

Intake of dietary salt and drinking water: Implications for the development of age-related macular degeneration

Andreas Bringmann,¹ Margrit Hollborn,¹ Leon Kohen,^{1,2} Peter Wiedemann¹

¹Department of Ophthalmology and Eye Hospital, University of Leipzig, Leipzig, Germany; ²Helios Klinikum Aue, Aue, Germany

Purpose: Systemic hypertension is a risk factor of age-related retinal diseases such as diabetic retinopathy and age-related macular degeneration. High intake of dietary salt and low intake of water increase extracellular osmolality resulting in hypertension, in particular in salt-sensitive individuals. This review summarizes the present knowledge regarding the impact of salt and water intake on the regulation of blood pressure, retinal function, and the development of age-related retinal diseases.

Methods: A literature search of the Medline database and a summary of recent studies that used human RPE cells.

Results: The salt sensitivity of the blood pressure and plasma osmolality increase with age, and body water deficits are common in older individuals. High plasma osmolality has adverse effects in the retina. In RPE cells, high osmolality induces expression and secretion of angiogenic factors, such as vascular endothelial growth factor (VEGF), placental growth factor, and basic fibroblast growth factor, and expression of aquaporin-5, a water channel implicated in transepithelial water transport. The transcriptional activities of hypoxia-inducible factor-1 (HIF-1) and nuclear factor of activated T cell 5 (NFAT5) are critical for the production of VEGF in response to salt-induced osmotic stress. Salt-induced osmotic stress also induces priming of the NLRP3 inflammasome and activates inflammatory enzymes in RPE cells.

Conclusions: Raised plasma osmolality may aggravate age-related retinal diseases by stimulation of local inflammation and angiogenic factor production in the RPE. Alterations in salt and water consumption, and of minerals that stimulate renal salt excretion, may offer nutritional approaches to prevent age-related retinal disorders, in particular in salt-sensitive individuals and individuals who show signs of body dehydration.

Despite new medical and surgical interventions, age-related macular degeneration (AMD) remains the leading cause of irreversible blindness in patients older than 65 years in developed countries [1]. The majority (about 90%) of patients suffer from dry AMD that is, in the early stage, characterized by the thickening of and structural changes in Bruch's membrane, basal laminar deposits (drusen), and lipofuscin accumulation in the RPE. In the advanced stage, dry AMD is characterized by geographic atrophy and choriocapillaris degeneration. The remaining patients suffer from exudative (neovascular) AMD characterized by choroidal neovascularization (CNV) and subretinal edema. Geographic atrophy, CNV, and edema are associated with photoreceptor damage resulting in progressive loss of visual acuity [2].

The pathogenesis of AMD is still incompletely understood. AMD is caused by age-dependent functional impairment and degeneration of RPE cells, and by a decrease in the choroidal blood flow; both result in hypoglycemia and hypoxia of the outer retina. A major function of the RPE is phagocytosis and digestion of membrane discs that are

shed from the tips of photoreceptor outer segments [3]. The discs contain high amounts of peroxidized lipids and protein adducts that are formed due to oxidative stress resulting from light exposure, the high oxygen consumption of photoreceptors, and the high polyunsaturated fatty acid content of photoreceptor membranes [4,5]. The age-dependent dysregulation of protein and lipid recycling and degradation pathways in RPE cells [6,7] impairs the regular degradation of peroxidized photoreceptor lipoproteins; this impairment results in lipofuscin accumulation in RPE cells and deposition of drusen in Bruch's membrane [8,9]. Accumulated lipoproteins constitute a hydrophobic barrier that adversely affects the transport of oxygen and nutrients from the choriocapillaris to the photoreceptors [10]. The resulting hypoglycemia and hypoxia of the outer retina induce overproduction of angiogenic factors by the RPE that stimulate the growth of choroidal vessels and increase the permeability of the outer blood-retinal barrier [10]. The toxic effects of peroxidized photoreceptor waste products and the decline in cellular clearance systems also cause chronic local inflammation associated with complement-mediated RPE cell injury, for example [5,9-12]. In addition to local inflammation, AMD is associated with systemic inflammation [13-16].

Vascular endothelial growth factor (VEGF) is the most relevant angiogenic factor induced by retinal hypoxia [17].

Correspondence to: Andreas Bringmann, Department of Ophthalmology and Eye Hospital, University of Leipzig, Liebigstrasse 10-14, D-04103 Leipzig, Germany; Phone: +49 (0) 341 9721557; FAX: +49 (0) 341 9721659; email: bria@medizin.uni-leipzig.de

In addition to glial cells and invading macrophages, RPE cells are an important source of VEGF in the outer retina [18]. The efficiency of the clinical use of anti-VEGF agents in the treatment of CNV [19] underlines the important role of VEGF in pathological neovascularization. However, in more than half of patients visual acuity does not improve after anti-VEGF therapy, and about 10% of patients do not respond to treatment [20]. Therefore, it was suggested that in addition to VEGF, further angiogenic factors are required to promote the development of CNV [10,21]. Such angiogenic factors produced by the RPE are, for example, placental growth factor (PIGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), heparin-binding epidermal growth factor-like growth factor (HB-EGF), and transforming growth factor- β (TGF- β) [10,22-27]. In addition, inflammatory and immune mediators (for example, complement proteins that accumulate in drusen and induce expression of VEGF in RPE cells) play a pathogenic role in CNV [9].

