J Cardiovasc Thorac Res, 2018, 10(3), 129-137
doi: 10.15171/jcvtr.2018.21
http://jcvtr.tbzmed.ac.ir

Review Article

New insights on atherosclerosis: A cross-talk between endocannabinoid systems with gut microbiota

Jalal Moludi[1,2], Mohammad Alizadeh[1*], Ned Lotfi Yagin[1], Yahiya Pasdar[3], Seyed Mostafa Nachvak[3], Hadi Abdollahzad[3], Ali Sadeghpour Tabaei[4]

[1]Nutrition Research Center, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Nutritional Sciences Department, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4]Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran

Article info

Article History:
Received: 14 October 2017
Accepted: 16 September 2018
epublished: 27 September 2018

Keywords:
Atherosclerosis
Endocannabinoids
Gastrointestinal Microbiome
Probiotics
Trimethylamine-N-Oxide

Abstract

The incidence of atherosclerosis is increasing rapidly all over the world. Inflammatory processes have outstanding role in coronary artery disease (CAD) etiology and other atherosclerosis manifestations. Recently attentions have been increased about gut microbiota in many fields of medicine especially in inflammatory diseases like atherosclerosis. Ineffectiveness in gut barrier functions and subsequent metabolic endotoxemia (caused by rise in plasma lipopolysaccharide levels) is associated with low-grade chronic inflammation i.e. a recognized feature of atherosclerosis. Furthermore, the role of trimethylamine-N-oxide (TMAO), a gut bacterial metabolite has been suggested in atherosclerosis development. On the other hand, the effectiveness of gut microbiota modulation that results in TMAO reduction has been investigated. Moreover, considerable evidence supports a role for the endocannabinoid system (ECS) in atherosclerosis pathology which affects gut microbiota, but their effects on atherosclerosis are controversial. Therefore, we presented some evidence about the relationship between gut microbiota and ECS in atherosclerosis. We also presented evidences that gut microbiota modulation by pre/probiotics can have significant influence on the ECS.

Please cite this article as: Moludi J, Alizadeh M, Lotfi Yagin N, Pasdar Y, Nachvak SM, Abdollahzad H, Sadeghpour Tabaei A. New insights on atherosclerosis: A cross-talk between endocannabinoid systems with gut microbiota. **J Cardiovasc Thorac Res** 2018;10(3):129-137. doi: 10.15171/jcvtr.2018.21.

Introduction

Cardiovascular disease (CVD) is one of the most common causes of mortality throughout the world with high social costs in terms of health care.[1] Atherosclerosis, the main cause of CVD development, which is considered as an inflammatory disease and created by arterial lesions containing cholesterol, immune infiltrates, and connective tissue elements.[2] In addition to the known risk factors such as hyperlipidemia and smoking which might raise endothelial injury, inflammatory processes may be involved in the development of atherosclerosis manifestations.[3] Recently attentions have been increased about gut microbiota in many fields of medicine especially in inflammatory diseases like atherosclerosis.[4] Furthermore, gut microbiota imbalance may be a key player in the inflammation onset in other organs which could alter the regular homeostasis.[5-7] However, the intestinal and molecular components involved in gut-to-organ dysregulation in atherosclerosis still remain unknown.[8] Moreover, our current understanding of the pathophysiology of atherosclerosis suggests that endocannabinoid signaling plays a critical role in pathogenesis of the atherosclerosis. The potential role of endocannabinoid in atherosclerosis seems to be their modulation of the chronic inflammatory response that occurs within the vascular wall.[9]

In the current review, we debate recent findings that reveal mechanisms connecting the gut microbiota to low-grade inflammation in context of the obesity. Additionally, we will discuss the potential relationships between the endocannabinoid systems (ECSs) with gut microbiota. Lastly, we talk over the potential modulation and their effects on gut microbiota and host metabolism in regard to development of the atherosclerosis.

Gut Microbiota

The human large intestine is consisted of large and various communities of microbial cells that create the

***Corresponding Author:** Mohammad Alizadeh, Email: mdalizadeh@tbzmed.ac.ir

gut microbiota. The whole microbial genome of the gut microbiota is known as the gut microbiome, which is 100 times greater than the human genome indicating the importance of gut microbiome.[8-10] There is no clear definition of healthy gut microbiota in human, but in healthy individuals, anaerobic *Bacteroidetes* and *Firmicutes* constituted more than 90% of the total bacterial species.[11,12]

Bacterial diversity among individuals is due to the differences in both host genomes and environmental factors, such as antibiotic usage, lifestyle, hygiene, and diet.[13-15] An altered gut microbial composition, known as dysbiosis, may lead to unfavorable effects in host and could predispose an individual to disease.[16]

Although, the increased microbial diversity contributes to greater stability of the gut environment and better health status, the mechanism is unknown.[17-19] Previous studies have shown that the gut microbiota can also affect a number of complex metabolic reactions in other organs possibly through gut permeability, fat storage and low-grade inflammation,[20,21] which subsequently affect atherogenesis. According to present knowledge, immune responses have a prominent role in atherosclerosis pathophysiology.[22,23] Considering the gut microbiota effects in immune systems, change in gut microbiota would have a valuable influence on atherosclerosis.[20] It seems that gut microbiota affects the atherosclerosis process in three ways. First, the chronic inflammation induced by metabolic endotoxemia, second by harmful metabolites such as trimethylamine-N-oxide (TMAO) due to the gut microbial dysbiosis and also because of the potential relationships between the ECSs with gut microbiota.

