Retinal vascular diseases (RVDs) 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 understand the gut microbiome and its impact on health and disease. Although 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. The gut microbiome consists of microbes including bacteria, archaea, viruses, and eukaryotes, and their microbial products and genes. It comprises more than a thousand different species of bacteria and carries about 150 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.\(^6\)\(^-\)\(^8\) 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. At the phylum level, Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia represent the majority of the human gut microbiome.\(^9\)

Associations between an imbalance in the gut microbiome’s composition, called dysbiosis, and various diseases such as inflammatory bowel disease, obesity, diabetes mellitus, atherosclerosis, alcoholic and non-alcoholic liver disease, cirrhosis, and hepatocellular carcinoma have been extensively researched. Dysbiosis, or imbalance in 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 occlusion, and retinal 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. (Am J Pathol 2023, 193: 1675–1682; https://doi.org/10.1016/j.ajpath.2023.02.017)
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. By contrast, production of microbial metabolites with protective effects, such as short-chain fatty acids (SCFAs) or bile acids (BAs), 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 over-activation 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.11,12,14–16 The connection between the gut microbiome and the retina has been termed the “gut-retina axis” (Figure 1).16

Although the role of the gut microbiome in the pathogenesis of diseases such as DR and RAO has been investigated recently, its influence on other diseases such as RVO remains 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 is elucidated in the first part. Associations between these RVDs, their risk factors, and the gut microbiome are discussed in the second part. Microbial metabolites are presented in the third part.

**Retinal Vascular Diseases**

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

**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. Microvascular retinal damage arises due to non- or inadequately controlled blood glucose levels. Although 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. 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 because 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 notice a deterioration of vision after development of macular edema. 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, such as proliferative DR, retinal hypoxia can lead to formation of new blood vessels, which can lead to various complications such as vitreous hemorrhage, glaucoma, or retinal detachment. There is a loss of retinal vessel autoregulation, basement membrane thickening, loss of pericytes, progressive nonperfusion of capillaries, and subsequent ischemia.22 The peripapillary capillary-free zone is much larger in individuals suffering from DR.23 This can lead to release of proangiogenic peptides such as vascular endothelial growth factor (VEGF), which is responsible for the formation of neovascularizations.24 End-stage disease (proliferative DR) 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 to those for DM and comprise a positive family history, age [younger for type 1 DM and older for type 2 DM (T2D)], 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 is of great interest.18

**Retinal Vein Occlusion**

The second most common RVD is 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). Impact on vision and complications differ depending on the affected area (central retinal vein versus smaller branch retinal vein). Smaller RVOs can 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 up-regulation of VEGF. Other complications are optic neuropathy, macular ischemia, vitreous hemorrhage, and retinal ischemia due to nonperfusion of retinal tissue. The latter can lead to choroidal neovascularization with potentially devastating visual outcomes.28 Because ischemic retinal areas are hard to identify upon ophthalmoscopy, they are best examined with fluorescein angiography, delineating areas of capillary nonperfusion. Fluorescein angiography also enables the detection of subclinical neovascularization. The mainstay of treatment is intravitreal anti-VEGF and steroid injections,
and retinal laser photocoagulation. Risk factors are arterial hypertension, dyslipidemia, obesity, atherosclerosis, and smoking.\textsuperscript{5,28} Interestingly, DM itself is an important risk factor for RVO and appears to be associated with the gut microbiome.\textsuperscript{29,30}

Retinal Artery Occlusion

With a worldwide annual incidence of about 1 to 15 per 100,000 people, RAO is the third most common RVD.\textsuperscript{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 a few hours. Non-perfusion of retinal arteries and arterioles, and consequent retinal hypoxemia, lead to nerve fiber swelling and breakdown of the metabolic cycle, ultimately leading to cell death (Figure 2C). Whereas most RAOs are caused by large vessel disease (macroangiopathy) such as embolization from atherosclerotic plaques, RAOs due to small vessel disease (microangiopathy) also exist. RAOs may be caused due to inflammatory or noninflammatory, purely embolic, pathophysiology. Vasculitis such as giant cell arteritis can cause occlusion of arterial vessels in RAO. A hypercoagulable state could point to other diseases affecting the coagulation cascade or malignancies.\textsuperscript{32} No formal treatment for RAO is available. Intravascular thrombolysis is a treatment option in case of onset of symptoms within 4.5 hours.\textsuperscript{5} In summary, in most cases, RAO can be considered an ischemic stroke.\textsuperscript{33} Patients at risk for vascular insults are also candidates for potential RAO. Inversely, patients are at elevated risk for a stroke in the weeks following a RAO.\textsuperscript{34} Risk factors include atherosclerosis, hypertension, DM, obesity, dyslipidemia, and thrombus-eliciting circumstances.