In addition to advanced age, race, and genetic factors, such as complement gene polymorphisms [28], lifestyle factors are associated with the risk of AMD. These factors include sunlight exposure, cigarette smoking, and nutrition [29]. Low levels of nutritional antioxidants and fat-rich nutrition increase the risk of AMD [1,30]. Plasma lipids, such as cholesterol and triglycerides, accumulate in Bruch's membrane and contribute to drusen formation [31,32]. Obesity is associated with AMD [33,34], possibly because obesity induces oxidative damage and systemic vascular inflammation.

AMD was suggested to represent an ocular manifestation of a systemic disease process [14]. Associations have been reported between AMD and hypertension, cardiovascular disease, cerebrovascular disease, dyslipidemia, chronic kidney disease, and neurodegenerative disorders [14]. Cardiovascular disease and AMD share common risk factors, such as atherosclerosis, systemic markers of inflammation, cigarette smoking, and hyperlipidemia [35-37]. An important aspect of cardiovascular disease is hypertension. Systemic hypertension confers increased risk of AMD. The association between high blood pressure and AMD has been well documented in various population-based studies [36,38-40]. Some studies described an association between neovascular AMD and hypertension, but not geographic atrophy [29,33,34,41,42]. The shared risk factors suggest that cardiovascular disease plays an etiological role in the development of AMD [34]. In the present article, we direct attention to two nutritional factors that may affect directly and indirectly (via the effect on blood pressure) the development of AMD: the intake of

dietary salt (sodium chloride) and drinking water. Alterations in salt and water consumption may offer nutritional approaches to prevent age-related retinal disorders, such as AMD.

Hypertension: Systemic hypertension affects a large proportion of the adult population and is a significant cause of morbidity worldwide. It was reported that 27% of adult Americans suffer hypertension ($\geq 140/90$ mmHg or use of antihypertensive medications) and another 31% suffer prehypertension (120–139/80–89 mmHg, no medication) [43]. Among adults older than 50 years, the lifetime risk of developing hypertension approaches 90% [44].

Blood pressure is proportional to cardiac output and vascular resistance to blood flow. Hypertension mainly results from an increase in the blood volume caused by increased osmotic pressure of the plasma (plasma osmolality); the expansion of the blood volume induces increases in cardiac output and in peripheral vascular resistance (Figure 1) [45-47]. Blood pressure increases with age [48] because of various age-related alterations. For example, plasma osmolality increases [49], and arterial walls become less elastic; the latter is caused by arteriosclerosis and atheromatosis. AMD is associated with increased systemic arterial stiffness [37].

Blood pressure can be lowered by decreases in cardiac output, vascular smooth muscle tonus, and plasma osmolality. This can be achieved with antihypertensive medications and with lifestyle modifications, such as increased physical activity, weight loss, and dietary alterations, including the Dietary Approaches to Stop Hypertension (DASH) diet (low-fat foods rich in fruits and vegetables and reduced in saturated fat and cholesterol), and moderation of alcohol intake [50,51]. Plasma osmolality can be decreased by reducing salt intake, increasing potassium intake, and increasing water consumption.

Plasma osmolality and hypertension: The role of salt intake: Plasma osmolality depends on the levels of glucose, lipids, and ions. Elevated plasma glucose frequently occurs during the postprandial phases and is common among elderly individuals because of the high prevalence of glucose intolerance [52,53]. However, the most relevant factor that determines plasma osmolality is the plasma salt level which is dependent on the balance between salt intake and excretion (Figure 1) [51,54]. Consumption of dietary salt is one cause of the age-related increase in blood pressure [48].

High intake of dietary salt is a main risk factor of cardiovascular disease. There is a linear relationship between salt consumption and blood pressure; however, the relationship