1) Systemic inflammation induced by dysbiosis

Several recent studies have proposed that mucosal barrier function disruption known as "dysbiosis" and subsequent gut microbiota-derived endotoxemia could lead to cardiometabolic diseases pathogenesis.[24,25] Increased levels of plasma endotoxin through chylomicrons absorption have been associated with CVD development.[26] Animal studies also revealed the accelerated atherosclerosis after endotoxins injection.[27] In this process, bacterial translocation and endotoxins are recognized by Toll-like receptors (TLRs) expressed on the host macrophages which in turn results in chronic inflammation. TLRs are family of membrane pattern-recognition receptors, which have a vital role in immune system and protect against many pathogens. It should be also mentioned that stimulation with lipopolysaccharides (LPS) recognized by TLRs (Figure 1) can lead to uncontrollable proinflammatory cytokines production, which can result in CVD.[28,29] In addition, this permits LPS leakage into the circulation activating systemic inflammation cytokines like tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) production.[30] Immunological and inflammatory processes probably play an important role in atherosclerosis pathogenesis and progression. Pro-inflammatory cytokines, such as IL-1, TNF-α, C-reactive protein (CRP), IL-2, and IL-6, all increase in patients with atherosclerosis.[31]

Some studies have demonstrated that probiotics can decrease the pro-inflammatory cytokines production.[32,33] Though in clinical trials, antibiotic therapy and subsequent gut microbiota alteration did not improve secondary cardiovascular outcomes,[34,35] it seems that, microflora intervention by diet modulation had better effects than antibiotic therapy. In both human and animal studies, probiotic supplementation was associated with reduced cholesterol profile and inhibition of atherosclerotic lesion composition and development.[36] For example, 8 weeks *Saccharomyces boulardii* supplementation in hypercholesterolemic adults led to decrease in lipoprotein particles and cardiovascular biomarkers.[37] It has been accepted that probiotics decrease inflammation and

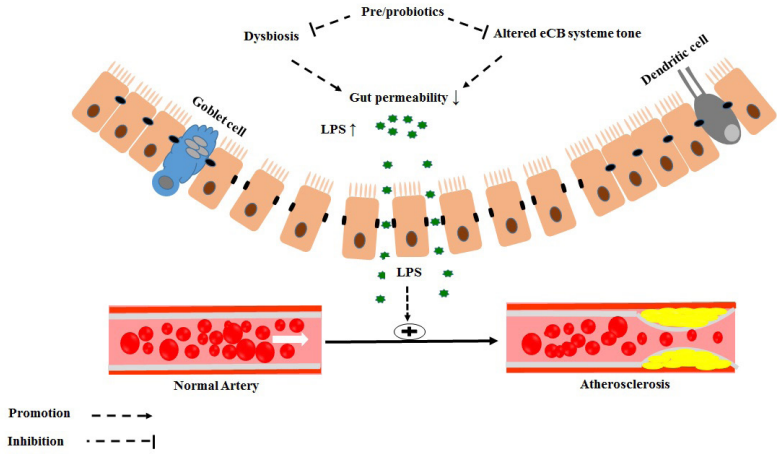

Figure 1. Endocannabinoid systems affect a number of complex metabolic reactions which might affect gut microbiota. According to current understanding, changes in gut microbiota have prominent role in the pathophysiology of atherosclerosis. Alter in ECS system tone increases gut permeability which enhances the LPS level. In fact there are two ways for increasing the LPS: 1) alter the tone of gut microbiota and 2) ECS system.

endotoxemia. However, the precious mechanism underlying remains unclear. But it is proposed that intestinal barrier plays an important role through lessening the translocation of microbiome-derived LPS into the bloodstream.

Obesity and dysbiosis

Obesity was already known as low-grade inflammation and some studies confirmed that obesity is linked with changes in gut microbiota in comparison with lean counterparts.[38,39] In fact, it can be assumed that the increased inflammation is the main reason of the CVD in obese individuals.[40] On the other hand, due to the impaired gut barrier function and altered gut microbiota, an obese person is more susceptible to inflammation.[41] For example, Cani et al, have shown that in obese mice, intestinal permeability and metabolic endotoxemia play major part in metabolic disorders pathogenesis. Moreover, they found that changes in gut microbiota through prebiotic supplementation increased endogenous glucagon like peptide (GLP-2) production, which in turn improved gut barrier functions, decreased inflammatory and oxidative stress markers.[5]

Gut microbiota modulation

To date, therapeutic methods which have been used to modify the microbiota include antibiotics, diet, prebiotics/probiotics.[34,42-44] While antibiotics therapy can alter the intestinal microflora, this method has failed to improve cardiovascular outcomes in clinical trial. Furthermore, considering the possible antibiotic resistance and microbial changes, this method has not been effective yet.[34,45] Prebiotics are non-digestible food elements that stimulate the growth activity of the gut bacteria.[22] Probiotics, the live microorganisms, have positive effects the host by producing vitamin K, B2, and short chain fatty acids such as acetate or propionate which are used as fuels for intestinal flora and coloncytes. Additionally, they decrease the epithelial barriers permeability in intestine, increase tight junctions' protein function, interact with host microbial community and compete with pathogens. Probiotics strains also interact with toll-like receptors in gut modifying the inflammatory responses.[43,46-48] A recent systematic review and meta-analysis of randomized controlled trials showed that probiotic supplementation did not only improve lipid profile and cardiovascular risk, but supplements with multiple probiotic strains was more useful rather than a single strain,[47] suggesting the importance of intestinal microflora diversity.

