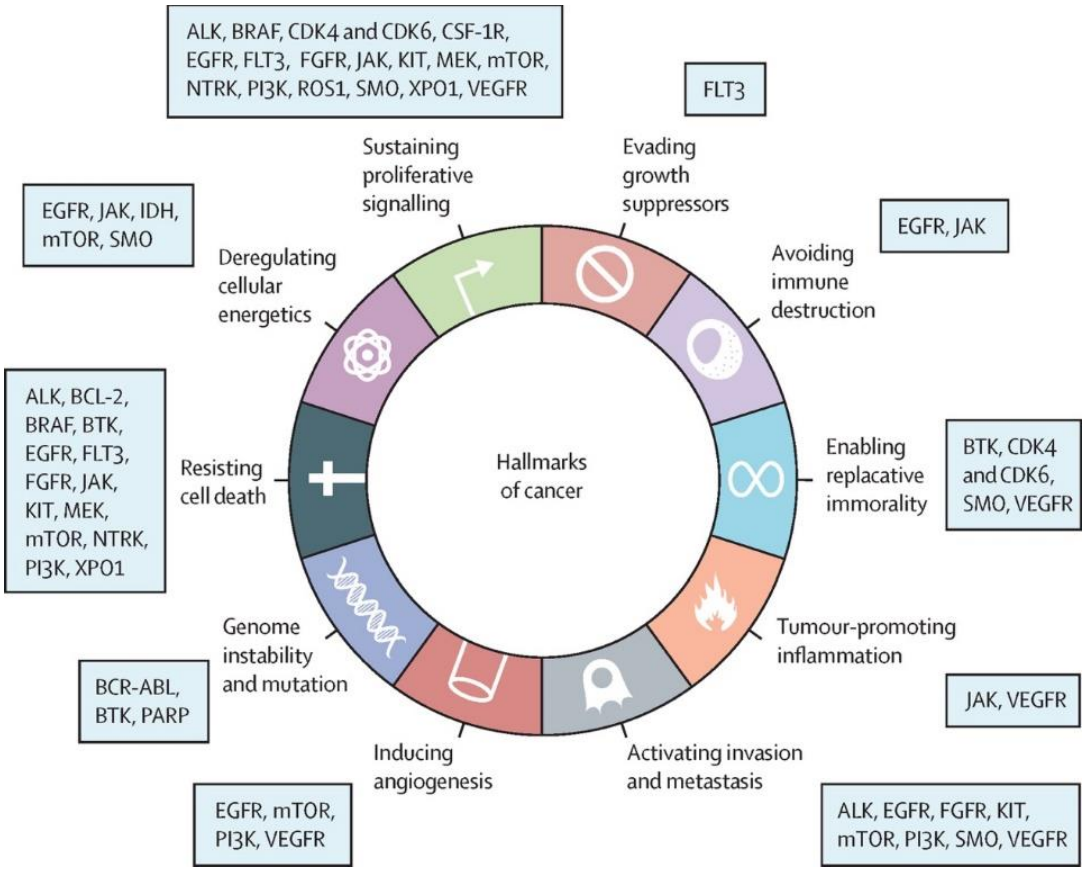


Figure: Personalised therapy in cancer, targets of approved small molecule inhibitors . Targets are mapped to the hallmarks of cancer as annotated by the Cancer Gene Census on the COSMIC website. The hallmarks of cancer are currently not annotated for CSF-1R, IDH, MEK, NTRK, PARP, and SMO in the Cancer Gene Census. These annotations have been added (Bedard et al., 2020)

To give an example, almost all cutaneous basal-cell carcinomas are characterised by the presence of genomic alternations that activate the Hedgehog signaling pathway, which can be irreversibly blocked by SMO inhibitors. Inhibition of the transmembrane protein Smoothened (SMO) prevents the induction of GLI transcriptional activity upon exposure of cancer cells to Shh ligands. Loss of induction of GLIs upon activation of Shh signaling inhibits the ability of Shh signaling to promote tumor progression and cancer stem cell maintenance. Therefore, SMO has been a primary target in the development of Hedgehog pathway inhibitors. Similarly, the activity of some of these inhibitors is dependent on specific signaling routes, even in the absence of genomic alterations within these

pathways. For example, the JAK1 and JAK2 inhibitor, ruxolitinib phosphate, improves survival in patients with myelofibrosis, regardless of the mutational status of the Janus kinase2 (*JAK2)* gene (Bedard et al., 2020).

Personalised precision medicine and markers: Personalized cancer therapy is a treatment strategy centered on the ability to predict which patients are more likely to respond to specific cancer therapies. This approach is founded upon the idea that tumor biomarkers are associated with patient prognosis and tumor response to therapy. In addition, patient genetic factors can be associated with drug metabolism, drug response and drug toxicity. Personalized tumor molecular profiles, tumor disease site and other patient characteristics are then potentially used for determining optimum individualized therapy options.

Tumor biomarkers can be DNA, RNA, protein and metabolomic profiles that predict therapy response. However, the most recent approach is the sequencing of tumor DNA, which can reveal genomic alterations that have implications for cancer treatment. This Personalized Cancer Therapy website was specifically developed as a tool for physicians and patients to assess potential therapy options based on specific tumor biomarkers.

Recent advances in precision medicine have resulted in national guidelines that require the implementation of precision approaches in clinical care. In cancer, precision medicine uses specific characteristics of patients and their tumors to develop tailored care plans and treatment regimens. Applications of precision medicine technologies include using blood-based protein markers to predict patient prognosis and monitor patients for recurrence, identifying patients who are at a high risk for cancer using germline genetic tests, and analysing molecular markers in tumor tissue to characterize patients' risk of recurrence and tumor susceptibility to specific targeted therapies. (Burnett-Hartman et al., 2019; Haslem et al., 2017; Kalia, 2013, 2015; Levit et al., 2019; Madhavan et al., 2018).

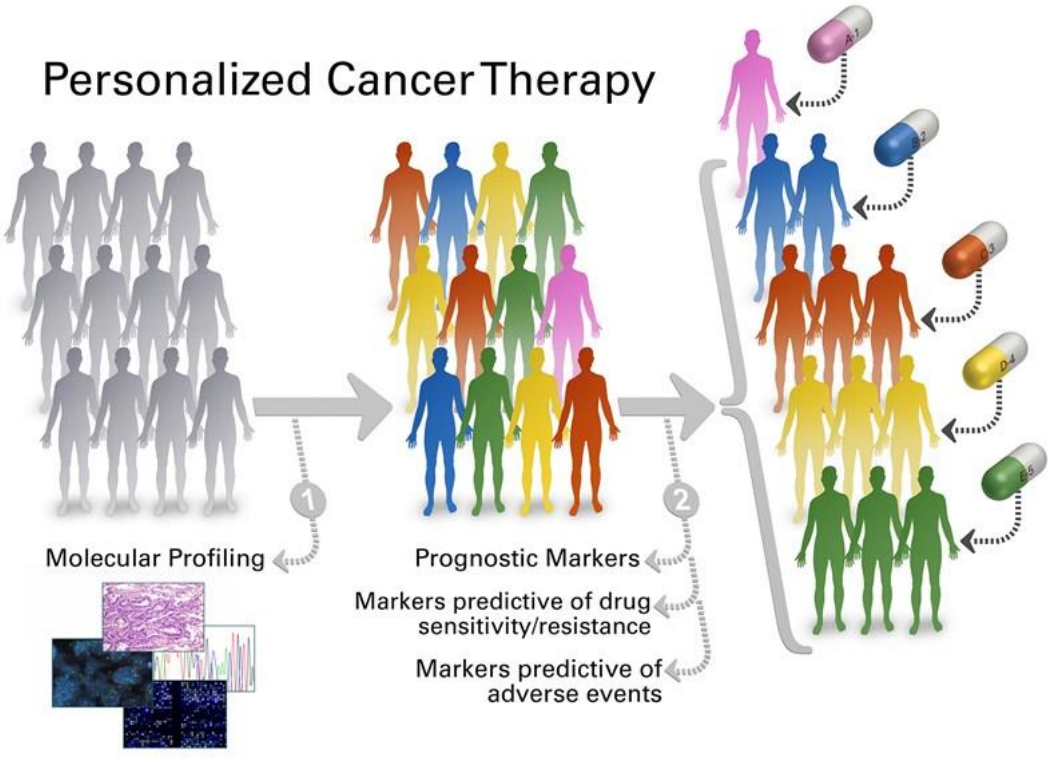


Fig personalised cancer therapy and markers.
http://www.novomics.com/eng/Research/precision.asp

For example, in breast cancer the benefit of a therapy with trastuzumab (Antibody against the extracellular domain of the growth factor receptor HER2, trade name Herceptin) combined with chemotherapy over chemotherapy alone as so remarkable for prolonging survival or reducing the likelihood of recurrence after surgery, that in 2013 the expert ASCO/CAP panel (and later the NCCN Breast Cancer Panel) expanded the 2007 definition of HER2-positive breast cancer to include patients who would now be identified as having "HER2-positive" disease based only on metrics such as identifying *HER2* gene amplification using "alternative probes" or using polysomy as a surrogate marker of HER2 positivity. https://www.ascopost.com/News/58977, (Dumitrescu, 2018).

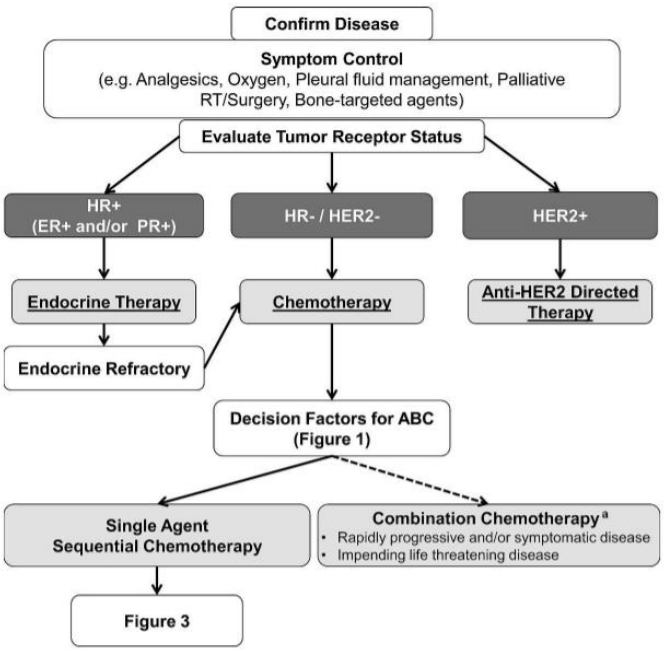


FIGURE 2 Approach to advanced breast cancer (ABC) treatment. RT = radiotherapy; HR = hormone receptor; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2. [a] Compared with single-agent chemotherapy, comes with an increased risk of treatment-related toxicity.

Fig Breast cancer and Her2. Treatments should be evaluated on an individualized basis in terms of evidence, targeted therapies for advanced disease, (Joy et al., 2015)

Epigenetic markers in precision medicine Conrad Waddington coined the term "epigenetics". In Waddington's developmental landscape, differentiating cells are "canalized" by specific environmental stimuli to follow different routes separated by mountain walls. The height of the walls increases during differentiation, symbolizing progressive loss of multi-potency and lineage restriction. Epigenetic and transcriptional regulators work in concert "adjusting the height of the walls" to restrict cells to a particular route. DNA methylation, histone modifications, chromatin remodelling, and microRNA—can be considered potential markers of cancer development and progression Endogenous and exogenous stimuli can deviate "the trajectory of cells," reorganizing the chromatin structure, and thus, leading to aberrant gene expression or repression, allowing them

to acquire the full set of so-called “cancer hallmarks”. The reversibility of these alterations by epigenetic therapies has far-reaching implications for clinical prevention and treatment. (Nebbioso et al., 2018)

The etiology of cancer is quite complicated and involves both environmental and hereditary influences. In cancer cells, the alteration of genomic information is usually detectable. Like genome instability and mutation, epigenome dysregulation is also pervasive in cancer. Some of the alterations determine cell function and are involved in oncogenic transformation. However, by reversing these mutations by drugs or gene therapy, the phenotype of cancer can revert to normal. The alteration of cellular methylation status by a specific methyltransferase might explain the differences in the probability of malignant transformation. In clinical settings totally different outcomes of therapies were noticed although cancer patients share the same staging and grade. In tumor tissues different tumor cells show various patterns of histone modification, genome-wide or in individual genes, indicating that epigenetic heterogeneity exists in tumors at a cellular level. Molecular biomarkers aree thought to be the method to explain observed different outcomes and to divide patients into different treatment groups (Cheng et al., 2019). There is also agreement that epigenetic , transcriptomic and metabolomic marker are needed in precision medicine. (Nebbioso et al., 2018) However, a number of promising epigenetic drugs directed against chromatin-regulatory molecules have recently been developed (Bauer et al., 2018).

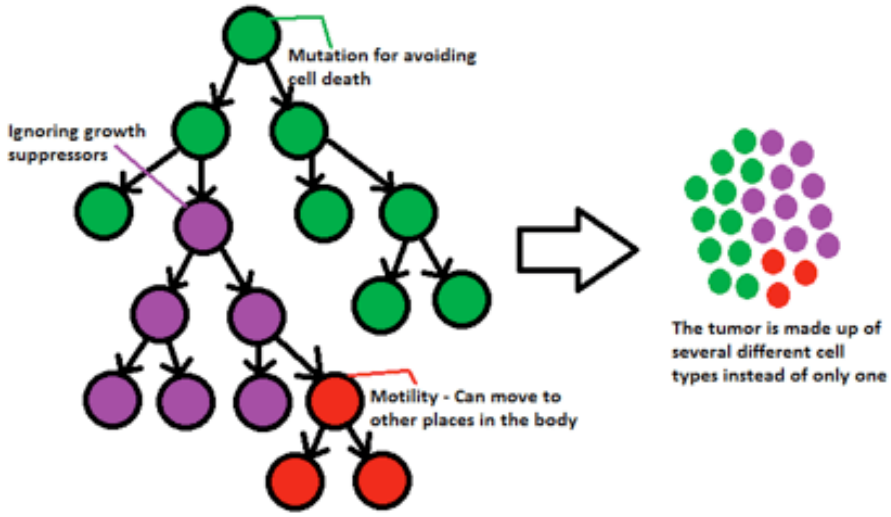


Fig A tumor is not one type of cell but many. The acquisition of mutations at different stages in the growth of the tumor allows for the existence of several subpopulations, which gives the tumor an advantage against drug treatments that only target a single type of mutation or epigenetic change http://sitn.hms.harvard.edu/flash/2012/issue118/

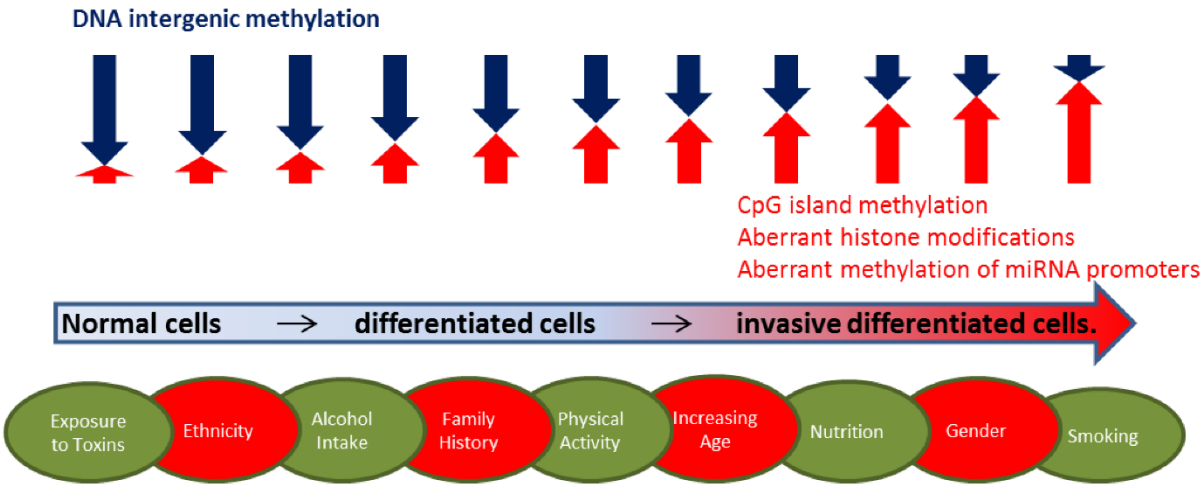


Fig epigenetic markers and epigenetic modifications that promote cancer risk and/or progression, and the modifiable (in green ovals) and non-modifiable (in red ovals) factors that may influence these epigenetic modifications. (Bishop & Ferguson, 2015)

Integration of markers; Integration of markers from different OMICS will further improve personalised medicine

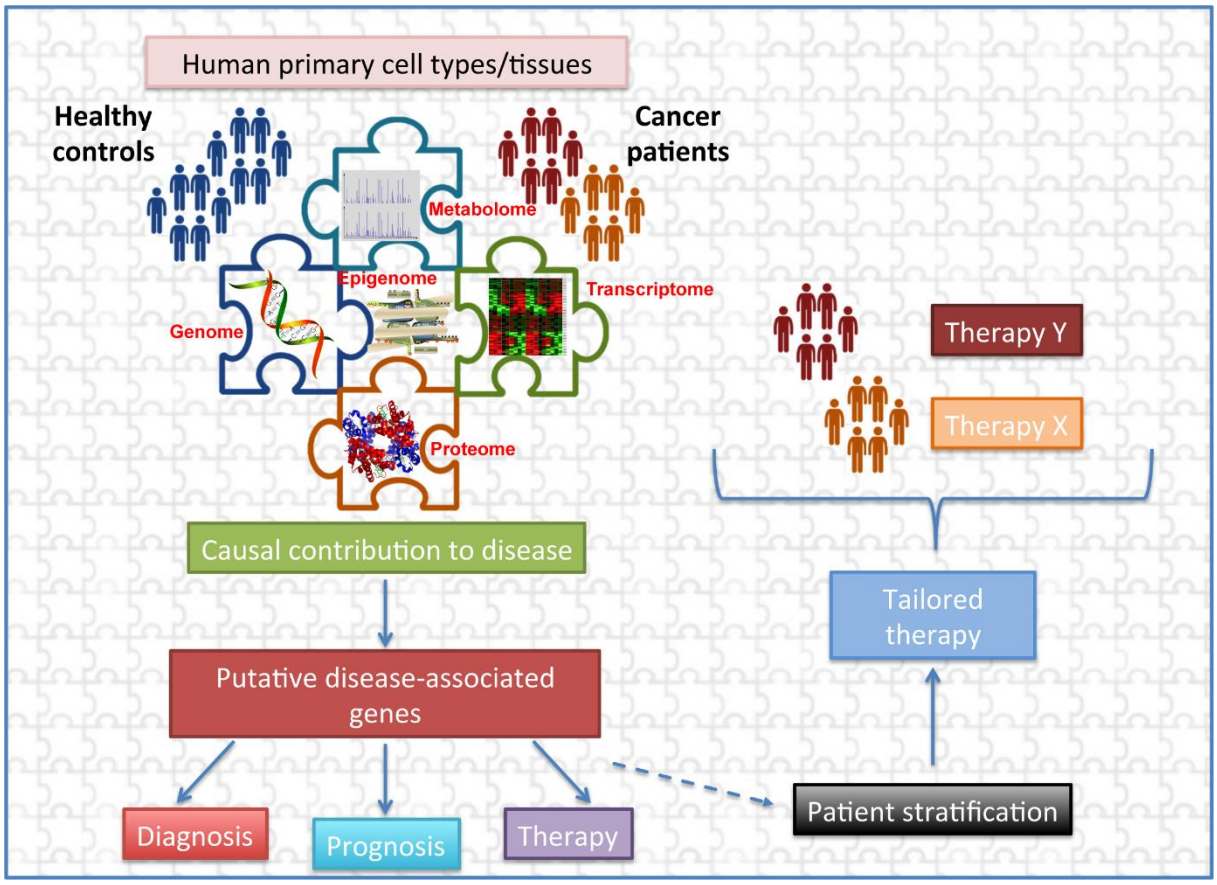


Fig Integrating and combining data from different platforms (genome-DNA sequence, transcriptome, proteome, metabolome, and epigenome) leads to a better understanding of the basis of cancer and paves the way toward personalized medicine. (Nebbioso et al., 2018)

Personalised Precision Nutrition

Lagging somewhat behind the development of precision medicine, precision nutrition follows similar concepts and wants to improve present serious metabolic problems. With nutrition-related diseases such as diabetes mellitus type II and obesity on the rise, it is for many experts unclear if the current efforts made to combat these diseases are effective or will be effective in the future.