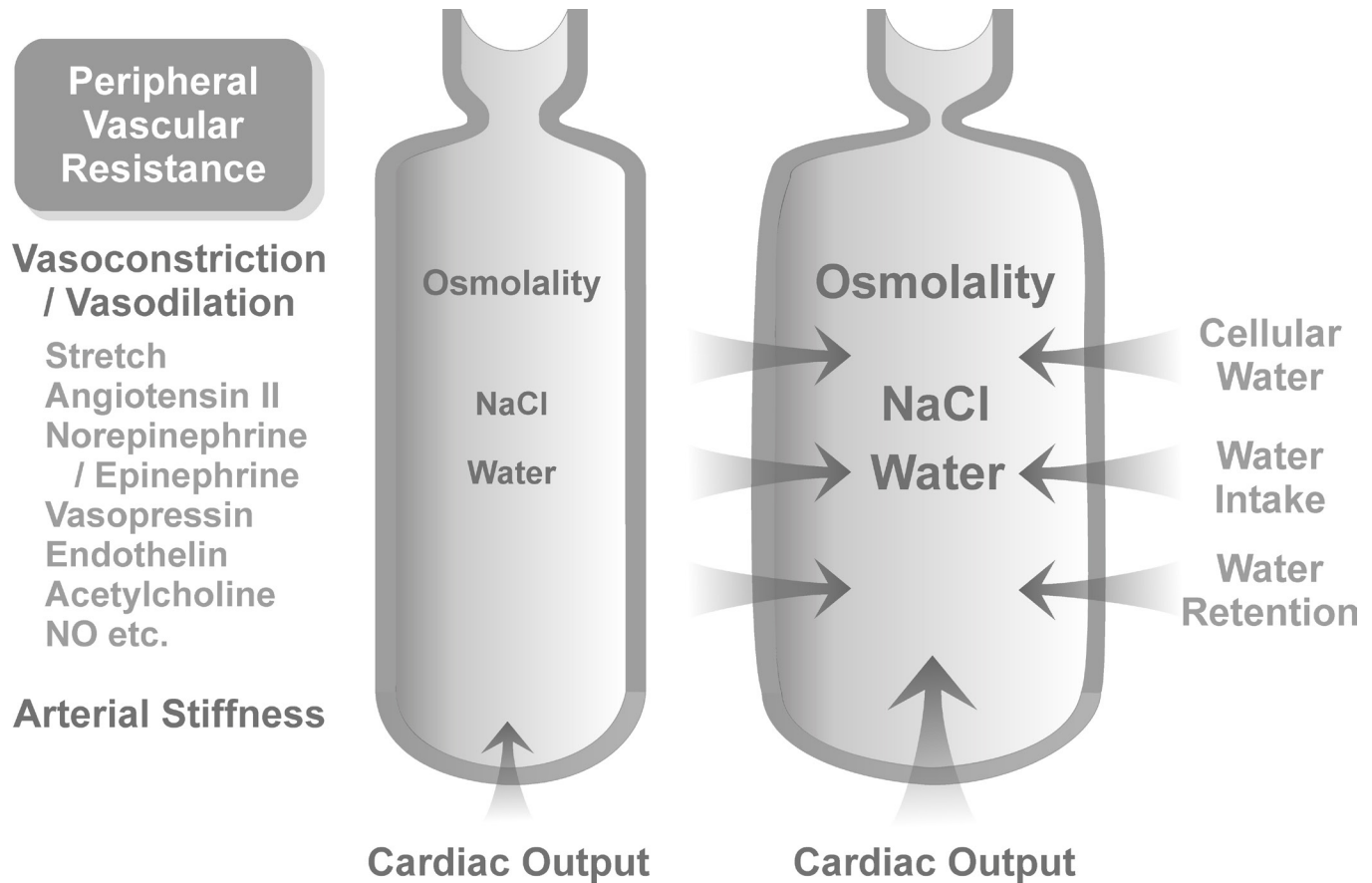


Figure 1. Factors that influence arterial blood pressure. Blood pressure is proportional to cardiac output and the total peripheral resistance. The latter is dependent on the stiffness of the arterial walls and the level of arteriolar constriction. High intake of dietary salt causes a rise in extracellular (including plasma) osmolality during the postprandial phases. The elevation of extracellular osmolality results in an increase in the extracellular fluid (including blood) volume by various mechanisms: osmotic movement of cellular water to extracellular fluids, stimulation of the thirst center resulting in increased fluid intake, stimulation of renal salt excretion, and secretion of vasopressin that produces increased renal water retention. All of these mechanisms reduce the plasma salt level. The blood volume expansion increases cardiac output and induces arteriolar constriction (to prevent tissue overperfusion); both result in hypertension. The level of arteriolar constriction is regulated by the activities of vasoconstrictory (stretch, sympathetic activity, angiotensin II, epinephrine, vasopressin, endothelin, etc.) and vasodilatory factors (acetylcholine, NO, etc.). Peripheral resistance increases with age mainly because the resistance vessels become thicker, stiffer, and narrowed due to arteriosclerosis (the replacement of the medial muscle fibers and intima by collagen) and atheromatosis (the deposition of lipids in vascular walls resulting in vascular inflammation and endothelial dysfunction). High salt also increases the blood pressure independent of blood volume expansion by direct effects on arterial smooth muscles, cardiac myocytes, hypothalamic control of blood pressure, and the local renin-angiotensin system. The blood pressure-raising effect of dietary salt increases with age mainly because of renal malfunction, which impairs salt excretion, and vascular alterations, such as arteriolar stiffening and decreased endothelial NO production. In addition, baseline plasma osmolality increases with age. The scheme shows the transition between an artery and an arteriole that controls tissue perfusion. In salt-resistant individuals, an increase in plasma osmolality induces a dilation of the artery (which prevents hypertension) while tissue perfusion remains constant due to arteriolar vasoconstrictory autoregulation. Age-related vessel stiffening impairs vessel dilation and contributes to the development of salt sensitivity of blood pressure.