As mentioned above, probiotics have beneficial effects on cardiovascular system. In this regard, a recent study revealed that *Lactobacillus rhamnosus,* a probiotic supplement decreased cardiac hypertrophy and improved ejection fraction at an animal model with myocardial infarction.[49] Therefore, regarding all these together the importance of probiotics effects in inflammation reduction as a main reason of atherosclerosis can be deduced.

2) TMAO as a marker of cardiovascular events

Although, bacterial components cause inflammation, certain bacterial metabolites such as trimethylamine[50] can also exert cytotoxicity and increase inflammation. Trimethylamine a biological compound is produced by gut microbiota from dietary phosphatidylcholine, choline, and carnitine. Liver flavin monooxygenase (FMO) enzymes oxidize TMA TMAO releasing it into the circulation.[50,51] TMAO concentrations are 3 μmol/L and 40 μmol/L in blood of healthy subjects and in renal failure patients respectively.[52] It should also be considered that TMAO levels may be affected by many factors including amount of dietary choline, carnitine, gut microbiota activity and kidney function.[53] Therefore, due to the considerable influence of TMAO in CVD, these factors especially kidney function should also be considered. In previous epidemiological studies, being vegetarian was associated with lower risk of CVD.[54-56] On the other hand, high content of TMAO precursors in omnivores diet with different intestine micro-flora (compared to vegetarians, omnivores had more *Prevotella* and lower *Bacteriodes* species in gut microbiome) led to higher susceptibility of these individuals to atherosclerosis.[57,58] It seems that TMAO might contribute to atherosclerosis development in part by activating macrophages to accumulate cholesterol to form foam cells in atherosclerotic lesions. Also, TMAO could change cholesterol metabolism in different organs such as liver and intestines inhibiting reverse cholesterol transport pathway.[59] As well, elevated TMAO levels have been shown to have prognostic value in patients with ischemic and non-ischemic cardiomyopathy.[60] Recent studies have assessed the effects of intestinal environment manipulation in reducing TMAO levels.[61,62] Wang et al demonstrated that in addition to decreased foam cell formation, TMAO production was prevented by 3, 3-dimethyl-1-butanol (DMB).[61] In another study Rong et al[62] presented that resveratrol supplementation inhibited the TMAO production by changing intestinal microflora, and supplementation also changed gut microbes type and increased Lactobacillus and Bifidobacterium species levels. In an animal study, probiotic usage changed TMAO levels which subsequently reversed atherosclerosis development.[63] Gut microbiota modulation in some patients may be the alteration of TMAO levels.[64-66] Wang et al[56] showed that gut microbiota plays a significant role in TMAO production in animal models. They indicated that treating mice with antibiotics changed the intestinal microflora and subsequently decreased TMAO production. Also, in a study conducted by Tripolt et al, daily supplementation of *Lactobacillus casei* Shirota (6.5×10^9 CFU) 3 times for 12 weeks in subjects with metabolic syndrome did not affect TMAO levels.[53] Consequently, it seems that well-designed clinical trials with different probiotic sources are required to determine the impact of

probiotics on TMAO production.

Effect of nutritional intervention on TMAO levels is inconsistent. For example, one study has found that while fish consumption was beneficial for CVD, the TMAO levels also increased concurrently.[52] In another study, oral L-carnitine supplementation significantly decreased vascular injury markers such as intercellular adhesion molecule-1 (ICAM), (vascular cellular adhesion molecule-1)VCAM-1 and malondialdehyde (MDA); however, at the same time plasma TMAO level increased significantly.[67] Information in this area is limited and need more research in order to find the best modulation in this direction. Moreover, a previous study has shown that oxidative stress plays an important role in cardiovascular and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.[68] Recent studies have highlighted TMAO positive role in CVD by reducing oxidative stress.[69,70] In a study, TMAO administration for about 12 weeks decreased endoplasmic reticulum stress and peripheral nerves dysfunction in streptozotocin-diabetic rats' models.[69] However, it is noteworthy to mention that it has not proved that TMAO promotes atherosclerosis and different food sources are more likely to be useful and confounding factors should be considered in the interpretation in this field.

3) Endocannabinoid systems

The ECS is an internal signaling system acts in various physiological functions, both in central and peripheral nervous systems and also in peripheral organs.[71] 2-arachidonyl glycerol (2-AG) and anandamide are main endocannabinoids which bind to cannabinoid receptors, named cannabinoid receptor 1 (CB 1) and CB 2, that are G-protein-coupled membrane receptors with an identical signaling mechanism (rReviewed elsewhere).[52] CB1 was first found in distinct areas of the brain as well as peripheral nerve terminals. CB2 is located in lymphoid tissues and myeloid cells and have role in immune response.[67] However, the expression of these receptors has not been restricted to such areas. For example, the CB2 receptor is also expressed in myocardium, coronary artery, endothelial and smooth muscle cells in cardiovascular system. Additionally, CB1 receptors have also been identified in myocardium, human coronary endothelial and smooth muscle cells.[50,70]

