Premise of Systems Microbiomics in Improving Health and Related Diagnostics for Human and Companion Animals

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Abstract

Systems microbomics — a comprehensive metabolic and microbiome phenotyping — has a high medical and public profile, as exemplified by the exponential growth of research publications and numerous lay press stories on microbiome-derived metabolites, probiotics and related topics. Research into the function of the host-microbiome interactions and the development of new microbiome-based nutritional products and therapeutics is a new horizon. The enormous diversity, functional capacity and age-associated dynamics of the host metabolome and microbiome, its association with nutrition, health maintenance and diseases ranging from localized gastroenterological disorders to inflammatory, metabolic and hepatic illnesses, make it a priority area of research and development at Nestlé and Nestlé Purina. Routine metabolome and microbiome analysis is poised to become a standard measure in following an individual's health status as well as measuring biomarkers for detecting or managing diseases.

Glossary of Abbreviations
CR: Caloric-Restricted
HDL: High-Density Lipoprotein

Introduction

Long-term restriction of energy intake without malnutrition is a robust intervention that has been shown to prolong life and delay age-related morbidity. However, modeling aging and age-related pathologies presents an analytical challenge due to the complexity of gene-nutrient and environment influences and interactions. Systems microbiomics approach was used to model serum and urinary metabolic phenotypes of caloric-restricted (CR) and pair-housed control-fed Labrador Retriever dogs. Alterations of amino acids, lipoproteins and glucose homeostasis provide further molecular evidence of the metabolic processes associated with the health benefits of long-term CR. Additionally, both aging and diet restriction altered populations of gut microbiota, manifested by variation of aromatic metabolites and aliphatic amine compounds. In summary, systems microbiomics combined with data modeling can lead to development of personalized nutrition that mimics the benefits of CR.