Figure 1 Illustration of the "gut-retina axis" with its different metabolites and pathways of the gut microbiome that may influence retinal vascular diseases and their risk factors. BMI, body mass index; LPS, lipopolysaccharides; SCFA, short-chain fatty acid; TMAO, trimethylamine N-oxide.
or events, such as atrial fibrillation, which appear to be influenced by the gut microbiome.\textsuperscript{5,11,28}

**RVDs, Associated Risk Factors, and the Microbiome**

The direct relationship between the gut microbiome and RVDs has not been studied comprehensively. To date, very few articles have addressed the influence of the gut microbiome on DR and RAO\textsuperscript{17,18,35,36} and no articles investigating the influence of the gut microbiome on RVO have been published.

The link between the gut microbiome and 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 the subject of extensive research. The following paragraphs describe 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.

**RAO and the Microbiome**

Compositional and functional shifts in the gut microbiome have been reported in RAO. Using whole-metagenome shotgun sequencing, the gut microbiome of 29 patients with nonarteritic RAO was compared with that of 30 healthy controls. The class \textit{Actinobacteria} and the species \textit{Bifidobacterium adolescentis}, \textit{Bifidobacterium bifidum}, \textit{Bacteroides stercoris}, and \textit{Faecalibacterium prausnitzii} were enriched in patients with RAO, whereas the family \textit{Lachnospiraceae}, and the genera \textit{Odoribacter} and \textit{Parasutterella} were enriched in controls.\textsuperscript{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 patients with RAO. Interestingly, the identified compositional and functional alterations of the gut microbiome were closely associated with atherosclerosis. A higher abundance of \textit{Collinsella} (belonging to the class of Actinobacteria) was found in patients with symptomatic atherosclerosis. DNA in atherosclerotic plaques originates primarily from \textit{Actinobacteria}. Furthermore, an enriched mevalonate pathway, also known as the target of statins, represents an important pathway in the pathogenesis of atherosclerosis.

**DM, DR, diet, and obesity**

DM is a metabolic disease characterized by impaired glucose tolerance. Its most common variant is T2D, affecting about 90% of all diabetic patients. Compositional and functional changes occur in the gut microbiome of diabetic individuals as compared to those in healthy controls.\textsuperscript{30} \textit{Escherichia coli}, \textit{Clostridium species}, \textit{Bacteroides caccae}, and \textit{Eggerthella lenta} were shown to be more abundant, whereas \textit{Eubacterium rectale}, \textit{Clostridiales sp. SS3/4}, \textit{Faecalibacterium prausnitzii}, and \textit{Roseburia intestinalis} were shown to be less abundant in T2D patients compared with healthy controls.\textsuperscript{30} Furthermore, several microbial pathways and metabolites were identified that may lead to the progression of diabetes. Gut bacteria—derived NOD1 ligands, acting as signal molecules between the gut and extraintestinal organs, modulate insulin trafficking in pancreatic beta cells.\textsuperscript{37} Indeed, treatment with fecal microbiota transplantation showed promising results in DM. The decline in endogenous insulin production was halted in recently diagnosed type 1 DM patients receiving allogenic fecal microbiota transplantations from healthy donors.\textsuperscript{38}

Although there are vast similarities in the gut microbiome composition changes in patients with DM and DR, the latter has been associated with lower bacterial diversity and specific compositional changes.\textsuperscript{18,35} At the phylum level, \textit{Bacteroidetes} were more abundant in diabetic patients with

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**Figure 2**  Representative fundus photography of diabetic retinopathy (A), retinal artery occlusion (B), and retinal vein occlusion (C).
DR compared with patients with only DM. Other bacteria such as the genera Blautia and Lactobacillus were more abundant in patients with DM, but not with DR. Abundance of Burkholderiaceae and unclassified Burkholderiales was increased in patients with DR, but was decreased in 22 families including Streptococcaceae, Coriobacteriaceae, and Veillonellaceae were noted. Furthermore, significant differences in lipid, amino, and nucleotide metabolism were observed in DR patients. Known mediators for DR development, such as arachidonic acid, hydroxyicosatetraenoic acids, and leukotriene, were increased in fecal samples of patients with DR.

DM and DR are associated with obesity. A high-caloric Western-style diet rich in saturated fat and sugar is one of the major risks for obesity and T2D. An increased intestinal permeability due to breakdown of the gut epithelium barrier was observed as a consequence of a Western-style diet. Diet modification is an important management tool in T2D treatment and is able to influence the composition of the gut microbiome. Increased Firmicutes and decreased Bacteroidetes were observed in diabetic mice fed with a compound dietary fiber and high-grade protein diet. An enrichment of Dubosiella, Parasutterella, Ruminococcaceae, Muribaculum, Allobaculum, and Bifidobacterium was observed after 4 weeks of the compound dietary fiber and high-grade protein diet. In addition, a decrease of hyperglycemia and insulin resistance, as well as a protective effect on the gut barrier in terms of reduced LPS and d-lactate levels, which are indicators for intestinal permeability, was also noted. This effect was attributed to a reduction of endotoxemia and an enhancement of mucin secretion.