The mechanisms by which endocannabinoids act as cardio protective components, include decreased neutrophil infiltration, inflammation, oxidative stress and increased activation of cardio protective signaling pathways through activation of CB1 and CB2.[68] Modulation of immunity and inflammatory response which occurs within the vascular wall appears to be the main role of endocannabinoid in atherosclerosis prevention.[69] The anti-inflammatory effects of CB2 activation are improvement of endothelial function, proliferation of vascular smooth muscle cell, plaque development, expression of adhesion molecules, decrease in oxidative stress and macrophage infiltration in in-vivo system.[70,72] Though, CB2 deficiency increased atherosclerosis susceptibility in mice,[73] due to the evidence of both pro- and anti-atherosclerotic effect of receptor activation, the role of CB1 in atherosclerosis is still controversial.[74,75] AEA and 2-AG release from endothelial due to the endocannabinoids administration had inconsistent effects ranging from increased heart rate and blood pressure to reduced atherosclerosis progression in mice.[76,77] In addition, low dose of anandamide lessened tumor necrosis factor a (TNFa) levels and induced ICAM-1, VCAM-1 expression in human coronary artery endothelial cell.[78] In contrast to theoretically beneficial effects of endocannabinoids in CVD, they might also demonstrate pro-thrombotic effects. As well, in previous animal and human studies it was observed that AEA and 2-AG activated platelet aggregation leading to atherosclerosis progression.[79,80] Considering the endocannabinoid side effects, it seems that their modulation with different method such as probiotics supplements might minimize their negative effects.[59] In addition to modulation of the host's immune responses and decline of harmful metabolite such as TMAO by probiotics, they might also stimulate CB1 and CB2 receptors.

Obesity and endocannabinoid system

As stated above, overweight and obesity are associated with increased gut permeability leading to metabolic endotoxemia and CVD. Also, obesity is associated with over activation of the ECS and gut microbiota modulation with prebiotics leads to normalization of ECS tone. On the other hand, gut-derived LPS contributes to altered endocannabinoid tone. Hence, the greater ECS tone which is found in obesity might also participate in CVD not only directly by acting on cardiovascular risk factors, but also indirectly by increasing plasma LPS levels, that consequently impair the gut permeability.

Interaction between Gut modulation and endocannabinoid systems

As discussed above, atherosclerosis is associated with altered ECS system tone.[81] Muccioli et al showed that gut microbiota selectively controls ECS system tone and also demonstrated that LPS is a potent stimulator of ECS synthesis. They also revealed that ECS system could regulate gut permeability and adipogenesis and gut microbiota modulation by probiotics strongly affects this pathway.[82] Dietary supplementation with probiotics decreased gut permeability, increased the expression of CB2 mRNA and lessened CB1 mRNA expression, AEA production and fat mass storage (Table 1). In fact, it can be said that gut microbiota and ECSs affect each other. In addition to direct role of ECS in cardiovascular system, it also plays a vital role in regulating gut-barrier function. The concept of gut-barrier is expressed by two concepts 'gate keepers' or 'gate openers'[83] and probiotics

Table 1. Effects of gut modulation on EC system

Subjects	Intervention	Effect	Reference
Mice	Feeding *L. acidophilus*	Increased expression of CB2 mRNA in colonic epithelial cells Analgesic functions in the gut comparable with the effects of endocannabinoid	Rousseaux et al[85]
Solea solea	Probiotic treatment with *Enterococcus faecium*	Up-regulated CB_1 mRNA Modulate energy homeostasis by inducing endocannabinoid signaling	Palermo et al[88]
Ob/ob mice	Prebiotic treatment with oligofructose	Lessen CB1 mRNA expression, AEA production and fat mass storage in prebiotics group LPS levels correlated with CB_1 mRNA expression and AEA levels	Muccioli et al[82]
Mice	Microbiota manipulations with antibiotics	2.5-fold decrease in total bacterial counts levels Up-regulation of CB2 expression	Aguilera et al[88]

generally mediate the gate keepers' role.[84] In fact, these functions in gut permeability control are not only related, they are identical. Rousseaux et al also showed a link between gut microbiota and ECS. They demonstrated that oral *Lactobacillus acidophilus* strain administration modulated cannabinoid receptors expression in rats' intestinal cells expression.[85] Furthermore, tight junction proteins (Zonulin and occluding) expression in Caco-2 cells were controlled by CB1-dependent mechanisms, but not by CB2.[82,86] Changes in CB1 and CB2 expression were associated with amount of bacterial translocation. Previous study showed that CB2 receptor activation, improved glucose tolerance and reduced permeability in an animal model study.[87]

One of the primary disadvantages of non-selective stimulation of CB receptor by endocannabinoid administration is its psychotropic side effects. However, pro/prebiotics administration seems to be well-tolerated without any harm. A recent study has shown that changing intestinal microflora with probiotics modified CB2 receptor expression. In this study, it was revealed that microbiota manipulations with antibiotics was associated with 2.5-fold decrease in the level of total bacterial counts and CB2 expression up-regulation in mice.[89] In another study it was also demonstrated that *Lactobacillus acidophilus* administration increased CB2 expression in mice colon, whereas some specific bacteria strain such as *Bifidobacterium* and *Escherichia coli* had no effect on CB2 expression.[85] Briefly, it seems that probiotics have undeniable role in ECS stimulation; however, according to the literature review, this area needs further investigation to exactly determine which bacteria strain has more beneficial effects depending on the different receptor. Further clinical studies are required to elucidate the cross-talk between probiotic of ECS system