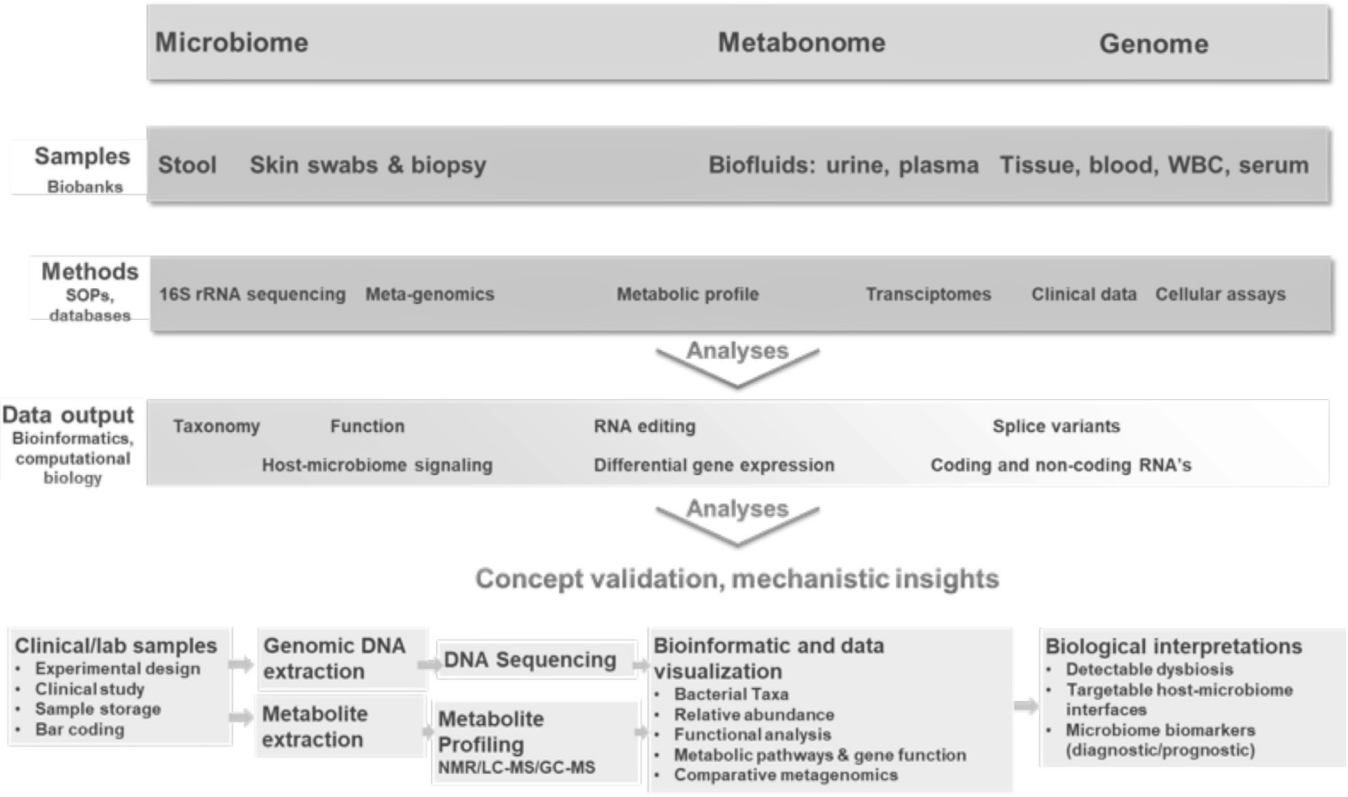
Systems Microbiomics

Scientists' interest in the human metabolome, microbiome and microbial metabolome has grown enormously in recent years. High-resolution ^{1}H NMR spectroscopy is a powerful tool for generating data on a multitude of metabolites in biofluids or tissues. Mass spectrometry when coupled to a liquid chromatography system provides a rapid platform for metabolite profiling at a concentration range of nM to pM. With the advent of ultra-performance liquid chromatograph hyphenated to a triple quad time-of-flight mass spectrometer equipped with an electrospray interface, complementary data to the ^{1}H NMR profile can be generated in 15 to 30 minutes per sample, thus enlarging the metabolite window. The acquired spectral profile of a biofluid, such as urine, plasma or saliva, reflects the metabolic status of the organism. ^{1}H NMR and/or MS spectroscopy of complex biological mixtures coupled with multivariate statistical analysis allow better visualization of the changing endogenous biological profile in response to a physiological challenge or stimulus, such as a disease process, administration of a xenobiotic, environmental stress, genetic modification, changes in nutrition, and other physiological effects.

Recent improvements in DNA sequencing, imaging, data analysis, and computing tools have begun to reveal the breadth of influence that microorganisms have on human and companion animal health. Microbiome analytics comprise standard routines for DNA/RNA preparations followed by 16S rRNA sequencing and/or metagenomics. The data is analyzed by specialized bioinformatics routines to decode genomic and microbiome profiles. Figure 1 describes the flow of the systems microbiomics including the key competencies needed.

Indigenous microbiota and its metabolic activity are essential components of the modern concept of human health, but the composition and functional characteristics of the microbiome/metabolome remain to be elucidated. Different patterns of microbial colonization or metabolic changes associated with disease states have been documented, but the patterns of microbial colonization and functional characteristics associated with health are less well-defined and vary with diet, environment and geography. Additionally, there is no widely accepted definition of a healthy microbiome or metabolome. It is important to point out that the healthy metabolic functions are preserved, even if the bugs themselves vary among otherwise healthy individuals.

Figure 1. Integrative analysis of host genome, metabolome and microbiome including a comprehensive systems microbiomics workflow.



Healthy microbiome could be described: (a) in terms of ecologic stability (i.e., the ability to resist community-structure change under stress or to rapidly return to baseline following a stress-related change), (b) by an idealized (presumably healthier) composition, or (c) by a desirable functional profile including metabolic and synthetic activity. Elucidation of the properties of a healthy metabolome and microbiota would provide a target for dietary interventions and microbial modifications aimed at sustaining health in generally healthy populations.

One major premise in the systems microbiomics research is to decipher host-microbiome metabolic, immune and neuronal signaling, thus allowing its reshaping with diet to improve health. This lends to basically three key research themes:

- Mapping of gut microbiota and metabolic status in healthy, subclinical and diseased subjects to understand its causal role in human health and the onset of disease
- Metabolome/microbiome-based predictive biomarkers of health and disease
- Modulations of gut microbial metabolites to deliver enhanced nutritional benefit

Figure 2. O-PLS-DA plots of ^{1}H NMR spectra of urine obtained from dogs fed with control and restricted diet at age of 13 weeks (A), 1.5 years old (B) and 9 years old (C).