between salt intake and the incidence of cardiovascular disease is nonlinear [54]. The optimal range of daily salt consumption is 2–3 g sodium/d, corresponding to about 5–8 g salt/d; higher and lower values increase the risk of cardiovascular disease [54]. Low salt consumption (<4 g/d) has various adverse consequences, such as increased insulin resistance, increased aldosterone secretion (which stimulates sodium

reabsorption), activation of the renin-angiotensin system, and increased sympathetic nerve activity; all of these effects increase blood pressure [55]. Human societies differ in levels of salt consumption. There are populations with moderate salt intake, such as in North America (about 5–8 g/d), Great Britain (about 9 g/d), and Germany (about 6–9 g/d), and populations with high salt consumption, such as in Finland

(about 11 g/d), South Korea (about 12 g/d), and Japan (about 14 g/d) [54]. The main source of salt in the diet (about 70%) is from processed foods [56]. In developed countries, the intake of dietary salt increased rapidly in the past along with the consumption of processed foods. Increased salt consumption may also represent an environmental risk factor for the progression of AMD [57].

Salt sensitivity of blood pressure: Human subjects differ in salt sensitivity of blood pressure. There are salt-resistant individuals; in these subjects, salt-induced variations in blood volume do not cause changes in plasma osmolality and blood pressure [47]. Salt resistance is mediated by effective renal salt excretion and endothelial production of vasodilatory nitric oxide (NO) [58,59]. Salt excretion restores normal blood volume within hours or days [47]. However, in salt-sensitive individuals, even small changes in plasma salt levels induce abnormal changes in blood pressure due to renal malfunction and impairment of endothelial NO production [60]. Thirty to fifty percent of hypertensive individuals, but also many normotensive individuals, are salt-sensitive [61,62]. Salt sensitivity of blood pressure increases with age [63].

Blood pressure–lowering minerals: In spite of the evidence that high salt intake is associated with hypertension, it was suggested that low consumption of minerals that reduce blood pressure (potassium, calcium, magnesium, phosphorus, bicarbonate) is a more important factor that induces hypertension; the blood pressure–lowering effect of these minerals is in part mediated by stimulation of renal salt excretion [64,65]. Potassium intake is inversely related to blood pressure [66]. The decreased content of blood pressure–lowering minerals is a characteristic of modern processed foods [67].

Dietary salt: Blood pressure–independent tissue damage: Excess salt also has direct harmful effects on tissues and vasculature, independent of blood pressure. The blood pressure–independent effects of high salt result in vascular remodeling, including thickening, stiffening, and narrowing of resistance arteries, and vascular fibrosis and inflammation [68,69]. Salt-induced vascular changes are mediated by various mechanisms, including stimulation of aldosterone synthesis [70] and oxidative stress [58]. High salt causes cellular perturbations, such as DNA double-strand breaks, oxidation of DNA and proteins, disruption of the mitochondrial structure and function, cytoskeletal alterations, and apoptotic cell death [71,72]. By inducing oxidative stress in RPE cells, high salt may also contribute to the development of AMD.

Plasma osmolality and hypertension: Role of water intake: Insufficient water intake increases the plasma salt level and osmolality. The baseline plasma osmolality increases, and the

total body water decreases with age [49,73]. Body water deficits are common in elderly individuals [74,75]. Among hospitalized patients and long-term care residents, prevalences of body dehydration as high as 80% have been reported [76]. Reduced water intake in aged individuals is a result of various factors, including decreased thirst sensation, poor appetite, decreased kidney function and vasopressin effectiveness, illness, disability, use of medications (e.g., diuretics), social isolation, and cognitive disorders [74]. Body dehydration is associated with chronic disease and functional impairment, including neurologic complications ranging from lethargy to coma, renal failure, cardiovascular disease, cataract, and dry eye [77-80].

Retinal effects of hypertension: Systemic hypertension has widespread effects in the sensory retina. Elevated blood pressure induces retinal vascular changes, including hypertensive retinopathy, choroidopathy, and optic neuropathy, and is associated with increased risks of further vascular complications, such as retinal vein and artery occlusion, arteriolar macroaneurysm, and embolic events [81,82]. Hypertensive retinopathy, a sign of systemic vascular disease [81], is common and seen in nearly 10% of the general adult nondiabetic population [83]. Arteriolar narrowing, the earliest manifestation of hypertensive retinopathy [82], is caused by functional (loss of NO-mediated vasodilation) and structural changes (arteriosclerosis) [84,85]. The resulting retinal malperfusion may contribute to degeneration of retinal neurons and photoreceptors [86].

