



## Nature has the answers: Discovering and validating natural bioactives for human health

Martin Kussmann<sup>1,2</sup>, David Henrique Abe Cunha<sup>3,4</sup>

<sup>1</sup>Kussmann-Biotech GmbH; Nordkirchen, Germany; <sup>2</sup>German Entrepreneurship; Cambridge, MA, USA; <sup>3</sup>Ideatomik Creative Industries, Botucatu, Brazil; <sup>4</sup>Sao Paulo State University, Institute of Biosciences, Rio Claro, Brazil

\*Corresponding Author: Martin Kussmann; Kussmann Biotech GmbH; Am Gorbach 18, 59394 Nordkirchen, Germany.

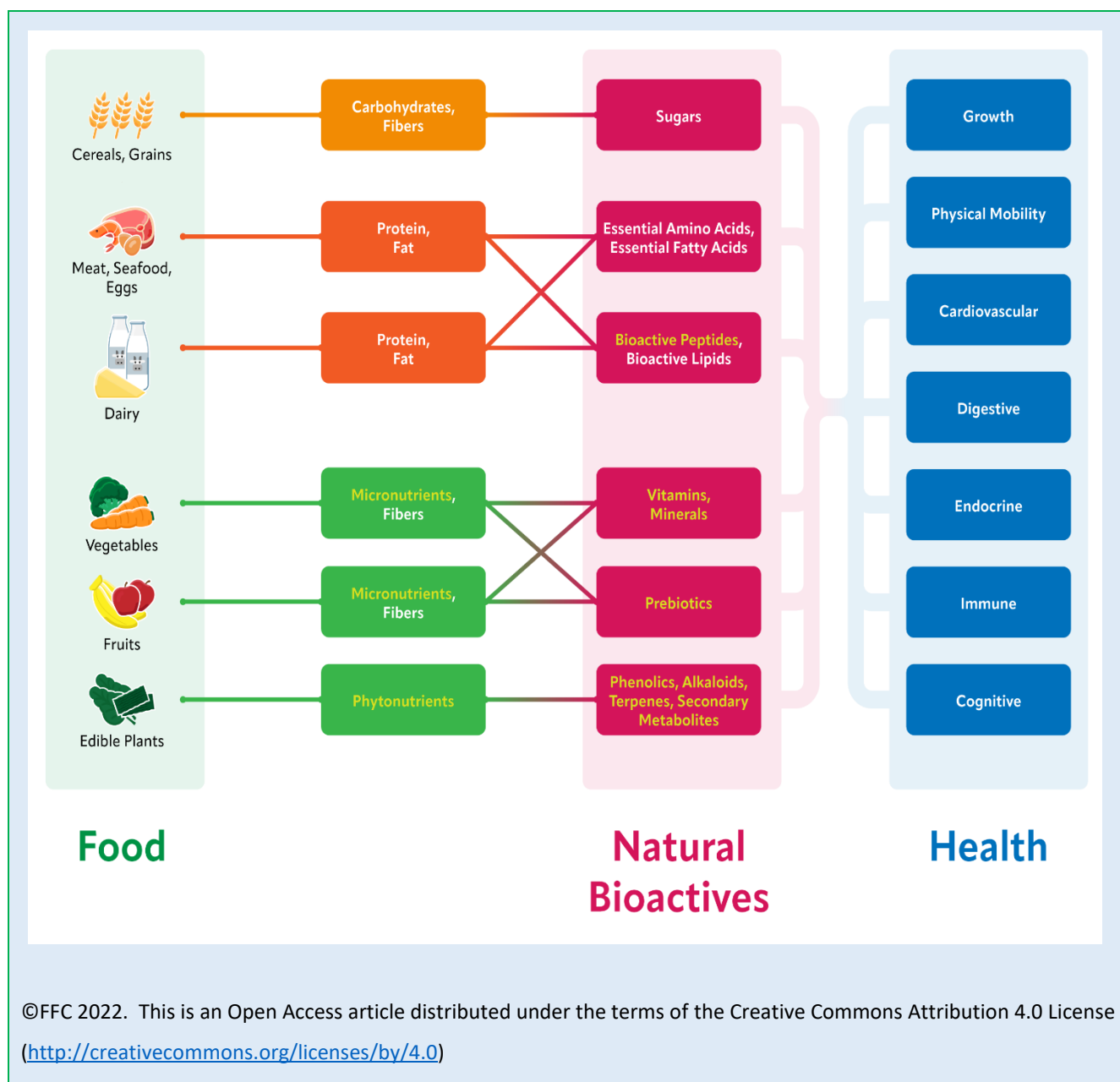
**Submission Date:** August 31<sup>st</sup>, 2022; **Acceptance Date:** October 28<sup>th</sup>, 2022; **Publication Date:** November 3<sup>rd</sup>, 2022

**Please cite this article as:** Kussmann M., Cunha D. H. A., Nature has the answers: Discovering and validating natural bioactives for human health. *Bioactive Compounds in Health and Disease* 2022; 5(10): 222-234. DOI: <https://www.doi.org/10.31989/bchd.v5i10.1000>

### ABSTRACT

Nature has the answer to many of our questions about human health. Natural bioactives, especially when derived from sustainable plant and food sources, provide a plethora of molecular solutions to nutritionally actionable, chronic conditions. The spectrum of these conditions, such as metabolic, immune, and gastrointestinal disorders, has changed with prolonged human life span, which should be matched with an appropriately extended health span: “adding years to life and adding life to years”. Natural bioactives can be classified into micronutrients (i.e., vitamins, and minerals), phytonutrients, bioactive peptides, and pre-, pro-, post- and synbiotics. Bioactives act in concert and interact with the human host and its (gut) microbiome. Therefore, the future of translational science on natural bioactives is being built on a: (a) systems-level rather than reductionist approaches to understanding their interdependent, and at times additive functions, as well as their interactions with the human host and (gut) microbiome; and (b) the leverage of artificial intelligence for discovery and validation, thereby substantially reducing the time from idea and concept to finished solutions for consumers and patients. Rather than focusing on a particular class of bioactive food molecules, as often done in molecular food and nutrition articles, this review embraces a broad range of these bioactives and puts them into perspective regarding a more efficient and sustainable leverage of the biochemical richness of plants and foods for its diverse positive effects on human health.

