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What connection is there between intestinal microbiota and heart disease?

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Information on the correlation between intestinal microbiota and cardiovascular risk is growing. Some species of the microbiota influence the metabolism of specific food components (such as carnitine, choline, phosphatidyl-choline), synthesizing the precursor of trimethylamine oxide, a molecule with documented harmful activity on the vascular wall. Other strains, on the other hand, metabolize dietary fibre by synthesizing short-chain fatty acids, which have a significant anti-inflammatory activity, or produce secondary metabolites originating from molecules present in food (such as enterodiol, which derives from lignin), characterized by a vascular protection activity. Prebiotic effects from plant compounds (such as berberine or resveratrol) are also documented, which would induce favourable changes in the composition of the microbiota. The possibility of influencing the composition and activity of the intestinal microbiota will probably represent, in the future, an important component of cardiovascular prevention strategies.

Introduction

An increasing number of studies, both from clinical and basic research, suggest the existence of a significant connection between the activities of the intestinal microbiota and cardiovascular risk. In this regard, the microbiota would act with different mechanisms: specific bacterial strains could, for example, have direct effects on atherogenesis; or instead modify, both in a potentially atherogenic and in a protective sense, compounds naturally present in food. This review will examine some of the microbiota–cardiovascular risk interactions that, in light of the available evidence, can already be considered to have a convincing scientific soundness.

A prime example of these interactions emerges from the link, documented in numerous observational studies, between the consumption of meat and eggs and an increase in cardiovascular risk. The classic explanation of this association refers, above all, to the presence, in these foods, of saturated fatty acids (especially in meat) and preformed cholesterol (especially in eggs). Both of these components would increase the levels of LDL cholesterol, and therefore cardiovascular risk. However, this interpretation has recently been challenged. A 2015 meta-analysis,[1] confirming evidence already present in the literature, has in fact significantly downsized the possible role of the saturated fats in increasing cardiovascular risk; the effect of dietary cholesterol on LDL cholesterol, on the other hand, is small, and probably of limited importance in a large part of the population.

It should therefore be considered possible that meats and eggs favour the formation of atheromatous lesions through a process not linked to their effect on cholesterolaemia. In this regard, the authors of a now classic paper have identified and characterized a well-defined metabolic mechanism which, starting from choline, phosphatidyl-choline, or carnitine (present in numerous foods of animal origin), leads to the formation of trimethylamine (TMA); TMA is then enzymatically oxidized by the liver, with the formation of trimethylamine oxide (TMAO).[2] Trimethylamine oxide would be responsible for arterial damage: if its blood levels are high, in fact, the risk of cardiovascular events increases by ~60%;[3] this increase is in any case independent of traditional cardiovascular risk factors.

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It is interesting to note that the conversion of carnitine, choline, or lecithin into TMA (which activates the subsequent metabolic processes, previously mentioned) takes place only in the presence, in the intestine, of bacterial strains characterized by a specific enzyme endowment. The determining role of the microbiota in this metabolic sequence is clearly documented in experimental animal models: the administration of carnitine in the mouse leads to a significant increase in the plasma levels of TMA and TMAO, but pre-treatment with antibiotics cancels this increase, blocking the conversion of carnitine to TMA.[2]

The practical implications of these findings are relevant. It is intuitive that, if the mechanism described is the one actually responsible of the vascular damage associated with the consumption of meat and eggs, this damage could also be controlled by changing the composition of the intestinal microbiota. Similarly, it is conceivable that subjects with different compositions of the microbiota will differently react to the dietary intake of carnitine and choline (and therefore of meat and eggs); these compounds would be converted into TMAO in subjects who host in their intestine bacterial strains relevant in this regard, while in other subjects (without such strains in the microbiota) such conversion would not take place. If this scenario were confirmed, changing the diet, or instead intervening on the microbiota, and therefore on the metabolic modifications induced by the microbiota on components of the diet, would become alternative or complementary procedures, to be carefully evaluated in their overall effects on cardiovascular risk.

Transferring these general concepts to operational recommendations is, however, complex, and probably for now premature. The variability of individual responses (in terms of TMAO production after an egg yolk load) is, for example, very high; we do not know, additionally, which species or strains of the microbiota play an essential role in these metabolic sequences, nor are we able to influence, through mechanisms of competition between the various bacterial species, the overall capacity of the microbiota itself to produce TMA. But it is reasonable to think that, in the future, we will be able to know and perhaps influence these processes: thus significantly changing—if the assumptions described will prove to be exact—the relationship between some important components of the diet and health.