Hypertension confers increased risk of glaucoma and is the main secondary risk factor of diabetic retinopathy [87]. Control of blood pressure reduces the risk of diabetic retinopathy and prevents microvascular complications independent of glycemia [88]. Systemic hypertension also confers increased risk of AMD. Hypertension may also contribute to age-related structural changes in Bruch's membrane [40]. However, antihypertensive medications do not alter the risk of AMD [41,89]. Several studies even described that the use of antihypertensive medications is associated with an increased incidence of AMD [90-92]. Differences between retinal and choroidal regulation of vessel perfusion [93] may explain the different associations between the use of antihypertensive medications and the risks of diabetic retinopathy and AMD.

Effects of hypertension on choroidal vasculature: Hypertension increases the risk of AMD via effects on choroidal circulation. Age- and AMD-related reduction in choroidal blood flow and the increase in choroidal blood velocity result from atrophy of the choriocapillaris and decreased parasympathetic perfusion regulation [94-98]. Among patients with AMD, there is a direct association between

blood pressure and choroidal blood velocity [99]. Inadequate choroidal perfusion can lead to hypoglycemia and hypoxia of the overlying RPE [100] and is associated with focal RPE hyperpigmentation, drusen formation, and development of CNV [99,101,102]. Choroidal blood flow is also reduced in diabetic retinopathy [103].

Choroidal vascular disease in AMD is caused by hypertension and additional conditions that drive vascular inflammation, such as obesity and complement-induced endothelial injury [104]. Patients with hypertensive AMD display a more severe decrease in choroidal blood flow compared with normotensive patients [99]. Inadequate choroidal blood flow was found in patients with a history of systemic hypertension; both parameters do not correlate with actual blood pressure when antihypertensive medications are used, suggesting that hypertension in the past produces chronic effects on the choroidal vasculature [99].

The cellular mechanisms by which hypertension increases the risk of AMD are little understood. Systemic hypertension increases retinal inflammation [105], and hypertension is associated with mechanical stress on vascular and retinal cells [106]. Raised choroidal blood velocity increases endothelial shear stress that induces fibrinoid necrosis of choroidal arterioles, oxidative stress, and vascular inflammation [58,82,107]. Mechanical stress induces expression of VEGF in retinal endothelial and RPE cells [106,108]. Because VEGF is the most relevant angiogenic factor in the retina [17], hypertension-induced mechanical stress will facilitate the development of neovascular retinal disorders. AMD is associated with a decrease in the expression of NO synthases in choroidal arteries and RPE; lower production of NO increases vasoconstriction and reduces choroidal blood flow [109]. Whether the AMD-related decrease in NO synthase expression is facilitated by high salt as in renal endothelia [58] remains to be clarified.

Retinal effects of osmotic stress: Retinal osmotic stress due to raised plasma osmolality is common in elder and hypertensive individuals. In diabetes, osmotic stress mainly results from hyperglycemia that entails increased systemic osmolality and intracellular sorbitol accumulation [53,110]. Osmotic stress has adverse effects on the retina. High plasma osmolality decreases the standing potential of the eye [111] that originates from the RPE [112]. The standing potential is reduced in retinal diseases that are associated with altered integrity of the RPE, such as chorioretinal dystrophies and diabetic retinopathy [113,114]. High extracellular osmolality also causes decreases in the electroretinogram b-wave and oscillatory potential [115], alters the membrane potential, resistance, and gene expression of the RPE [116,117], induces transmitter

release from synaptic terminals [118], and increases the adhesion of neutrophils to vascular endothelia [119] that represents an early step of vascular injury in diabetic retinopathy [120]. Exudative AMD is associated with a breakdown of the outer blood–retinal barrier constituted by the RPE [2]. Osmotic conditions regulate the tightness of this barrier. High osmolality at the basal side of the RPE induces a breakdown of the barrier, while low osmolality increases the tightness of the barrier [121]. Mannitol infusion in human subjects increases plasma osmolality and results in opening of the blood–brain and blood–retinal barriers [122].

Retinal effects of high salt: High salt consumption has various effects in the retina. In treatment-resistant hypertensive patients, salt consumption increases the wall thickness of retinal arterioles independent of blood pressure [123]. In salt-sensitive rats, high salt consumption induces retinal arteriolar spasm and ischemia [124].

Osmotic gradients between the retina and the blood are resolved by glial and RPE cells via mediating transmembrane ion and water fluxes [3,125]. Transmembrane water transport is facilitated and directed by aquaporin (AQP) water channels [126,127]. AQPs are a family of proteins that facilitate the bidirectional movement of water across membranes in dependence on the osmotic gradient and hydrostatic pressure [128]. High salt loading in rats results in retinal glial cell activation, intracellular edema, and mitochondrial swelling in retinal ganglion and glial cells, and increased expression of AQP1 in glial membranes [129]. Similar alterations in glial AQP expression have been observed in experimental ischemic and diabetic retinopathies [130,131]. High salt intake aggravates the changes in glial AQP expression associated with diabetic retinopathy independent of the blood pressure [132]. It is likely that alterations in the expression of additional AQP subtypes, for example, of AQP11 that is upregulated in retinal inflammation [133], may be implicated in the salt- and high osmolality-induced responses of glial and RPE cells.