Worldwide 40% of the adult population are overweight and numbers are rising. The obesity epidemic is not only affecting rich countries anymore but low- and middle-income countries as well. The problem has become global. With the burden of obesity comes a higher risk for noncommunicable diseases (NCD) such as diabetes, cardiovascular disease, and some cancers ("WHO Obesity," 2014). National and international efforts to reverse this trend have not been effective enough. Many countries have implemented nutritional guidelines to help prevent disease and provide optimal nutrition for individuals. The US has had many different forms of nutritional guidelines in the past decades, the most recent one being the "My plate" food-based dietary guideline. A systematic review overlooking the adherence and knowledge of the US nutrition guidelines since 1992 found low adherence to the guidelines and that knowledge did not lead to action (Haack & Byker, 2014). It is alarming that that decades of research, education and policymaking have not been able to decipher the problem of nutrition-related diseases. This may indicate that novel approaches are needed to solve this epidemic.

Dietary guidelines are an extremely important instrument, but even a very recent overview "A **Global Review of Food-Based Dietary Guidelines**"(Herforth et al., 2019) addresses the problem of important regional disparities. E.g. "Meat, particularly red meat, is treated differently across countries. Although a few countries describe meat as nonsubstitutable, 23% recommend limits on meat intake, most commonly in Europe. These recommendations seem to contrast, but meat consumption can have different nutritional and environmental consequences depending on the type of meat and level of intake, and countries' key messages might relate to where the majority of their populations stand with regard to typical levels of meat consumption".

Another problem of **dietary guidelines** is that they reflect the daily intake required to meet the **nutrient requirements** of 97,5% of the healthy population, meaning they are not geared towards specific individual needs or towards sick individuals. More so, these guidelines are based on statistical evidence of epidemiologic studies and not on interventional trials. Even though the European Food Safety Authority (EFSA) acknowledges that "the physiological requirement varies between individuals dependent upon genetic and epigenetic **differences**, age, sex [and] physiological state" it is presumed that nutrient requirements follow a normal distribution (EFSA, 2010).

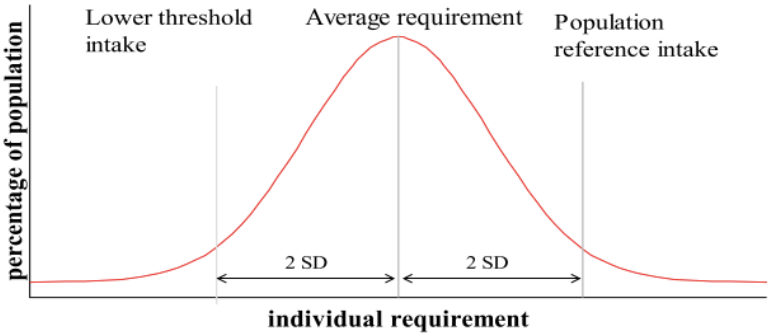


Fig. Normal distribution of nutrient requirements, (EFSA, 2010).

As the EFSA correctly states, the individual nutrient requirement is affected by genetic and epigenetic differences, as well as by the gut microbiota. These factors are not included in current nutritional guidelines as it is impossible to construct general guidelines that incorporate such personal data.

The field of **personalised preventative nutrition** tries to incorporate these factors to better advise patients on their nutritional needs (de Toro-Martín et al., 2017) (Ordovas et al., 2018b)

Personalised precision nutrition is based on the idea that individualising nutritional advice, products, or services will be more effective than more generic approaches. Personalisation can be based on:

• Biological evidence of differential responses to foods/nutrients dependent on genotypic or phenotypic characteristics

• Analysis of current behaviour, preferences, barriers, and objectives and subsequent delivery of interventions, which motivate and enable each person to make appropriate changes to his or her eating pattern (Ordovas et al., 2018a)

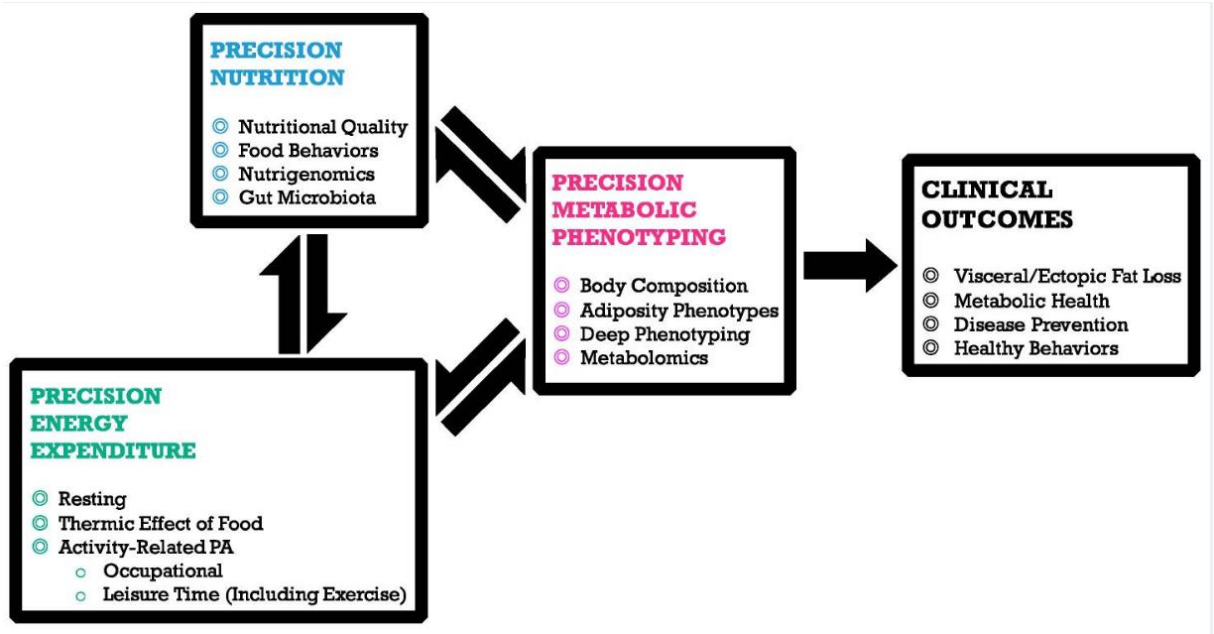


Fig Precision nutrition features and their relationships. PA: physical activity, (de Toro-Martín et al.,

Nutrigenetics, Nutrigenomis, Nutriepigenetics, Nutriepigenomic

The scientific literature does not always differentiate between the term genetic, genomic, epigenetic, epigenomic as we as nutrigenetic, nutrigenomic or nutriepigenetics and nutriepigenomics in a harmonised way. E.g. often literature includes both genetic and epigenetics under nutrigenomics.

The **main difference** between genomics and genetics is that genetics scrutinizes the functioning and composition of the single gene whereas genomics addresses all genes and their interrelationships in order to identify their combined influence on the growth and development of the organism. Epigenomics refers to global analyses of **epigenetic** changes across the entire **genome**.

Nutrigenetics term was used first time by Dr R.O Brennan in 1975 in his book Nutrigenetics (Simopoulos, 2010). Nutrigenetics points to understanding how the genetic background of an individual impact to the diet (Mutch et al., 2005).

Nutrigenomics aims to identify the effects of several nutrients, including macronutrients and micronutrients on the genome (Mutch et al., 2005) and explores the interaction between genes and nutrients or food bioactives and their effects on human health. The influence of nutrients on the transcription activity, gene expression, and heterogeneous response of gene variants is also referred to as “Nutrigenomics”. (Farhud & Zarif Yeganeh, 2010)

Nutrigenomics also describes the use of functional genomic tools to study a biological system to understanding of how nutritional molecules affect metabolic pathways and homeostatic control. This branch of science will reveal the optimal diet form within a series of nutritional changes, whereas Nutrigenetics will yield critically important information that assists clinicians in identifying the optimal diet for a given individual, i.e. personalised nutrition. Transcriptomics, proteomics, and metabolomics are also technologies that apply in Nutrigenomics research (Farhud & Zarif Yeganeh, 2010).

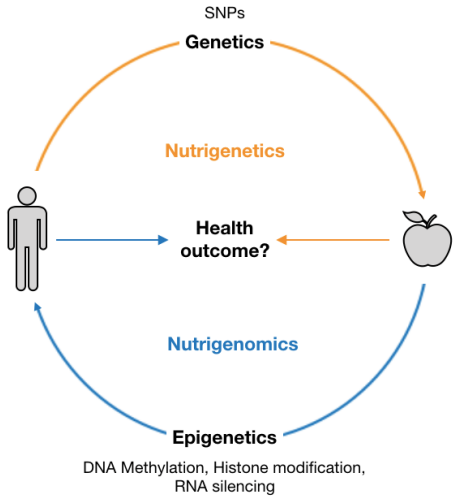


Fig. Influence of genetic and epigenetic mechanisms on health outcomes, (Farhud & Zarif Yeganeh, 2010).

Personal Nutrition and genetic mutations, SNPs

All human genomes are the same except for few mutations that form our individual DNA. Different nucleotide sequences even within a family determine for example the difference in eye color between siblings. If such variations in the nucleotide sequence occur in less than one percent of the population it is referred to as a mutation if they occur in more than one percent their considered a polymorphism.

Polymorphisms are not directly responsible for certain diseases, but they may increase the risk of developing one as they can for example have a negative effect on one's metabolism. Single nucleotide polymorphisms (SNPs) are polymorphisms where only one nucleotide in the gene is altered. Many specific SNPs are gaining relevance in the scientific community as they are connected to drug response, nutrient bioavailability, metabolism, and disease risk. If a SNPs is found in a gene encoding an enzyme that is vital for the metabolism of a nutrient, that SNP may promote or inhibit the enzymes activity and thereby alter the nutrient requirement (Ermini et al., 2014).

Since 2007 genome-wide association studies (GWASs) have identified hundreds of common genetic variants (usually single nucleotide polymorphisms (SNPs) associated with common diseases and traits. This information is catalogued online (see www.ebi.ac.uk/fgpt/gwas for an interactive diagram). To date, there are few examples where GWAS findings have translated into useful tests that can help individual patients.

GWASs typically involve the direct genotyping of several hundred thousand SNPs in hundreds to thousands of DNA samples using microarray technology. After stringent quality control procedures, each variant is analysed against the trait of interest. Typically, researchers will collaborate and combine data from studies with the same disease or trait available. As more studies have performed genome-wide genotyping GWASs using individuals of European ancestry have reached, for example, more than 250,000 individuals for height and BMI, 32,000 Europeans for type 2 diabetes,[3] and 190,000 individuals for lipid levels.- Similar GWAS efforts have been performed in other major ethnic groups

Typically, the frequency of a risk allele is >5%, meaning that more than 1 in 10 people will often carry the disease risk allele. As even common diseases typically affect only 1–5% of the population, the vast majority of risk allele carriers are unaffected and, because common diseases are polygenic, many affected individuals do not carry any risk allele. These common variant associations especially SNPs currently have limited relevance to clinical decision-making. There are few examples where GWAS findings have proven useful to individual patients. The vast majority of variants identified by GWASs are common and of subtle effect.

The risks (usually expressed as odds ratios) associated with common alleles are <2.0, and for continuous traits such as body mass index (BMI) usually <0.1 standard deviation (SD), e.g. the most strongly associated variants associated with type 2 diabetes (the variant in *TCF7L2*) and coronary heart disease (the variant near *CDKN2A*/*B*) confer risks, expressed as odds ratios of approximately 1.4 per risk allele. The variants most strongly associated with BMI and height do so with per allele effects of 0.1 SD (approximately 0.4 kg/m^2) and 1 cm respectively. Most of the variants confer much smaller effects than these examples. Many studies have combined information from multiple associated SNPs and shown that these explain more of the phenotype. However, even when combined, the variants rarely provided sufficient statistical power to offer any predictive value, e.g. when considering the sensitivity and specificity of information from common genetic variants, the 40 strongest type 2 diabetes variants have a receiver operator curve (ROC) area under the curve (AUC)

value of 0.63, where 0.5 is the same as flipping a coin and 0.8 is considered clinically useful. However, here are some common diseases where directly genotyping sets of common variants could be useful to individual patients (Timothy M. Frayling et al., 2007b)(Abraham et al., 2014; Allen, Estrada, et al., 2010; Allen, Johansson, et al., 2010; Cho et al., 2012; Church et al., 2010; Dina et al., 2007; T. M. Frayling, 2014; Timothy M. Frayling et al., 2007b; *Genome-wide association studies: the good, the bad and the ugly*, n.d.; Gerken et al., 2007; Han et al., 2014; Journals, 2008; Kilpeläinen et al., 2011; Kooner et al., 2011; Kramer et al., 2013; Langothe et al., 2008; McMurray et al., 2013; A. P. Morris et al., 2012; Mulligan et al., 2011; Owens et al., 2007; Pasquali et al., 2014; Rung et al., 2009; Samani et al., 2007; Scuteri et al., 2007b; Smemo et al., 2014; Speliotes et al., 2010; Teslovich et al., 2010; Weedon et al., 2007; Willer et al., 2013; Williams Amy et al., 2014).

SNPs also have importance in the development of personalised nutrition. They are the tool that is used to identify a patient's individual genetic need and disease risk and provide the basis to build individualized diets and therapies. Indeed, SNPs (or more generally speaking, SNV, single nucleotide variants, a term that comprises both common and low-frequency alleles) are by far the most widely studied genetic variation in the field of precision nutrition. In this sense, several SNPs have been associated with common chronic diseases through interactions with the intakes of macro and micronutrients, or with the consumption of particular foods and dietary patterns (Table 1). Examples include polymorphisms in genes related to taste perception including the sweet taste receptor (TAS1R2) and a cluster of differentiation 36 (CD36), which were associated with dyslipidemia in Mexican subjects consuming high amounts of carbohydrates and fats, respectively. Common variants in genes regulating homocysteine metabolism, such as methylenetetrahydrofolate reductase (MTHFR, and methionine synthase (MTR), have been linked to increased risk for breast cancer in individuals with low intakes of folate, vitamin B 6, and vitamin B 12. Also, it has been reported that in addition to sunlight, vitamin D status can also be influenced by several polymorphisms in vitamin D pathway genes, thereby modulating its biological functions in the organism. Interestingly, SNPs in the vitamin D receptor (VDR) gene, which affect vitamin D availability . have been associated with osteoporosis predisposition in postmenopausal women with low calcium intakes. Moreover, SNPs in genes encoding lipid proteins such as apolipoprotein C3 (APOC3) and apolipoprotein A1 (APOA1) conferred a higher risk of metabolic syndrome in subjects with a Western dietary patter. Likewise, a genetic variant in the cytochrome P450 family 1 subfamily A member 2 (CYP1A2) gene was associated with an increased risk of hypertension and CVD in moderate and heavy coffee drinkers. Additionally, studies using genetic risk scores (GRS) have examined the cumulative effect of SNPs on diet interactions and disease susceptibility (Ahn et al., 2010; Cornelis et al., 2006; Desmarchelier et al., 2016; Ferrari et al., 1998; Hosseini-Esfahani et al., 2014; Stathopoulou et al., 2011).

Nutrigenetic examples of SNPs-diet interactions involved in disease risk

Genes	Polymorphisms	Alleles	Diet interactions	Putative disease risks	Ref.
TAS1R2	rs35874116	G	High carbohydrate	Hypertriglyceridemia	[16]
CD36	rs1761667	A	High fat, SFA	Hypercholesterolemia	[17]
MTHFR	rs1801133	T	Low folate, vitamin B_6, and vitamin B_{12}	Breast cancer	[18]
MTR	rs1805087	G	Low folate, vitamin B_6, and vitamin B_{12}	Breast cancer	[18]
VDR	rs1544410	A	Low calcium	Osteoporosis	[22]
APOC3	rs5128	C	Western dietary pattern	Metabolic syndrome	[23]
APOA1	rs670, rs5069	A, T	Western dietary pattern	Metabolic syndrome	[24]
CYP1A2	rs762551	C	Moderate and heavy coffee drink	Hypertension, CVD	[25, 26]
FTO	rs9939609	T	Low adherence to Mediterranean diet	T2DM	[106]
MC4R	rs17782313	T	Low adherence to Mediterranean diet	T2DM	[106]
FTO	rs9939609	A	High fat	Obesity	[107, 108]
FTO	rs8050136	A	High carbohydrate	Obesity	[109]
MC4R	rs12970134	A	Western dietary pattern and high SFA	Metabolic syndrome	[110]
APOB	rs512535	G	High fat	Metabolic syndrome	[111]
TCF7L2	rs7903146	T	High dessert and milk	T2DM	[112]
TCF7L2	rs7903146	T	High SFA	Metabolic syndrome	[113]
LCT	rs4988235	T	High dairy products	Obesity	[114]
PPARG	rs1801282	G	High fat	Obesity	[115]
PNPLA3	rs739409	G	High carbohydrate	NAFLD	[116]
TXN	rs2301241	T	Low vitamin E	Abdominal obesity	[117]

MTHFR, methylenetetrahydrofolate reductase; *MTR*, methionine synthase; *FTO*, fat mass and obesity associated; *MC4R*, melanocortin 4 receptor; *APOC3*, apolipoprotein C3; *APOA1*, apolipoprotein A1; *APOB*, apolipoprotein B; *CD36*, cluster of differentiation 36; *TCF7L2*, transcription factor 7 like 2; *LCT*, lactase; *PPARG*, peroxisome proliferator activated receptor gamma; *PNPLA3*, patatin like phospholipase domain containing 3; *TAS1R2*, taste 1 receptor member 2; *VDR*, vitamin D receptor; *CYP1A2*, cytochrome P450 family 1 subfamily A member 2; *TXN*, thioredoxin; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; T2DM, type 2 diabetes mellitus; CVD, cardiovascular disease.