**Keywords:** bioactive, micronutrient, phytonutrient, prebiotic, probiotic, synbiotic, peptide, artificial intelligence, discovery, validation



**INTRODUCTION:** Comprehensive investigation of human nutritional health effects at the molecular level requires the understanding of the composition of and the interplay between the genomes of food, human gut microbiome, and human host (1). Food genomes are researched for discovery and exploitation of macro- and micronutrients, as well as specific bioactives aiming at genes coding for bioactive proteins, peptides, and synthetic capacity for phytonutrients and prebiotics. The human gut microbiome encompasses a complex

ecosystem in the intestine with profound impact on host metabolism of natural bioactives. Modern nutrition science explores health-related aspects of bioactive food components, thereby promoting health, preventing or delaying the onset of disease, optimizing performance, and assessing benefits and risks in individuals and (sub-) populations.

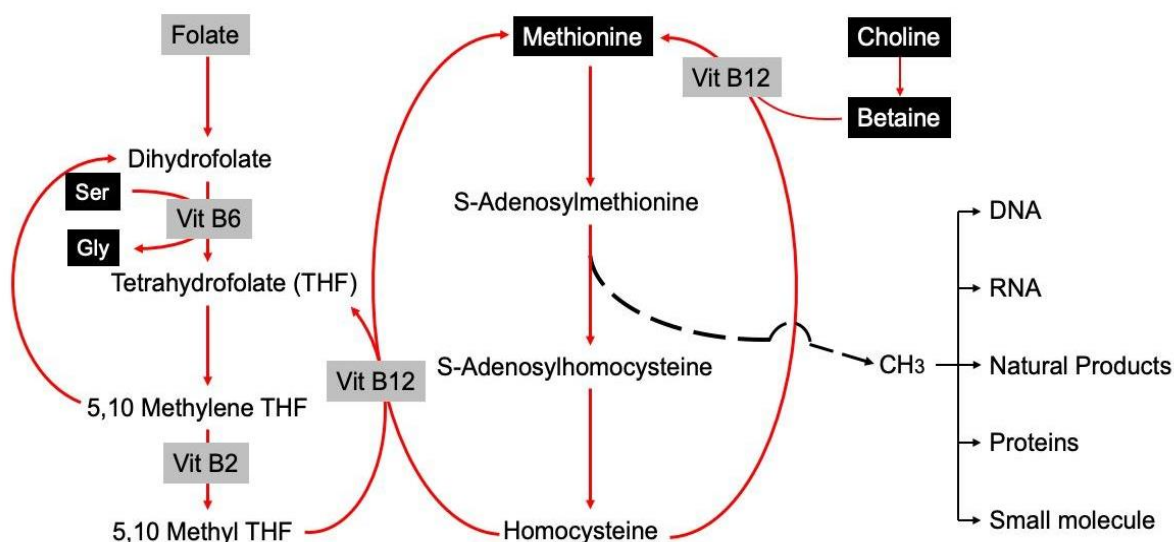
**Natural Bioactives:** Natural bioactives can be classified into (a) micronutrients (i.e., vitamins, essential fatty and

amino acids, minerals) (2); (b) phytonutrients (e.g., polyphenols) (3); (c) bioactive peptides (4); and (d) pre-, pro-, post- and synbiotics (5). While the discovery and characterization of single micronutrients have been largely accomplished in the 20<sup>th</sup> century, their interactive functions and holistic benefits for health have only become recently appreciated (6), within the concept of 'systems health': "we are not consuming single molecules, but complex diets". Phytonutrients form an established class of (e.g., immune and metabolic) health-beneficial compounds, yet the vast majority of these bioactives remains to be discovered, largely because of the immense biosynthetic reservoir of the plant kingdom. Likewise, bioactive peptides have remained largely underexploited as molecular means for health promotion, mainly due to the following reasons (7): poor bioavailability after oral consumption due to breakdown along the gastrointestinal tract; limited transport from gut to blood; and inefficient discovery and translation based on serendipitous research and/or high-throughput screening. Pre-, pro-, syn- and postbiotics, as parts of the intestinal microbiome, are increasingly being appreciated as molecular or bacterial health promoters (5). There are many associations between population census-based microbiome sequencing studies and various human organ systems and health conditions that have been established. However, the complexity of the (human) microbiome requires an ecosystem-level understanding and establishing directional relationships between microbiome composition, including probiotic players and prebiotic substrates, and health effects has been challenging.

**Micronutrients:** Micronutrients- or essential nutrients- encompass vitamins, essential fatty and amino acids, as

well as minerals (trace elements). They typically function as co-factors of enzymes or key building blocks in metabolic pathways and are essential to sustain (human) life. In contrast to macronutrients (bulk carbohydrates, fat, and protein), they are not required in large daily amounts (grams), rather the small, required quantities (mg to mg) need to be provided through proper dietary intake or supplementation daily because the human body cannot synthesize these micronutrients in sufficient amounts (8). Other nutrients may also become essential under certain conditions, such as in diseased states, when the body cannot maintain sufficient levels of micronutrient synthesis.

