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Microbiome and Ocular Health: Insights and Perspectives Theme Issue

Microbiome and retinal vascular diseases

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Abstract

The gut microbiome consists of more than thousand different microbes and their associated genes and microbial metabolites. It influences various host metabolic pathways and is therefore important for homeostasis. In recent years, its influence on health and disease was extensively researched. In case of a microbiome disequilibrium called dysbiosis, the gut microbiome is associated with several diseases. Consequent chronic inflammation may lead to or promote inflammatory bowel disease, obesity, diabetes mellitus, atherosclerosis, alcoholic and non-alcoholic liver disease, cirrhosis, hepatocellular carcinoma and other diseases. The pathogenesis of the three most common retinal vascular diseases, diabetic retinopathy, retinal vein and artery occlusion, may also be influenced by an altered microbiome and associated risk factors such as diabetes mellitus, atherosclerosis, hypertension and obesity. Direct cause-effect relationships remain less well understood. A potential prevention or treatment modality for these diseases could be targeting and modulating the individual's gut microbiome.

Introduction

Retinal vascular diseases (RVD) refer to a range of diseases affecting the blood vessels in the eyes and represent a major part of ophthalmic disease burden in the general population. Diabetic retinopathy (DR) is the most common RVD, followed by retinal vein occlusion (RVO) and retinal artery occlusion (RAO).[1, 2] Due to the large number of affected patients and the immense disease burden, it is of medical and economic interest to find measures of improving treatment and ultimately preventing these diseases. While the three entities DR, RVO and RAO have different pathophysiologic characteristics, they share common risk factors such as atherosclerosis, arterial hypertension, dyslipidemia and obesity.[3-5]

In recent years, extensive research has been done to understand the gut microbiome and its impact on health and disease. It influences metabolic processes and the immune system of its host through microbial-derived metabolites. Our gut microbiome consists of microbes including bacteria, archaea, viruses and eukaryotes and their microbial products and genes. It comprises more than thousand different species of bacteria and carries about one hundred fifty times more genes than found in the entire human genome. This leads to the assumption that this “essential organ” is of utmost importance for homeostasis and health.[6] With the introduction of modern high-throughput sequencing techniques, such as 16S rRNA gene sequencing and whole-metagenome shotgun sequencing, the taxonomic composition of the gut microbiome has been described. On the phylum level, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria, Fusobacteria* and *Verrucomicrobia* represent the main part of the human gut microbiome.[7]

Associations between an imbalance in the gut microbiome’s composition, called dysbiosis, and various diseases such as inflammatory bowel disease, obesity, diabetes mellitus (DM), atherosclerosis, alcoholic and non-alcoholic liver disease, cirrhosis and hepatocellular carcinoma have been found.[6] Under dysbiotic conditions, the equilibrium shifts toward bacteria with pathogenic characteristics. This may lead to the breakdown of the intestinal epithelial barrier and subsequently to translocation

of microbes and their products into the systemic circulation. These microbial products may have various effects on tissue and cells in all organs of the body, including the eye.

Certain microbial products such as lipopolysaccharides (LPS) lead to inflammation. In contrast, production of microbial metabolites with protective effects such as short-chain fatty acids (SCFA) or bile acids (BA), may be inhibited in case of gut dysbiosis. Furthermore, epigenetic programming through histone acetylation and deacetylation by commensal bacteria, which promotes or represses the expression of certain genes, may have an influence on pathogenesis through overactivation of the immune system leading to chronic low-grade inflammation.[8] Associations between an altered gut microbiome and the pathogenesis of ophthalmic diseases as well as their risk factors have been suggested.[9-15] The connection between the gut microbiome and the retina has been termed the "gut-retina axis" (Figure 1).[16]

While the role of the gut microbiome in the pathogenesis of some diseases such as DR and RAO, has been investigated in recently published studies, its influence on other diseases such as RVO remain less understood.[17, 18] This review aims to summarize the current knowledge linking the gut microbiome to the most common retinal vascular diseases. The pathophysiology of the three main RVDs are elucidated in the first part. In the second part, associations between these RVDs, their risk factors and the gut microbiome are discussed. In the last part the microbial metabolites are presented.

Retinal vascular diseases

The following paragraphs depict the three most common RVDs including their epidemiology and pathophysiology.