Table Nutrigenetic examples of SNPs-diet interactions involved in disease risk (Ramos-Lopez et al., 2017)

Certain nutrigenetic trials analyzing SNPs-diet interactions involved in the differential responses to nutritional interventions

Genes	Polymorphisms	Alleles	Diet interactions	Dietary responses	Ref.
FTO	rs1558902	A	High protein	Greater weight loss	[118]
FTO	rs1558902	A	Low fat	Less reductions in insulin and HOMA-IR	[119]
TCF7L2	rs7903146	T	High fat	Smaller weight loss and HOMA-IR	[120]
APOA5	rs964184	G	Low fat	Greater reduction in TC and LDL-c	[121]
GIPR	rs2287019	T	Low fat	Greater weight loss and greater decreases in glucose, insulin and HOMA-IR	[122]
CETP	rs3764261	C	High fat	Larger increases in HDL-c and decreases in triglycerides	[123]
DHCR7	rs12785878	T	High protein	Greater decreases in insulin and HOMA-IR	[124]
LIPC	rs2070895	A	Low fat	Higher decreases in TC and LDL-c and a lower increase in HDL-c	[125]
PPM1K	rs1440581	C	High fat	Less weight loss and smaller decreases in insulin and HOMA-IR	[126]
TFAP2B	rs987237	G	High protein	Higher weight regains	[127]
IRS1	rs2943641	C	High carbohydrate	Greater decreases in insulin, HOMA-IR and weight loss	[128]
PCSK7	rs236918	G	High carbohydrate	Higher decreases in insulin and HOMA-IR	[129]
MTNR1B	rs10830963	G	High protein	Lower weight loss in women	[130]
IL6	rs2069827	C	Mediterranean diet	Lower weight gains	[131]

FTO, fat mass and obesity associated; *TCF7L2*, transcription factor 7 like 2; *APOA5*, apolipoprotein A5; *GIPR*, gastric inhibitory polypeptide receptor; *CETP*, cholesteryl ester transfer protein; *DHCR7*, 7-dehydrocholesterol reductase; *LIPC*, lipase C, hepatic type; *PPM1K*, protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1K; *TFAP2B*, transcription factor AP-2 beta; *IRS1*, insulin receptor substrate 1; *PCSK7*, proprotein convertase subtilisin/kexin type 7; *MTNR1B*, melatonin receptor 1B; *IL6*, interleukin-6; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance.

Fig Polymorphisms and dietary consequences, (Ramos-Lopez et al., 2017)

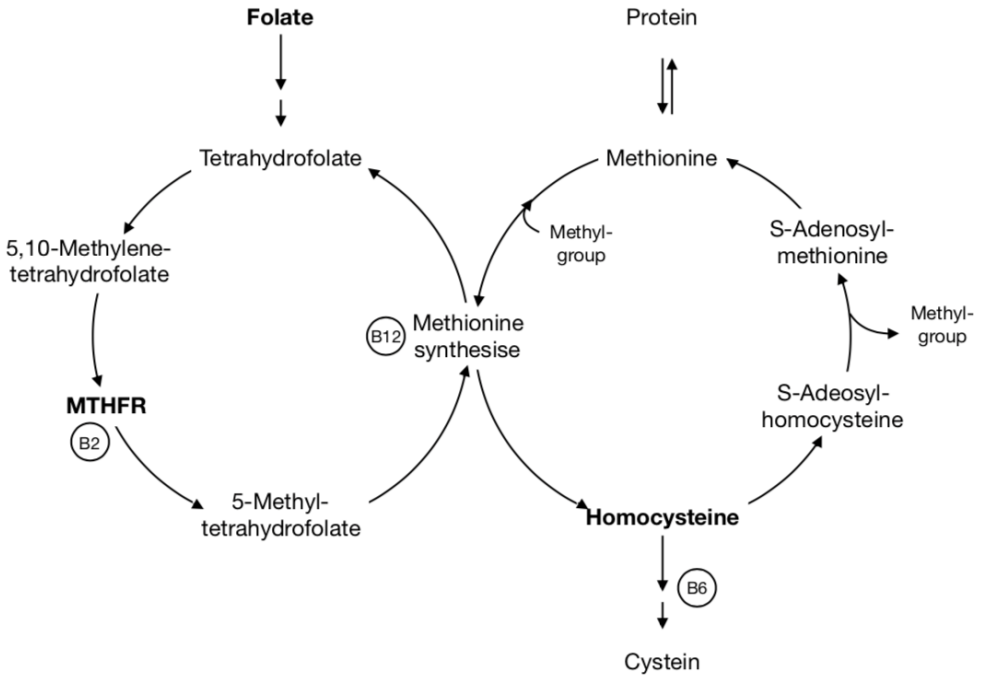
There has been some controversy on the effectiveness of SNPs to predict disease risk. Genome-wide association studies (GWAS) as the basis for the use of these SNPs indicate low odds ratios. (Hodge & Greenberg, 2017). Use of SNPs only for personalised nutrition have been also critically discussed because they are only based on correlation between gene and disease, and they don't include interactions between genes and environmental factors , and lastly they fail to explain their heritability (Prasad et al., 2016).

Case study The MTHFR C677T polymorphism

One of the most researched polymorphisms is the Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, which has been linked to cardiovascular disease, cancer, diabetes, and psoriasis.

The MTHFR enzyme is involved in the folate metabolism which plays an important role in DNA- and purine synthesis and methylation. Figure 4 shows the exact mechanism by which MTHFR converts 5,10-Methylenetetrahydrofolate (5,10-THF) to 5-Methyltetrahydrofolate (5-THF), which is then able

to donate a methyl group for the conversion of homocysteine to methionine via the methionine synthetase. Methionine is the precursor of S-Adenosyl-methionine (SAM) which is a crucial methyl donator for many reactions and also for the conversion of methionine back to homocysteine (Goyette et al., 1998).



One carbon pathway and methionine cycle

The MTHFR gene contains 15 mutations that are linked to a defect in enzyme activity: 14 rare mutations and one common mutation C677T. The MTHFR locus is mapped on chromosome 1, the common mutation is located on Exon 4 at position 677 where a thymine is built in instead of a cysteine. This results in a valine instead of an alanine being positioned within the final MTHFR enzyme. The polymorphism reduces the enzymes stability to heat, which results in a decreased activity of the enzyme at 37°C or higher. Such deficiencies in the function of the MTHFR enzyme result in higher homocysteine levels in individuals with homozygous mutations compared to individuals with no mutation. The higher homocysteine levels are due to the decreased conversion of 5,10-THF to 5-THF and thus less conversion of homocysteine to methionine, leaving homocysteine to accumulate (Rozen, 1997).

Studies have shown that individuals with a TT genotype in their MTHFR gene have higher homocysteine levels and lower plasma folic acid levels as individuals with a CC genotype. It has also been shown that the homocysteine concentration is inversely correlated with vitamin B12 and folic acid levels. Low vitamin B12 and folic acid levels have been linked to hyperhomocysteinaemia (high plasma homocysteine level). Supplementation with vitamin B12 and folic acid has been shown to reduce plasma homocysteine levels (Liew & Gupta, 2015).

Hyperhomocysteinaemia itself has been linked to cardiovascular-related diseases, although the mechanisms and the evidence is still unclear. Studies investigating the MTHFR C677T polymorphism, hyperhomocysteinemia and cardiovascular disease (CVD) have come to contradicting conclusions:

A meta-analysis by Kang et. al. suggested an increased risk in hemorrhagic stroke in individuals with the MTHFR C677T polymorphisms and concluded that the TT genotype may be and important risk factor for hemorrhagic stroke (Kang et al., 2013).

A mendelian randomization meta-analysis by Clarke et. al. showed that lifelong elevated plasma homocysteine (within the normal range) had no or little effect on coronary heart disease (CHD). The study also found no evidence of the TT genotype in the MTHFR C667 polymorphism increasing CHD risk compared to the CC genotype. The authors of the study indicate that the discrepancy to studies with positive outcomes may be due to publication bias, systematic errors, differences between ethnic groups and different genotyping methods (Clarke et al., 2012).

A cohort study published in the American Journal of Clinical Nutrition by Yang et. al. found an inverse association of MTHFR and CVD. The aim on the study was to examine the association between MTHFR and CVD in the US adult population by analyzing the NHANES III (1991-1994) Linked Mortality File until 2006. They confirmed that the MTHFR polymorphisms was associated with lower folate and higher serum homocysteine levels. A high folate concentration was associated with lower risk of all-cause mortality and CVD, whereas a higher homocysteine concentration was not. The authors also emphasized that the inverse correlation was only significant after the introduction of folic acid fortification in 1998. This indicates that adequate folic acid levels may be important in controlling homocysteine levels and CVD risk (Q. Yang et al., 2012).

An observational study conducted by Husemoen et. al. in Denmark confirmed higher homocysteine level in individuals with the MTHFR C677T polymorphism with low folate and vitamin B12 levels. In contrast to the previous study mentioned the authors found no association between the MTHFR polymorphism and all-cause mortality, stroke, or hypertension regardless of folic acid levels. This could suggest the unclear role of folic acid in regard to homocysteine concentrations and disease risk. In contrast to the US, Denmark has no mandatory folic acid fortification policy. The authors also found a positive association between the TT genotype and ischemic heart disease (IHD) regardless of vitamin B12 or folic acid status (Husemoen et al., 2014).

Contradicting results were also found in studies with different ethnic groups. Zhang et. al. concluded that the TT genotype of the MTHFR gene is associated with an increased risk of myocardial infarction in the Chinese population. (Wenbin Zhang et al., 2016) A meta-analysis by Lewis et. al. indicated no evidence for the association of the MTHFR C667T polymorphism and cardio vascular disease (CVD) in the European, North American, and Australian population. The authors suggested that this could be due to sufficient folate intake in these counties (Lewis et al., n.d.).

The MTHFR polymorphisms has been linked to other diseases such as psoriasis, neurological and psychiatric diseases, diabetes mellitus, cancer, and infertility. These associations will not be discussed in this paper (Liew & Gupta, 2015).

In summary, the evidence is not clear whether the MTHFR C667T polymorphism is associated with CVD. The studies all seem to conclude that the MTHFR gene is linked to higher homocysteine levels and lower folate levels. There are contradicting findings regarding the significance of folate. Studies have shown that folate can reduce homocysteine levels, but whether this influences disease risk is unclear. Another factor is ethnicity; studies have shown different outcomes for different ethnic groups or even within the same ethnic group. Such discrepancies make it difficult to reach a unified conclusion. Lastly, none of the studies mentioned possible mechanisms of how homocysteine may affect CVD but are solely based on statistical correlations. It is also to be said that most studies show a positive correlation are older and not meta-analysis.

What implications such associations could have for nutritional therapy is unclear. Studies indicate that the MTHFR polymorphism raises homocysteine level and that folate and vitamin B12 could lower homocysteine levels to potentially reduce the risk of certain diseases. The question remaining is whether individuals with this mutation need higher folate doses than the recommended amount?

A US dose-response trial done by Anderson et. al. concluded that there was no dose-response relation between folate supplementation and homocysteine levels in older, healthy adults. They indicated that higher folate supplementation does increase folate status, but in the age of mandatory folate fortification folate supplementation does not decrease homocysteine concentrations. Only adults with a low folate status may benefit from folate supplementation (C. A. M. Anderson et al., 2010).

This study did not differentiate if individuals where affected by the MTHFR C677T polymorphism, but it indicates that individuals with low folate levels (such as those effected by the MTHFR polymorphism) may benefit from folate supplementation.

An interventional study conducted on 932 northern Chinese women showed no improvements in reducing high homocysteine levels in individuals with the MTHFR TT genotype when folate supplements where given for 6 months. Folate supplementation raised plasma folate levels in the TT genotype, but even at a high-dose supplementation of 4000 µg folic acid per week the TT genotype still had significantly lower plasma folate and higher plasma homocysteine levels than the CC genotype. The study concluded that the TT genotype affected the response to folic acid supplementation at any folic acid dose (100 µg/d, 400 µg/d and 4000 µg/w). The authors also indicated that a 4000 µg/week supplementation does not have greater benefits than 400 µg/d. The study was conducted in a population that does not have mandatory folic acid food fortification (Crider et al., 2011).

These studies both conclude that folic acid supplementation increases plasma folate levels both in individuals with and without the TT genotype. Whether higher plasma folate levels help to decrease homocysteine concentrations is unclear. It may help individuals with a low folate status (with no TT genotype), but individuals with the MTHFR polymorphism may not redeem the benefits (even at high dose supplementation) due to their defect enzyme activity.

For nutritional therapy we can conclude: Even though the science is clear that MTHFR C677T individuals have lower folate and higher homocysteine levels, the question remains whether folate supplementation may help decrease homocysteine levels. Can folate (or a combination of folate, vitamin B12 and B6) supplementation help individuals with the TT genotype? Another open question is whether one should be worried about higher homocysteine levels, as they have been associated with various diseases, but the evidence does not seem conclusive (as we have seen for CVD).

It seems that there are many studies investigating the MTHFR polymorphism, folate, and different diseases, but each study only identifies one part of the whole picture. No study was found that looks at the MTHFR TT genotype in combination with folate supplementation and association to a certain disease. Differences in mandatory folate fortification in different countries and diverse ethnic groups also make the analysis more difficult.

Nevertheless, it is still important to know an individual's genetic footprint. MTHFR individuals are at risk of low plasma folate levels, which can cause birth defects such as (neural tube defects), developmental defects and anemia. It is therefore crucial that individuals keep track of their folate status (especially women) and try to reach optimal folate levels though nutrition or supplementation. Preventative personalised nutrition could thus play an important role in helping individuals establish

their personal/genetic dietary needs and possibly prevent disease through optimal nutritional status. It is important to keep in mind not only the genotype but also the phenotype of the patient. The MTHFR polymorphism only indicated a risk of low folate plasma concentrations and is not to be interpreted as a diagnosis. Laboratory testing is required to verify the effects of the MTHFR genotype.

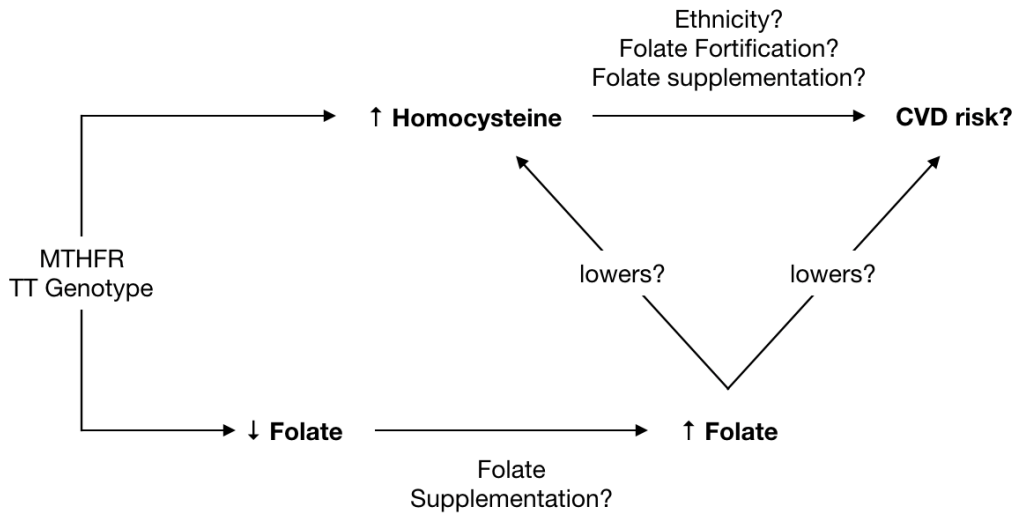


Fig. Summary of MTHFR polymorphism open questions

Case study The FTO polymorphism and obesity

Obesity is becoming a burden on public health as it raises the risk for other diseases, such as diabetes mellitus. Preventing obesity therefore has a high priority in health care.

The FTO (fat mass and obesity associated) gene has been associated with the body mass index (BMI) and is thought to predispose to obesity and diabetes. Individuals with the homozygous variant of the gene weighed 3kg more and had a 1.6-fold increased chance in developing obesity compared to non-homozygous individuals. A GWAS of Europeans found SNPs of the FTO gene to be linked to type 2 diabetes and BMI, but when adjusted for BMI the association with type 2 diabetes disappeared. This suggests that the SNPs directly affect BMI and indirectly raise the type 2 diabetes risk through higher BMI.

The FTO SNP rs9939609 located in the first intron on chromosome 16 occurs in 40% of the adult population. Studies showed that for each additional rs9939609 risk allele the BMI increased by 0.4 kg/m^2, increasing the odds of being overweight or obese (31% increased risk). Further research confirmed that the SNP is associated with increased waist circumference (~1cm) and subcutaneous fat (14% difference across the three genotype groups). This was not only observed on adults but the FTO SNP also affects children from the age of 7. These changes in BMI persist in puberty and beyond (Timothy M Frayling et al., 2009).

Even though the effect of the FTO gene may seem ignorable, scientists have persisted on identifying the exact biological mechanisms as the polymorphism affects nearly half of the European population. Further studies have pointed out increased energy intake, reduced satiety and possibly increased fat intake as mechanisms underlying the effects of the FTO gene (Tung & Yeo, 2011).

The role of FTO is still under examination. Mouse models suggest a potential role in nucleic acid repair and modification. Total lack of FTO in humans has been associated with growth retardation, brain deficiencies, cardiac abnormalities, and premature death.

Interesting results have also been found regarding FTOs food intake regulating effects. FTO is expressed amongst other tissues in the hypothalamus, a brain area which helps regulate energy homeostasis. Mouse models showed that food intake decreases when FTO is overexpressed and that food intake increases when FTO is knocked down. Fasting also decreased FTO expression, while high-fat diets increased FTO expression (Tung & Yeo, 2011),

More recent studies have suggested that the FTO gene executes its effect on body weight by modulating the expression of neighboring genes. Iroquois homeobox 3 (IRX3) and Retinitis pigmentosa GTPase regulator-interacting protein-1-like (RPGRIP1L) are neighboring genes that are functionally connected to the FTO gene (See fig. 6). The rs1421085 region of the FTO variant functions as an enhancer for the IRX3 gene. Overexpression of IRX3 has been associated with reduced browning capacity of white adipocytes. The RPGRIP1L gene is regulated by the rs8050136 FTO variant; it functions as a promotor for the RPGRIP1L gene. The gene is involved in leptin control in the hypothalamus. Mouse studies have shown that low RPGRIP1L expression reduced leptin signaling and thereby increased food intake and body weight.

These findings raise the question of whether FTOs obesity-inducing effects arise from the FTO protein itself or from its interactions with neighboring genes (J. Y. Chang et al., 2018).

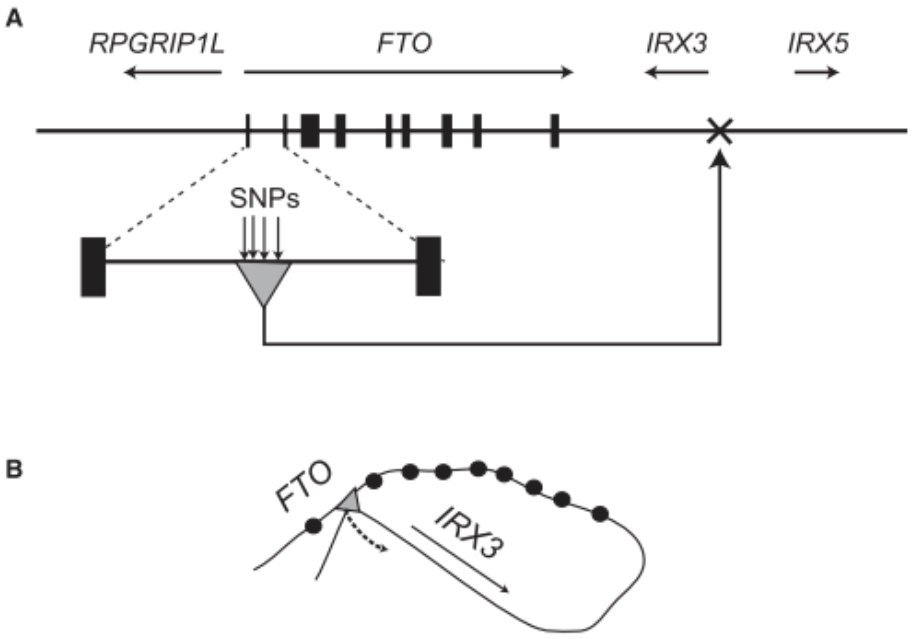


Fig. Gene regulation influenced by genetic variants at the FTO locus. (A) Genetic view at the FTO locus, including a putative enhancer covering single-nucleotide polymorphisms (SNPs) located in the first intron. (B) Possible mechanism for transcriptional regulation of homeobox genes IRX3 and IRX5, neighboring the FTO gene.