Interestingly, TMAO would also possess a specific pro-inflammatory effect, which would be relevant in determining its contribution to cardiovascular risk. A state of chronic micro-inflammation, which can generally be identified by a modest but stable increase in plasma levels of C-reactive protein, is in fact regularly associated, in prospective studies, with an increased risk to develop cardiovascular diseases. Recently it has been shown that some compounds of bacterial synthesis (short-chain fatty acids or SCFAs) have an interesting anti-inflammatory action.[4] The intestinal microbiota produces these compounds mainly from fermentable fibres (such as pectins, beta-glucans, guar gum, inulin, and phospho-oligosaccharides), essentially contained in fruit, vegetables, and whole grains. Non-fermentable fibres (such as cellulose and lignin), on the opposite, do not contribute significantly to SCFA synthesis.

These fatty acids are important, as previously mentioned, also for their role in modulating inflammatory processes, through different mechanisms.

On the one hand, SCFA improves the barrier effect of the intestinal mucosa (e.g. by stimulating the production of mucin), limiting the passage of molecules and cells in the circulatory system, and thus contributing to the reduction of the overall inflammatory stimulus to the body. But SCFAs also have more direct effects: many cells involved in the immune response are in fact equipped with specific receptors for these fatty acids, which if activated affect their functions, mainly in an inhibitory way.[5] These receptors are mostly G protein-coupled receptors, which generally have cyclic AMP as the second mediator. They are widespread in many areas of the body (e.g. they are also found on the surface of the cells of the intestinal epithelium, or fat cells, where they influence, e.g. lipolysis).

In general, SCFAs (and especially butyrate) seem to inhibit the activation of the nuclear factor NF-kB and the activity of histone deacetylases (HDAC),[6] two mechanisms that act on biochemical processes that are central, among other things, in inflammatory responses. Their overall effect, as we remembered, is to attenuate the inflammatory responses to various stimuli. Through these mechanisms, butyrate, for example, inhibits the proliferation of T lymphocytes, and at high doses induces apoptosis of activated T lymphocytes.[7] T-reg cells, characterized by regulatory or suppressive activity on immunity mechanisms, are instead activated by SCFAs, probably always through the inhibition of HDAC.[8] Inhibition of activation of the nuclear factor NF-kB and of the activity of HDAC also seems to be the mechanism behind the suppression of the release of TNF-alpha (one of the most important inflammatory cytokines) by granulocytes, monocytes, and macrophages after contact with bacterial membrane lipopolysaccharides.

These anti-inflammatory actions probably explain the protective role of SCFA in inflammatory bowel diseases, now well documented, but they also allow to hypothesize a possible preventive action of atherosclerosis and its clinical manifestations, characterized, as mentioned, by a activation of inflammatory processes. Short-chain fatty acids also seem to counteract the accumulation of triglycerides in fat cells; contrasting the development of overweight and obesity and thus also limiting the pro-inflammatory action of excess weight. These anti-inflammatory effects of SCFA can help to understand the well-recognized association, between the dietary consumption of dietary fibre and the reduction of plasma levels of PCR or interleukina-6 or TNF-alpha. The systemic anti-inflammatory effect of the fibre, not easy to understand for a non-absorbable compound, becomes in fact better explained if it is associated with instead well absorbable metabolites of the fibre, such as SCFA. And this anti-inflammatory action of fibre can help justify the favourable results that fibre consumption plays, according to a recent meta-analysis, on both cardiovascular and neoplastic risk, as well as on all-cause mortality.[9]

Another possible level of intervention of the microbiota in these phenomena can also be represented by an increase in the bioavailability of antioxidant molecules, which amplifies their protective effect; some compounds of vegetable origin can also have a prebiotic effect on specific strains.

Berberine, present in many food supplements, seems, for example, able to stimulate the growth of *Akkermansia muciniphila*, which would significantly contribute to the vascular protection effect associated with the use of berberine itself in experimental models.[10] Similarly, many foods rich in polyphenols are able to influence intestinal bacterial growth, also representing the metabolic basis for the synthesis of secondary metabolites, sometimes of potential health interest (such as enterodiol produced from lignin).[11] Resveratrol, by changing the profile of the intestinal microbiota, would instead reduce, according to some preliminary data obtained in the animal, the conversion of choline into TMA and therefore in the corresponding oxidized compound (TMAO),[12] thus weakening one of the possible mechanisms, previously mentioned, of the atherogenicity of meat and eggs.

Some authors have also wondered if some of the health benefits typical of the Mediterranean diet, especially on cardiovascular risk, can actually derive from the specific microbiota associated with this diet.