The advent and development of the disciplines 'Molecular Nutrition' and 'Nutrigenomics' revealed that micronutrients can also influence epigenetic status of the host. Epigenetic processes encompass DNA methylation, post-translational histone modifications, and regulatory microRNAs. Methylation in CpG-enriched regions can regulate gene expression and is essential for cell differentiation and tissue integrity. The effect of methylation on gene expression depends on location: high methylation levels in promoters normally repress gene transcription; methylation within introns and exons typically enhance gene expression. The connection between micronutrients and epigenetics is the one carbon pathway (Fig. 1), which produces the methyl group donor for more than 150 transmethylation enzymes, including DNA methyltransferases and histone protein transmethylases, which are centrally involved in epigenetic processes. This pathway is fueled at multiple steps by dietary components including essential nutrients (vitamins and choline) and amino acids (Fig. 1) (9).



**Figure 1.** One-carbon and tetrahydrofolate cycles. Dietary components are depicted in boxes and consist of vitamins (grey boxes) and other organic molecules (black boxes). Methyltransferases use S-adenosylmethionine (SAM) to transfer a methyl group to macro- and small molecules [reproduced from (9)].

The further conceptual development of ‘Molecular Nutrition’ and ‘Nutrigenomics’ into ‘Systems Nutrition’ (10) expanded from the previous reductionist approaches and binary relationships of single micronutrients and their association with single genes and conditions (e.g. deficiencies): it became apparent that micronutrients also interact, i.e. the combination of micronutrient levels matters more than concentration values of individual compounds, and both bioavailability and bioefficacy of micronutrients depend on ethnic ancestry and individual genetic profile (11), as well as the individual (gut) microbiome (12). Moreover, micronutrients require variable amounts of time to see changes in health and risk for disease (13). This advancement also incorporated the appreciation of single ‘parent’ vitamins encompassing a range of ‘vitamers’. For example, different sub-forms or metabolites derived from the parent molecule upon intake and metabolism, with specific bioavailability and bioefficacy properties (14). These principles were incorporated into an intervention study designed to

develop better recommendations for food bioactives for populations and individuals (6).

**Phytonutrients:** Plants have played a vital role in human health for thousands of years and have yet to be used to their full potential. Phytonutrients are natural compounds found in plant foods, such as vegetables, fruit, whole grain products and legumes. Phytonutrients include carotenoids, flavonoids, and isothiocyanates. These plant compounds can exert beneficial health effects in cooperation with other essential nutrients. These effects range from antioxidative to anti-inflammatory and immune-modulatory, via neuroprotective to hormone-controlling (3).

Polyphenols are a large family of plant-derived antioxidants involved in defense against ultraviolet radiation or pathogens. Polyphenols from cocoa, mainly flavanols, may affect risk factors for chronic conditions in humans, including high blood pressure, dyslipidemia, inflammation, insulin resistance, vascular reactivity, and other oxidative stress-related conditions. Health benefits

of cocoa polyphenols are widely documented in epidemiological and experimental studies and robust evidence supports their effects on cardiovascular, immunological, and digestive health (15).

For example, a randomized, controlled, double-blind, cross-over, clinical trial in healthy young adults who consumed a single dose of high-polyphenols cocoa, followed by a one-week washout period, was performed (16). Analysis of circulating metabolites, plasma antioxidant capacity and gene expression changes in PBMCs were performed under fasting conditions and two hours after treatment. While no association was found between conjugated metabolites in plasma and antioxidant capacity, the PBMC gene expression changes indeed indicated anti-inflammatory effects. With the bioavailability of cocoa polyphenols, particularly of the monomer (-)-epicatechin, having been investigated after a single-dose intake, the effect of sustained cocoa consumption on the metabolic profile of the structurally related (-)-epicatechin metabolites (SREMs) was investigated in a related randomized, controlled crossover clinical trial in healthy young adults after consumption of a single-dose and after daily consumption of 1.3 g of polyphenol-rich cocoa powder for 28 days. The individual SREMs profile and concentrations after a 28-day supplementation were found comparable to those after a single dose (15).

While phytonutrients form an established class of health-beneficial compounds, the vast majority of these bioactives remains to be discovered, largely because of the immense biosynthetic reservoir of the plant kingdom. In addition, the for-long inefficient discovery and translation, historically based upon *ad hoc* research and later accelerated and scaled up with bottom-up high-throughput screening. However, while HTS clearly accelerated compound discovery, it didn't widen the bottleneck from compound identification to health benefit association and clinical translation. This may be

due in part to the "bottom-up design of discovery", in which the (vast) natural product space is harvested, fractionated, and characterized biochemically and the isolates/fractions/compounds are tested for bioactivity *in vitro* before assigning potential benefits to them and proceeding to (pre-)clinical investigation.

The development and application of artificial intelligence, enabling the computational prediction of structure, function, and source of natural bioactives and the design-for-benefit approach is emerging as a game-changer that can significantly accelerate discovery and translation of bioactives and thereby dramatically reduce the time from concept to (prototype) product (17). This approach is not only being pursued in academic settings, but also being developed by biotechnology companies. Brightseed, for example, deploys a proprietary digital platform that elucidates the interaction between plant bioactives and human biology by predicting bioactive plant compounds and how these impact specific health conditions. Using artificial intelligence, they accelerate the discovery of natural health compounds (especially phytonutrients), detect new benefits for existing plant-based ingredients, and identify plant sources that contain the highest abundance of beneficial bioactives.

**Bioactive peptides:** Natural bioactive plant and food peptides are safe and efficacious means for molecular delivery of health benefits, plus they are laid up for *in silico* discovery and prediction, for several pertinent reasons (18):

- Peptides are "small proteins" and the "language of nature": living systems use peptides to communicate and regulate their functions.
- Peptides co-evolved with mankind as regulators and modulators of human physiology.
- Peptides exert highly specific biological functions.