A randomized clinical trial investigated the influence of the rs9939609 FTO gene in combination with a Mediterranean diet on weight loss. Study participants were divided into a nutritional intervention group following a Mediterranean diet and a control group, body composition and weigh loss was compared after 4 weeks. The authors found that the Mediterranean diet had a positive effect on weight loss and multiple body composition parameters, while the FTO did not seem to influence weigh loss. FTO was observed to only influence total body water significantly. The A allele of the FTO gene is associated with higher BMI. This study confirmed that participants with the A allele lost less body weight compared to the TT genotype, but the results were not significant. Although the authors concluded that the FTO genotype did not influence the nutritional intervention outcome, they emphasized that understanding FTOs role in fat-loss interventions is crucial for developing better preventative strategies and difficulties during weight loss. Furthermore, more studies are needed to clarify the interaction between genetic variation and nutritional therapies (Di Renzo et al., 2018).

The FTO variant is a highly researched polymorphism in the nutrigenetic community. Much is known about its biological function in different tissues and there seems to be scientific consensus on its effects on body weight, but how relevant this effect is still unclear. Nevertheless, the interest in this gene have not only brought useful findings for the field of nutrigenetics but also for the general understanding of FTOs meaning in humans.

What consequences this has for personalised nutrition is unclear. Studies showed that the influence of FTO is marginal, increasing body weight by only 3kg on average. Interventional studies did not see benefits in weight loss interventions when knowing the FTO genotype. Nevertheless, it cannot be ignored that almost half of the population could potentially be affected by this polymorphism. More studies are needed to understand FTOs role in personalised nutrition and dietary interventions. A minor change does not mean its impact is insignificant.

Case study: discussions on nutritional SNP testing

Genetic testing, Update on Genetic Testing , *Is it ready for prime time to inform personalised nutrition advice? A recherche by* Jill Weisenberger, MS, RDN, CDE, CHWC, FAND, *Today's Dietitian*, Vol. 21, No. 5, P. 36, May 2019 Issue

Dietitians routinely encourage clients to make reasonable diet and lifestyle changes, and they base their recommendations on the client's current diet, health status, health goals, income, cultural and food preferences, health literacy, willingness to make changes, and more. Genetic testing may provide additional information on which to base personalised recommendations.

Research studies examining weight loss, insulin sensitivity, risk factors for CVD, and more demonstrate outcomes of various dietary interventions on average, but there is a wide variation in response. Some participants are responders to the intervention, and others are nonresponses. Why? Genetic differences may explain part of it. With the sequencing of the human genome in 2003, the idea that providing actionable lifestyle and nutrition guidance based on one's own genetic makeup looked to be a possibility.

When dietitians or other health care providers gather information about a client's family history of obesity or disease, they are glimpsing genetic data by inference. However, it is impossible to know the role of shared genetics vs the role of shared environment. Actual gene testing might uncover genetic variants that influence how dietary components are absorbed, metabolized, and used, and whether individuals are at risk of obesity or other health conditions.

Increasing interest in personalised health care and piqued curiosity about ancestry has led to a boom in direct-to-consumer (DTC) genetic testing, as well as nutrition-related genetic testing interpreted by dietitians. In recent years, improvements in testing technologies have significantly reduced the expense and turnaround time of genetic tests. "While it used to take months to genotype dozens of SNPs, millions of SNPs can now be genotyped rather quickly," says Scott Thompson, chief operating officer of Genetic Direction, a genetic testing provider in Dallas. He says the cost of genotyping has dropped industrywide and, in the last five years, the cost at Genetic Direction has decreased by more than 50%. Thus, testing is available to more people. According to the Centers for Disease Control and Prevention, millions of people have participated in DTC genetic testing to learn ancestry data, which frequently is coupled with health data.

Genetic testing companies offer a host of programs for weight loss, heart health, athletic performance, macronutrient, and micronutrient metabolism, and more. Consumers access genetic tests directly through the internet, weight loss programs, and dietitians.

How the Tests Are Done: Typically, the consumer puts a sample of saliva or cells from the inside of the cheek into a package provided by the testing company and mails it to the lab. In the lab, the DNA is extracted from the sample, isolated, and analysed to determine the consumer's genotype for a specific set of SNPs. Finally, the company interprets the SNP genotypes and generates a report, which goes directly to the consumer for self-interpretation, or the report may be sent to a dietitian or weight loss counsellor for discussion with the consumer.

The content of a report and actionable advice will vary by genetic testing company. One company may test 45 genetic markers, while another may test 50 or only 30 markers. In addition, each company may look at different markers for the same health trait. For example, one company may examine seven genes to assess how the body metabolizes fat, and another will look at more or fewer. This can lead to differences in the interpretation of a health trait and subsequent dietary recommendations. Even if two companies look at the same genes, their proprietary algorithms may weigh the genes differently and yield different recommendations.

Scientific advisors review the literature to determine which genetic markers to consider for specific traits and create algorithms when more than one marker is used to assess the same trait, e.g., the ability to lose and maintain weight. A variety of studies may provide these data. For example, large studies including the Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial have identified genes that play a role in weight loss, says Mark Sarzynski, PhD, FACSM, FAHA, an assistant professor at the Arnold School of Public Health at the University of South Carolina and the director of genomics research at Genetic Direction. In the POUNDS LOST study, 811 subjects with obesity or overweight were assigned to one of four heart-healthy eating plans with varying levels of macronutrients for two years. Though weight losses were similar among the various plans, later analysis suggested that individuals with a particular genotype dropped more pounds on a high-carbohydrate diet while those with a different genotype fared better on a high-protein diet. Some observational studies also have found significant gene-diet interactions. Though research is clear that a variety of eating plans facilitates weight loss, it's the goal of genetic testing companies to identify an optimal focus for each person and provide DNA-based diet and lifestyle advice in general.

Yet not all experts agree that the science the DNA testing companies provide is ready for widespread use. According to George P. Patrinos, PhD, a professor at the University of Patras department of pharmacy in Patras, Greece, and full member and national representative for the European Medicines Agency's CHMP Pharmacogenomics Working Party in Amsterdam, the Netherlands, there's insufficient evidence to support that diet can be individualized based on a panel of a few genomic variants. Patrinos and others conducted a meta-analysis of approximately 1,200 studies involving 38 gene variants offered by commercial nutrigenetic testing companies. They concluded that these 38 gene variants weren't associated with the occurrence of nutrient-related conditions such as obesity and diabetes.6 Genetic testing companies often exploit the public's lack of genetic literacy and provide them with expensive reports of no value, Patrinos says.

Timothy Caulfield, LLM, FRSC, FCAHS, Canada Research Chair in Health Law & Policy and a professor and faculty of law and of the School of Public Health at the University of Alberta, also finds the research surrounding nutrition-related genetic testing underwhelming. "For me, the data isn't definitive enough to justify the kind of marketing and pop culture noise we are seeing. Not even close," he says.

How Dietitians Use Genetic Testing: Experts agree that physicians should order tests to diagnose or treat a disease. Disease risk genes are ones that directly predict the likelihood of developing a disease. They're called high-penetrance genes if there's a very high likelihood of developing the condition just by having the genetic variant, explains Ahmed El-Sohemy, PhD, a professor and Canada Research Chair in Nutrigenomics at the University of Toronto and founder and chief scientific officer of Nutrigenomix Inc, a multinational genetic testing company with more than 8,000 practitioners in 35 countries. An example of a high-penetrance gene is BRCA1, which is a rare genetic variant with a very high likelihood of developing breast cancer. Fortunately, these types of genetic variants are not common, but they "require guidance from a medical doctor or genetic counsellor," he says.

Nutrigenomix, Genetic Direction, and many other companies typically test for and provide reports for low-penetrance genes, which are genes that suggest increased disease risk, or modifier genes. Modifier genes affect the metabolism or response to nutrients and various dietary factors such as caffeine, sodium, and gluten, El-Sohemy explains. For example, having a risk variant of the CYP1A2 gene does not influence the known risk of any condition by itself. “But having the risk variant means you’re inefficient at metabolizing or detoxifying caffeine. So being a slow metabolizer of CYP1A2 will increase your risk of heart disease if you drink more than two cups of coffee,” he adds. However, research linking coffee consumption to CVD risk based on genetic variants is mixed.7 Another modifier gene is the ACE gene, which directs the body to produce the angiotensin-converting enzyme. Individuals with a risk variant for the ACE gene are at risk of high blood pressure in the presence of a high-sodium diet.

Dietitians are implementing genetic testing in a variety of settings. Cindy H. Carroll, MS, RD, LDN, RN, IFNCP, of Bedford, Massachusetts, has used genetic testing in her private practice for about five years within a variety of clinical areas, including weight management. She frequently provides tests through PureGenomics. One interesting application is an SNP linking weight gain to saturated fat intake. “Not everyone is sensitive to saturated fat, in terms of their weight, but some people with this SNP may be,” she explains. High saturated fat intake may stall weight loss even when calorie intake is reduced, she adds.

According to Kristin Kirkpatrick, MS, RDN, consultant of wellness nutrition services at Cleveland Clinic Wellness Institute, many patients more fully embrace dietary changes based on the results of genetic testing. For example, among other changes, one client included more omega-3 fatty acids based on a gene related to triglycerides and more whole grains based on a gene associated with increased risk of type 2 diabetes.

Kassandra Gyimesi, RDN, owner of Personalised Nutrition Concierges, LLC, a virtual practice based in Denver, provides nutrition-related genetic testing through Nutrigenomix. She notes that clients want dietary guidance unique to them. After seeing the cardiometabolic results of her DNA report, one recent client with hypertension and no genetic family history to draw on was motivated to cut back her caffeine and sodium intake. Both the client and her doctor were ecstatic with the results, Gyimesi says.

Nanci Guest, PhD, RD, CSCS, who sits on the scientific advisory board of Nutrigenomix, uses genetic testing in her private practice in Toronto, Ontario. Guest and colleagues conducted a randomized controlled trial of more than 100 athletes. They found that both 2 mg/kg and 4 mg/kg caffeine improved 10-km cycling time, but only in those with a specific genotype of the CYP1A2. Increased caffeine had no effect in athletes with a different genotype and even hurt performance in those with a third genotype. “CYP1A2 genotype should be considered when deciding whether an athlete should use caffeine for enhancing endurance performance,” she explains.

Nutrisystem integrated an optional genetic testing component to their weight loss program last year. According to Courtney McCormick, MPH, RDN, LDN, manager of clinical research and nutrition at Nutrisystem, survey data showed that “3 out of 4 diet intenders believed the knowledge provided by genetic testing would increase their motivation for both dieting and exercise.” Nutrisystem, in partnership with Genetic Direction, developed a genetic-based report that includes data associated with weight management, eating behaviours, response to exercise, caffeine metabolism, and metabolism of specific micronutrients.

Best Practices for RDs: When it comes to interpreting genetic testing results to counsel clients, it’s important for dietitians to first be well trained, says Martin Kohlmeier, MD, PhD, a professor of

nutrition and director of the Human Research Core and Nutrigenetics Laboratory at the University of North Carolina (UNC) Nutrition Research Institute. Just as there are nuances in appropriately interpreting lab results and recommending treatment, there are careful distinctions that must be made with the review of genetic tests and the guidance that stems from them. So properly using genetic information cannot occur without additional effort and training, he adds. Both the UNC Nutrition Research Institute and the International Society of Nutrigenetics/Nutrigenomics offer training programs for health professionals. Dietitians should take the following guidance into consideration:

Research the genetic testing company. Before integrating nutrition-related genetic testing in their practices, dietitians should research genetic testing partners. Start by making sure the lab is either certified by CLIA (Clinical Laboratory Improvement Amendments) or accredited by the CAP (College of American Pathologists), Thompson says. And be sure that both the genes tested, and the research used to make assessments are publicly available in the report, he adds.

Avoid companies that attempt to diagnose clients. Research the credentials and experience of the company's scientific advisory board and review a sample report for thoroughness and ease of use. Some companies couple their reports with recommendations for their own brand of supplements, which some dietitians will find helpful, but others will view as a conflict of interest.

Interpret DNA tests cautiously. Mascha Davis, MPH, RDN, works with Pathway OME in her Los Angeles–based private practice. She emphasizes the importance of understanding that these tests are not diagnostic. They show predispositions only, and clients should understand that. Carroll points out that DNA testing must be used in conjunction with traditional assessment measures and clinical presentation. For example, a gene SNP predisposition to a nutrient deficiency should not lead to supplements without checking for an actual deficiency. But certain SNPs may help explain why some people need supplementation, she explains. Likewise, genetic tests should be combined with other proven strategies such as medications or calorie control, as indicated, Thompson adds. McCormick suggests using the DNA report as an opportunity to kick off the motivational interviewing process. Find out what areas the client wants to start with and plan small behavioural changes, she adds.

A client may bring in an unfamiliar report from an outside testing company. Recognize that some DTC testing companies have little science behind their reports, and it may be best to guide the client to other foundations for behaviour change. As an extreme example, one DNA report noted a possibility of a choline deficiency. The user was directed to consume at least 550 mg choline daily, which, according to the report, could be met by consuming three cups of raw lentils or three items from a fast food restaurant daily—recommendations dietitians would be unlikely to make.

Patrinos emphasizes that much of the nutrition and lifestyle advice generated from DNA testing is similar or identical to general health guidance, such as to consume more fruits, vegetables, and whole grains, and reduce sodium intake. No one needs to pay extra money to get this advice, he says. Caulfield says that "given that a big hunk of the population doesn't come close to meeting the basic nutrition recommendations, it seems absurd to focus on a high-tech niche technology backed by underwhelming science." In addition, he worries that taking an individualized approach to nutrition will shift the policy focus away from the type of population

Personalised nutrition and gene expression

Nutrition may exert an impact on health outcomes by directly affecting the expression of genes that regulate critical metabolic pathways (Fenech et al., 2011). To date, a large number of studies have evaluated the effect of different dietary factors on gene expression profiles, which are related to disease susceptibilities. With regard to dietary patterns, subjects following a Western dietary pattern, characterized by high intakes of refined grain products, desserts, sweets, and processed meats, showed a gene expression profile associated with inflammatory response and cancer signaling compared to those who consumed high amounts of vegetables, fruits, and whole-grain products (Bouchard-Mercier et al., 2013). Similarly, pathway analyses revealed that high meat consumption was associated with gene networks linked to cancer in colon tissue (Bouchard-Mercier et al., 2013). High-fat diets, especially rich in saturated fatty acids, have induced gene expression profiles related to inflammation, glucose intolerance, and liver lipid accumulation, as well as upregulation of neuropeptide expression involved in obesity development. On the other hand, low-protein diets enhanced hepatic gluconeogenic gene expression with subsequent glucose intolerance (Bouchard-Mercier et al., 2013).

Nutrigenomic examples of interactions between dietary intakes and gene expression profiles involved in disease risk

Dietary factors	Target genes	Expression changes	Putative disease risks	Ref.
Low protein	*NR1H3*	–	T2DM	[47]
Low protein	*HSD11B1, PCK1*	+	T2DM	[47]
Choline and folate deficiency	*PPARGA*	–	NAFLD	[48]
Chromium deficiency	Insulin signaling genes	–	T2DM	[49]
Selenium deficiency	*TLR2, ICAM1*	+	CVD	[50]
Vitamin B_{12} deficiency	*SREBF1, LDLR*	+	Dyslipidemia	[51]
Vitamin A deficiency	*GATA4*	–	CVD	[52]
High fat and high sugar	*LEP, SREBF1, PLIN*	+	Obesity	[65]
High fat	*OPRM1, PENK, DAT*	+	Obesity	[74]
Low protein	*CYP7A1*	–	Dyslipidemia	[75]
Selenium deficiency	*VHL*	–	Cancer	[79]
Vitamin D deficiency	*NFKBIA*	–	T2DM	[80]
High SFA	*TNFA, IL6*	+	CVD	[132]
High SFA	Proinflammatory "obesity-linked" genes	+	Obesity-related inflammation	[133]
High SFA	*PPARGC1A*	–	NAFLD	[134]
High SFA	*ADGRE1*	+	Obesity-related inflammation	[134]
High fat	*LEPR, NPY*	+	Obesity	[135]
High fat	*TH, DRD4*	+	Obesity	[136]
High fat rich in lard	*OPN, ADGRE1, TNFA, NFKB1*	+	Obesity-related inflammation and insulin resistance	[137]
High fat rich in lard	*OPN, TLR2, TLR4, TNFA*	+	Obesity-related inflammation and insulin resistance	[138]
High fat and high sugar	*DRD2*	–	Obesity	[139]
High fat and high sugar	*NPY*	+	Obesity	[140]
High fat and high sugar	*POMC*	–	Obesity	[140]
High carbohydrate	*FGF21*	+	NAFLD	[141]
Low folate and choline	Genes involved in cellular proliferation	+	Liver cancer	[142]
Western diet plus vitamin D deficiency	*TLR2, TLR4, TLR9, IL1B, IL4, IL6, RETN*	+	NAFLD	[143]
Choline and folate deficiency	*APOE, FOXA1, FOXA2*	–	NAFLD	[144]

SFA, saturated fatty acids; *TNFA*, tumor necrosis factor alpha; *IL6*, interleukin-6; *PPARGC1A*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; *ADGRE1*, adhesion G protein-coupled receptor E1; *LEPR*, leptin receptor; *NPY*, neuropeptide Y; *TH*, tyrosine hydroxylase; *DRD4*, dopamine receptor D4; *OPRM1*, opioid receptor, mu 1; *PENK*, preproenkephalin; *DAT*, dopamine transporter; *OPN*, osteopontin; *NFKB1*, nuclear factor kappa B subunit 1; *TLR2*, toll-like receptor 2; *TLR4*, toll-like receptor 4; *DRD2*, dopamine receptor D2; *POMC*, proopiomelanocortin; *LEP*, leptin; *SREBF1*, sterol regulatory element binding transcription factor 1; *PLIN*, perilipin; *FGF21*, fibroblast growth factor 21; *CYP7A1*, cytochrome P450 family 7 subfamily A member 1; *NR1H3*, nuclear receptor subfamily 1 group H member 3; *HSD11B1*, hydroxysteroid 11-beta dehydrogenase 1; *PCK1*, phosphoenolpyruvate carboxykinase 1; *TLR9*, toll-like receptor 9; *IL1B*, interleukin-1 beta; *IL4*, interleukin-4; *RETN*, resistin; *APOE*, apolipoprotein E; *FOXA1*, forkhead box A1; *FOXA2*, forkhead box A2; *PPARA*, peroxisome proliferator activated receptor alpha; *LDLR*, low-density lipoprotein receptor; *NFKBIA*, NFKB inhibitor alpha; *GATA4*, GATA binding protein 4; *ICAM1*, intercellular adhesion molecule 1; VHL, von Hippel-Lindau; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; NAFLD, nonalcoholic fatty liver disease.

Fig nutrigenomic examples, (Ramos-Lopez et al., 2017).

Experimental studies have shown the beneficial effects of nutrients and bioactive food compounds as a result of the regulation of critical gene expressions. In this sense, it has been reported that consuming a Mediterranean diet reduces the postprandial expression of genes that encode proteins related to inflammation, endoplasmic reticulum stress, atherogenesis, and oxidative stress (Camargo et al., 2012a, 2012b; Yubero-Serrano et al., 2013a, 2013b). Also, high intakes of monounsaturated fatty acids through the consumption of olive oil have been associated with a low expression of genes involved in inflammation and abnormal lipid storage (Camargo et al., 2012a)(Varela et al., 2013). Diets with a high content of polyunsaturated fatty acids favorably regulate the expression of neuropeptide genes involved in energy homeostasis (Dziedzic et al., 2007). Moreover, energy-restricted diets supplemented with eicosapentaenoic acid, and α-lipoic acid have been associated with the upregulation of fatty acid-oxidizing genes, as well as downregulation of lipogenic and proinflammatory genes (Huerta et al., 2017). In contrast, high-protein diets prevent and reverse NAFLD by modulating the expression of genes involved in liver lipid metabolism (Garcia-Caraballo et al., 2013; Garcia Caraballo et al., 2014). Concerning the effects of bioactive food compounds on gene expression, those most widely studied include green tea, theaflavin (black tea), sulforaphane (cruciferous vegetables), resveratrol (grapes and red wine), curcumin (turmeric), genistein (soy bean), and several apple polyphenols. Thus, epigallocatechin-3-gallate, theaflavin, curcumin, sulforaphane, and genistein may exert anticancer properties by upregulating tumor suppressor genes and conversely, downregulating tumor-promoting genes. In addition, curcumin and resveratrol have shown antiatherogenic effects by decreasing the expression of matrix metalloproteinases, which are involved in plaque formation and progression (Cao et al., 2014)(Z. Huang et al., 2008). Of note, apple polyphenols apparently prevented diet-induced obesity through the regulation of genes involved in adipogenesis, lipolysis, and fatty acid oxidation (Boqué et al., 2013).