Systemic hypertension aggravates experimental diabetic retinopathy by increasing angiotensin II and sympathetic activities [134]. A low-salt diet modulates the retinal renin-angiotensin-aldosterone system that prevents experimental ischemic retinopathy and reduces vascular leakage and production of angiogenic and inflammatory factors, such as VEGF [135]. However, in human subjects, the use of angiotensin converting enzyme inhibitors is associated with a higher risk of AMD [90,91].

High salt: Induction of angiogenic growth factors in RPE cells: The development of CNV is stimulated by angiogenic factors [10]. The expression of VEGF, TGF- β , and bFGF is elevated in retinal tissues and CNV membranes of patients

with AMD [136,137]. High extracellular osmolality may facilitate the development of neovascular AMD via direct effects on the RPE. Whether salt-induced high extracellular osmolality induces angiogenic factor expression in RPE cells has been recently investigated in cultured cells. High salt activates RPE cells, as indicated by the activation of mitogen-activated protein kinase (MAPK) pathways (Figure 2A) [57]. This is associated with increased expression of various angiogenic growth factor genes, including the *VEGF*, *bFGF*, *PIGF*, and *HB-EGF* genes (Figure 2B,C) [57,138,139]. The time-dependency of *VEGF* gene expression is different between salt- and sucrose-induced high osmolality (Figure 2B,C) [57]. This suggests that high salt induces VEGF expression by two different mechanisms: via the increase in extracellular osmolality and by alteration of the transmembrane sodium chloride gradient. High salt also induces secretion of VEGF, bFGF, and PIGF proteins from RPE cells [57,138,139]. The salt-induced expression of VEGF, bFGF, and HB-EGF is dependent on autocrine or paracrine activation of growth factor receptors, such as TGF- β and FGF receptors [139]. This suggests that

high salt also induces a release of TGF- β and FGF from the cells. The expression of the *VEGF* gene induced by hypoxia and high salt is not additive (Figure 3A) [57]; this suggests that high extracellular salt may stimulate the expression of VEGF in RPE cells to a similar degree as hypoxia. Triamcinolone acetonide, an anti-inflammatory steroid clinically used for the rapid resolution of retinal edema, completely inhibits the salt-induced expression and secretion of VEGF in RPE cells [57]. Because a decrease in extracellular osmolality results in downregulation of *VEGF* and *PIGF* gene expression [57,138], the production of VEGF and PIGF in RPE cells is directly related to extracellular osmolality.

High salt: Induction of AQP5 in RPE cells: The development of retinal edema is an important vision-threatening condition of exudative AMD [140]. Normally, RPE cells clear the excess fluid from the subretinal space [3] by transcellular transport of ions and water; water transport is facilitated by AQP water channels [126]. The higher permeability of the outer blood-retinal barrier induced, for example, by VEGF