- The presence in natural sources (e.g., plant and food) and the biological function of peptides can be largely predicted *in silico*.
- From a food perspective, peptides are considered nutrients--the only nutrients that are directly encoded in the genome.
- Orally administered peptides typically have a short half-life across ingestion and digestion: the challenge of using peptides as orally delivered bioactives lies in their stability, bioavailability and bioefficacy, rather than in their safety.
- Food peptides are components of long-term consumed food sources and can therefore be "generally recognized as safe" (GRAS).
- However, bioactive peptides have remained largely underexploited as molecular means for health promotion, mainly due to the following reasons (7):
  - general poor bioavailability after oral consumption due to breakdown along the gastrointestinal tract.
  - Limited transport from gut to blood
  - Inefficient discovery and translation based on *ad hoc* research and/or high-throughput screening.

While peptides are present and used abundantly in foods, they have typically been discovered and identified serendipitously or through traditional use. Currently, the most obvious applications of peptides in food are in infant formula, sports nutrition foods and dietary supplements (7). Fermented dairy products provide health benefits due to their peptide content. For example, peptides (in the form of hydrolyzed vegetable proteins) provide savory flavors for traditional and processed foods, while other (anti-microbial) peptides produced by the cheese-making process protect the food from spoilage. Furthermore, food-derived peptides are widely thought to not only be a source of additional health benefits but to be a potential solution to the removal or

replacement of artificial preservatives (19), reduction of sugar and salt, and to aid production of cultured meat proteins.

Protein hydrolysates containing food peptides also have a long history of global consumption. For example, in infant formulae based on soy or rice protein hydrolysates to avoid cow's milk allergy and in fermented foods (yogurt, kefir, kimchi, tempeh, tofu, natto, pickled vegetables) containing bioactive peptides with known health benefits. The safety of food protein hydrolysates has been extensively assessed in pre-clinical and clinical studies and they are regulated as food hydrolysates rather than drugs (20).

After the initial period of serendipitous discovery of natural bioactives (including peptides), high-throughput screening (HTS) has greatly advanced the speed and efficiency of the process. It remains the attempt to capture and leverage the molecular complexity and diversity of nature by large scale and high automation. However, the translation of these molecular discoveries into practice for consumer and patient care has been limited-- especially in nutrition. In contrast to pharmaceutical applications, it was found that compound purity does not necessarily correlate with compound bioactivity and efficacy. Food bioactives exert multiple, individually subtle effects that -- only when combined -- may converge to a profound and lasting health benefit (1). Hence, it is often a blend of many bioactives that makes the difference. Moreover, HTS takes a brute-force approach to the harvesting, fractionation, and characterization of bioactives without much upfront guidance for possible translation and application, thereby resulting in a (very) high number of required bioactivity and bioefficacy tests, which are typically lower in throughput than the chemical screening (21).

By placing artificial intelligence (AI) in front of the peptide discovery and development process, the path

from idea to solution can be shortened from decades to a few years. The number of “wet laboratory” experiments and (pre-)clinical studies, typically being the time- and cost-determining factors, can be dramatically reduced by upfront intelligent *in silico* design (22): starting with a desired and defined benefit, be it for human/animal health or a healthier and more sustainable food solution, bioactive peptides can be predicted to exert such function, and then be tested *in vitro*; the iterative and integrated cyclic process of such prediction and testing can readily generate – *via* a refined search – a good number of potent bioactive candidates to be finally validated *in vivo*, often directly in human (23), or in a food technology setting (19). Consequently, AI can indeed empower bioactive peptide discovery, food ingredient and dietary supplement design, manufacturing, and clinical validation (24). An AI-discovered, natural bioactive peptide was recently taken from concept to a clinically proven product (25,26): the target benefit was the promotion of muscular health in humans, specifically improved muscle recovery after immobilization or strenuous exercise. Peptides were predicted to positively modulate the molecular pathways involved in muscle atrophy, muscle protein synthesis and controlling inflammation. The peptides were tested individually *in vitro*. Then, the best natural (plant/food) source containing these peptides was identified and a hydrolysis process was designed to free these peptides from their parent proteins. The final hydrolysate was administered as a powder in human clinical trials on muscle strength recovery post-immobilization and after strenuous exercise, comparing the designed bioactive peptide blend to a milk protein concentrate, i.e., the current standard-of-care.

#### **THE HUMAN MICROBIOME AND PRE-, PRO-, POST-, AND SYNBIOTICS**

**Human microbiome:** Human microbiotas are communities of bacteria, fungi, protists, archaea and viruses (referred to as the bacteriome, mycobiome,

protistome, archaeome, and virome, respectively) that live on and/or inside the human body. These microbial communities exist on every mucosal surface in and on the human body, and each body site within the same person has an own ecology. Likewise, everyone’s microbial community is different from other humans. Human-resident micro-organisms encode an estimated two to twenty million genes, whereas the human genome encodes only an estimated twenty to twenty-five thousand. The microbiome represents up to 99.9% of the genetic capacity in the human body. Microbial communities are dynamic and change within and between each stage of life, from birth to death (27). By far, the most heavily colonized organ is the gastrointestinal tract (GIT), which harbors a complex microbial ecosystem. Surprisingly, the colon is estimated to contain over 70% of all microbes in the human body. The human gut is dominated by several bacterial phyla including *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. Investigating, understanding, and leveraging the natural and induced changes in our microbiotas can revolutionize our comprehension of human biology (28).