Interaction between atherosclerosis, endocannabinoid systems and gut microbiota

Gut microbiota also regulates gut permeability and inflammatory response through LPS-ECS system regulatory loops.[82,90] On the other hand, LPS stimulates ECS synthesis,[77,91] hyper-activates it in the intestines leading to increase in gut permeability and systemic inflammation.[90] The crosstalk between gut microbiota and ECS system might contribute to atheroscrosis development directly by acting on adipose tissue and indirectly by increasing plasma LPS levels.[82] Probiotic may also alter gut microbiota tone which in turn decreases both intestinal and adipose tissue ECS system responsiveness, improving gut barrier and stabilize adipogenesis. It has been shown that blocking CB_1 receptor protects against low-grade inflammation which is promising evidence that the ECS system, inflammation, and atherosclerosis are interrelated.[91] Therefore, we suggest that there might be similar molecular mechanisms underlying the ECS and gut barrier function related to endotoxemia and inflammation.

Conclusion

Even though there are many questions which have been unanswered, studies demonstrated that mucosal barrier function disruption and subsequent gut microbiota-derived endotoxemia could contribute to cardiometabolic diseases pathogenesis. As well, number of studies revealed that TMAO in systemic circulation can activate macrophages which lead to cholesterol accumulation and subsequent foam cells formation in atherosclerotic lesions. On the other hand, accumulating evidence proposes that ECS involved in many physiological processes that are related to maintenance of gut-barrier function and inflammation regulation. Hence, although present literature review provides beneficial evidence in support

Key points

- Gut microbiota interact with host via several mechanisms such as control of intestinal permeability, prevention of endotoxin (LPS) absorption and subsequent inflammation
- ECS involved in many biological processes, ranging from the regulation of energy homeostasis to inflammation and intestinal barrier function
- Gut microbiota regulates gut permeability and inflammatory response through LPS-eCB system regulatory loops
- Gut microbiota modulation by pre/probiotics can have significant influence on the ECS

of crosstalk between ECS and gut microbiota, additional studies are needed to clarify whether gut microbiota modulation can alter ECS tone and inflammation levels or not.

Ethical approval

Not applicable.

Competing interests

All authors declare no competing financial interests exist.

Acknowledgments

The authors would like to thanks, Rajaie Heart Hospital doctors who helped us.

References

1. Yusuf S, Reddy S, Ôunpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. **Circulation** 2001;104(22):2746-53.
2. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. **N Engl J Med** 2005;352(16):1685-95. doi: 10.1056/NEJMra043430
3. Moludi J, Keshavarz S, Tabaee AS, Safiri S, Pakzad R. Q10 supplementation effects on cardiac enzyme CK-MB and troponin in patients undergoing coronary artery bypass graft: a randomized, double-blinded, placebo-controlled clinical trial. **J Cardiovasc Thorac Res** 2016;8(1):1. doi: 10.15171/jcvtr.2016.01
4. Zaman A, Helft G, Worthley S, Badimon J. The role of plaque rupture and thrombosis in coronary artery disease. **Atherosclerosis** 2000;149(2):251-66.
5. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. **Gut** 2009. doi: 10.1136/gut.2008.165886
6. Cani PD, Osto M, Geurts L, Everard A. Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. **Gut Microbes** 2012;3(4):279-88. doi: 10.4161/gmic.19625
7. Caesar R, Fåk F, Bäckhed F. Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism. **J Intern Med** 2010;268(4):320-8. doi: 10.1111/j.1365-2796.2010.02270.x.
8. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. **J Clin Invest** 2011;121(6):2126-32. doi:10.1172/JCI58109.
9. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. **Science** 2005;308(5728):1635-8. doi: 10.1126/science.1110591
10. Griffin JL, Wang X, Stanley E. Does our gut microbiome predict cardiovascular risk? A review of the evidence from metabolomics. **Circ Cardiovasc Genet** 2015;8(1):187-91. doi: 10.1161/CIRCGENETICS.114.000219
11. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al. Structure, function and diversity of the healthy human microbiome. **Nature** 2012;486(7402):207. doi: 10.1038/nature11209
12. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. **Nature** 2006;444(7122):1022. doi: 10.1038/nature11209
13. Mariat D, Firmesse O, Levenez F, Guimarães V, Sokol H, Doré J, et al. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. **BMC Microbiol** 2009;9(1):123. doi: 10.1186/1471-2180-9-123
14. Hébuterne X. Gut changes attributed to ageing: effects on intestinal microflora. **Curr Opin Clin Nutr Metab Care** 2003;6(1):49-54. doi: 10.1097/01.mco.0000049038.06038.57
15. Greenwood C, Morrow AL, Lagomarcino AJ, Altaye M, Taft DH, Yu Z, et al. Early empiric antibiotic use in preterm infants is associated with lower bacterial diversity and higher relative abundance of Enterobacter. **J Pediatrics** 2014;165(1):23-9. doi: 10.1016/j.jpeds.2014.01.010
16. Tamboli C, Neut C, Desreumaux P, Colombel J. Dysbiosis in inflammatory bowel disease. **Gut** 2004;53(1):1-4.
17. Pflughoeft KJ, Versalovic J. Human microbiome in health and disease. **Annu Rev Pathol** 2012;7:99-122. doi:10.1146/annurev-pathol-011811-132421
18. Aziz Q, Doré J, Emmanuel A, Guarner F, Quigley E. Gut microbiota and gastrointestinal health: current concepts and future directions. **Neurogastroenterol Motil** 2013;25(1):4-15. doi: 10.1111/nmo.12046
19. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. **Cell** 2006;124(4):837-48. doi: 10.1016/j.cell.2006.02.017
20. Maslowski KM, Mackay CR. Diet, gut microbiota and immune responses. **Nature Immunol** 2010;12(1):5. doi: 10.1038/ni0111-5.
21. Hakansson A, Molin G. Gut microbiota and inflammation. **Nutrients** 2011;3(6):637-82.
22. Palinski W, Witztum J. Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. **J Intern Med** 2000;247(3):371-80. doi: 10.1046/j.1365-2796.2000.00656.x
23. Greaves DR, Channon KM. Inflammation and immune responses in atherosclerosis. **Curr Atheroscler Rep** 2002;23(11):535-41.
24. Deitch EA. Bacterial translocation of the gut flora. **Indian J Med Microbiol** 1990;30:184. doi:10.4103/0255-0857.118870
25. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. **Diabetes** 2007;56(7):1761-72. doi: 10.2337/db06-1491
26. Wiedermann CJ, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F, et al. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. **J Am Coll Cardiol** 1999;34(7):1975-81.
27. Westerterp M, Berbée JF, Pires NM, van Mierlo GJ, Kleemann R, Romijn JA, et al. Apolipoprotein CI is crucially involved in lipopolysaccharide-induced atherosclerosis development in apolipoprotein E–knockout mice. **Circulation** 2007;116(19):2173-81. doi: 10.1161/CIRCULATIONAHA.107.693382
28. Deitch EA, Berg R, Specian R. Endotoxin promotes