Interestingly, gene expression profiles have also been used to predict the responsiveness to nutritional treatments. In this area, it has been reported that, prior to the consumption of a low-fat diet, adipose gene expression profiling was able to differentiate responders from non-responders, as well as serve as a weak predictor of subjects predisposed to lose weight (Mutch et al., 2011). Also, the analysis of gene expression in subcutaneous adipose tissue revealed that genes regulating fatty acid metabolism, citric acid cycle, oxidative phosphorylation, and apoptosis were differentially regulated during a low-calorie diet between weight maintainers and weight regainers after weight loss (Mutch et al., 2005)(Mutch et al., 2011) . Moreover, expression levels of pro-inflammatory genes were higher at the end of a low-calorie diet in subjects who after dietary-induced weight loss subsequently regained weight (Goyenechea et al., 2009). Differentially expressed genes in adipose tissue were also observed between successful and unsuccessful subjects after an energy restriction-induced weight loss program(Márquez-Quiñones et al., 2010). In this study, pathway analyses revealed that the main biological processes represented in adipose tissue from subjects who regained weight included cellular growth and proliferation, cell death, cellular function, and maintenance, whereas mitochondrial oxidative phosphorylation was the major network associated with continued weight loss.

Certain nutrigenomic studies assessing gene expression profiles associated with nutritional interventions

Nutritional interventions	Target genes	Expression changes	Potential health effects	Ref.
Mediterranean diet	*NFKB1, IKBKB, MMP9, IL1B, MAPK8, XBP1*	–	Anti-inflammatory, antiatherogenic	[53]
Mediterranean diet plus olive oil	*NFKB1, MMP9, TNFA*	–	Anti-inflammatory, antiatherogenic	[55]
Mediterranean diet	*NFE2L2, SOD1, SOD2, TXNRD1*	–	Anti-inflammatory, antioxidant	[54]
High MUFA	*APOBR*	–	Antilipidemic, antiatherogenic	[56]
Energy-restricted diet plus EPA	*IL10*	+	Anti-inflammatory	[58]
High PUFA	*POMC, GALP*	+	Antiobesity	[57]
High PUFA	*HCRT, MCH*	–	Antiobesity	[57]
Energy-restricted diet plus EPA and α-lipoic acid	Lipid catabolism genes	+	Antilipidemic	[59]
Energy-restricted diet plus EPA and α-lipoic acid	Lipid storage genes	–	Anti-lipidemic	[59]
High protein	*PPARGC1A, PCK1, GSTA, CPT1A*	+	Antisteatotic	[60, 61]
High protein	*FGF21, SCD1*	–	Antisteatotic	[60, 61]
Curcumin	*MMP-9, MMP-13, EMMPRIN*	–	Antiatherogenic, anticancer	[62, 63]
Resveratrol	*EMMPRIN*	–	Antiatherogenic	[64]
Apple polyphenols	*LEP, SREBF1, PLIN*	–	Antiobesity	[65]
Apple polyphenols	*PPARGC1A, AQP7, AEBP1*	+	Antiobesity	[65]
Flavonoid-fish oil supplement	Phagocytosis-related inflammatory genes	–	Anti-inflammatory	[145]
High n-3/n-6 PUFA ratio	*TLR4, TNFA, IL6, CRP*	–	Anti-inflammatory, antidiabetic	[146]
EGCG	*MMP9, MMP2*	–	Antitumorigenic	[147, 148]
Theaflavin	*MMP2*	–	Antitumorigenic	[149]
Resveratrol	*FASN*	–	Antisteatotic	[150]
Sulforaphane	*EGR1*	+	Anticancer	[151]
Genistein	*P21, P16*	+	Anticancer	[152]
Genistein	*BMI1, c-MYC*	–	Anticancer	[152]

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; *NFKB1*, nuclear factor kappa B subunit 1; *IKBKB*, inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta; *MMP9*, matrix metallopeptidase 9; *IL1B*, interleukin 1 beta; *MAPK8* (*JNK1*), mitogen-activated protein kinase 8; *XBP1*, X-box binding protein 1; *TNFA*, tumor necrosis factor alpha; *APOBR*, apolipoprotein B receptor; *NFE2L2*, nuclear factor, erythroid 2 like 2; *SOD1*, superoxide dismutase 1; *SOD2*, superoxide dismutase 2; *TXNRD1*, thioredoxin reductase 1; *IL10*, interleukin 10; *POMC*, proopiomelanocortin; *GALP*, galanin like peptide; *HCRT*, hypocretin neuropeptide precursor; *MCH*, melanin-concentrating hormone; *PPARGC1A*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha; *PCK1*, phosphoenolpyruvatecarboxykinase1;*GSTA*,glutathioneS-transferasecluster;*CPT1A*,carnitinepalmitoyltransferase 1A; *FGF21*, fibroblast growth factor 21; *SCD1*, stearoyl-coenzyme A desaturase 1; *TLR4*, toll-like receptor 4; *IL6*, interleukin 6; *CRP*, C-reactive protein; *MMP2*, matrix metallopeptidase 2; *MMP13*, matrix metallopeptidase 13; *EMMPRIN*, extracellular matrix metalloproteinase inducer; *FASN*, fatty acid synthase; *EGR1*, early growth response 1; *LEP*, leptin; *SREBF1*, sterol regulatory element binding transcription factor 1; *PLIN*, perilipin; *AQP7*, aquaporin 7; *AEBP1*, adipocyte enhancer binding protein 1.

Fig Nutritional interventions, gene expression and health effects, (Ramos-Lopez et al., 2017)

Personalised nutrition and nutriepigenetics

Epigenetic modifications do not only occur “naturally” as a regulation mechanism of gene expression, they are also responsible for certain diseases such as autoimmune and neurodegenerative disorders or cancer. Bioactive food components have been shown to influence epigenetic modifications and thus modulate gene expression. Such regulators of gene expression may have the ability to reverse or inhibit the epigenetic changes that occur during disease. Preventative personalised nutrition therefore may provide an easy and inexpensive approach to prevent and potentially cure certain diseases by using the gene expression regulating capacity of certain bioactive compounds (Stefanska et al., 2012),

This is precisely what the field of nutriepigenetics investigates. It studies the interaction between nutrients and epigenetic modification, questioning what effect they may have on health outcomes.

The epigenetic control of gene expression is involved in critical biological and physiological processes, such as imprinting, silencing of specific chromosomal domains, embryonic development, cellular differentiation, and organogenesis. However, dysregulation of epigenetic phenomena can alter phenotype and cell function, leading to the onset and progression of diverse chronic diseases (Campión et al., 2010)[. In this sense, complex interactions among nutritional factors and DNA methylation, covalent histone modifications and noncoding RNAs, including microRNAs (miRNAs), have been implicated in obesity, dyslipidemia, T2DM, NAFLD, cancer, and CVD. For example, high-fat and sugar diets have been related to abnormal methylation patterns of neuropeptide genes controlling food intake, which may contribute to the development of obesity (Vucetic et al., 2010). Low-protein diets induced glucose and lipid alterations by disrupting histone modifications in key regulatory genes (Vo et al., 2013). Also, choline and folate shortages enhanced miRNAs changes responsible for the progression of NAFLD (Tryndyak et al., 2016). Different micronutrient deficiencies such as folate, vitamin A, vitamin B, potassium, iron, and selenium correlated with hypermethylation of tumor suppressor genes, demonstrating a role in cancer (Uthus et al., 2011). Deprivations of vitamin D , calcium, magnesium , and chromium could increase the risk of developing T2DM through promoting aberrant methylation patterns in genes involved in glucose homeostasis, insulin signaling and inflammatory response (Takaya et al., 2011, 2013) . Additionally, deficits of selenium and vitamin A were associated with the pathogenesis of CVD by affecting the DNA methylation status of critical genes (G. Yang et al., 2014).

Nutriepigenetic examples of interactions between dietary intakes and epigenetic modifications involved in disease risk

Dietary factors	Epigenetic signatures	Modification types	Putative disease risks	Ref.
Low protein	*NR1H3* acetylation	–	T2DM	[47]
Chromium deficiency	Methylation of insulin signaling genes	+	T2DM	[49]
Selenium deficiency	*TLR2*, *ICAM1* methylation	–	CVD	[50]
Vitamin B_{12} deficiency	*SREBF1*, *LDLR* methylation	–	Dyslipidemia	[51]
Vitamin A deficiency	*GATA4* methylation	+	CVD	[52]
High fat and high sugar	*LEP* methylation	+	Obesity	[65]
High fat	*OPRM1*, *PENK*, and *DAT* methylation	–	Obesity	[74]
Low protein	*CYP7A1* acetylation	–	Dyslipidemia	[75]
Choline and folate deficiencies	miR-134, miR-409-3p, miR-410 and miR-495 expressions	+	NAFLD	[76]
Choline and folate deficiencies	miR-34a, miR-122, miR-181a, miR-192, and miR-200b expressions	+	NAFLD	[77]
Low folate, vitamin A, vitamin B_1, potassium, iron	*P16*, *P14*, and *hMLH1* methylation	+	Cancer	[78]
Selenium deficiency	*VHL* methylation	+	Cancer	[79]
Vitamin D deficiency	*NFKBIA* methylation	+	T2DM	[80]
Calcium deficiency	*HSD11B1* methylation	–	T2DM	[81]
Magnesium deficiency	*HSD11B2* methylation	+	T2DM	[82]
High fat and high sugar	*FASN* methylation	–	Obesity, NAFLD	[88]
Choline and folate deficiencies	*APOE*, *FOXA1*, and *FOXA2* methylation	+	NAFLD	[144]
High fat and high sugar	*FASN* methylation	–	Obesity, NAFLD	[153]
Low fruit consumption and folate deficiency	*LINE-1* methylation	–	Cancer	[154]

LEP, leptin; *FASN*, fatty acid synthase; *OPRM1*, opioid receptor, mu 1; *PENK*, preproenkephalin; *DAT*, dopamine transporter; *CYP7A1*, cytochrome P450 family 7 subfamily A member 1; *NR1H3*, nuclear receptor subfamily 1 group H member 3; *LINE-1*, long interspersed element-1; *MLH1 (HMLH1)*, mutL homolog 1; *APOE*, apolipoprotein E; *FOXA1*, forkhead box A1; *FOXA2*, forkhead box A2; *SREBF1*, sterol regulatory element binding transcription factor 1; *LDLR*, low-density lipoprotein receptor; *NFKBIA*, NFKB inhibitor alpha; *GATA4*, GATA binding protein 4; *TLR2*, toll-like receptor 2; *ICAM1*, intercellular adhesion molecule 1; *VHL*, von Hippel-Lindau; *HSD11B1*, hydroxysteroid 11-beta dehydrogenase 1; *HSD11B2*, hydroxysteroid 11-beta dehydrogenase 2; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; NAFLD, nonalcoholic fatty liver disease.

Fig Nutriepigenetic examples of interactions between dietary intakes and epigenetic modifications involved in disease risk, (Ramos-Lopez et al., 2017)

The reversible feature of epigenetic marks has given rise to the design of specific nutritional interventions aimed at reversing epigenetic alterations that might have a significant impact on preventing and treating human chronic diseases (nutriepigenetics) (S.-W. Choi & Friso, 2010). Thus, several experimental studies have investigated the epigenetic mechanisms underlying the health effects of certain nutrients and bioactive food components. For instance, it was found that the anti-inflammatory effects of consuming a Mediterranean diet were related to hypermethylation of proinflammatory genes (Nicoletti et al., 2016). The administrations of polyunsaturated fatty acids positively modulated the expression of several miRNAs, which suppressed oncogenic and lipogenic genes (Gil-Zamorano et al., 2014). Also, the anticancer properties of resveratrol, epigallocatechin-3-gallate, curcumin, sulforaphane, and genistein have been associated with some epigenetic modifications including hypomethylation and acetylation of tumor suppressor genes, and an increase in miRNAs targeting oncogenes. Likewise, apple polyphenols and pterostilbene (a derivate of resveratrol), prevented diet-induced obesity by regulating the methylation status of genes involved in lipid metabolism (Gracia et al., 2014). Furthermore, it was reported that curcumin exerted protective effects against liver injury and heart failure through modulating DNA methylation patterns and histone modifications of key genes (P. Wu et al., 2016). Based on this evidence, it has been proposed that the introduction of these dietary compounds into an “epigenetic diet” could serve as an effective strategy for reducing the incidence of obesity and associated comorbidities (T. M. Hardy & Tollefsbol, 2011). Additionally, studies have shown that some of the health benefits of energy restriction are mediated partially by epigenetic mechanisms including the prevention of aberrant DNA methylation patterns and chromatin alterations (Yuanyuan Li et al., 2011). Thus, it has been reported that moderate energy reductions might contribute to delay the onset of some aging-related diseases and extend lifespan through epigenetic mechanisms (S. Martin et al., 2013).

Epigenetic marks have also been found to modulate the effect of nutritional treatments on weight loss and changes in metabolic profiles, which could be used as biomarkers to predict the responsiveness to dietary prescriptions (F. I. Milagro et al., 2013). For example, methylation levels of circadian genes correlated with the magnitude of weight loss and circulating blood lipids after a nutritional program based on a Mediterranean dietary pattern (Fermín I. Milagro et al., 2012). Similarly, methylation patterns of appetite-regulatory genes were associated with the success in weight loss or the risk of weight regain (Cordero et al., 2011). Moreover, reductions of body fat and serum lipids were related to changes in the methylation status of genes involved in inflammatory response and fatty acid metabolism (Cordero et al., 2011)(Aumueller et al., 2015). Furthermore, differential baseline expression of several miRNAs was found between responders and non-responders to a weight-loss trial that consisted of following an energy-restricted treatment(Fermín I. Milagro et al., 2013).

As a final point, it is important to highlight that there may be interactions between the different genetic/epigenetic approaches, which may modulate the effectiveness of precision nutrition on the treatment of some chronic diseases.

Certain nutriepigenetic studies evaluating epigenetic modifications related to diverse nutritional interventions

Nutritional interventions	Epigenetic signatures	Modification types	Potential health effects	Ref.
Apple polyphenols	*SREBF1* methylation	–	Antiobesity	[65]
Apple polyphenols	*PPARGC1A* methylation	+	Antiobesity	[65]
Mediterranean diet	*EEF2*, *IL4I1* methylation	–	Anti-inflammatory	[84]
Mediterranean diet	*MAPKAPK2* methylation	+	Anti-inflammatory	[84]
Mediterranean diet	*IL6* methylation	+	Anti-inflammatory	[85]
Fish oil and pectin	miR-19b, miR-26b, miR-203 expressions	+	Anticancer	[86]
DHA	miR-192, miR-30c expressions	+	Antilipidemic	[87]
Pterostilbene	*FASN* methylation	+	Antiobesity	[88]
Curcumin	p300 HAT activity	–	CVD prevention	[89]
Curcumin	*FGFR3*, *FZD10*, *GPX4*, *HOXD3* methylation	–	Antifibrotic	[90]
Resveratrol	miR-129, miR-328-5p, miR-539-5p		Antilipidemic	[149]
Genistein	P21, P16 chromatin activators	+	Anticancer	[151]
Genistein	P21, P16 chromatin repressors	–	Anticancer	[151]
Methyl donor supplementation	*FASN* methylation	+	Antisteatotic	[155]
Extra-virgin olive oil	CNR1 (CB1) methylation	–	Anticancer	[156]
PUFA	Global DNA methylation	+	Anticancer	[157]
Resveratrol	*BRCA-1* methylation	–	Anticancer	[158]
Resveratrol	miR-101b, miR-455 expressions	+	Anti-inflammatory, anticancer	[159]
Resveratrol	Sirt1 activation	+	Anti-inflammatory, anticancer	[160, 161]
EGCG	*RXRA* methylation	–	Anticancer	[162]
EGCG	miR-16 expression	+	Anticancer	[163]
Green tea polyphenols and EGCG	EZH2, class I HDAC activity	–	Anticancer	[164]
Green tea polyphenols and EGCG	P53 acetylation	+	Anticancer	[165]
Curcumin	miR-22 expression	+	Anticancer	[166]
Sulforaphane	HDAC activity	–	Anticancer	[167, 168]
Sulforaphane	P21 acetylation	+	Anticancer	[168]
Genistein	P21, P16 acetylation	+	Anticancer	[169]

DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; EGCG, epigallocatechin-3-gallate; *EEF2*, eukaryotic translation elongation factor 2; *IL4I1*, interleukin-4 induced 1; *MAPKAPK2*, mitogen-activated protein kinase-activated protein kinase 2; *IL6*, interleukin-6; CNR1 (CB1), cannabinoid receptor 1; *BRCA1*, DNA repair associated; sirt1, sirtuin 1; *FASN*, fatty acid synthase; *RXRA*, retinoid X receptor alpha; EZH2, enhancer of zeste homolog 2; *FGFR3*, fibroblast growth factor receptor 3; *FZD10*, frizzled class receptor 10; *GPX4*, glutathione peroxidase 4; *HOXD3*, homeobox D3; HATs, acetyltransferases; HDACs, histone deacetylases; H3, histone 3; *ERS1*, estrogen receptor 1 (alpha); *SREBF1*, sterol regulatory element binding transcription factor 1; *PPARGC1A*, peroxisome proliferative activated receptor, gamma, coactivator 1 alpha.

Fig Nutritional interventions and epigenetic modifications, (Ramos-Lopez et al., 2017)

Personalised nutrition and microbiota

The gut microbiota is composed of a community of microorganisms that inhabit our digestive tract. These microorganisms' ferment dietary fibres that reach the colon and thereby produce short chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. The distribution of SCFAs is dependent on the substrate used. For example, acetate production is increased during fat oxidation, while propionate production dominates in the presence of branch chained amino acids. Furthermore, each fermentation product has its own function. Butyrate is mainly used as an energy source for epithelial colon cells, acetate and propionate enter the peripheral blood and execute their functions there (W. Huang et al., 2016).

SCFAs have been extensively researched and regulatory mechanisms have been found. They have been shown to play a role in the regulation of the immune system, glucose and lipid metabolism and blood pressure. Furthermore, they modulate epigenetic mechanisms and cell signaling pathways. For these reasons SCFAs have been proposed as novel therapeutic strategies for inflammatory diseases.

SCFAs play out their regulatory role by binding to different G-coupled protein receptors (GCPRs). The free fatty acid receptor 3 (FFAR3) for example has been linked to regulating insulin secretion and appetite, which suggests the importance of SCFAs in satiety and energy balance control. Another GCPR involved in regulating immune function and blood pressure has been related to SCFAs. Butyrate and propionate are non-competitive HDAC inhibitors. This exhibits their role in epigenetic gene regulation by influencing histone acetylation. SCFAs gene expression has been linked to nuclear factor-κB (NF-κB), p53 and nuclear factor of activated T-cell modulation. SCFAs are also involved in regulating immunity, by suppressing the production of pro-inflammatory mediators and enhancing the release of anti-inflammatory cytokines (W. Huang et al., 2016).