Metagenomic analysis of the human gut microbiome has revealed millions of genes and evidence for beneficial roles of the intestinal microbiome in human health and disease is rapidly increasing. Perturbation of the intestinal microbiota may lead to chronic conditions, such as autoimmune diseases, colon cancers, gastric ulcers, bowel diseases, cardiovascular and metabolic diseases. *Via* the gut-brain axis, the intestinal microbiome has also been associated with neurological and brain disorders. Association is critical, as many of the large-scale clinical studies revealed associations between gut microbial communities (“population census”) and conditions, rather than directional causality, which refers to the microbiome either giving rise to or being a consequence of the condition (29).

With the understanding and definition of a 'healthy (gut) microbiome' still pending, restoration of a healthy microbiome is difficult to accomplish without knowing the target(s). However, the administration of probiotics has shown encouraging results in (clinical) studies regarding enhancing the prevalence of health-beneficial bacteria within the gut microbiome (5). Microbiomics has triggered increasing scientific, industrial, and public interest in probiotics and prebiotics as possible agents for gut microbiome management and control. Genomic and bioinformatic tools facilitate the establishment of mechanistic relationships within the gut microbial ecosystem and their effects on the host's health status. As a consequence, several biotech companies have emerged in the area of personalized gut microbiome management (e.g., MRM Health WildBio Tech Seres Therapeutics).

**Probiotics:** The term probiotic is derived from the two Greek words, "pro" and "bios", meaning "for life". An expert panel was established in 2013 by the International Scientific Association for Probiotics and Prebiotics (ISAPP) to structure the field of probiotics. According to the FAO/WHO, a probiotic is defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" the FAO/WHO definition has been widely adopted by organizations and agencies, such as Codex (under the FAO/WHO umbrella), Health Canada, the World Gastroenterology Organization, the European Food Safety Authority (EFSA) and the Institute of Food Technologists. This definition is inclusive of a broad range of microbes and applications, whilst capturing the essence of probiotics: microbial, viable, and beneficial to host health.

**Prebiotics:** In 1920, Cheplin et al. reported that after consumption of carbohydrates the human intestinal

microbiota was enriched with lactic bacteria (30). Consistent with the original embodiment of prebiotics, yet in view of the latest scientific and clinical developments, the ISAPP updated the definition of a prebiotic in 2017 (31): "a substrate that is selectively utilized by host micro-organisms conferring a health benefit". This definition expands the term 'prebiotics' to possibly include non-carbohydrate substances, applications to body sites outside of the gastrointestinal tract, and diverse categories beyond food. However, the requirement for specific microbiome-mediated mechanisms was conserved and beneficial health effects must be demonstrated for a substance to be considered a prebiotic.

**Synbiotics:** In 2019, the ISAPP panel updated the definition of a synbiotic to "a mixture comprising live microorganisms and substrate(s) selectively utilized by host micro-organisms that confers a health benefit on the host" (32). It was clarified that a complementary synbiotic, not designed for its components to function cooperatively, must be composed of a probiotic and a prebiotic. A synergistic synbiotic is a synbiotic for which the substrate is designed to be selectively utilized by the co-administered microorganisms.

A particularly well-investigated field of synbiotics has been established around human milk oligosaccharides (HMOs) and early, health-beneficial colonizers of the intestine of the newborn, which is sterile at birth (33). Human milk is uniquely rich in complex carbohydrates (34). *Lactobacilli* and *Bifidobacteria* have been identified as the pioneers of infant gut colonization. Combining *in vitro* microbiology with high-resolution mass spectrometry, specific HMOs were identified substrates and nutrients for specific bacteria, thereby molecularly establishing a synbiotic relationship (35). Such HMOs are meanwhile synthesized from cow's milk oligosaccharides (CMOs) for use in infant

formulae to better match the nutritive value of breast milk (36).

**Postbiotics:** The ISAPP also reviewed the definition and scope of postbiotics, considering the scientific, commercial, and regulatory parameters encompassing this emerging term. The panel defined a postbiotic as a “preparation of inanimate micro-organisms and/or their components that confers a health benefit on the host” (37). Effective postbiotics must contain inactivated microbial cells or cell components, with or without metabolites, that contribute to observed health benefits. The health benefits conferred by pro-, pre-, syn- and postbiotics have been extensively investigated. These biotic food supplements have been demonstrated to modify or restore the pre-existing intestinal microbiome and to facilitate smooth functioning of the intestinal environment. Commonly used probiotic strains are *Bifidobacteria* and *Lactobacilli*. Prebiotics like FOS, GOS, XOS, inulin, and fructans are the most used fibers which, when used in combination with probiotics, can work as synbiotics that can enhance the viability of the probiotics. The aforementioned biotics have been identified, investigated, and mainly applied to improve gut, immune, and metabolic health.

A conceptually and strategically new approach to discovery and design of pre-, pro- and synbiotic blends has recently been undertaken with the objective of developing an immune-beneficial weaning diet for infants (38). Weaning is a period of marked physiological change. The introduction of solid foods and the changes in milk consumption are accompanied by significant gastrointestinal, immune, developmental, and microbial adaptations (39). Defining a reduced number of infections and a better vaccine response as desired health benefit for infants around weaning, the group identified *in silico* (i.e. by advanced public domain mining) infant gut microbes as potential deliverers of

these benefits. Using public domain literature mining and *in silico* reverse metabolic engineering, the team constructed probiotic-prebiotic-food associations, which can guide targeted feeding of immune health-beneficial microbes by weaning food. Competition and synergy for prebiotic nutrients between selected microbes were also analyzed. Finally, this information was translated into designing an experimental complementary feed for infants enrolled in a pilot clinical trial (40), which was followed by a full trial analyzing the gut microbial changes in the infants subjected to designed complementary feeding (41).

