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Microbiota and Cardiovascular Risk: The Missing and Found Link

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Microbiota and us

Our body is inhabited by trillions of bacteria. The term microbiota refers to the myriad of microorganisms that coexists with their hosts. In mammals, they colonize mainly the gastrointestinal tract in mostly anaerobic and rich nutrient environment. The gut microbiota codevelops with the host in a complex interplay between host genome, nutrition, and life-style. The role of gut microbiota in the regulation of multiple host metabolic pathways arise from interactive host-microbiota metabolic, signaling, and immune-inflammatory axes which in turn connect the gut, liver, muscle, and brain [1].

Gut microbiota and host immune system interact from birth. The microbiota shapes the development of the host immune system, and this in turn shapes the composition of the microbiota. This crosstalk is transmitted through hundreds of signaling pathways and different classes of molecules. The effects extend beyond the immune system and act upon multiple organs such as the gut, liver, muscle and brain through host-microbe metabolic axes, exemplified by production of bile acids, choline, and short-chain fatty acids (SCFAs)

that are essential for host health [2]. The production of these metabolites by microbes contributes to the host metabolic phenotype and hence to disease risk. The profound influence of the gut microbiota on the host immune system is strongly associated with long-term health prospects. Although the composition of the core gut microbiota is essentially stable throughout adulthood, there are components that are biologically and metabolically flexible, responding by alteration in species composition to different factors such as environmental stresses or changes in diet. The final effects of these changes may influence health or disease risk [3].

A second genome

Recent studies estimate that the microbiota genome contains 100-fold more genes than the host genome [4]. The development of efficient methods for genome sequencing and bioinformatics analysis enables fast and accurate analysis of the microbiome. The integrated analysis of metagenomic data and metabolic processes provides deeper understanding of the metabolic impact of the metagenome. This integrated analysis shows that microbiome act as a second genome to the host modulating not only metabolic process but extending to host physiology in the most general sense. In addition, this second genome can be transferred between individuals with profound impacts on host phenotype. From this transplantable second genome, some causal mechanisms for metabolic disease have been characterized. For example, the transplanted microbiota from obese to lean mice promoted absorption of monosaccharides from the gut lumen, selectively suppressed the production of fasting-induced adipocyte factor (Fiaf) and induced de novo hepatic lipogenesis and deposition of triglycerides in adipocytes and the liver [5]. On the contrary, germ-free lean mice lacking gut microbiota were resistant to becoming obese on a fat-enriched diet. Phosphorylated adenosine monophosphate – activated protein kinase (AMPK) was increased in skeletal muscle and liver of these mice. These examples show that there is a well established link between gut microbiome and human metabolic processes.

Gut microbiota and metabolic diseases

Over decades, the prevalence of metabolic diseases has steadily increased in developed countries [6]. Poor diet and lack of exercise are behind this phenomenon. Given that gut microbiota is an important environmental factor involved in the regulation of body weight and energy homeostasis, its role in metabolic disease has been explored. Studies in monozygotic and dizygotic twin pairs concordant for leanness or obesity showed that the gut microbiome is shared between the twin pairs in a great proportion [7]. In addition, the intestinal microbiota can cause metabolic disease in mice in relation to their genetic background [8,9]. Although many studies analyzed the microbiota and microbiomes of obese and lean individuals, there is a lack of consensus about specific bacterial species associated to leanness or obesity. However, a central study demonstrated that the intestinal microbiota of obese individuals differed in microbial diversity compared with that of lean persons, with a lower prevalence of Bacteroidetes and a higher prevalence of Firmicutes [10]. Moreover, later studies suggest that gut bacterial richness, expressed as bacterial gene count and regardless of exact composition, associates to metabolic parameters and body weight stability over time. Individuals with a low bacterial richness show more overall adiposity, insulin resistance and dyslipidemia and a more pronounced inflammatory phenotype when compared with high bacterial richness individuals [11]. The obese individuals among the lower bacterial richness group also gain more weight over time. A small double-blinded randomized controlled trial in insulin-resistant males with metabolic syndrome showed that intestinal infusions of allogenic or autologous microbiota from lean donors increase insulin sensitivity of recipients after six weeks. This change was accompanied by a significant increase in intestinal microbial diversity [12].

The origin for dysbiosis and loss of bacterial richness seems to be a complex interplay between diet, inherited microbiota, antibiotic treatments and clinical history, among others. Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria, thanks to their largely impermeable cell wall. High-fat diet also increases the proportion of Gram-negative to Gram-positive microbes in the gut by favoring their growth. Lipopolysaccharide (LPS), a component of the outer membranes of Gram-negative bacteria, generates low-grade chronic inflammation (metabolic endotoxemia) in mice. Metabolic endotoxemia results in insulin resistance [13].

Gut microbiota, diet and atherosclerosis

Causal links between microbiome and cardiovascular disease (CVD) often include host-microbiota co-metabolites involving dietary intake, gut microbiota and liver metabolism. The best studied example is the pro-atherogenic and prothrombotic plasma metabolite trimethylamine N-oxide (TMAO). TMAO is shown to be formed through a cross-organism pathway involving nutrient pre-cursors abundant in a red meat (choline,

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phosphatidylcholine and L-carnitine) and the sequential action of both gut microbiota, initially forming trimethylamine (TMA), and host liver converting TMA into TMAO. Numerous studies reveal an association between systemic TMAO levels and cardiovascular risk in both humans and animals[14]. The concentration of TMAO is elevated in patients with atherosclerosis and directly correlates with pathology. TMAO induces platelet hyper-reactivity increasing thrombosis potential. It also reduces reverse cholesterol transport and induces pro-inflammatory cytokines expression and leukocyte recruitment promoting cholesterol accumulation in the foam cells of atheroma [15]. Interestingly, plasma TMAO levels among patients presenting with acute coronary syndrome may predict both near and long-term adverse cardiovascular events [16]. In addition, patients with heart failure (HF) have high levels of TMAO, which also associates to poorer long-term survival regardless of underlying etiology [17]. Otherhost-microbiota co-metabolites involved in CVD include branched chain amino acids and short chain fatty acids.