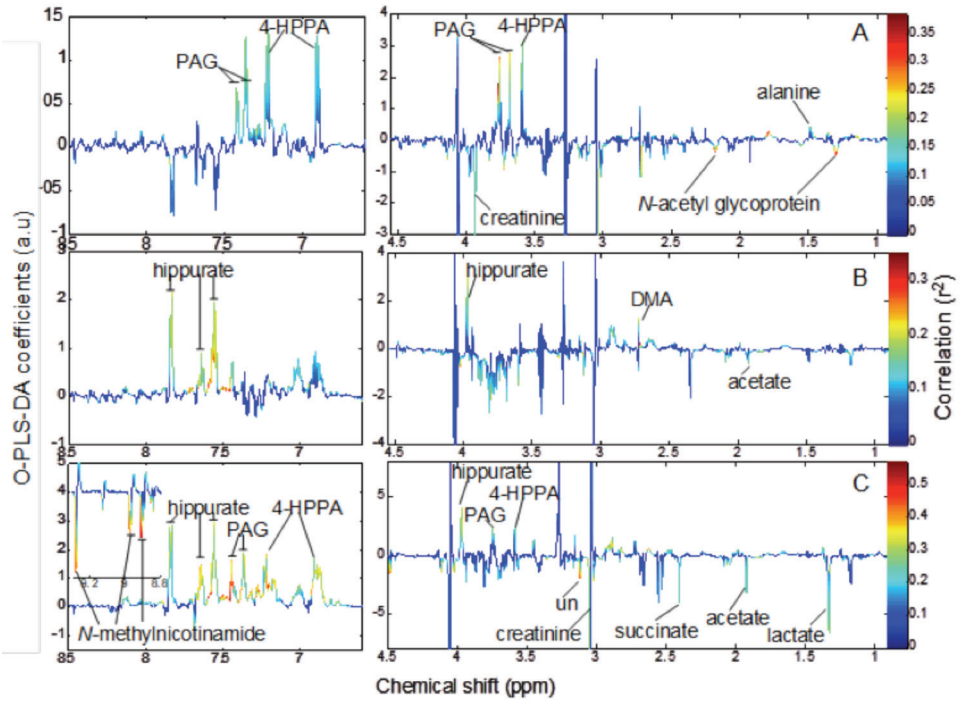
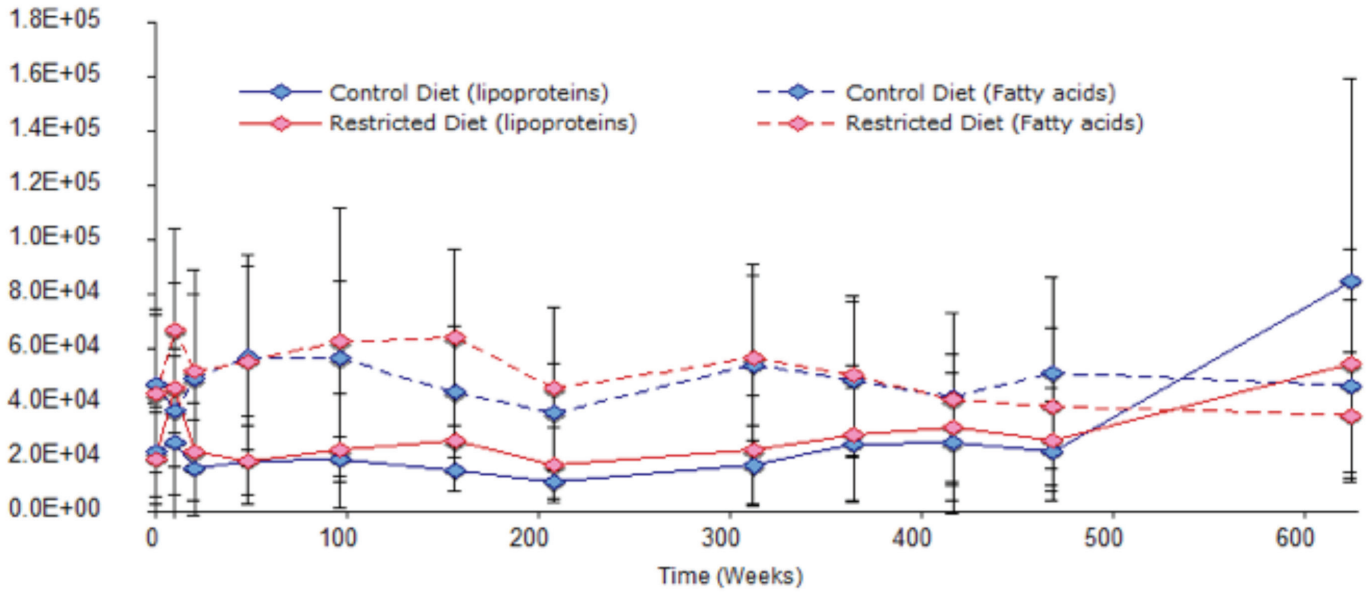