Diabetic Retinopathy

Diabetic Retinopathy (DR) is the most common retinal vascular disease and a major ocular complication of DM. The pathophysiology of DR is dominated by microvascular pathology. The microvascular retinal damage arises due to non- or inadequately controlled blood glucose levels. While patients usually do not notice any symptoms in the early stages, later stages of the disease are often more devastating and can ultimately end in complete blindness, making it a very insidious disease. This is troublesome alone due to the epidemiologic numbers of patients affected by DM. Today, there are roughly 285 million people affected by DM worldwide of which one third suffers from DR.[19]

The most important aspect of treatment is early screening for DR in diabetic patients as well as control and regulation of blood glucose levels, anticipating hyperglycemia as it has a direct effect on DR.[20] Early stages are characterized by vascular retinal changes such as dot and blot hemorrhages, cotton-wool spots, venous beading and intraretinal microvascular anomalies (Figure 2A). Patients often only notice a deterioration of vision if macular edema develops. Macular edema is characterized by retinal thickening and accumulation of intraretinal fluid in the central macula, due to breakdown of the blood-retinal-barrier.[21] In advanced disease stages, retinal hypoxia can lead to formation of new blood vessels, which can lead to various complications such as vitreous hemorrhage, glaucoma and retinal detachment. This stage is called proliferative diabetic retinopathy. There is a loss of retinal vessel autoregulation, basement membrane thickening, loss of pericytes, progressive non-perfusion of capillaries and subsequent ischemia.[22] The periarterial capillary free zone is much larger in individuals suffering from DR.[23] This can lead to release of pro-angiogenic peptides such as vascular endothelial growth factor (VEGF), which is responsible for the formation of neovascularisations.[24] End-stage disease (proliferative diabetic retinopathy) is associated with repeated hemorrhages from these incompetent vessels and fibrosis of the retinal surface leading to tractional retinal detachments.[25] Treatment includes retinal laser photocoagulation of ischemic areas and altered vasculature as well as intravitreal anti-VEGF injections, steroid injections and vitreoretinal surgery. Risk factors for DR are identical with those for DM and comprise a positive family history, age (younger for type 1 DM and

older for type 2 DM), ethnicity, sedentary lifestyle, obesity and diet. Due to the large and continuously growing number of patients affected with DM and DR and accumulating evidence of associations with the gut microbiome, targeting these diseases by microbiome-altering interventions are of great interest.[18]

Retinal Vein Occlusion

The second most common RVD is retinal vein occlusion (RVO). It is defined as a vascular insult of the retina based on impeded venous outflow, usually due to embolism in the venous vasculature (Figure 2B). Depending on the affected area (central retinal vein versus smaller branch retinal vein), impact on vision and complications differ. Smaller RVOs can even go unnoticed by the patient if only a peripheral area of retinal tissue is affected. Globally, about 28 million people suffer from the consequences of RVO.[26] The most common complication of RVO is macular edema usually causing a reduction in visual acuity.[27] Development of macular edema is the consequence of increased vascular permeability due to vascular congestion and upregulation of vascular endothelial growth factor (VEGF). Other complications are optic neuropathy, macular ischemia, vitreous hemorrhage and retinal ischemia due to non-perfusion of retinal tissue. The latter can lead to choroidal neovascularization with potentially devastating visual outcomes.[28] Since ischemic retinal areas are hard to identify upon ophthalmoscopy, they are best examined with fluorescein angiography (FA), delineating areas of capillary non-perfusion. FA also enables the detection of subclinical neovascularization. The mainstay of treatment are intravitreal anti-VEGF and steroid injections and retinal laser photocoagulation. Risk factors are arterial hypertension, dyslipidemia, obesity, atherosclerosis and smoking.[5, 28] Interestingly, DM itself is an important risk factor for RVO and seems to be associated with our gut microbiome[29, 30]

Retinal Artery Occlusion

Third most common RVD is retinal artery occlusion (RAO) with a worldwide annual incidence of about 1-15 per 100'000 people.[31] RAO occurs due to occlusion of an arterial vessel supplying the retina. Due to the very high metabolic demand of the retina and absence of vascular bypasses, RAO usually leads to severe visual impairment within few hours. Because of non-perfusion of retinal arteries and arterioles and consequent retinal hypoxemia, nerve fiber swelling and breakdown of the metabolic visual cycle occurs, ultimately leading to cell death (Figure 2C). While most RAO are caused by large vessel disease (macroangiopathy) such as embolization from atherosclerotic plaques, cases due to small vessel disease (microangiopathic) also exist. An inflammatory cause must be separated from a non-inflammatory, purely embolic cause. Vasculitis such as giant cell arteritis can cause occlusion of arterial vessels as in RAO. Furthermore, a hypercoagulable state must be excluded. This could point to other diseases affecting the coagulation cascades or malignancies.[32] No formal treatment for patients who suffered from a RAO is available. If the onset of symptoms is less than 4.5 hours ago, intravascular thrombolysis is a treatment option.[5] In summary, in most of the cases of RAO, the disease can be considered as an ischemic stroke.[33] Patients at risk for vascular insults are also candidates for potential RAO. Inversely in the weeks following a RAO, patients are at elevated risk for a stroke.[34] Risk factors are atherosclerosis, hypertension, DM, obesity, dyslipidemia and thrombus eliciting circumstances or events, such as atrial fibrillation, whereby these risk factors seem to be influenced by the gut microbiome.[5, 11, 28]

Retinal vascular diseases, associated risk factors and the microbiome

The direct relationship between the gut microbiome and retinal vascular diseases has not been studied comprehensively. To date,there are only a few articles addressing the influence of the gut microbiome on DR and RAO [17, 18, 35, 36] and no article investigating the influence of the gut microbiome on RVO.