#### ***Pro-, pre-, syn- and postbiotics in the human microbiome***

**context:** Current applications of pro- and prebiotics to induce functional changes in the microbiome and its crosstalk with the host to improve insulin sensitivity and lipid metabolism are promising options for diabetes and obesity management. The gut–brain axis encompasses interactions between the enteric and autonomic nervous systems, pituitary and gut hormones, and gut inflammatory status. Together, this system can influence nerve function and ultimately, behavior, in early life. Examples include probiotic modulation of the microbiome to influence cholecystokinin (CCK) output (which in turn regulates vagal nerve transmissions involved in satiety regulation and prebiotics-mediated, fermentative production of SCFAs and, thereby, gut hormone release, which in turn modulate hypothalamic neuronal activity involved in appetite regulation (5).

Future applications of pro- and prebiotics may well extend beyond management of gastrointestinal conditions, as the role of the microbiome in other organs such as the peripheral and central nervous system is better understood. These new avenues may include chronic immune disorders, psychological behaviors, or psychiatric conditions. Standardized pro-/prebiotic study designs and protocols and defined test agent

characteristics are crucial. Essentially, the quality of pro- and prebiotic research must meet the standards for either evidence-based nutrition or medicine (5).

Meanwhile, there are many biotech start-ups and SMEs developing pre- and probiotics-based solutions for consumer and patient care. The research portfolio of these companies ranges from pre-/probiotics prediction and discovery, via *in vitro* and *in vivo* validation to specialist formulation and production. The application formats span from dietary supplements (typically as pills) to capsules for fecal transplantation. For example, Wild Biotech Ltd. explores the microbiome of wild animals with particularly resilient phenotypes to mine, predict and discover microbial bioactives that may/can confer this resilience and translate these discoveries into human health applications. MRM Health specializes in designing defined blends of probiotics with combined human health benefits, which are tested in an advanced *in vitro* human GI system and then validated in human trials.

## CONCLUSION

Nature holds an enormous reservoir of bioactives that can be leveraged for humans and animal health and food sustainability. Plants and foods constitute a vast and sustainable resource for micronutrients, phytonutrients, bioactive peptides, and prebiotics. While the possibilities seem unlimited, the leverage has been limited, mainly due to the complexity of the natural product space and inefficient discovery, validation, and translation. Artificial intelligence-based, benefit-directed design approaches are emerging and future means for accelerating the leverage of natural bioactives.

Natural bioactives in (plants) foods come in blends, interact with each other, the host and its microbiome, and typically exert subtle long-term effects. Their health benefits depend on the compound type, the delivery format (food ingredient or dietary supplement), the oral delivery mode and, eventually, on compound

bioavailability and bioefficacy. *In vivo* bioavailability and bioefficacy are in turn subjected to population and individual genetics. In view of this complexity in biochemical space and over biological time, the 'systems nutrition' approach is required to better understand and leverage the food bioactive space, rather than reductionist studies of seeming binary relationships between single or a few bioactives, genes, and conditions.

In the long-term, comprehensive leverage of the natural product space from sustainable plants and foods bears the potential to contribute significantly to more preventive health care, a more sustainable health care system, better population and individual health, and a more prudent use of our planetary resources.

**Abbreviations:** AI: artificial intelligence, CCK: cholecystokinin, CMO: cow's milk oligosaccharide, EFSA: European Food Safety Authority, FAO: Food and Agriculture Organization of the United Nations, FOS: fructo-oligosaccharide, GOS: galacto-oligosaccharide, GIT: gastrointestinal tract, GRAS: generally recognized as safe, HTS: high-throughput screening, HMO: human milk oligosaccharide, ISAPP: International Scientific Association for Probiotics and Prebiotics, PBMC: peripheral blood mononuclear cell, SAM: S-adenosylmethionine, SCFA: short-chain fatty acid, SMEs: small and medium-sized enterprises, SREM: structurally related (-)-epicatechin metabolite, WHO: World Health Organization, XOS: xylo-oligosaccharide.

**Author contributions:** MK has conceptualized and drafted the manuscript. DHAC provided the illustrations and contributed to the concept, writing, and finalizing of the manuscript.