Figure 3. Blood serum metabolic profile of young and old dogs.



Proof of Concept Studies on Caloric Restriction Applying Systems Microbiomics Approach

We have applied systems microbiomics strategy to study the changes in urinary metabolic signatures for the duration of the lives of paired sibling dogs fed as controls or with 25% CR. Age was the dominating factor influencing the metabolic trajectory and was mainly associated with the increased excretion of creatinine up to adulthood, followed by a decrease in later life that occurred roughly in parallel with declining lean body composition. In addition, relatively high excretion of glycoproteins was noted in dogs at early ages. Changes in gut microbiota were associated with both aging and dietary restriction. Additional effects of dietary restriction were associated with reduced energy expenditure manifested by depleted levels of creatine, 1-methylnicotinamide, lactate, acetate, and succinate in urine of dogs fed with CR (see Figure 2). This study also has highlighted the benefits of using systems micobiomics for the detection of subtle physiological changes and dietary effects on mammalian metabolism. The role that gut microflora plays in longevity and quality-of-life responses to CR is potentially important.

^{1}H NMR of blood serum profile of the young and older dogs revealed aging metabolic phenotypes independent of diet characterized by high levels glutamine, creatinine, methylamine, dimethylamine N-oxide, and glycerophosphocholine and by decreasing levels of glycine, aspartate, creatinine, and citrate indicative of metabolic changes associated largely with muscle mass (see Figure 3). We have carried out similar CR studies in mice.

Our work demonstrates the strong potential of systems microbiomics to reveal a global snapshot of the highly dynamic and interconnected metabolic processes of various tissues of an animal, while including the often-ignored interactions with the gut microflora. This provides a valuable tool for the study of aging and its retardation by CR. Our findings provide a view of the changes in energy metabolism and consequential changes in lipoprotein metabolism associated with aging and CR. Beyond their role in lipid transport and metabolism, lipoproteins, especially high-density lipoproteins (HDL), regulate immune processes that impact the development or prevention of many aging-associated diseases.

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References for Further Reading

Lindon JC, Holmes E, Nicholson JK. Metabonomics Techniques and Applications to Pharmaceutical Research and Development. *Pharm Res*. 2006;23:1075-1088.

Martin FP, Wang Y, Sprenger N, et al. Probiotic Modulation of Symbiotic Gut Microbial-Host Metabolic Interactions in a Humanized Microbiome Mouse Model. *Mol Syst Biol*. 2008;4:157.

Rezzi S, Ramadan Z, Fay LB, Kochhar S. Nutritional Metabonomics: Applications and Perspectives. *J Proteome Res*. 2007;6:513-525.

Rezzi S, Ramadan Z, Martin FP, Fay LB. Human Metabolic Phenotypes Link Directly to Specific Dietary Preferences in Healthy Individuals. *J Proteome Res*. 2007;6:4469-4477.

Richards SE, Wang Y, Claus, SP, et al. Metabolic Phenotype Modulation by Caloric Restriction in a Lifelong Dog Study. *J Proteome Res*. 2013;12:3117-3127.

Wang Y, Lawler D, Larson B, et al. Metabonomic Investigations of Aging and Caloric Restriction in a Life-Long Dog Study. *J Proteome Res*. 2007;6:1846-1854.

Metabonomics and Gut Microbiota in Nutrition and Disease. In: Molecular and Integrative Toxicology Series. Kochhar S, Martin FP (eds). New York: Springer Publishing. 2015.