The link between the gut microbiome and the RVDs may be explained by risk factors that partially overlap between the different entities. Most of these risk factors and their associations with the gut microbiome have already been subject of extensive research. In the following paragraphs, we will review the associated changes of the gut microbiome in RAO and DR and clarify how the risk factors for RVDs are connected with the gut microbiome.

Retinal artery occlusion and the microbiome

In RAO, compositional and functional shifts in the gut microbiome were reported. Using whole metagenome shotgun sequencing, the gut microbiome of 29 patients with non-arteritic RAO was compared to 30 healthy controls. The class *Actinobacteria* and the species *Bifidobacterium adolescentis, Bifidobacterium bifidum, Bacteroides stercosis* and *Faecalibacterium prausnitzii* were enriched in RAO patients, whereas the family *Lachnospiraceae*, the genera *Odoribacter* and *Parasutterella* were enriched in controls.[17]

In addition to these taxonomic differences, there were differences in functional features of the gut microbiome. The mevalonate pathway and methylerythritol phosphate pathway, both involved in cholesterol metabolism, were enriched in RAO patients. Interestingly, the identified compositional and functional alterations of the gut microbiome were closely associated with atherosclerosis. In patients with symptomatic atherosclerosis, a higher abundance of *Collinsella* (belonging to the class of *Actinobacteria*) was found. DNA found in atherosclerotic plaques mainly originates from *Actinobacteria*. Furthermore, an enriched mevalonate pathway, also known as the target of statins, represents an important pathway in the pathogenesis of atherosclerosis.

Diabetes mellitus, diabetic retinopathy, diet and obesityDM is a metabolic disease characterized by impaired glucose tolerance. Its most common variant is type 2 diabetes mellitus (T2D), affecting about 90% of all diabetic patients. There is evidence that in diabetic individuals compared to healthy controls, compositional and functional changes occur in the gut microbiome.[30] The abundances of

182 *Escherichia coli, Clostridium species, Bacteroides caccae* and *Eggerthella lenta* were higher, while the
183 abundances of *Eubacterium rectale, Clostridiales sp. SS3/4, Faecalibacterium prausnitzii* and *Roseburia*
184 *intestinalis* were lower in T2D patients compared to healthy controls.[30] Furthermore, several microbial
185 pathways and metabolites were identified that may lead to the progression of diabetes. Gut bacteria
186 derived NOD1 ligands, acting as signal molecules between the gut and extra-intestinal organs,
187 modulate insulin trafficking in pancreatic beta cells.[37] Indeed, treatment with fecal microbiota
188 transplantation (FMT) showed promising results in DM. In recently diagnosed type 1 DM patients
189 receiving allogenic FMTs from healthy donors, a decline in endogenous insulin production was
190 observed.[38]
191
192 While there are vast similarities in the gut microbiome composition changes in patients with DM and
193 DR, the latter has been associated with lower bacterial diversity and specific compositional changes.[18,
194 35] At the phylum level *Bacteroidetes* were more abundant in diabetic patients with DR compared to
195 patients with only DM.[18] Other bacteria such as the genera *Blautia* and *Lactobacillus* were more
196 abundant in patients with DM but without DR.[18] In another study increased abundance of
197 *Burkholderiaceae* and *Burkholderiales_unclassified* were found in patients with DR while significantly
198 less abundances in 22 families including Streptococcaceae, Coriobacteriaceae and Veillonellaceae
199 were noted.[35] Furthermore significant differences in lipid-, amino- and nucleotide metabolism were
200 observed in DR patients.[35] In addition, known mediators for DR development, such as arachidonic acid,
201 hydroxyeicosatetraenoic acids and leukotriene were increased in fecal samples of patients with DR.[35]
202
203 DM and DR are associated with obesity.[39] A high-caloric western style diet rich in saturated fat and
204 sugar is one of the major risks for obesity and T2D. As a consequence of a western style diet, an
205 increased intestinal permeability due to breakdown of the gut epithelium barrier was observed.[10, 40]
206 Diet modification is an important management tool in T2D treatment and is able to influence the
207 composition of the gut microbiome. In diabetic mice fed with compound dietary fiber and high-grade

protein (CFP) diet, increased *Firmicutes* and decreased *Bacteroidetes* abundances were observed. An enrichment of *Dubosiella, Parasutterella, Ruminococcaceae, Muribaculum, Allobaculum* and *Bifidobacterium* was observed after 4 weeks of CFP diet. In addition, a decrease of hyperglycemia and insulin resistance as well as a protective effect on the gut barrier in terms of reduced lipopolysaccharide and D-lactate levels, which are indicators for intestinal permeability, was noted. This effect was attributed to a reduction of endotoxemia and an enhancement of mucin secretion.[41]