the translocation of bacteria from the gut. **Arch Surg** 1987;122(2):185-90.
29. Cani PD, Rodrigo B, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. **Diabetes** 2008. doi: 10.2337/db07-1403.
30. Popa C, Netea MG, Van Riel PL, Van Der Meer JW, Stalenhoef AF. The role of TNF-α in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. **J Lipid Res** 2007;48(4):751-62. doi: 10.1194/jlr.R600021-JLR200
31. Libby P. Inflammation in atherosclerosis. **Arterioscler Thromb Vasc Biol** 2012;32(9):2045-51. doi:10.1161/atvbaha.108.179705
32. Lin YP, Thibodeaux CH, Peña JA, Ferry GD, Versalovic J. Probiotic Lactobacillus reuteri suppress proinflammatory cytokines via c-Jun. **Inflamm Bowel Dis** 2008;14(8):1068-83. doi: 10.1002/ibd.20448.
33. Rodes L, Khan A, Paul A, Coussa-Charley M, Marinescu D, Tomaro-Duchesneau C, et al. Effect of probiotics Lactobacillus and Bifidobacterium on gut-derived lipopolysaccharides and inflammatory cytokines: an in vitro study using a human colonic microbiota model. **J Microbiol Biotechnol** 2013;23(4):518-26.
34. Grayston JT, Kronmal RA, Jackson LA, Parisi AF, Muhlestein JB, Cohen JD, et al. Azithromycin for the secondary prevention of coronary events. **N Engl J Med** 2005;352(16):1637-45.doi: 10.1056/NEJMoa043526
35. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helø OH, et al. Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. **BMJ** 2006;332(7532):22. doi: 10.1136/bmj.38666.653600.55
36. Ghanavati M, Hosseini SA, Alipour M, Ashtari-larky D, Cheraghpour K. The role of probiotics in the management of cardiovascular disease risk factors. **Clin Exc** 2015; 4 (S1):156-140.
37. Ryan JJ, Hanes DA, Schafer MB, Mikolai J, Zwickey H. Effect of the probiotic Saccharomyces boulardii on cholesterol and lipoprotein particles in hypercholesterolemic adults: a single-arm, open-label pilot study. **J Altern Complement Med** 2015;21(5):288-93. doi: 10.1089/acm.2014.0063.
38. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. **Science** 2013;341(6150):1241214. doi: 10.1126/science.1241214.
39. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded?. **Diabetes Care** 2010;33(10):2277-84. doi: 10.2337/dc10-0556
40. Wang Z, Nakayama T. Inflammation, a link between obesity and cardiovascular disease. **Mediators inflamm** 2010;2010. doi: 10.1155/2010/535918.
41. Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. **Mol Aspects Med** 2013;34(1):39-58. doi: 10.1016/j.mam.2012.11.001.
42. Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, et al. Evidence for a link between gut microbiota and hypertension in the Dahl rat. **Physiol Genomics** 2015;47(6):187-97. doi: 10.1152/physiolgenomics.00136.2014.
43. Ebel B, Lemetais G, Beney L, Cachon R, Sokol H, Langella P, et al. Impact of probiotics on risk factors for cardiovascular diseases. A review. **Crit Rev Food Sci Nutr** 2014;54(2):175-89. doi: 10.1080/10408398.2011.579361.
44. Kim MS, Hwang SS, Park EJ, Bae JW. Strict vegetarian diet improves the risk factors associated with metabolic diseases by modulating gut microbiota and reducing intestinal inflammation. **Environ Microbiol Rep** 2013;5(5):765-75. doi: 10.1111/1758-2229.12079.
45. Cannon CP, Braunwald E, McCabe CH, Grayston JT, Muhlestein B, Giugliano RP, et al. Antibiotic treatment of Chlamydia pneumoniae after acute coronary syndrome. **N Engl J Med** 2005;352(16):1646-54. doi: 10.1056/NEJMoa043528.
46. Corthésy B, Gaskins HR, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. **J Nutr** 2007;137(3):781S-90S. doi: 10.1093/jn/137.3.781S.
47. Ettinger G, MacDonald K, Reid G, Burton JP. The influence of the human microbiome and probiotics on cardiovascular health. **Gut Microbes** 2014;5(6):719-28. doi: 10.4161/19490976.2014.983775.
48. Dylag K, Hubalewska-Mazgaj M, Surmiak M, Szmyd J, Brzozowski T. Probiotics in the mechanism of protection against gut inflammation and therapy of gastrointestinal disorders. **Curr Pharm Des** 2014;20(7):1149-55.
49. Gan XT, Ettinger G, Huang CX, Burton JP, Haist JV, Rajapurohitam V, et al. Probiotic administration attenuates myocardial hypertrophy and heart failure after myocardial infarction in the rat. **Circ Heart Fail** 2014;7(3):491-9. doi: 10.1161/CIRCHEARTFAILURE.113.000978.
50. Jie L, Bin G, MIRSHAHI F, SANYAL AJ, KHANOLKAR AD, MAKRIYANNIS A, et al. Functional CB1 cannabinoid receptors in human vascular endothelial cells. **Biochem J** 2000;346(3):835-40.
51. Ufnal M, Zadlo A, Ostaszewski R. TMAO: A small molecule of great expectations. **Nutrition** 2015;31(11-12):1317-23. doi: 10.1016/j.nut.2015.05.006.
52. Howlett AC. The cannabinoid receptors. **Prostaglandins Other Lipid Mediat** 2002;68:619-31. PMID:12432948.
53. Tripolt NJ, Leber B, Triebl A, Köfeler H, Stadlbauer V, Sourij H. Effect of Lactobacillus casei Shirota supplementation on trimethylamine-N-oxide levels in patients with metabolic syndrome: An open-label, randomized study. **Atherosclerosis** 2015;242(1):141-4. doi: 10.1016/j.atherosclerosis.
54. Szeto Y, Kwok TC, Benzie IF. Effects of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. **Nutrition** 2004;20(10):863-6. doi: 10.1016/j.nut.2004.06.006.
55. Tang WW, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. **J Clin Invest** 2014;124(10):4204-11. doi: 10.1172/JCI72331.
56. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. **Nature** 2011;472(7341):57.
57. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. **Science** 2011;334(6052):105-8. doi: 10.1126/science.1208344.
58. Matijašić BB, Obermajer T, Lipoglavšek L, Grabnar I,