**Competing Interests:** There are no conflicts of interest to declare.

**Acknowledgements/Funding:** N.A. Manuscript publication costs covered by Kussmann Biotech GmbH.

## REFERENCES

- Kussmann M, van Bladeren PJ. The extended nutrigenomics - understanding the interplay between the genomes of food, gut microbes, and human host. *Front Genet.* 2011;2(MAY). DOI: <https://doi.org/10.3389/fgene.2011.00021>.
- Monteiro JP, Kussmann M, Kaput J. The genomics of micronutrient requirements. Vol. 10, *Genes and Nutrition*. Springer Verlag; 2015. DOI: <https://doi.org/10.1007/s12263-015-0466-2>
- Hoang T, Kim J. Phytonutrient supplements and metabolic biomarkers of cardiovascular disease: An umbrella review of meta-analyses of clinical trials. *Phytotherapy Research.* 2021 Aug 16;35(8):4171–82. DOI: <https://doi.org/10.1002/ptr.7079>.
- Sun X, Udenigwe CC. Chemistry and Biofunctional Significance of Bioactive Peptide Interactions with Food and Gut Components. *J Agric Food Chem.* 2020 Nov 18;68(46):12972–7. DOI: <https://doi.org/10.1021/acs.jafc.9b07559>.
- Petschow B, Doré J, Hibberd P, Dinan T, Reid G, Blaser M, et al. Probiotics, prebiotics, and the host microbiome: The science of translation. *Ann N Y Acad Sci.* 2013;1306(1):1–17. DOI: <https://doi.org/10.1111/nyas.12303>.
- Mathias MG, Coelho-Landell C de A, Scott-Boyer MP, Lacroix S, Morine MJ, Salomão RG, et al. Clinical and Vitamin Response to a Short-Term Multi-Micronutrient Intervention in Brazilian Children and Teens: From Population Data to Interindividual Responses. *Mol Nutr Food Res.* 2018 Mar 1;62(6). DOI: <https://doi.org/10.1002/mnfr.201700613>.
- Hayes M. Food proteins and bioactive peptides: New and novel sources, characterization strategies and applications. Vol. 7, *Foods*. MDPI Multidisciplinary Digital Publishing Institute; 2018. DOI: <https://doi.org/10.3390/foods7030038>.
- Barkhidarian B, Roldos L, Iskandar MM, Saedisomeolia A, Kubow S. Probiotic Supplementation and Micronutrient Status in Healthy Subjects: A Systematic Review of Clinical Trials. *Nutrients.* 2021 Aug 28;13(9):3001. DOI: <https://doi.org/10.3390/nu13093001>.
- Kaput J, Monteiro JP, Morine MJ, Kussmann M. Personalized nutrition. In: *Reference Module in Biomedical Sciences*. Elsevier; 2022. DOI: <https://doi.org/10.1016/j.copbio.2008.02.005>.
- Kaput J, Perozzi G, Radonjic M, Virgili F. Propelling the paradigm shift from reductionism to systems nutrition. Vol. 12, *Genes and Nutrition*. BioMed Central Ltd.; 2017. DOI: <https://doi.org/10.1186/s12263-016-0549-8>
- Fuzo CA, da Veiga Ued F, Moco S, Cominetti O, Métairon S, Pruvost S, et al. Contribution of genetic ancestry and polygenic risk score in meeting vitamin B12 needs in healthy Brazilian children and adolescents. *Sci Rep.* 2021 Dec 1;11(1). DOI: <https://doi.org/10.1038/s41598-021-91530-7>
- Barone M, D'Amico F, Brigidi P, Turrone S. Gut microbiome–micronutrient interaction: The key to controlling the bioavailability of minerals and vitamins? *BioFactors.* 2022 Mar 16;48(2):307–14. DOI: <https://doi.org/10.1002/biof.1835>.
- Mathias MG, Coelho-Landell C de A, Scott-Boyer MP, Lacroix S, Morine MJ, Salomão RG, et al. Clinical and Vitamin Response to a Short-Term Multi-Micronutrient Intervention in Brazilian Children and Teens: From Population Data to Interindividual Responses. *Mol Nutr Food Res.* 2018;62(6). DOI: <https://doi.org/10.1002/mnfr.201700613>.
- Andraos S, Jones B, Wall C, Thorstensen E, Kussmann M, Smith DC, et al. Plasma b vitamins: Population epidemiology and parent-child concordance in children and adults. *Nutrients.* 2021 Mar 1;13(3):1–15. DOI: <https://doi.org/10.3390/nu13030821>
- Barrera-Reyes PK, Cortés-Fernández de Lara J, Poquet L, Redeuil K, Kussmann M, Silva-Zolezzi I, et al. Circulating structurally related (-)-epicatechin metabolite species and levels after sustained intake of a cocoa powder high in polyphenols are comparable to those achieved after a single dose. *Nutrients.* 2021 Nov 1;13(11). DOI: <https://doi.org/10.3390/nu13113829>
- Barrera-Reyes PK, Hernández-Ramírez N, Cortés J, Poquet L, Redeuil K, Rangel-Escareño C, et al. Gene expression changes by high-polyphenols cocoa powder intake: a randomized crossover clinical study. *Eur J Nutr.* 2019;58(5). DOI: <https://doi.org/s00394-018-1736-8>.
- Schlöpfer P, Zhang P, Wang C, Kim T, Banf M, Chae L, et al. Genome-wide prediction of metabolic enzymes, pathways, and gene clusters in plants. *Plant Physiol.* 2017 Apr 1;173(4):2041–59.