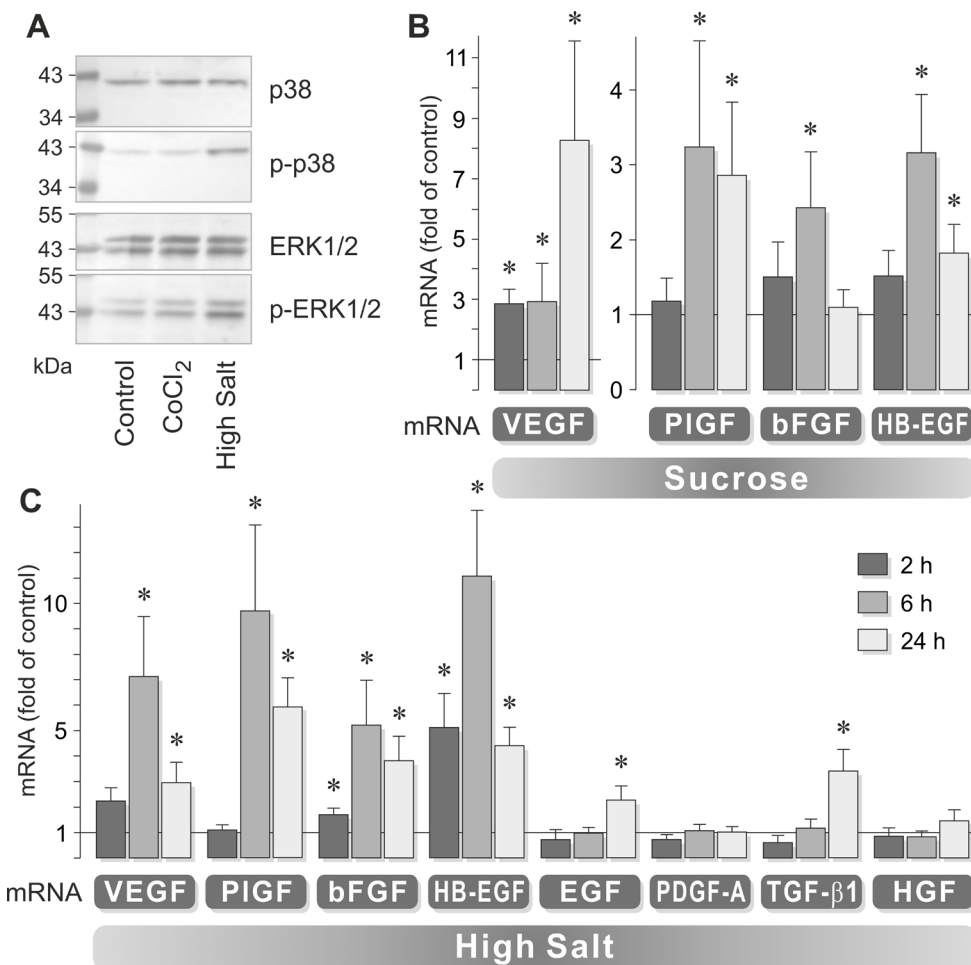


Figure 2. High extracellular osmolality activates human RPE cells in vitro and stimulates gene expression of angiogenic factors. **A:** Western blot analysis shows that the addition of 100 mM salt (NaCl) to the culture medium for 20 min induces increased phosphorylation levels of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1/2 proteins. Phosphorylation of p38 MAPK and ERK1/2 is not stimulated by chemical hypoxia (induced by addition of 150 μ M CoCl₂) for 20 min. Amounts of total proteins are shown above; amounts of phosphorylated proteins are shown below. **B, C:** Relative mRNA levels of growth factors in cells cultured in media with high osmolality for 2, 6, and 24 h. High osmolality was induced by the addition of 100 mM sucrose (**B**) and 100 mM salt (**C**), respectively, to the culture medium. EGF = epidermal growth factor; HGF = hepatocyte growth factor. * $p < 0.05$ vs. isoosmotic control. Modified after Hollborn et al. [57,138] and Veltmann et al. [139].