Moreover, an excessive amount of body fat (linked with a high caloric intake) influences both, the development of T2D and the composition of the gut microbiome. In animal studies, gut microbes had an influence on inflammatory pathways and host gene expression, promoting weight gain. Germ free (GF) mice are known to keep a low body fat percentage, even when fed with a high-caloric diet. After fecal microbiota transplantation from normal mice, a significant increase in body fat percentage was observed. This may be due to different absorption and metabolization of dietary intake by the modified gut microbes and activation of different pathways involved in triglyceride production and insulin resistance.[11]

In human obese subjects, increased amounts of *Bacteroidetes* were found, though there are studies disputing this observation.[11] Beli et al. showed that db/db mice (a genetically engineered mouse model for diabetes research) on an intermittent fasting regime displayed a reduction in DR features and compositional changes in the gut microbiome. Increased levels of *Firmicutes* and decreased levels of *Bacteroidetes* and *Verrucomicrobia* were observed. The authors hypothesized that intermittent fasting prevents DR by altering the gut microbiome toward species producing neuroprotective agents such as the bile acid “tauroursodeoxycholate”.[42] To prevent end-organ damage such as DR, treatment of T2D through the modification of the gut microbiome by dietary interventions may therefore be an interesting approach.

Hypertension and Atherosclerosis

Hypertension and atherosclerosis are known risk factors for cardiovascular disease and RVD. The elevation of the arterial blood pressure is one of the factors leading to atherosclerosis. In atherosclerosis, the subendothelial accumulation of cholesterol, lipids and elastin forms plaques in arteries. These plaques can narrow the vessels, reducing the perfusion of subsequent organs and in case of plaque rupture and thrombus formation, lead to thromboembolism and vessel occlusion. Factors contributing to hypertension and atherosclerosis arise from a combination of genetic and environmental causes. The gut microbiome represents the intersection between the environment and the host and seems to play a role in their pathophysiology.

Various gram-negative bacteria such as *Klebsiella*, *Prevotella*, *Desulfibrio* and *Parabacteroides* are associated with hypertension. In a rodent model for hypertension, fecal microbiome transplantation from a hypertensive mouse to a GF mouse induced hypertension in the GF mouse. Furthermore, the gut microbiome from hypertensive rats was different compared to normotensive control rats.[10] Different pathways linking the gut microbiome to the development and progression of atherosclerosis have been studied. Since bacterial DNA, most often from *Chlamydia pneumoniae*, has been isolated from atherosclerotic plaques, bacteria may have an influence in plaque development, progression and/or rupture. It is assumed that infection of the vessel wall or at a distant site can lead to plaque formation. Indeed, compositional changes of the gut microbiome were found in patients with symptomatic atherosclerosis. Whereas *Collinsella* was enriched, *Eubacterium* and *Roseburia* were decreased in atherosclerotic patients compared to healthy controls.[43]

In the last paragraphs, we outlined the connection between the risk factors of RVD and an altered microbiome. In the following paragraphs, the most important microbial metabolites will be presented.

Microbial metabolites

Various microbial metabolites are produced by the gut bacteria. These metabolites have signaling properties and enable the communication between the gut microbiome and its host by means of

influencing various pathways. While some have detrimental effects such as causing inflammation (lipopolysaccharide and trimethylamine N-oxide), others have protective effects such as maintaining homeostasis (neuroprotective bile acids and short-chain fatty acids). In case of gut dysbiosis, the equilibrium of metabolites with harmful and protective effects shifts leading to chronic inflammation associated with several diseases.

Lipopolysaccharide

Lipopolysaccharide (LPS), a bacterial surface glycolipid and part of the outer membrane of most gram-negative bacteria, is associated with an acute and chronic inflammation. It is recognized through the Toll-like receptor 4 and triggers an immediate response by the innate immune system, resulting in an acute inflammation via the release of pro-inflammatory cytokines.In case of an altered epithelial gut membrane (e.g. through dybiosis) combined with a translocation and persistent release of LPS into the systemic circulation, LPS results in a chronic low-grade inflammation. It is involved in the pathogenesis of many diseases, such as obesity, cardiovascular disease, chronic kidney disease, inflammatory bowel disease and diabetes.[44,45] Furthermore, it was shown that LPS accelerates neurodegeneration by activating microglia. This may play a role in retinal diseases such as age-related macular degeneration.[46] Moreover, the persistent low-grade inflammation was linked to the development of diabetic microvascular complications such as DR.[47] Higher levels of LPS and pro-inflammatory cytokines were found in individuals with DR. Demonstration of inflammation leading to DR was provided by diabetic patients who also suffered from rheumatoid arthritis, taking salicylates to treat this condition. In these patients, the incidence of DR was found to be significantly lower.[47]

Trimethylamine N-oxide

Trimethylamine N-oxide (TMAO) is a microbial metabolite produced by microbes in the gastrointestinal tract from dietary choline, phosphatidylcholine, betaine and L-carnitine and subsequent oxidation. Various bacteria have different capacities to produce TMAO. Therefore, depending on the individual composition of the gut microbiome, TMAO levels may be different. It has been suggested that TMAO is important in the pathophysiology of cardiovascular diseases (CVD) with higher TMAO levels correlating with worse outcome.[43] The role of TMAO in the pathogenesis of atherosclerosis is not fully understood. It has been shown that TMAO modulates several metabolic pathways, including the cholesterol and sterol metabolism. By changing the expression of cholesterol transporters, TMAO may inhibit reverse cholesterol transport.