In most of the published studies, in fact, the consumption of a Mediterranean type diet is associated with a specific microbiota, different from that associated with a western-style dietary pattern. The 'Mediterranean type' microbiota is first of all characterized, generally speaking, by a greater biodiversity (i.e. by a greater number of bacterial species identified);[13] a characteristic considered positive from the point of view of the effects on health. More specifically, the western diet is, for example, associated with high levels of *Bacteroides*, while *Prevotella* are more represented in the Mediterranean diet. Gutierrez-Diaz *et al.*[14] have identified higher levels of *Clostridium* cluster XIVa and *Faecalibacterium prausnitzii* in subjects with a high adherence score to the Mediterranean diet. The same score, in another publication of the group, was associated with a greater abundance of Bacteroidetes, Prevotellaceae, and *Prevotella* and a lower presence of Firmicutes and Lachnospiraceae. Even a lower Firmicutes-Bacteroidetes ratio has been associated with high adherence to the Mediterranean diet.[15]

Typical components of the Mediterranean diet also seem to be associated with the presence of specific strains in the intestinal microbiota. Cereals, for example, with the presence of Bifidobacterium and Faecalibacterium, Tenericutes and *Dorea* with olive oil, red wine again with Faecalibacterium, vegetables with Rikenellaceae, *Dorea*, *Alistipes*, and *Ruminococcus*, legumes with *Coprococcus*.[14] The same authors have observed a correlation between the polyphenol content of the diet (typically high in the Mediterranean diet) and the presence of specific clostridium clusters (XIVa) and Faecalibacterium, capable of synthesizing butyrate and probably endowed with an anti-inflammatory action (like *Akkermansia*, also more represented in association with the Mediterranean diet). The combination of the dietary components typical of the diet and the specific prevalent microbiota leads to the production of specific metabolites, such as the SCFA, previously mentioned, more represented in the faeces of subjects with a Mediterranean diet as opposed to the TMAO, more associated with a western-type diet.

Conclusion

In conclusion, accumulating evidence indicates a direct involvement of the gut microbiota in cardiovascular risk. This area will be further explored in the coming years, and it is easy to predict that this will also allow to face cardiovascular risk through innovative strategies, based on the modification of the microbiota itself.

Conflict of interest: none declared.

References

1. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budylowski P, Schünemann H, Beyene J, Anand SS. Intake of saturated and trans unsaturated fatty acids and risk of all-cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ* 2015;**351**: h3978.
2. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WHW, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;**19**: 576–585.
3. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut microbiota metabolites and risk of major adverse cardiovascular disease events and death: a systematic review and meta-analysis of prospective studies. *J Am Heart Assoc* 2017;**6**:e004947.
4. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987;**28**:1221–1227.
5. Stoddart LA, Smith NJ, Milligan G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, -2, and -3: pharmacology and pathophysiological functions. *Pharmacol Rev* 2008;**60**: 405–417.
6. Silva JPB, Navegantes-Lima KC, de Oliveira ALB, Rodrigues DVS, Gaspar SLF, Monteiro VVS, Moura DP, Monteiro MC. Protective mechanisms of butyrate on inflammatory bowel disease. *Curr Pharm Des* 2019;**24**:4154–4166.
7. Bailón E, Cueto-Sola M, Utrilla P, Rodríguez-Cabezas ME, Garrido-Mesa N, Zarzuelo A, Xaus J, Gálvez J, Comalada M. Butyrate in vitro immune-modulatory effects might be mediated through a proliferation-related induction of apoptosis. *Immunobiol* 2010;**215**: 863–873.
8. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013;**504**:451–455.
9. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* 2019;**393**:434–445.
10. Zhu L, Zhang D, Zhu H, Zhu J, Weng S, Dong L, Liu T, Hu Y, Shen X. Berberine treatment increases Akkermansia in the gut and improves high-fat diet-induced atherosclerosis in Apoe-/-mice. *Atherosclerosis* 2018;**268**:117–126.
11. Rienks J, Barbaresko J, Nöthlings U. Association of polyphenol biomarkers with cardiovascular disease and mortality risk: a systematic review and meta-analysis of observational studies. *Nutrients* 2017;**9**: E415.

12. Chen M-L, Yi L, Zhang Y, Zhou X, Ran L, Yang J, Zhu J-D, Zhang Q-y, Mi M-T. Resveratrol attenuates trimethylamine-N-oxide (TMAO)-induced atherosclerosis by regulating TMAO synthesis and bile acid metabolism via remodeling of the gut microbiota. *MBio* 2016;**7**:e02210.
13. Jin Q, Black A, Kales SN, Vattem D, Ruiz-Canela M, Sotos-Prieto M. Metabolomics and microbiomes as potential tools to evaluate the effects of the Mediterranean diet. *Nutrients* 2019;**11**:207.
14. Gutierrez-Diaz I, Fernandez-Navarro T, Sanchez B, Margolles A, González S. Mediterranean diet and faecal microbiota: a transversal study. *Food Funct* 2016;**7**:2347–2356.
15. Garcia-Mantrana I, Selma-Royo M, Alcantara C, Collado MC. Shifts on gut microbiota associated to Mediterranean diet adherence and specific dietary intakes on general adult population. *Front Microbiol* 2018;**9**:890–895.