Avguštin G, Rogelj I. Association of dietary type with fecal microbiota in vegetarians and omnivores in Slovenia. **Eur J Nutr** 2014;53(4):1051-64. doi: 10.1007/s00394-013-0607-6.
59. Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. **Cell Metab** 2013;17(1):49-60. doi: 10.1016/j.cmet.2012.12.011.
60. Wang Z, Tang WW, Buffa JA, Fu X, Britt EB, Koeth RA, et al. Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. **Eur Heart J** 2014;35(14):904-10.
61. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. **Cell** 2015;163(7):1585-95. doi: 10.1016/j.cell.2015.11.055.
62. Chen M-l, Yi L, Zhang Y, Zhou X, Ran L, Yang J, et al. 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(2):e02210-15. doi: 10.1128/mBio.02210-15.
63. Martin FPJ, Wang Y, Sprenger N, Yap IK, Lundstedt T, Lek P, et al. Probiotic modulation of symbiotic gut microbial–host metabolic interactions in a humanized microbiome mouse model. **Mol Syst Biol** 2008;4(1):157. doi: 10.1038/msb4100190.
64. Costanza AC, Moscavitch SD, Neto HCF, Mesquita ET. Probiotic therapy with Saccharomyces boulardii for heart failure patients: a randomized, double-blind, placebo-controlled pilot trial. **Int J Cardiol** 2015;179:348-50. doi: 10.1016/j.ijcard.2014.11.034
65. Lam V, Su J, Koprowski S, Hsu A, Tweddell JS, Rafiee P, et al. Intestinal microbiota determine severity of myocardial infarction in rats. **FASEB J** 2012;26(4):1727-35. doi: 10.1096/fj.11-197921.
66. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. **Nat Med** 2013;19(5):576. . doi: 10.1038/nm.3145.
67. Porter AC, Felder CC. The endocannabinoid nervous system: unique opportunities for therapeutic intervention. **Pharmacol Ther** 2001;90(1):45-60.
68. Tuma R, Steffens S. Targeting the endocannabinod system to limit myocardial and cerebral ischemic and reperfusion injury. **Curr Pharm Biotechnol** 2012;13(1):46-58.
69. Dewald O, Duerr GD. The role for the endocannabinoid system in cardioprotection and myocardial adaptation. **Intech Open** 2016. doi: 10.5772/62485.
70. Steffens S, Pacher P. Targeting cannabinoid receptor CB2 in cardiovascular disorders: promises and controversies. **Br J Pharmacol** 2012;167(2):313-23. doi:10.1111/j.1476-5381.2012.02042.x.
71. Boyd ST. The endocannabinoid system. Pharmacotherapy. **Alcohol Alcohol** 2006;26(12P2):218S-21S. doi:10.1093/alcalc/agh110.
72. Carbone F, Mach F, Vuilleumier N, Montecucco F. Cannabinoid receptor type 2 activation in atherosclerosis and acute cardiovascular diseases. **Curr Med Chem** 2014;21(35):4046-58.
73. Netherland CD, Pickle TG, Bales A, Thewke DP. Cannabinoid receptor type 2 (CB2) deficiency alters atherosclerotic lesion formation in hyperlipidemic Ldlr-null mice. **Atherosclerosis** 2010;213(1):102-8. doi:10.1016/j.atherosclerosis.2010.07.060
74. Dol-Gleizes F, Paumelle R, Visentin V, Marés A-M, Desitter P, Hennuyer N, et al. Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor–deficient mice. **Arterioscler Thromb Vasc Biol** 2009;29(1):12-8. doi:10.1161/ATVBAHA.108.168757.
75. Nissen SE, Nicholls SJ, Wolski K, Rodés-Cabau J, Cannon CP, Deanfield JE, et al. Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. **JAMA** 2008;299(13):1547-60. doi:10.1001/jama.299.13.1547.
76. Roth MD. Pharmacology: marijuana and your heart. **Nature** 2005;434(7034):708.
77. Maccarrone M, De Petrocellis L, Bari M, Fezza F, Salvati S, Di Marzo V, et al. Lipopolysaccharide downregulates fatty acid amide hydrolase expression and increases anandamide levels in human peripheral lymphocytes. **Arch Biochem Biophys** 2001;393(2):321-8..doi:10.1006/abbi.2001.2500.
78. Bátkai S, Rajesh M, Mukhopadhyay P, Hasko G, Liaudet L, Cravatt BF, et al. Decreased age-related cardiac dysfunction, myocardial nitrative stress, inflammatory gene expression, and apoptosis in mice lacking fatty acid amide hydrolase. **Am J Physiol Heart Circ Physiol** 2007;293(2):H909-H18. doi:10.1152/ajpheart.00373.2007.
79. Braud S, Bon C, Touqui L, Mounier C. Activation of rabbit blood platelets by anandamide through its cleavage into arachidonic acid. **FEBS Lett** 2000;471(1):12-6. doi: 10.1016/S0014-5793(00)01359-4.
80. Bátkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, Harvey-White J, et al. Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. **Circulation** 2004;110(14):1996-2002. doi:10.1161/01.CIR.0000143230.23252.D2.
81. Matthews AT, Ross MK. Oxyradical stress, endocannabinoids, and atherosclerosis. **J Neuroendocrinol** 2015;3(4):481-98. doi:10.1111/j.1365-2826.2008.01685.x.
82. Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM, et al. The endocannabinoid system links gut microbiota to adipogenesis. **Mol Syst Biol** 2010;6(1):392. doi:10.1038/msb.2010.46.
83. Cani PD, Plovier H, Van Hul M, Geurts L, Delzenne NM, Druart C, et al. Endocannabinoids—at the crossroads between the gut microbiota and host metabolism. **Nat Rev Endocrinol** 2016;12(3):133. doi:10.1038/nrendo.2015.211.
84. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. **Proc Natl Acad Sci U S A** 2013;110(22):9066-71. doi:10.1073/pnas.1219451110.
85. Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, et al. Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors. **Nat Med** 2007;13(1):35. doi:10.1038/nm1521.
86. Moreira APB, Texeira TFS, Ferreira AB, Peluzio MdCG, Alfenas RdCG. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. **Br J Nutr** 2012;108(5):801-9. doi:10.1017/S0007114512001213.
87. Bermudez-Silva FJ, Sanchez-Vera I, Suárez J, Serrano A,