In flavin monooxygenase 3 (enzyme involved in TMAO production) knockdown mice, reduced atherosclerotic lesion size was observed.[43] Furthermore, there is evidence that TMAO is involved in the development of diabetes mellitus through immunological and inflammatory pathways.[44] Moreover in RAO patients, TMAO levels were increased compared to healthy individuals and a positive correlation of TMAO levels with the abundance of *Akkermansia* was found, while a negative correlation with the abundance of *Parasutterella* and *Lachnospiraceae* was noted.[17] Although TMAO is generally accepted to be pro-atherogenic, a recent study found no increase in TMAO levels in asymptomatic patients with atherosclerosis and even lower levels in patients following stroke and transitory ischemic attacks.[48] Thus, further research is needed to identify the mechanisms influencing CVD through TMAO and to explore the role of the gut microbiome in regulating TMAO plasma levels.

Bile Acids

Bile acids (BA) are important signaling molecules binding to cellular receptors such as the farnesoid X receptor, G protein-coupled bile acid receptor 1 and Vitamin D Receptor, which are expressed in different tissues. Importantly, the BA metabolism is influenced by the gut microbiome composition. Bacteria convert conjugated BAs into unconjugated BAs in the small intestine. About 5% of BA do not

enter the enterohepatic cycle and reach the colon, where they are further metabolized into secondary BAs by gut bacteria. They influence various physiological processes such as the cholesterol and lipid metabolism, immune regulation and neuroprotection. Therefore, they are assumed to play an important role in the pathophysiology of various diseases including diabetes. In T2D and obesity, characteristic BA profiles were found. Depending on the individual gut microbiome composition, the BA profile differs vastly.[49] In DR, a better outcome was speculated to be associated with the increase of the neuroprotective BA tauroursodeoxycholate (TUDCA) whose metabolism may be modulated by *Firmicutes* bacteria. Interestingly, retinal primary ganglion cells express the TUDCA receptor TGR5 and its activation by pharmacological agents prevented DR in a mouse model.[42]

Short-chain fatty acids

Short-chain fatty acids (SCFA) are produced by bacterial fermentation of dietary fibers and act as signaling molecules by binding to G-protein coupled receptors.[10]. Various bacteria such as *Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus bromii, Eubacterium halii* and *Bifidobacterium adolescentis* have the ability to produce SCFAs. The most common SCFAs are butyrate, propionate and acetate acids. These SCFAs have immunoregulatory effects by inducing the release of anti-inflammatory cytokines. They regulate the lipid and glucose metabolism, gut motility and vasoreactivity as well as influence energy harvesting. Furthermore, they act as an energy source for the gut epithelium.[6] They were shown to have a hypotensive effect, preventing CVD and reducing target organ damage when supplemented to a low fiber diet.[50] Moreover, SCFAs have neuroprotective effects as has been shown in a model of T-cell mediated autoimmunity used for experimental autoimmune encephalomyelitis. In this model, the disease course is dependent on the balance of pro-inflammatory Th1 and Th17 immune cells and neuroprotective Treg cells. By administration of dietary

SCFAs, a stimulatory effect on differentiation to and proliferation of neuroprotective Treg cells was shown and the disease course was ameliorated.[51]

Conclusion and outlook

Evidence for an influence of the gut microbiome on retinal health is accumulating mainly through the prism of its risk factors. Dysbiosis may lead to the development of RVDs through promotion of CVDs, such as atherosclerosis, hypertension as well as DM and obesity. In a single study the direct influence on pathways favoring the pathogenesis of RAO was described. As we deepen our understanding of the implications of the gut microbiome on retinal pathology, targeted therapeutic approaches may be developed to achieve personalized treatment options.[52] Another effective approach to change the gut microbiome composition is the short-term administration of antibiotics.[53, 54] However, if continuously administered, adverse side effects make this approach unfeasible. To restore homeostasis from a dysbiotic state, probiotic supplementation can be used. For instance, through the administration of *Lactobacillus rhamnosus GG,* which is commonly used in probiotic formulations, retinal dysfunction was restored and adverse vascular remodeling mitigated in $CBS^{+/-}$ mice.[55] A more natural and sustainable long-term approach could be based on the targeted modification of our diet. It has been shown that a healthy diet with abundant fiber intake is advantageous, since fibers are further processed into SCFAs with health promoting effects. Although there is a lot of research describing the associations between the gut microbiome and several diseases including RVDs, direct cause-effect relationships are still an active topic of research and need further investigation.