- DOI: <https://doi.org/10.1104/pp.16.01942>
18. Daliri EBM, Lee BH, Oh DH. Current trends and perspectives of bioactive peptides. Vol. 58, Critical Reviews in Food Science and Nutrition. Taylor and Francis Inc.; 2018. p. 2273–84. DOI: <https://doi.org/10.1080/10408398.2017.1319795>.
  19. Mohan NM, Zorgani A, Earley L, Chauhan S, Trajkovic S, Savage J, et al. Preservatives from food—For food: Pea protein hydrolysate as a novel bio-preservative against *Escherichia coli* O157:H7 on a lettuce leaf. Food Sci Nutr. 2021 Nov 1;9(11):5946–58.  
DOI: <https://doi.org/10.1002/fsn3.2489>
  20. Schaafsma G. Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. Vol. 63, European Journal of Clinical Nutrition. 2009. p. 1161–8. DOI: <https://doi.org/10.1038/ejcn.2009.56>.
  21. Beutler JA. Natural Products as a Foundation for Drug Discovery. Curr Protoc Pharmacol. 2019 Sep 26;86(1). DOI: <https://doi.org/10.1002/cpph.67>
  22. Doherty A, Wall A, Khaldi N, Kussmann M. Artificial Intelligence in Functional Food Ingredient Discovery and Characterization: A Focus on Bioactive Plant and Food Peptides. Vol. 12, Frontiers in Genetics. Frontiers Media S.A.; 2021. DOI: <https://doi.org/10.3389/fgene.2021.768979>.
  23. Kennedy K, Keogh B, Lopez C, Adelfio A, Molloy B, Kerr A, et al. An Artificial Intelligence Characterized Functional Ingredient, Derived from Rice, Inhibits TNF- $\alpha$  and Significantly Improves Physical Strength in an Inflammaging Population. Foods. 2020 Sep 1;9(9).  
DOI: <https://doi.org/10.3390/foods9091147>
  24. Casey R, Adelfio A, Connolly M, Wall A, Holyer I, Khaldi N. Discovery through machine learning and preclinical validation of novel anti-diabetic peptides. Biomedicines. 2021 Mar 1;9(3).  
DOI: <https://doi.org/10.3390/biomedicines9030276>.
  25. Corrochano AR, Cal R, Kennedy K, Wall A, Murphy N, Trajkovic S, et al. Characterising the efficacy and bioavailability of bioactive peptides identified for attenuating muscle atrophy within a *Vicia faba*-derived functional ingredient. Curr Res Food Sci. 2021 Jan 1;4:224–32. DOI: <https://doi.org/10.1016/j.crf.2021.03.008>
  26. Cal R, Davis H, Kerr A, Wall A, Molloy B, Chauhan S, et al. Preclinical evaluation of a food-derived functional ingredient to address skeletal muscle atrophy. Nutrients. 2020 Aug 1;12(8):1–17.
  - DOI: <https://doi.org/10.2174/1566523214666141127095336>
  27. Martino C, Dilmore AH, Burcham ZM, Metcalf JL, Jeste D, Knight R. Microbiota succession throughout life from the cradle to the grave. Nat Rev Microbiol. 2022 Jul 29; DOI: <https://doi.org/10.1038/s41579-022-00768-z>.
  28. Kelly P, Alderton G, Thomas Scanlon S, Ash C. A MULTIPLICITY OF MICROBIOMES. Science (1979). 2022 May 27;376(6596):932–3.  
DOI: <https://doi.org/10.1126/science.adc9690>.
  29. Surana NK, Kasper DL. Moving beyond microbiome-wide associations to causal microbe identification. Nature. 2017 Dec 14;552(7684):244–7.  
DOI: <https://doi.org/10.1038/nature25019>.
  30. Cheplin HA, Rettger LF. Studies on the Transformation of the Intestinal Flora, with Special Reference to the Implantation of *Bacillus Acidophilus*. Proceedings of the National Academy of Sciences. 1920 Dec;6(12):704–5.  
DOI: <https://doi.org/10.1073/pnas.6.12.704>.
  31. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Vol. 14, Nature Reviews Gastroenterology and Hepatology. Nature Publishing Group; 2017. p. 491–502.  
DOI: <https://doi.org/10.1038/nrgastro.2017.75>.
  32. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Vol. 17, Nature Reviews Gastroenterology and Hepatology. Nature Research; 2020. p. 687–701. DOI: <https://doi.org/10.1038/s41575-020-0344-2>.
  33. de Leoz MLA, Kalanetra KM, Bokulich NA, Strum JS, Underwood MA, German JB, et al. Human milk glycomics and gut microbial genomics in infant feces show a correlation between human milk oligosaccharides and gut microbiota: a proof-of-concept study. J Proteome Res. 2015 Jan 2;14(1):491–502.  
DOI: <https://doi.org/10.1021/pr500759e>.
  34. Xu G, Davis JC, Goonatilake E, Smilowitz JT, German JB, Lebrilla CB. Absolute Quantitation of Human Milk Oligosaccharides Reveals Phenotypic Variations during

- Lactation. *J Nutr.* 2017;147(1):117–24. DOI: <https://doi.org/10.3945/jn.116.238279>.
35. Underwood MA, Gaerlan S, de Leoz MLA, Dimapasoc L, Kalanetra KM, Lemay DG, et al. Human milk oligosaccharides in premature infants: absorption, excretion, and influence on the intestinal microbiota. *Pediatr Res.* 2015 Dec;78(6):670–7. DOI: <https://doi.org/10.1038/pr.2015.162>.
  36. Weinborn V, Li Y, Shah IM, Yu H, Dallas DC, German JB, et al. Production of functional mimics of human milk oligosaccharides by enzymatic glycosylation of bovine milk oligosaccharides. *Int Dairy J.* 2020 Mar;102. DOI: <https://doi.org/10.1016/j.idairyj.2019.104583>.
  37. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Vol. 18, *Nature Reviews Gastroenterology and Hepatology.* *Nature Research*; 2021. p. 649–67. DOI: <https://doi.org/10.1038/s41575-021-00440-6>.
  38. Michelini S, Balakrishnan B, Parolo S, Matone A, Mullaney JA, Young W, et al. A reverse metabolic approach to weaning: In silico identification of immune-beneficial infant gut bacteria, mining their metabolism for prebiotic feeds and sourcing these feeds in the natural product space. *Microbiome.* 2018;6(1). DOI: <https://doi.org/10.1186/s40168-018-0545-x>
  39. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. *Nat Med.* 2016 Jul 7;22(7):713–22. DOI: <https://doi.org/10.1038/nm.4142>.
  40. Lovell AL, Eriksen H, McKeen S, Mullaney J, Young W, Fraser K, et al. ‘Nourish to Flourish’: complementary feeding for a healthy infant gut microbiome—a non-randomised pilot feasibility study. *Pilot Feasibility Stud.* 2022 May 18;8(1):103. DOI: <https://doi.org/10.1186/s40814-022-01059-3>
  41. McKeen S, Roy NC, Mullaney JA, Eriksen H, Lovell A, Kussman M, et al. Adaptation of the infant gut microbiome during the complementary feeding transition. *PLoS One.* 2022;17(7):e0270213. DOI: <https://doi.org/10.1371/journal.pone.0270213>