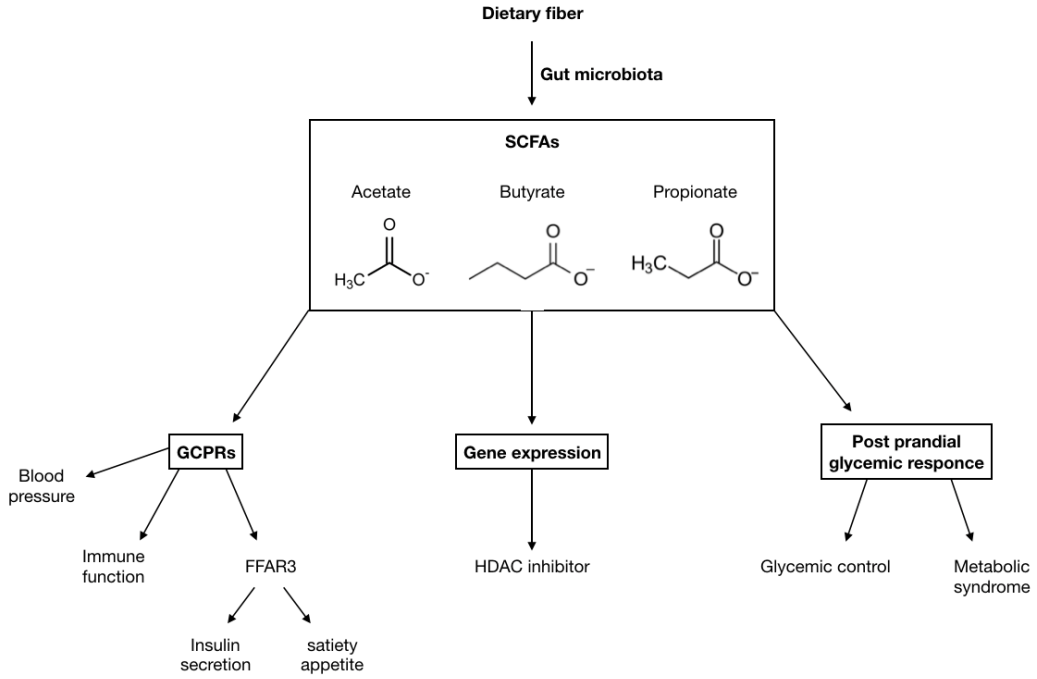


Fig. Summary of the effects of dietary fibers on gut microbiota and SCFAs, (Venegas et al., 2019)

The fermentation byproducts of gut microbiota play a vital role in the hosts homeostasis. By effecting immunity, gene regulation, appetite and lipid and glucose metabolism SCFAs are key components in individual's health. As mentioned, diet is the biggest contributor to SCFA production and regulates microbiota composition. Therefore, personalised nutrition may benefit from incorporating microbiota information into their recommendations. A few studies have shown promising results:

Zeevi et. al. conducted various studies on the prediction of glycemic response in combination with microbiota composition and personalised nutrition. In their first study they successfully showed that the post prandial glucose response (PPGR) varied significantly in individuals when consuming the same standardized meal. Opposite PPGR were also found in different individuals consuming the same meal. The different PPGRs were associated with individual microbiota composition. Taxa such as the *Enterobacteriaceae* were positively linked to PPGR, the same taxa have been associated with poor glycemic control and metabolic syndrome. The second study found that an algorithm containing the patients clinical and microbial data can accurately predict PPGRs. In the final study Zeevi et. al. tested whether personalised nutrition interventions could improve glycemic response. The authors found

lower PPGRs and positive gut microbiota changes after the intervention. For example, *Bifidobacterium adolescentis*, which low levels have been linked to greater weight loss, decreased during the dietary intervention.

The authors concluded that standardized glycemic indexes may have limited utility for the individual, as PPGRs vary greatly between individuals consuming the same meal. This itself also questions commonly used standardized dietary advice, which may not benefit every individual. Instead personalised dietary interventions including microbiota data and predictive algorithms may allow health professionals to more precisely tailor interventions towards their patients individual needs (Zeevi et al., 2015).

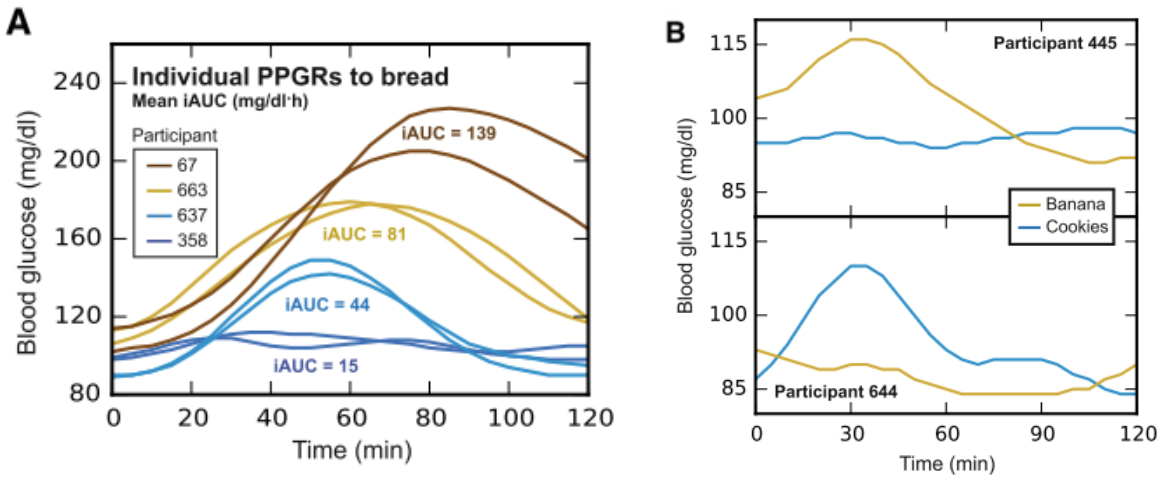


Fig.High variability in PPGRs between individuals consuming the same meal; (B) Opposite PPGRs in different individuals consuming the same meal, (Zeevi et al., 2015)

An interventional study analyzing the effects of SCFAs on the epigenetic regulation of FFAR3 in type 2 diabetes and obesity found significantly lower diversity in the microbiota of diabetics and obese individuals compared to the lean control group. For example, lower quantities of *F. prausnitzii* were found in type 2 diabetics suggesting the presence of low-level inflammation, as *F. prausnitzii* is negatively correlated with inflammation.

Furthermore, a negative correlation between promotor methylation of the FFAR3 gene and BMI was shown. Both type 2 diabetics and obese individuals showed lower methylation in comparison to lean controls. After the intervention (nutritional consulting for four months) only obese patients showed an increase in methylation, but not enough to reach the lean group.

The key factor in FFAR3 regulating satiety may be the regulation of leptin expression. Leptin is a hormone produced mainly in adipocytes that contributes to the suppression of appetite and fat metabolism. In the presence of FFARs propionate can induce leptin expression in adipocytes. Studies have shown that higher plasma leptin levels correlate with body fat mass, suggesting that in obese individuals, who have higher leptin levels, leptin cannot execute its role as a satiety hormone and regulate appetite.

The findings of this study elaborate the importance of gut microbiota on gene expression, BMI and satiety, which could be a helpful tool to better understanding and intervening in diseases such as type 2 diabetes or obesity (Marlene Remely et al., 2014a).

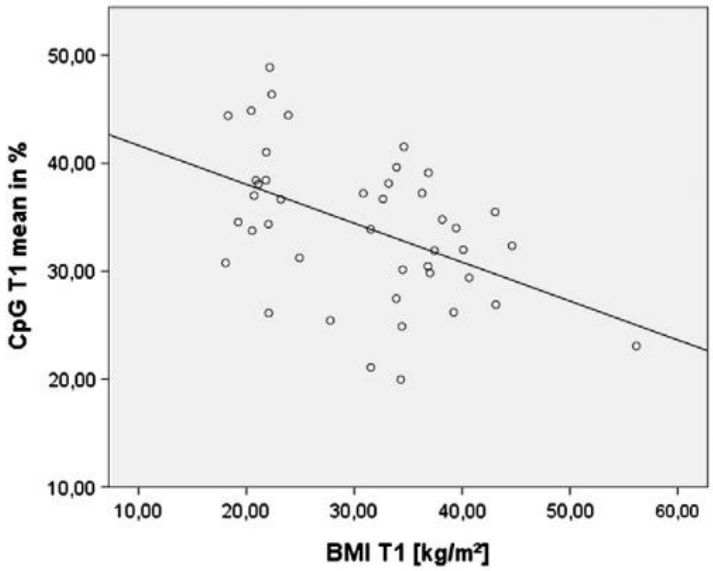


Fig. Correlation between BMI and FFAR3 methylation-% at time point 1 (T1) , (Marlene Remely et al., 2014a)

It is evident that personalised nutrition needs to include individual microbiota composition to fully understand the patients' needs. Variation in microbiota and it is impacts on health are further evidence of the lack of utility of current dietary recommendations and an appeal for novel ways to do nutritional counseling. Gut microbiota can function not only as a good predictor for health and nutritional intervention outcomes but can also be a parameter to measure changes in health. Personalised nutrition could use microbiota composition data to dive even further into the personalization of an individual's diet and combined with genetic and epigenetic information have a complete picture of a patient's health status and nutritional needs.

The microbiota modulates the pathogenesis, progression, and treatment of diseases, ranging from metabolic disorders to neurological diseases. Reshaping host–microbiota interactions through personalised nutrition is a new therapeutic avenue for both disease control and prevention. Gut microbiota composition and function is shaped from infancy, when the individual is colonized by bacteria from caregivers and the surrounding environment, a process that strongly influences the composition of the microbiota in adulthood Although early life events — including the mode of birth, type of feeding and complementary diet (MC Arrieta, 2014) (Laursen, 2017) have strong effects on the microbiota, it does retain some degree of flexibility and can be modulated through exposure to a variety of environmental factors (Yatsunenko, 2012). Of these, diet is the key determinant of the microbiota configuration, through modulation of the abundance of specific species and their individual or collective functions(Claesson, 2012). Furthermore, the effects of a particular diet on individuals in the population differ from person to person and may be influenced by a combination of host and microbiome features, the latter influence mostly being determined by the environment

rather than the genetic background, and thus is potentially more amenable to intervention (Rothschild, 2018).

Collectively, three chemically and biologically complex systems function together and influence each other to determine an individual's dietary responses: diet, the microbiota, and host physiology and metabolism, as well as immune regulation in response to the bacterial colonization of body surfaces (Zmora et al., 2019). These three systems are highly interconnected and interdependent.

Non-westernized populations such as the Hadza consume mainly raw or wild foods, resulting in a gut microbiota with higher diversity than in Western populations, whose diet derives almost entirely from commercial agricultural products (Yatsunenko, 2012; Zeevi et al., 2015) (Obregon-Tito, 2015; Schnorr, 2014). A rural diet leads to enrichment in Bacteroidetes (including the genera *Prevotella* and *Xylanibacter*), allowing rural populations to maximize energy intake from fibres, which is concordant with a depletion in Firmicutes(Filippo, 2010). Strikingly, the loss of diversity seen in westernized populations was also found to occur in individuals who migrated from developing nations to the United States as early as six to nine months after arrival. In the guts of these immigrants, the Western-associated genus *Bacteroides* started to displace the non-Western-associated genus *Prevotella* (Vangay, 2018). Importantly, in comparison to the simpler and more homogeneous diets in rural areas, urban environments offer a large variety of foods, which leads to greater inter-individual Vriation.

In humans, a high intake of dietary fat (mainly saturated fatty acids) is associated with reduced microbiota richness and diversity in both adults and infants (Wolters et al., 2019). A recent intervention study showed that a high-fat diet in healthy adults is associated with increased levels of *Alistipes* and *Bacteroides* species, a decrease in *Faecalibacterium* species and elevation of the faecal cometabolites p-cresol and indole, all changes that are associated with cardiovascular and metabolic disorders

Variability of gut microbiomes. The consumption of omega-3 polyunsaturated fatty acids (PUFAs) leads to an increased abundance of several butyrate-producing bacteria, in line with the known anticancer and anti-inflammatory effects of omega-3 PUFAs (Watson, 2018).

In humans, a long-term animal protein-rich diet is associated with the Bacteroides enterotype (G. Wu, 2011). A short-term animal protein-rich diet consistently increases the level of bile-tolerant bacterial species (including *Alistipes*, *Bilophila* and *Bacteroides*), while decreasing the abundance of saccharolytic microorganisms (including *Roseburia* species, *rectale* and *Ruminococcus bromii*) (David, 2014). By contrast, consumption of a plant protein diet, based on glycated pea proteins, significantly increases the levels of commensal lactobacilli and bifidobacteria and elevates short-chain fatty acid (SCFA) production in humans (D Swiatecka, 2011) (Wan, 2019).

The long-term consumption of complex carbohydrates has been shown to promote the *Prevotella* genus (G. Wu, 2011). Dietary fibre impacts human gut microbial ecology, resulting in a high abundance of Bacteroidetes (*Prevotella* species). (Filippo, 2010).

α-Diversity (intra-individual) is a predictor of the extent of microbiota composition change upon the short-term consumption of different protein sources (red meat, white meat and nonmeat sources) in healthy subjects. Importantly, changes are also highly variable between individuals, without strong population-level trends (Lang, 2018).

In overweight people, diets that are high in non-digestible carbohydrates result in a significant increase in bacteria within the phylum Firmicutes, including *Ruminococci* species, *Roseburia* species

and *Eubacterium rectale* (Walker, 2011). By contrast, diets poor in fermentable carbohydrates in obese individuals result in a significant reduction of butyrate-producing Firmicutes and a decline in faecal butyrate levels (Duncan, 2007).

Although response to fibre has a common signature within the population, heterogeneous and highly personalised shifts in the human microbiota have also been detected in response to carbohydrates, including dietary fibre (Tap, 2015; Walker, 2011), resistant starches, and carbohydrate-containing prebiotics (Bouhnik, 2004; LM Davis, 2011). Consumption of a high-fibre weight-stabilization or weight-loss diet in obese individuals affects the intestinal microbiota composition with significant interpersonal variation(Salonen, 2014) (Korpela, 2014). Although faecal butyrate levels generally increase upon indigestible carbohydrate consumption, the response also varies widely among individuals (McOrist, 2011). The microbiome response to dietary carbohydrates can be predicted from the baseline microbial diversity (Salonen, 2014). This dietary intervention is less efficient in improving clinical phenotypes in individuals with lower microbial gene richness. In addition, prior dietary habits could also potentially influence the gut microbiota response to dietary interventions. For example, healthy individuals with habitual high fibre intake exhibit greater gut microbiota responses to an inulin-type fructan prebiotic than those with low fibre intake (Healey, 2018), highlighting the importance of considering habitual dietary patterns when aiming to modulate gut microbiota through dietary interventions.

Microbiota responses to diet: There is emerging evidence that the changes that dietary interventions elicit in host metabolism are person-specific, and that this heterogeneity stems from unique microbiota signatures, in addition to host physiology (Zeevi et al., 2015).

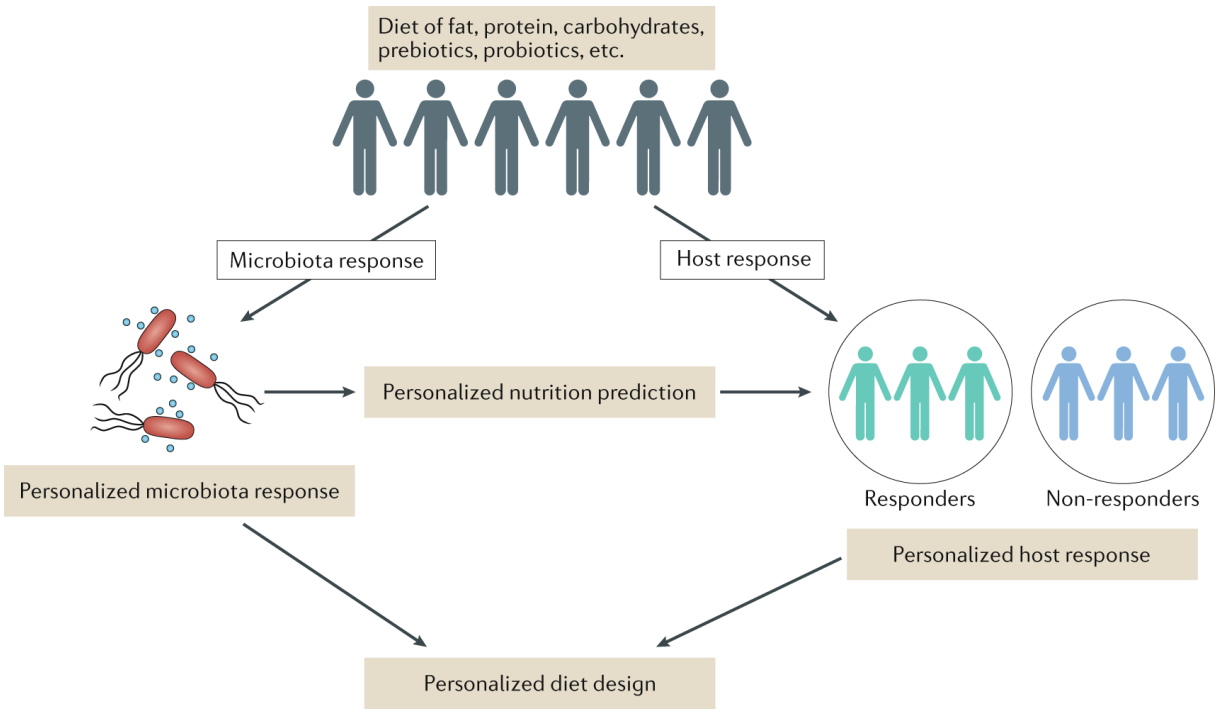


Fig Diet changes the gut microbiome composition and function in a person-specific manner, which is associated with the specific pre-intervention microbiome profile. Diet also results in highly individualized variation in host responses (for example, glycaemic response), which can be accurately predicted by the host's unique microbiome signatures. By utilizing both aspects, personalised

nutritional strategies can be developed in order to modify an individual's microbiome and further improve the response to a specific diet (Kolodziejczyk et al., 2019).

The level of one particular bacterial species may be a predictor of the response to a particular diet. Intake of whole grains induced anti-inflammatory responses and blood glucose level changes of different magnitudes in healthy subjects; those with greater improvements in blood IL-6 levels had higher levels of *Dialister* and lower levels of Coriobacteriaceae species in their stools, whereas *E. rectale* was correlated with postprandial glycaemic and insulin responses (Martinez, 2013). In overweight and obese adults on a calorie-restricted diet, individuals with higher levels of baseline *Akkermansia muciniphila* exhibited a greater improvement in insulin sensitivity and lipid metabolism, as well as a greater reduction in body fat, suggesting a predictive role of *A. muciniphila* in assessing response to dietary interventions (Dao, 2016).

Individuals can be classified into responders and non-responders on the basis of the outcomes of dietary interventions. For example, in childhood inflammatory bowel syndrome (IBS), individuals who respond to a low fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet have higher proportions of Bacteroidaceae, Erysipilotrichaceae and Clostridiales species, with a greater capacity for saccharolytic metabolism, whereas non-responders harbour higher levels of bacteria belonging to the genus *Turicibacter*. Similarly, relative to non-responders, individuals who respond to a low fermentable substrate diet in the management of childhood IBS are characterized by higher levels of taxa belonging to the genera *Sporobacter* and *Subdoligranulum*, and by a lower abundance of taxa belonging to *Bacteroides* (Chumpitazi, 2014, 2015).