References

[1] Jampol LM, Glassman AR, Sun J: Evaluation and Care of Patients with Diabetic Retinopathy. N Engl J Med 2020, 382:1629-37.

[2] Leasher JL, Bourne RRA, Flaxman SR, Jonas JB, Keeffe J, Naidoo K, Pesudovs K, Price H, White RA, Wong TY, Resnikoff S, Taylor HR, Study obotVLEGotGBoD: Global Estimates on the Number of People Blind or Visually Impaired by Diabetic Retinopathy: A Meta-analysis From 1990 to 2010. Diabetes Care 2016, 39:1643-9.

[3] Lin KY, Hsih WH, Lin YB, Wen CY, Chang TJ: Update in the epidemiology, risk factors, screening, and treatment of diabetic retinopathy. J Diabetes Investig 2021, 12:1322-5.

[4] Risk factors for branch retinal vein occlusion. The Eye Disease Case-control Study Group. Am J Ophthalmol 1993, 116:286-96.

[5] Scott IU, Campochiaro PA, Newman NJ, Biousse V: Retinal vascular occlusions. Lancet 2020, 396:1927-40.

[6] Wang B, Yao M, Lv L, Ling Z, Li L: The Human Microbiota in Health and Disease. Engineering 2017, 3:71-82.

[7] Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC: What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms 2019, 7:14.

[8] Nayyar A, Gindina S, Barron A, Hu Y, Danias J: Do epigenetic changes caused by commensal microbiota contribute to development of ocular disease? A review of evidence. Human Genomics 2020, 14:11.

[9] Karlsson FH, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, Bäckhed F, Nielsen J: Symptomatic atherosclerosis is associated with an altered gut metagenome. Nature Communications 2012, 3:1245.

[10] Avery EG, Bartolomaeus H, Maifeld A, Marko L, Wiig H, Wilck N, Rosshart SP, Forslund SK, Müller DN: The Gut Microbiome in Hypertension. Circulation Research 2021, 128:934-50.

[11] John GK, Mullin GE: The Gut Microbiome and Obesity. Curr Oncol Rep 2016, 18:45.

[12] Zysset-Burri DC, Morandi S, Herzog EL, Berger LE, Zinkernagel MS: The role of the gut microbiome in eye diseases. Prog Retin Eye Res 2022:101117.

[13] Zinkernagel MS, Zysset-Burri DC, Keller I, Berger LE, Leichtle AB, Largiadèr CR, Fiedler GM, Wolf S: Association of the Intestinal Microbiome with the Development of Neovascular Age-Related Macular Degeneration. Sci Rep 2017, 7:40826.

[14] Zysset-Burri DC, Keller I, Berger LE, Largiadèr CR, Wittwer M, Wolf S, Zinkernagel MS: Associations of the intestinal microbiome with the complement system in neovascular age-related macular degeneration. NPJ Genom Med 2020, 5:34.

[15] Napolitano P, Filippelli M, Davinelli S, Bartollino S, dell'Omo R, Costagliola C: Influence of gut microbiota on eye diseases: an overview. Ann Med 2021, 53:750-61.

[16] Nadeem U, Xie B, Movahedan A, D'Souza M, Barba H, Deng N, Leone VA, Chang E, Sulakhe D, Skondra D: High Throughput RNA Sequencing of Germ-Free Mouse Retina Reveals Metabolic Pathways Involved in the Gut-Retina Axis. bioRxiv 2020:10.01.318949.

[17] Zysset-Burri DC, Keller I, Berger LE, Neyer PJ, Steuer C, Wolf S, Zinkernagel MS: Retinal artery occlusion is associated with compositional and functional shifts in the gut microbiome and altered trimethylamine-N-oxide levels. Sci Rep-uk 2019, 9:15303.

[18] Huang Y, Wang Z, Ma H, Ji S, Chen Z, Cui Z, Chen J, Tang S: Dysbiosis and Implication of the Gut Microbiota in Diabetic Retinopathy. Front Cell Infect Microbiol 2021, 11:646348.

[19] Lee R, Wong TY, Sabanayagam C: Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye Vis (Lond) 2015, 2:17.

[20] Aiello LP, Group ftDER: Diabetic Retinopathy and Other Ocular Findings in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study. Diabetes Care 2013, 37:17-23.

[21] Ford JA, Lois N, Royle P, Clar C, Shyangdan D, Waugh N: Current treatments in diabetic macular oedema: systematic review and meta-analysis. BMJ Open 2013, 3:e002269.