Fuentes E, Juan-Pico P, et al. Role of cannabinoid CB2 receptors in glucose homeostasis in rats. **Eur J Pharmacol** 2007;565(1-3):207-11. doi:10.1016/j.ejphar.2007.02.066.

88. Palermo FA, Mosconi G, Avella MA, Carnevali O, Verdenelli MC, Cecchini C, et al. Modulation of cortisol levels, endocannabinoid receptor 1A, proopiomelanocortin and thyroid hormone receptor alpha mRNA expressions by probiotics during sole (Solea solea) larval development. **Gen Comp Endocrinol** 2011;171(3):293-300. doi:10.1016/j.ygcen.2011.02.009.
89. Aguilera M, Vergara P, Martinez V. Stress and antibiotics alter luminal and wall-adhered microbiota and enhance the local expression of visceral sensory-related systems in mice. **Neurogastroenterol Motil** 2013;25(8):e515-e29. doi:10.1111/nmo.12154.
90. 90. Alhamoruni A, Lee A, Wright K, Larvin M, O'sullivan S. Pharmacological effects of cannabinoids on the Caco-2 cell culture model of intestinal permeability. **J Pharmacol Exp Ther** 2010;335(1):92-102. doi:10.1124/jpet.110.168237.
91. Liu J, Bátkai S, Pacher P, Harvey-White J, Wagner JA, Cravatt BF, et al. Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-κB independently of platelet-activating factor. **J Biol Chem** 2003;278(45):45034-9. doi:10.1074/jbc.M306062200.