More accurate personalised prediction methods that differentiate responders from non-responders have been developed by combining baseline microbiome signatures with other important individual traits. In an 800-person cohort comprising overweight or obese non-diabetic individuals in Israel, high interpersonal variability in the postprandial glycaemic response (PPGR) to identical foods was predicted accurately by gut microbiome, dietary habits, blood parameters and anthropometrics using a machine-learning approach. Different dietary components, age, serum parameters and the microbiome all exhibited relative contributions to the personalised predictions, showing either beneficial or non-beneficial but person-specific predictive effects. More specifically, 21 beneficial and 28 non-beneficial microbiome-based features were identified, in line with their relative contributions to the algorithm-based predictions. More strikingly, short-term personalised dietary interventions based on these predictions resulted in consistent gut microbiota alterations and a lower PPGR (Zeevi et al., 2015). The levels of contribution of the microbiome and of discrete clinical and laboratory features to the predictability may vary and merit further examination in diverse populations. This personalised approach to predicting the PPGR to food was recently validated in a non-diabetic population in the United States. (Mendes-Soares, 2019)More recently, a large-scale twin study revealed high interpersonal variability in postprandial responses (glycaemic, insulinaemic and lipemic responses) to diets, highlighting that even genetically similar twins respond differently to identical meals (Berry et al., 2019). This suggests that, rather than genetic make-up, non-genetic factors, including gut microbiome, host metabolism, meal timing, nutritional content, and exercise, have a fundamental role in determining the response to food. This further supports the notion that to achieve the same result in different individuals, personalised approaches to diet need to be employed. Nevertheless, such a 'tailored nutritional approach' is in its infancy, and more feasible, sustainable personalised nutritional strategies need to be developed to optimize one's gut microbiome and improve host responsiveness (Kolodziejczyk et al., 2019). Still, in the same manner as personalised medicine, personalised nutrition approaches aim to identify key microbiome

features that predict the response to particular food components, which can then inform the design of a diet leading to favourable outcomes.

Recently an algorithmus was proposed after collection of gut microbiome activity (i.e., metatranscriptomic) data and measured the glycemic responses of 550 adults who consumed more than 27,000 meals from omnivore or vegetarian/gluten-free diets. Gut microbiome activity makes a statistically significant contribution to individual variation in glycemic response, in addition to anthropometric factors and the nutritional composition of foods. A predictive model (multilevel mixed-effects regression) of variation in glycemic response among individuals ingesting the same foods was designed. Functional features were aggregated from microbial activity data as candidates for association with mechanisms of glycemic control. (Tily et al., n.d.)

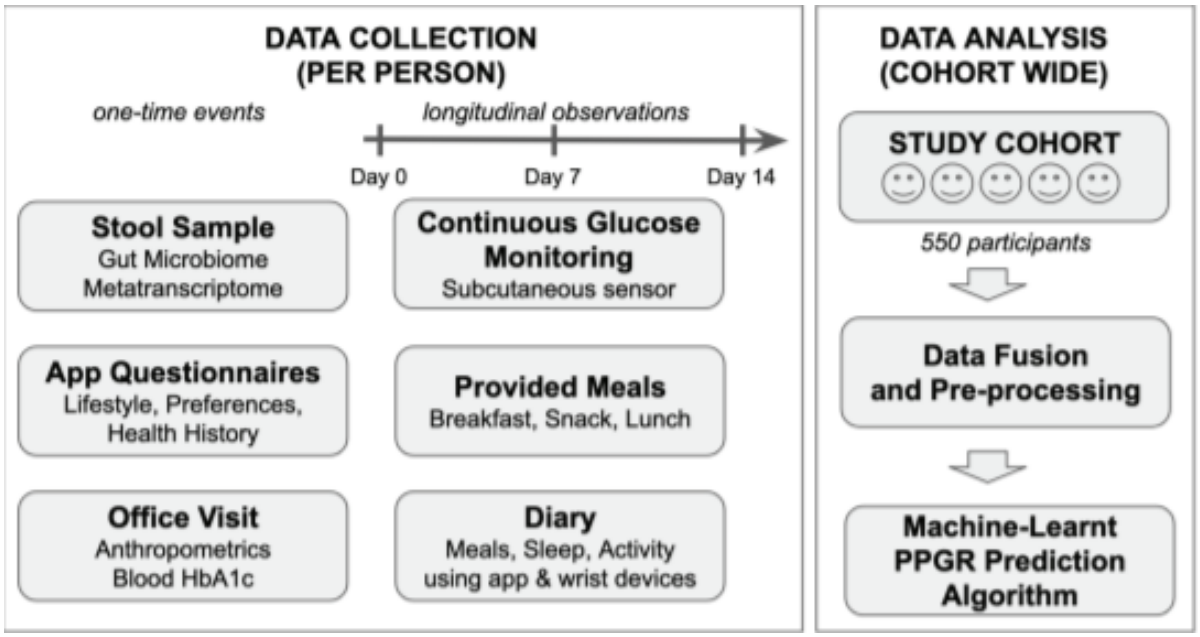


Fig design of an algorithm for postprandial glycemic response (PPGR) (Tily et al., n.d.)

Integration of data from multiple OMICS for personalised precision nutrition

Experiences from various OMIC layers, such as genetic, epigenetic, gene expression, metabolic or microbiota have been shown to give information about personal responses to foods and nutrition as well as personal requirements for diets. In many situations, there will also be interactions between molecular pathways involving elements of these layers. Transcription is regulated by two major mechanisms. On the one hand, changes in DNA sequence are responsible for genetic gene regulation. On the other hand, chromatin structure regulates gene activity at the epigenetic level. Given the fundamental participation of these mechanisms in transcriptional regulation of virtually any gene, they are likely to co-regulate a significant proportion of the genome. The simple concept behind this idea is that a mutation may have a significant impact on local chromatin structure by modifying DNA methylation patterns or histone type recruitment. Elucidating how genetic and epigenetic mechanisms co-participate in regulating transcription may assist in some of the unresolved cases of genetic variant-phenotype association. One example is loci that have biologically

predictable functions but genotypes that fail to correlate with phenotype, particularly disease outcome. Conversely, a crosstalk between genetics and epigenetics may provide a mechanistic explanation for cases in which a convincing association between phenotype and a genetic variant has been established, but the latter does not lie in a promoter or protein-coding sequence. DNA methylation, histone marks but also miRNAs have found to be modified by SNPs (Fermín I. Milagro et al., 2013).

Another example is the intense interaction between microbiota, their metabolites and epigenetics. Changes in gut microbial composition affect various epigenetic patterns (comprising DNA methylation, histone modifications, and chromatin remodelling, which orchestrate a seemingly infinite variety of molecular and cellular processes. Alterations in gut microbiota e.g. due to obese phenotype induce metabolic changes. Possible mechanisms are signaling mediated by bacterial components via pattern-recognition receptors: Toll-like receptor 2 (TLR2) and TLR4 (including NF-κB or the signaling of SCFAs (short-chain fatty acids) produced by the microbiota via GPRs (G-protein coupled receptors) and via HDAC (histone deacetylases) (Bäckhed et al., 2004; Cani et al., 2008; Cani & Delzenne, 2009; Caricilli et al., 2011; Clemente et al., 2012; M. Remely et al., 2014; Marlene Remely et al., 2014b).

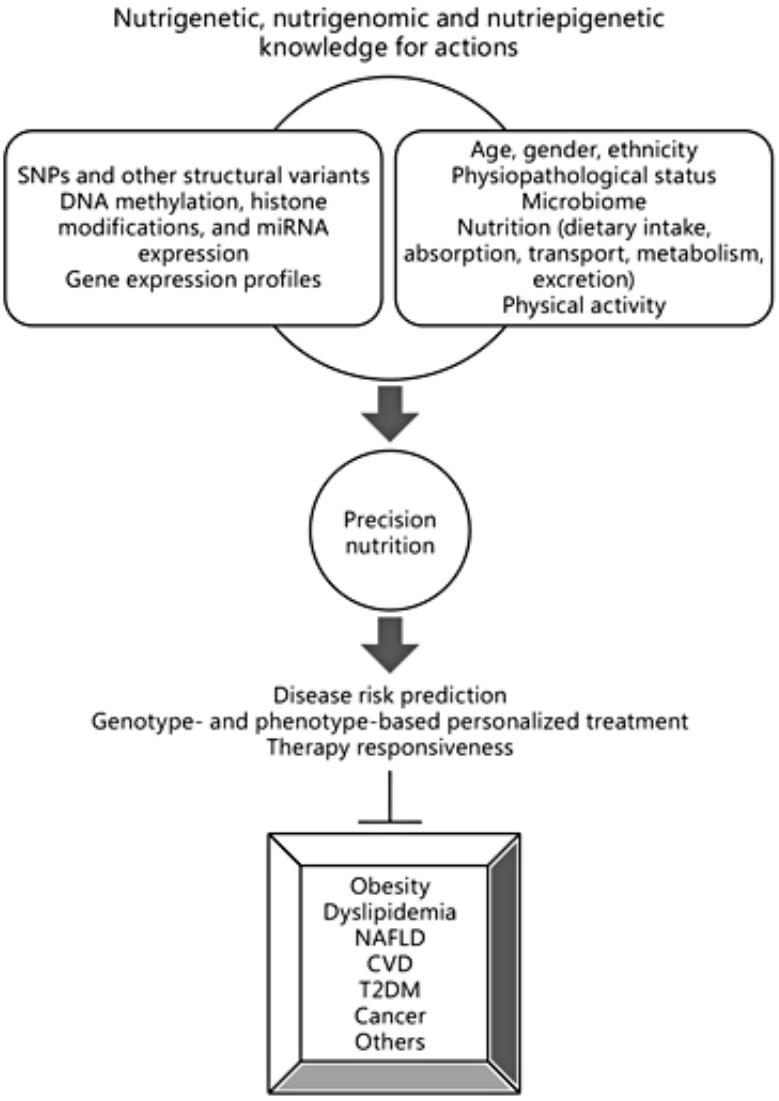
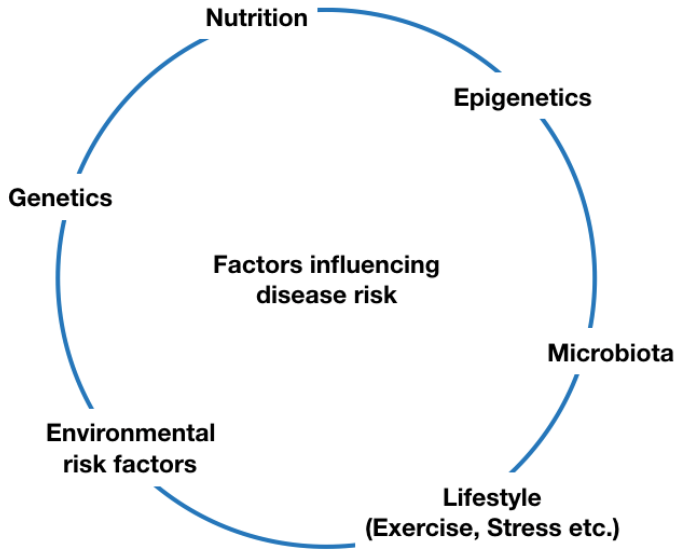


Fig Nutrigenetic, nutrigenomic, and nutriepigenetic approaches for precision nutrition to the prevention and management of obesity and associated chronic diseases.
https://zhuanlan.zhihu.com/p/80750035

The integration of information derived from markers of different OMICS is required for nutritional advice based on personal precision nutrition. The combination of markers from multiple OMIC layers could offer a significantly better understanding of an individual's well-being and nutritional needs. Furthermore, the importance of physical activity for health should not be undermined. Personalised nutrition recommendations should include physical activity as well as other lifestyle-related recommendations. Only by seeing the complete picture of a disposition personal and all the factors that may influence it can we give the best nutritional advice (Brettfeld et al., 2017; Brettfeld & Maver, 2016; A. G. Haslberger et al., 2015). In the area of metabolic diseases often FTO SNP mutations are analysed, but do not reflect the complex interactions of FTO genes and BMI. Future developments of integration of information of different omics, such as epigenetics, microbiota metabolites and environment may enable improved information for health professionals guiding consumers (Lilja et al., 2017). Unfortunately, most companies presently offer only tests for genetic SNP markers for metabolic advice.



Factors influencing disease risk, (A. G. Haslberger et al., 2015)

Translation of personalised precision nutrition into praxis

Personalised nutrition can be applied in two broad areas: firstly, for the dietary management of people with specific diseases or who need special nutritional support—for example, in pregnancy or old age, and, secondly, for the development of more effective interventions for improving public

health. It has traditionally focused on maximising the benefits and reducing the adverse effects of dietary changes for the individual. However, this focus on the individual may have limited impact on populations. To have a wider impact, it must be deployed at a scale and in a way that reduces (rather than increases) health disparities. Individuals may also wish to use personalised nutrition to achieve personal goals/ambitions that are less directly related to health—for example, to deal with preferences for, and dislikes of, specific foods, to attempt to achieve a desired body size or shape, or for competitive sports (Ganio et al., 2009).

Personalised nutrition is based on the idea that individualising nutritional advice, products, or services will be more effective than more generic approaches.

Personalisation can be based on: Biological evidence of differential responses to foods/nutrients dependent on genotypic or phenotypic characteristics or the analysis of current behaviour, preferences, barriers, and objectives and subsequent delivery of interventions, which motivate and enable each person to make appropriate changes to his or her eating pattern.

Differences in the response of people to dietary components have been well documented for almost a century. This provides the basis, and motivation, for developing personalised nutrition strategies. The trend towards personalisation is the result of: firstly, nutrition research that provides a better understanding of how diet affects health; secondly, new technology that enables better and continuous measurements of markers of individual health and fitness; and thirdly, new analytical tools that interpret this flow of data and transform it into user friendly practical information. (Andraos et al., 2019; de Roos & Brennan, 2017; Fallaize et al., 2013; Ganio et al., 2009; German & Young, 2004; Głąbska et al., 2019; C. Morris et al., 2013; O'Donovan et al., 2017; Ordovas et al., 2018b; *Personalised nutrition: Food4Me project: (EUFIC)*, n.d.; *Science and PoliticS of nutrition*, n.d.; Petre & Vatasescu, 2019; San-Cristobal et al., 2013; Stewart-Knox et al., 2015; Sweeney, 1927; Tamburrano et al., 2019).

Most researchers, and other stakeholders in personalised nutrition, have focused on the capture of genotypic or phenotypic characteristics. The implicit assumption is that the more we can measure, the more effective will be the outcomes of personalisation. There is increasing realisation that, unlike with medication, dietary changes require individuals to make daily, sometimes hourly, choices. The adoption of these lifestyle changes (including but not limited to changes in dietary patterns) is highly dependent on effective collaboration with participants who are being helped to take responsibility for their behaviour, and, ultimately, health. Increasing technology is available that can motivate healthy eating. However, such applications usually adopt a "one-size-fits-all" approach that is biased towards specific cultures or population subgroups. Evidence suggests that it is possible to facilitate a change in behaviour using genetic testing or personalised advice as to the catalysts. More emphasis is needed to develop behavioural approaches that will best motivate particular individual and cultural groups.

There may be benefits in moving from a decision framework based on health professionals' perspectives of effectiveness to one of shared decision making. An intervention based on shared decision making between the provider and the recipient becomes personalised and may increase acceptance and adherence. In this regard, the **Food4Me Study** stands out. It was a EU funded randomised controlled trial (RCT) involving >1600 participants from seven European countries. The project studied the effects of internet-based personalised nutrition advice on lifestyle changes. Their recent 6-month randomized control trail compared behavioral changes in individuals who received personalised nutrition (PN) advice or conventional dietary advice. Personalised nutrition advice was

further categorized in individual diet intake, individual diet intake and phenotypic data and individual diet intake, phenotypic genotypic data and medical practice (Price et al., 2017).

The study asked two key questions:

- Is personalised nutrition more effective in changing diet than a conventional one-size-fits-all approach?
- Does the basis used for personalisation matter? (With particular interest in the benefit of personalisation based on phenotypic and genotypic characteristics)

After 6 months, the answer was clear. Personalisation of dietary advice assisted and/or motivated consumers to eat a healthier diet and follow a healthier lifestyle (in comparison with "impersonal" (conventional) dietary advice). The "Healthy Eating Index" was used as the global measure of "healthfulness" of eating patterns and change was measured after 3 and 6 months.

Personal nutrition was more effective in improving lifestyle changes compared to conventional advice and showed that personalised nutrition was more effective than a conventional one-size-fits-all approach as control. These finding should come to no surprise, as conventional dietary advice is geared towards the general population and may be inadequate for the individual. The study also showed that internet-based advice can be more effective than face-to-face interventions (less dropouts, high compliance for blood sampling and completion of follow up), which could be promising for future online-based interventions.

Different levels of recommendation for women (not pregnant or lactating)

Global recommendation	Personalised dietitian recommendation based on an individual's history and preferences	Personalised recommendation based on individual history, preferences, and genetic information
Zn (8 mg/day): Consume a wide variety of foods containing zinc. Red meat and poultry provide the majority of zinc in the American diet. Other good food sources include beans, nuts, certain types of seafood, whole grains, fortified breakfast cereals, and dairy products	Recommendations vary according to age, sex, pregnancy and lactation (2-13 mg). Personalisation will account for these individual characteristics. In addition, consideration should be given to: • People who have had gastrointestinal surgery, such as weight loss surgery, or who have digestive disorders, such as ulcerative colitis or Crohn's disease. Both these conditions can decrease the amount of zinc that the body absorbs and increase the amount lost in the urine • Vegetarians, because they do not eat meat, which is a good source of zinc. Also, the beans and grains they typically eat contain compounds that prevent complete absorption of zinc by the body. For this reason, vegetarians might need to eat as much as 50% more zinc than the recommended amounts • Older infants who are breastfed because breast milk contains insufficient zinc for infants aged >6 months. Infants taking formula receive sufficient zinc. Older infants who do not take formula should be given foods that contain zinc, such as pureed meats • Alcoholics, because alcoholic beverages decrease the amount of zinc absorbed by the body and increase the amount lost in the urine. Also, many alcoholics eat a limited amount and variety of food, so they may not get enough zinc • People with sickle cell disease, because they might need more zinc	SLC30A8: Carriers of the A allele at the rs11558471 SLC30A8 (zinc transporter) variant need supplements containing zinc in addition to a healthy diet to maintain proper glucose homoeostasis.[55] Knowledge of this genetic information will trigger a recommendation for Zn supplementation
Dietary fat and cholesterol : Choose a diet low in fat, saturated fat, and cholesterol	Use fats and oils sparingly. Use the nutrition facts label to help you choose foods lower in fat, saturated fat, and cholesterol Eat plenty of grain products, vegetables, and fruits Choose low fat milk products, lean meats, fish, poultry, beans, and peas to get essential nutrients without substantially increasing calories and intake of saturated fat	*TCF7L2:* For carriers of the T allele at the TCF7L2-rs7903146 polymorphism a Mediterranean diet reduces its adverse effect on cardiovascular risk factors and incidence of stroke, but not so a low fat diet. Therefore carriers of the T allele will be recommended to: • Eat primarily plant based foods, such as fruits and vegetables, whole grains, legumes, and nuts • Replace butter with healthy fats such as extra virgin olive oil • Use herbs and spices instead of salt to flavour foods • Limit red meat to a few times a month • Eat fish and poultry at least twice a week • Drink red wine in moderation (optional)[39]
Vitamin B2 (riboflavin): Consume the appropriate recommended dietary allowance (RDA) from a variety of foods	Recommendations vary according to age, sex, pregnancy, and lactation (0.3-1.6 mg/day) Personalisation will take account of these individual characteristics. In addition, consideration should be given to: • Vegetarian athletes, as exercise produces stress in the metabolic pathways that use riboflavin People who are vegan or consume little milk, or both, are also at risk of riboflavin inadequacy	*MTHFR:* Carriers of the TT genotype at the MTHFR C677T polymorphism are at higher risk of hypertension, which may not reach targets (systolic blood pressure <120 mmHg) with medication. However, they particularly benefit from riboflavin supplementation (~1.6 mg/day)[56] *SLC52A3:* Brown-Vialetto-Van Laere syndrome is caused by mutations in the *SLC52A3* gene, which encodes the intestinal riboflavin transporter. As a result, these patients have riboflavin deficiency. Riboflavin supplementation can be life saving in this population[57]

Fig: Example for recommendations from a global , personalised dietitian based on individuals history and preference as well as personalised dietitian based on individuals history, preference and genetic information (Ordovas et al., 2018a).