[22] Ogura S, Kurata K, Hattori Y, Takase H, Ishiguro-Oonuma T, Hwang Y, Ahn S, Park I, Ikeda W, Kusuhara S, Fukushima Y, Nara H, Sakai H, Fujiwara T, Matsushita J, Ema M, Hirashima M, Minami T, Shibuya M, Takakura N, Kim P, Miyata T, Ogura Y, Uemura A: Sustained inflammation after pericyte depletion induces irreversible blood-retina barrier breakdown. JCI Insight 2017, 2:e90905.

[23] Li H, Ding X, Lu L, Yang J, Ma J: Morphometry of the normal retinal periarteral capillary-free zone and changes during severe nonproliferative diabetic retinopathy. Clin Hemorheol Microcirc 2019, 72:169-78.

[24] Lechner J, O'Leary OE, Stitt AW: The pathology associated with diabetic retinopathy. Vision Research 2017, 139:7-14.

[25] Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, Gardiner TA, Lyons TJ, Hammes HP, Simó R, Lois N: The progress in understanding and treatment of diabetic retinopathy. Prog Retin Eye Res 2016, 51:156-86.

[26] Song P, Xu Y, Zha M, Zhang Y, Rudan I: Global epidemiology of retinal vein occlusion: a systematic review and meta-analysis of prevalence, incidence, and risk factors. J Glob Health 2019, 9:010427.

[27] Rogers S, McIntosh RL, Cheung N, Lim L, Wang JJ, Mitchell P, Kowalski JW, Nguyen H, Wong TY: The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and Australia. Ophthalmology 2010, 117:313-9.e1.

[28] Rehak M, Wiedemann P: Retinal vein thrombosis: pathogenesis and management. J Thromb Haemost 2010, 8:1886-94.

[29] Wang Y, Wu S, Wen F, Cao Q: Diabetes mellitus as a risk factor for retinal vein occlusion: A meta-analysis. Medicine (Baltimore) 2020, 99:e19319.

[30] Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J: A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012, 490:55-60.

[31] Feltgen N, Neubauer A, Jurklies B, Schmoor C, Schmidt D, Wanke J, Maier-Lenz H, Schumacher M: Multicenter study of the European Assessment Group for Lysis in the Eye (EAGLE) for the treatment of central retinal artery occlusion: design issues and implications. EAGLE Study report no. 1 : EAGLE Study report no. 1. Graefes Arch Clin Exp Ophthalmol 2006, 244:950-6.

[32] Hayreh SS: Acute retinal arterial occlusive disorders. Prog Retin Eye Res 2011, 30:359-94.

[33] Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, Elkind MS, George MG, Hamdan AD, Higashida RT, Hoh BL, Janis LS, Kase CS, Kleindorfer DO, Lee JM, Moseley ME, Peterson ED, Turan TN, Valderrama AL, Vinters HV: An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013, 44:2064-89.

[34] Callizo J, Feltgen N, Pantenburg S, Wolf A, Neubauer AS, Jurklies B, Wachter R, Schmoor C, Schumacher M, Junker B, Pielen A: Cardiovascular Risk Factors in Central Retinal Artery Occlusion: Results of a Prospective and Standardized Medical Examination. Ophthalmology 2015, 122:1881-8.

[35] Ye P, Zhang X, Xu Y, Xu J, Song X, Yao K: Alterations of the Gut Microbiome and Metabolome in Patients With Proliferative Diabetic Retinopathy. Front Microbiol 2021, 12:667632.

[36] Bai J, Wan Z, Zhang Y, Wang T, Xue Y, Peng Q: Composition and diversity of gut microbiota in diabetic retinopathy. Front Microbiol 2022, 13:926926.

[37] Zhang Q, Pan Y, Zeng B, Zheng X, Wang H, Shen X, Li H, Jiang Q, Zhao J, Meng Z-X, Li P, Chen Z, Wei H, Liu Z: Intestinal lysozyme liberates Nod1 ligands from microbes to direct insulin trafficking in pancreatic beta cells. Cell Research 2019, 29:516-32.

[38] de Groot P, Nikolic T, Pellegrini S, Sordi V, Imangaliyev S, Rampanelli E, Hanssen N, Attaye I, Bakker G, Duinkerken G, Joosten A, Prodan A, Levin E, Levels H, Potter van Loon B, van Bon A, Brouwer C, van Dam S, Simsek S, van Raalte D, Stam F, Gerdes V, Hoogma R, Diekman M, Gerding M, Rustemeijer C, de Bakker B, Hoekstra J, Zwinderman A, Bergman J, Holleman F, Piemonti L, De Vos W, Roep B, Nieuwdorp M: Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. Gut 2021, 70:92-105.

[39] Jensen BA, Marette A: Microbial translocation in type 2 diabetes: when bacterial invaders overcome host defence in human obesity. Gut 2020, 69:1724-6.

[40] Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T, Liao W: Influence of diet on the gut microbiome and implications for human health. J Transl Med 2017, 15:73.