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 mice are known to have 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 metabolism of dietary intake by the modified gut microbes and activation of different pathways involved in triglyceride production and insulin resistance.

Increased numbers of Bacteroidetes were found in obese humans, though some studies dispute this observation. 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. The mice had increased levels of Firmicutes and decreased levels of Bacteroidetes and Verrucomicrobia. The investigators hypothesized that intermittent fasting prevents DR by altering the gut microbiome toward species producing neuroprotective agents such as the BA tauroursodeoxycholate. 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 (CVD) and RVD. The elevation of 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, Desulfovibrio, and Parabacteroides are associated with hypertension. In a rodent model for hypertension, fecal microbiome transplantation from a hypertensive mouse to a germ-free mouse induced hypertension in the germ-free mouse. Furthermore, the gut microbiome from hypertensive rats was different compared with normotensive control rats. Different pathways linking the gut microbiome to the development and progression of atherosclerosis have been studied. Because bacterial DNA, most often from Chlamydia pneumoniae, has been isolated from atherosclerotic plaques, bacteria may have an influence on 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 with healthy controls.

The paragraphs above outlined the connection between the risk factors of RVD and an altered microbiome. The following paragraphs present the most important microbial metabolites.

**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. Whereas some [LPS and trimethylamine N-oxide (TMAO)] have detrimental effects, such as causing inflammation, others have protective effects such as maintaining homeostasis (neuroprotective BAs and SCFA). The equilibrium of metabolites with harmful and protective effects shifts in
dysbiosis, leading to chronic inflammation associated with several diseases.

Lipopolysaccharide

LPS, a bacterial surface glycolipid and part of the outer membrane of most Gram-negative bacteria, is associated with acute and chronic inflammation. It is recognized by the toll-like receptor 4 and triggers an immediate response by the innate immune system, resulting in an acute inflammation via the release of proinflammatory cytokines. In case of an altered epithelial gut membrane (eg, through dysbiosis) 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, CVD, chronic kidney disease, inflammatory bowel disease, and diabetes. It also accelerates neurodegeneration by activating microglia. This may play a role in retinal diseases such as age-related macular degeneration. Moreover, persistent low-grade inflammation was linked to the development of diabetic microvascular complications such as DR. Higher levels of LPS and proinflammatory 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.

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 CVD, with higher TMAO levels correlating with worse outcome. The role of TMAO in the pathogenesis of atherosclerosis is not fully understood. TMAO modulates several metabolic pathways, including cholesterol and sterol metabolism. By changing the expression of cholesterol transporters, TMAO may inhibit reverse cholesterol transport.

Reduced atherosclerotic lesion size was observed in flavin monoxygenase 3 (enzyme involved in TMAO production) knockdown mice. Furthermore, there is evidence that TMAO is involved in the development of DM through immunological and inflammatory pathways. In patients with RAO, TMAO levels were increased compared with those in healthy individuals, and a positive correlation was noted between TMAO levels and the abundance of Akkermansia, whereas a negative correlation was noted between TMAO and the abundance of Parasutterella and Lachnospiraceae. Although TMAO is generally accepted to be proatherogenic, 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. 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

BAs 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, BA metabolism is influenced by the gut microbiome composition. Bacteria convert conjugated BAs into unconjugated BAs in the small intestine. About 5% of BAs 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 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. Characteristic BA profiles have been found in T2D and obesity. Depending on the individual gut microbiome composition, the BA profile differs vastly. 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.

Short-Chain Fatty Acids

SCFAs are produced by bacterial fermentation of dietary fibers and act as signaling molecules by binding to G protein-coupled receptors. Various bacteria such as Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus bromii, Eubacterium hallii, 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 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. They were shown to have a hypotensive effect, preventing CVD and reducing target organ damage when supplemented to a low-fiber diet. 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 proinflammatory Th1 and Th17 immune cells and neuroprotective Treg cells.
Administration of dietary SCFAs stimulated differentiation and proliferation of neuroprotective Treg cells, and ameliorated the disease course.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 and hypertension, as well as DM and obesity. The direct influence of gut microbiome on pathways favoring the pathogenesis of RAO has been described. Understanding of the implications of the gut microbiome on retinal pathology has the potential to help develop targeted therapeutic approaches to achieve personalized treatment.52 The gut microbiome composition may be changed by the short-term administration of antibiotics.53,54 However, if continuously administered, adverse side effects make this approach unfeasible. Probiotic supplementation can be used to restore homeostasis from a dysbiotic state. 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 diet. It has been shown that a healthy diet with abundant fiber intake is advantageous, because 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.

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