Developments of personalised health prevention and precision nutrition

Personalised nutrition can nowadays be seen at three levels: the first, least personal level is personalised (often Internetbased) nutritional advice based on individual lifestyles (including nutritional data)—this is currently the dominant approach in the marketplace. A substantial proportion of cases involve personalised nutrition based on phenotypic information (e.g., anthropometry, clinical parameters, biochemical markers of nutritional status), and it is only the third level of personalised nutrition using genomic data which is still the exception rather than a mainstream activity in the market (Rimbach & Minihane, 2009).

Personal precision nutrition aims to prevent and manage chronic diseases by tailoring dietary interventions or recommendations to one or a combination of an individual's genetic and epigenetic background, microbiota structure, metabolic profile, and environmental exposures. Dietary interventions have been successful in altering abundance, composition, and activity of gut microbiota that are relevant for food metabolism and glycaemic control.

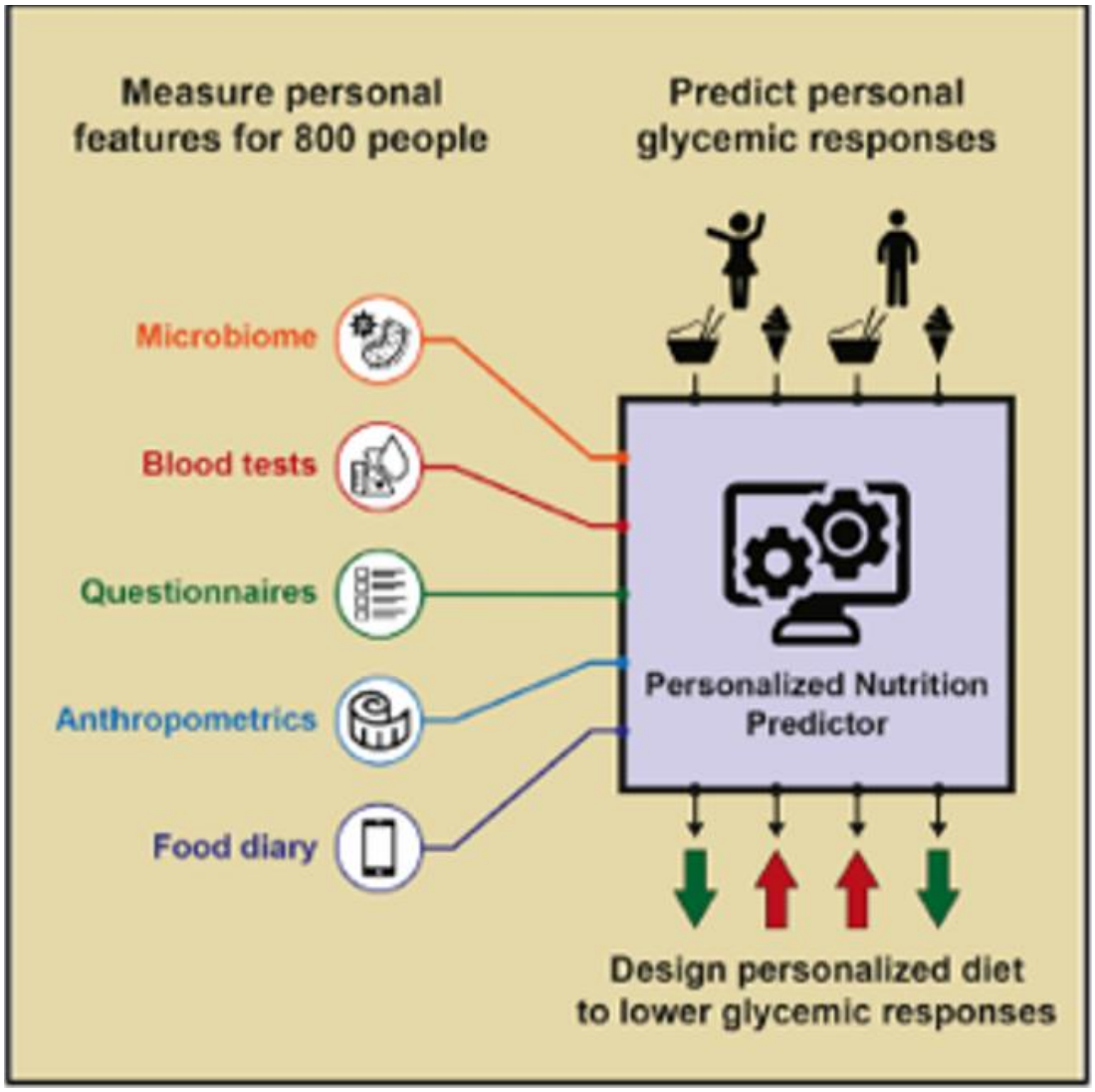
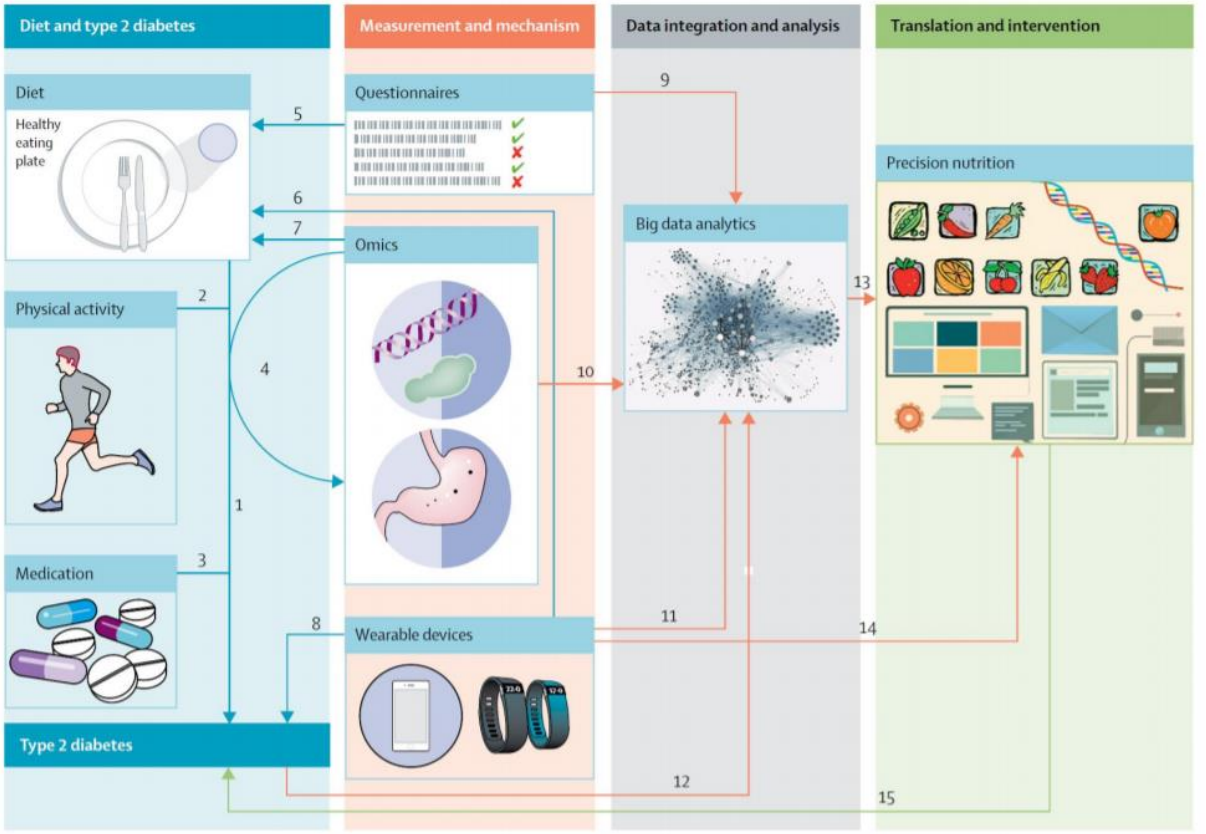


Fig prediction of glycemic responses for the design of a personalised diet (Zeevi et al., 2015)

In addition, mobile apps and wearable devices facilitate real-time assessment of dietary intake and provide feedback which can improve glycaemic control and diabetes management. By integrating these technologies with big data analytics, precision nutrition has the potential to provide personalised nutrition guidance for more effective prevention and management of complex metabolic diseases such as type 2 diabetes (D. D. Wang & Hu, 2018).



Precision nutrition for prevention and management of type 2 diabetes (D. D. Wang & Hu, 2018).

Advancement of personalised precision nutrition will be facilitated by a number of factors. Firstly, the development of a strong theoretical basis, including identification of the most important individual characteristics on which to base personalisation. Secondly, the evidence for efficacy and cost effectiveness from well- designed intervention studies. Thirdly, the introduction of a regulatory framework designed to protect the public and to give confidence to health professionals and policy makers. This will require a substantial increase in the scientific evidence. This implies more robust study designs ranging from randomized controlled trials (RCTs) enrolling participants based on preselected genotypes, to n-of-1 trials and aggregated n-of-1 trials. Such research will benefit from multidisciplinary research teams, comprising, for example, behavioural psychologists, computer scientists, biomedical scientists, and nutritionists. Integration of other “omics” to provide greater mechanistic interpretation of the evidence. This is likely to include emphasis on epigenomics, metabolomics, and microbiomics. In this respect, proof of principle of the role of the microbiome in shaping interindividual variability in response to diet has been established (Ordovas et al., 2018b).

Nutri(epi) genetic testing can be a promising tool to complement dietary advise in both clinical and preventative settings. It may reduce public health costs by providing personalised dietary advice for

disease management and prevention (San-Cristobal et al., 2013). With adequate scientific evidence nutritional tests can provide an early screening opportunity and could increase the demand for health-related consultations and screenings, which will help prevent disease (Covolo et al., 2015).

It may be only a matter of time until the research is mature enough to guarantee accurate test results and adequate recommendations that may be applied in the nutritional and medical field.

Preventative personalised precision nutrition has the potential to revolutionize current nutrition therapy methods and health care system, by providing individualized dietary advice based on the patients genetic, epigenetic, and even microbiota information. Yet it still faces crucial challenges before it can widely be implemented in clinical practice and possibly in online consulting.

Once there is consensus on effective genetic, epigenetic and microbiota biomarkers researchers and health professionals can focus on further aspects of personalised nutrition, such as educating health professionals to correctly interpret genetic and epigenetic data, creating ways to motivate positive behavior change in patients and correctly implementing personalised nutrition into medical practice. Furthermore, ethical, and legal guidelines, as well as standardized regulations for tests, need to be put in place to assure patients health is not being harmed. (Ferguson et al., 2016)

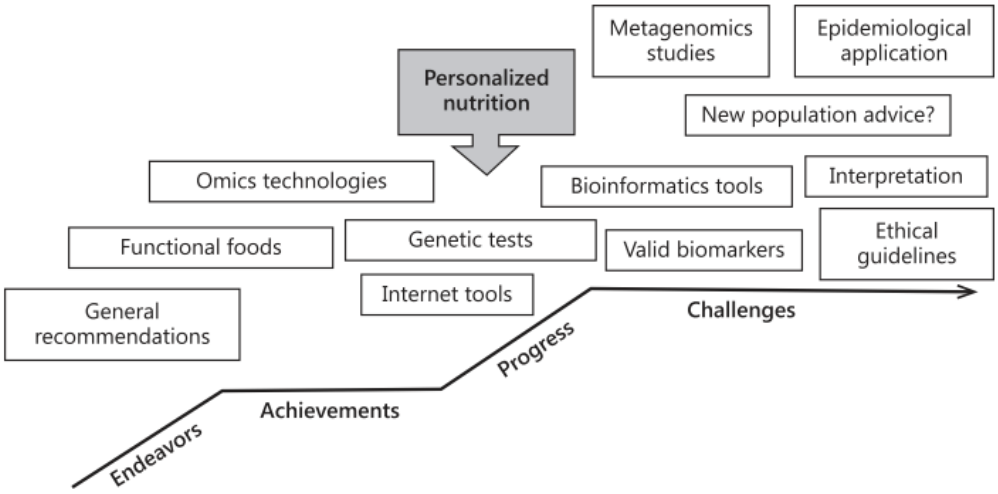


Fig. Achievements already made and challenges faced by personalised nutrition (Prasad et al., 2016)

In conclusion, the way to personalised precision nutrition developed from simple genetic SNP testing to addition of lifestyle and environmental data, epigenetic, microbiota and metabolic marker already reflecting impacts of lifestyle and nutrition to the integration of data from markers of multiple OMICS.

Personalised precision nutrition and consumer aspects

Consumer attitudes toward genetic tests aiming to reveal the risks of a predisposition to various illnesses have already been examined by several papers; consumer acceptance of nutrigenomics-based personalised nutrition, however, has only been examined by a few. The most important

motivator to have a genetic test done was the consumers' own health and the health of their family members. Whereas early genetic testing, especially direct to consumer testing encountered substantial criticism, modern ways of a personalised precison nutrition has reflected these restrictions and gets a much broader approval.

Consumer attitude and behavioral change: A systematic review analyzing consumer attitudes towards direct to consumer genetic testing (DTC. GT) DTC-GT showed that there is generally low awareness of DTC-GT in the general population, even though most study participants had high education levels. Nevertheless, participants in most studies were interested in knowing their disease risk, especially if they were parents (to know their child's risk) or if they had a higher disease risk (e.g. family history). Interest increased when the test results were positive and decreased with price of genetic testing and knowing the risks of DTC-GT (less regulated and accurate results). It was also found that participants preferred genetic testing to be performed by a health professional, instead of it being marketed towards consumers (Covolo et al., 2015):

The impact of genetic testing results on lifestyle changes is fragmentary. Some individuals expressed concern and the intention to change their lifestyle, while others showed indifference to the test results even though they were at a higher risk for disease. Moderate lifestyle changes could be observed, though a 3 month follow up showed no impact on user behavior. Furthermore, one year follow-ups showed no difference in concern compared to individuals who were not tested for disease risk (Covolo et al., 2015):

An interventional study assessed the impact of receiving genetic information without personalised dietary advice on health behavior changes. Participants were given information about their apoE genotype, which is thought to effect lipid metabolism and cholesterol absorption and may play a role in CVD. 122 participants were categorized in high risk, low risk and control group. During the one-year intervention individuals received mandatory and elective lectures by qualified nutritionist, emails, and voluntary personal discussions with a doctor to inform them about a healthy lifestyle and the apoE genotype. The researchers concluded that dietary fat quality improved significantly in the high-risk group compared to the control, but only for a short period of time. Positive behavior changes diminished once active communication stopped, indicating that behavioral change is largely due to outside stimuli such as reminders of the health implications of a disease (Hietaranta-Luoma et al., 2014):

Related to genetic tests, considerable concerns were consistently raised about internet privacy, data security, data use, and data destiny; participants articulated their fears about the potential for information to be used by companies for commercial gain or to fall into the hands of insurers, employers, or government agencies. In contrast with the more numerous research studies examining consumer judgements related to genetic tests, only a few studies examined consumer preferences for genetic based personalised nutrition. Based on both qualitative and quantitative studies consumers usually show positive attitudes toward genetic-based personalised nutrition; about one third to a half of respondents would use a service of this kind and would follow a personalised diet, although there are significant international differences in this area. For example, in the research conducted by among the six European countries examined the proportion of individuals who would follow a personalised diet is the highest in the United Kingdom and Italy (38.7% and 38.3%, respectively), and the lowest in Germany (13.4%) (Abrahams et al., 2018). Ultimately, consumer acceptance of personalised nutrition is determined either by consumer perception of the rational trade-off between the technology's benefits and costs, or more emotional perceptions of risk and uncertainty. In fact, the costs and benefits of personalised nutrition turned out to be of primary importance in consumer judgements. Interestingly, determinants of consumer acceptance identified

in the literature are better predictors of consumer rejection than acceptance. (Szakály et al., 2019). A driver of personalised nutrition is the increasing awareness of consumers of their individuality Personalised nutrition fits well into the current marketing trend of moving the consumer company relationship from the mass model toward a customized model. Consumer goods have become increasingly personalised, particularly during the second half of the 20th century. Individualized products appeal to today's sophisticated consumers and allow them to feel empowered and to stand out from the crowd.

A special ethical question concerns how access to personalised nutrition could be ensured for lower-income classes which could have a greater need for it but lack the means to (A. R. H. Fischer et al., n.d.) adopt it.

Discussion and conclusions

Our review shows the strong molecular, often epigenetic, interactions of the individual lifestyle and nutrition with health. Personal values contribute to result in quite different lifestyles with different requirements for health conservation. The strong demand for improved opportunities for disease prevention will pave the way for individualised, preventive health concepts.

Fast scientific and technological developments will provide options for improved health preservation and an extended lifespan even in scenarios of a more "competitive, stressed lifestyle (Kopp, 2019)", often correlating with unbalanced nutrition. Approved panels of molecular markers from different OMICS need to be established for the monitoring of health or upcoming riks. Functional foods in combination with adequate lifestyles are a promising instrument for health conservation but need to be more extensively analysed in the light of quality, stability, and bioavailability.

Some citizens, however, may prefer more relaxed lifestyles also focusing on "holistic ways of nutrition" without strong demands for options from modern science or technology. Social science and politics will need to continue to develop health concepts and international harmonized regulations to meet the needs of groups of citizens with quite different expectations.

Ecosystem resilience is a major value of human nutrition and health care and needs to be a central goal in regulations. Information and education of citizens about different scenarios of health conservation and disease prevention which enable informed decisions for personal concepts or products need to sysnergize with regulations based on often difficult scientific criteria.

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Abbreviations:

DNA (deoxyribonucleic acid)

cDNA (complementary DNA)

RNA (ribonucleic acid)

cRNA (complementary RNA)

RNase (ribonuclease)

DNase (deoxyribonuclease)

rRNA (ribosomal RNA)

mRNA (messenger RNA)

tRNA (transfer RNA)

AMP, ADP, ATP, dAMP, ddATP, and GTP, etc. (for the respective 5' phosphates of adenosine and other nucleosides)

I.S. Immune System

ATPase and dGTPase, etc. (adenosine triphosphatase and deoxyguanosine triphosphatase, etc.)

NAD (nicotinamide adenine dinucleotide)

NAD^+ (nicotinamide adenine dinucleotide, oxidized)
NADH (nicotinamide adenine dinucleotide, reduced)

NADP (nicotinamide adenine dinucleotide phosphate)

NADPH (nicotinamide adenine dinucleotide phosphate, reduced)

$NADP^+$ (nicotinamide adenine dinucleotide phosphate, oxidized)
poly(A) and poly(dT), etc. (polyadenylic acid and polydeoxythymidylic acid, etc.)

oligo(dT), etc. (oligodeoxythymidylic acid, etc.)

UV (ultraviolet)

PFU (plaque-forming units)

CFU (colony-forming units)

MIC (minimal inhibitory concentration)

Tris [tris(hydroxymethyl)aminomethane]

DEAE (diethylaminoethyl)

EDTA (ethylenediaminetetraacetic acid)

EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*',*N*'-tetraacetic acid]

HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid)

PCR (polymerase chain reaction)

AIDS (acquired immunodeficiency syndrome)