[41] Ni Y, Zheng A, Hu Y, Rong N, Zhang Q, Long W, Yang S, Nan S, Zhang L, Zhou K, Wu T, Fu Z: Compound dietary fiber and high-grade protein diet improves glycemic control and ameliorates diabetes and its comorbidities through remodeling the gut microbiota in mice. Frontiers Nutrition 2022, 9:959703.

[42] Beli E, Yan Y, Moldovan L, Vieira CP, Gao R, Duan Y, Prasad R, Bhatwadekar A, White FA, Townsend SD, Chan L, Ryan CN, Morton D, Moldovan EG, Chu F-I, Oudit GY, Derendorf H, Adorini L, Wang XX, Evans-Molina C, Mirmira RG, Boulton ME, Yoder MC, Li Q, Levi M, Busik JV, Grant MB: Restructuring of the Gut Microbiome by Intermittent Fasting Prevents Retinopathy and Prolongs Survival in db/db Mice. Diabetes 2018, 67:1867-79.

[43] Jonsson AL, Bäckhed F: Role of gut microbiota in atherosclerosis. Nature Reviews Cardiology 2017, 14:79-87.

[44] Tanase DM, Gosav EM, Neculae E, Costea CF, Ciocoiu M, Hurjui LL, Tarniceriu CC, Maranduca MA, Lacatusu CM, Floria M, Serban IL: Role of Gut Microbiota on Onset and Progression of Microvascular Complications of Type 2 Diabetes (T2DM). Nutrients 2020, 12:3719.

[45] Candelli M, Franza L, Pignataro G, Ojetti V, Covino M, Piccioni A, Gasbarrini A, Franceschi F: Interaction between Lipopolysaccharide and Gut Microbiota in Inflammatory Bowel Diseases. Int J Mol Sci 2021, 22:6242.

[46] Noailles A, Maneu V, Campello L, Lax P, Cuenca N: Systemic inflammation induced by lipopolysaccharide aggravates inherited retinal dystrophy. Cell Death Dis 2018, 9:350.

[47] Fernandes R, Viana SD, Nunes S, Reis F: Diabetic gut microbiota dysbiosis as an inflammaging and immunosenescence condition that fosters progression of retinopathy and nephropathy. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 2019, 1865:1876-97.

[48] Yin J, Liao SX, He Y, Wang S, Xia GH, Liu FT, Zhu JJ, You C, Chen Q, Zhou L, Pan SY, Zhou HW: Dysbiosis of Gut Microbiota With Reduced Trimethylamine-N-Oxide Level in Patients With Large-Artery Atherosclerotic Stroke or Transient Ischemic Attack. J Am Heart Assoc 2015, 4:e002699.

[49] Joyce SA, Gahan CG: Bile Acid Modifications at the Microbe-Host Interface: Potential for Nutraceutical and Pharmaceutical Interventions in Host Health. Annu Rev Food Sci Technol 2016, 7:313-33.

[50] Kaye DM, Shihata WA, Jama HA, Tsyganov K, Ziemann M, Kiriazis H, Horlock D, Vijay A, Giam B, Vinh A, Johnson C, Fiedler A, Donner D, Snelson M, Coughlan MT, Phillips S, Du X-J, El-Osta A, Drummond G, Lambert GW, Spector TD, Valdes AM, Mackay CR, Marques FZ: Deficiency of Prebiotic Fiber and Insufficient Signaling Through Gut Metabolite-Sensing Receptors Leads to Cardiovascular Disease. Circulation 2020, 141:1393-403.

[51] Haghikia A, Jörg S, Duscha A, Berg J, Manzel A, Waschbisch A, Hammer A, Lee D-H, May C, Wilck N, Balogh A, Ostermann Annika I, Schebb NH, Akkad Denis A, Grohme Diana A, Kleinewietfeld M, Kempa S, Thöne J, Demir S, Müller Dominik N, Gold R, Linker Ralf A: Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. Immunity 2015, 43:817-29.

[52] Tang WH, Hazen SL: The contributory role of gut microbiota in cardiovascular disease. J Clin Invest 2014, 124:4204-11.

[53] Patangia DV, Anthony Ryan C, Dempsey E, Paul Ross R, Stanton C: Impact of antibiotics on the human microbiome and consequences for host health. Microbiologyopen 2022, 11:e1260.

[54] Ianiro G, Tilg H, Gasbarrini A: Antibiotics as deep modulators of gut microbiota: between good and evil. Gut 2016, 65:1906-15.

[55] Singh M, George AK, Homme RP, Majumder A, Laha A, Metreveli N, Pushpakumar SB, Tyagi SC: Probiotic Supplementation Mitigates Vascular Remodeling in the Retina. The FASEB Journal 2019, 33:484.11-.11.

Figure Legends

Fig 1 – Illustration of the “Gut-Retina Axis” with its different metabolites and pathways of the gut microbiome which may influence retinal vascular diseases and their risk factors

Fig 2 – Representative fundus photography of A: DR, B: RAO and C: RVO

The Gut-Retina Axis