Although host-microbiota co-metabolism is at the core of cardiovascular health, other potential mechanisms may also be involved. Gut microbiota endotoxins, such as LPS, may translocate into the bloodstream and start an inflammatory cascade that eventually promotes atherosclerosis. Patients with symptomatic atherosclerosis, high cardiovascular risk or coronary artery disease (CAD) exhibit unique microbiome patterns with potential pro-inflammatory characteristics [18, 19, 20]

Therapeutic possibilities

Clinical and animal studies have demonstrated that the gut microbiota and their imbalance state, either because of the bacterial richness or because of specific bacterial composition, are associated with metabolic and cardiovascular disease. Modulation of gut microbiota composition and function through diet, antibiotics, prebiotics and probiotics may enable, in the long term, the capacity to alter host metabolism for health benefits. However, the understanding of the causal links between gut microbiota and CVD is limited. The evidence from animal studies may help in delineating specific therapeutic approaches. Researchers managed to prevent atherosclerosis in a mouse model by decreasing plasma TMAO levels[21]. Vancomycin reduced myocardial infarctions and increased post ischaemic mechanical function recovery in a Dahl S rat model of ischaemia/reperfusion injury of the heart [16]. This effect was associated with a change in the gut microbiota composition and a reduction of plasma leptin. The administration of the leptin-suppressing probiotic *Lactobacillus plantarum* 299v confirmed the role of leptin in this effect [22]. Interestingly, L. plantarum PH04 (another strain of this probiotic) also exhibit cholesterol-lowering capabilities in hypercholesterolemic mice. The administration of L. plantarum PH04 was associated with a 10-fold increase in fecal lactic acid bacteria [23].

However, the evidence from human studies is contradictory. A meta-analysis of clinical trials of antibiotic therapy in patients with CAD failed to demonstrate any benefit with regard to mortality or cardiovascular events. This result suggests that gut microbiota modification by antibiotics does not modify the evolution of CAD [24]. Probiotics seem to decrease low density lipoproteins (LDL)-cholesterol and improve the LDL/high density lipoproteins (HDL) ratio, as well as lower blood pressure, inflammatory mediators, blood glucose levels and body mass index [25]. However, clear definitions of exact strains and dosages of the probiotics that will bring about positive health effects are lacking. In addition, factors like immunity and genetics of the host may largely influence the efficacy of probiotics. There is a need for further studies to understand the mechanisms by which probiotics may beneficially affect the cardiovascular system and to rule out negative effects on health.

Conclusion and future

The growing evidence from animal and human studies shows that gut microbiota influence host health and disease. However, we need major advances in our mechanistic understanding of how gut microbiota convert dietary and endogenous molecules into metabolites and how it communicates with peripheral organs in the host. The recent discoveries open the possibility for numerous microbial pathways as potential pharmacological targets for the treatment of cardiometabolic diseases. Our understanding of the interactions among gut microbiota organization and function, host genome and environmental factors would provide more personalized and tailored therapeutic interventions.

- Daniel Monleón

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Obituary: Cinzia Tiberi

All those who have submitted papers to the Journal of Hypertension for the last twenty-two years, and all those who have been contacted to review papers for it, the majority – I think – of the readers of the Journal of Hypertension have had numerous chances of corresponding with Cinzia and know how heavily she has contributed to the spreading of knowledge on hypertension.

Since 1995 she was the thread connecting the members of the Editorial Office in Milan. She was the liaison between the Editor in Milan and the Publisher in London and she was the reference point for all those corresponding with the Journal. She knew how to combine effectiveness with kindness, firmness with friendliness, urgency with leisure. She had met most of the protagonists of hypertension research in person as she had been responsible for the organization of the scientific programme of all the European Meetings on Hypertension held in Milan since the first one in 1983, and attended all these meetings providing help, wisdom and a smile.

Cinzia was fluent in foreign languages, particularly English and Spanish, the latter learnt at high school and college in Lima, Peru, when her father was director of an Italian bank there. Back in Italy, she gained a university degree in foreign languages in Milan, and in 1981 joined the staff of the Centro di Fisiologia Clinica e Ipertensione to help us organize the 1981 Meeting of the International Society of Hypertension in Milan.

She soon became an invaluable collaborator and in 1995 took charge of running the Editorial Office of the Journal of Hypertension in Milan, a job she continued tirelessly until Spring 2016 when she started another struggle, this time against illness, with the same determination, trust and optimism that she had used in her work for hypertension. She did not deserve to lose this battle but unfortunately she eventually did, and passed away on the 17th of July 2017.

With Cinzia, all of us, the group of the Editorial Office in Milan, have lost more than just one of us, we have lost the best part of us. We worked with Cinzia for many years and there is no risk we will forget her and her help, but we would like the ISH members consulting their Journal papers from 1995 to 2016 to be aware of how much of that huge body of information and knowledge is the result of Cinzia's silent but heartfelt work.

- Alberto Zanchetti