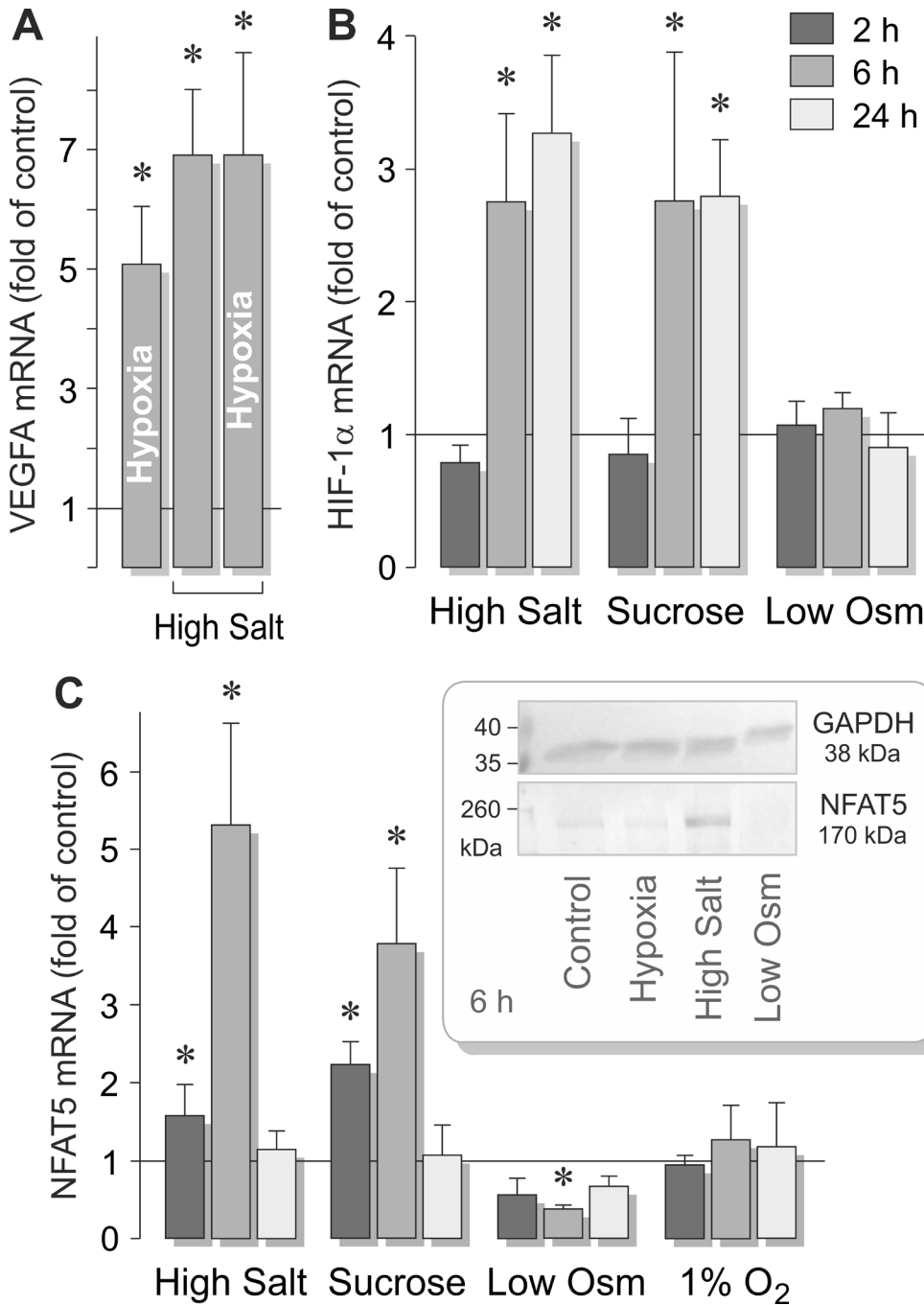


Figure 3. High osmolality-induced expression of transcription factors in RPE cells. **A:** Chemical hypoxia (induced by the addition of 150 μ M CoCl₂) and high salt (+ 100 mM NaCl) do not additively increase the expression of the *VEGF* gene. **B:** High osmolality, induced by the addition of 100 mM salt (NaCl) or 100 mM sucrose to the culture medium, induces gene expression of hypoxia-inducible factor-1 alpha (HIF-1 α). Low osmolality (Low Osm; 60% osmolality) has no effect. **C:** High osmolality increases, and low osmolality decreases, the gene expression of nuclear factor of activated T cell 5 (NFAT5). Hypoxia (1% O₂) has no effect. *Inset:* The NFAT5 protein level in RPE cells determined with western blot analysis. High salt increases and low osmolality decreases the cellular NFAT5 protein level. Hypoxia (+ 150 μ M CoCl₂) has no effect. *p<0.05 vs. control. Modified after Hollborn et al. [57].

allows increased fluid flux from choroidal vessels into the subretinal space. The fluid clearance capacity of RPE cells can be exceeded when excess blood-derived fluid enters the subretinal space and when the cells alter the expression of ion channels, transporters, and AQPs. RPE cells freshly isolated from human post-mortem donor eyes contain gene transcripts of various AQP subtypes; high salt induces expression of the *AQP3*, *AQP5*, and *AQP8* genes in RPE cells [57]. Throughout

the body, AQP5 plays an important role in transporting water across epithelia. In RPE cells, the expression of the *AQP5* gene is directly related to extracellular osmolality; *AQP5* gene expression is increased by high osmolality and decreased by low osmolality [57]. *AQP5* gene expression in RPE cells is also reduced under hypoxic conditions and in the presence of blood serum [57]. Triamcinolone acetonide does not prevent high salt-induced expression of AQP5 [57].

High salt: Activation of transcription factors in RPE cells: Hypoxia and high salt induce non-additive expression of the *VEGF* gene (Figure 3A), suggesting the involvement of common mechanisms of transcriptional activation of the *VEGF* gene under both conditions [57]. Cellular adaptation to hypoxia is mainly mediated by transcriptional activation of target genes by the hypoxia-inducible factor-1 (HIF-1) resulting in increased production of proteins that improve oxygen and nutrient supply [141]. These genes include *VEGF*, *glucose transporter*, and *erythropoietin genes* [142]. HIF-1 also triggers the expression of inducible NO synthase [143]; NO induces vasodilation but also causes nitrosative stress and may contribute to photoreceptor degeneration [144]. HIF-1-induced transcriptional activation of VEGF plays a critical role in the development of experimental CNV [145].

High extracellular salt induces increased transcriptional activation of HIF-1 α in RPE cells (Figure 3B) [57]. Inhibition of HIF-1 activity decreases salt-induced transcription of the *VEGF* gene by approximately 50% and abrogates almost completely salt-induced secretion of VEGF from RPE cells [57]. The salt-induced transcriptional activation of AQP5 is, in part, dependent on the activity of nuclear factor kappa beta (NF- κ B) activity, but not on HIF-1 [57]. NF- κ B is the main transcription factor that regulates the gene expression of proteins involved in inflammatory cell responses.

Normally, high extracellular osmolality causes water flux from cells resulting in cell shrinkage, an early event of apoptotic cell death [146]. However, cells possess several adaptive mechanisms that allow them to survive in osmotic stress by restoration of the osmotic balance. Cellular survival under high-salt conditions is initially maintained by activation of ion transport systems and thereafter by intracellular accumulation of small organic osmolytes and increased abundance of heat shock proteins and AQPs [71,72]. The classical cellular response to high extracellular osmolality involves the transcription of target genes by the nuclear factor of activated T cell 5 (NFAT5), also known as tonicity-responsive enhancer binding protein (TonEBP/OREBP) [72]. This transcription factor has a large C-terminal region that harbors a high osmolality-sensitive transactivation domain. NFAT5 binds to the tonicity responsive enhancer elements (TonEs) in the 5' region of the target genes. The target genes of NFAT5 include genes for enzymes and transporters that are implicated in intracellular accumulation of organic osmolytes, such as sorbitol, myo-inositol, betaine, and taurine (aldose reductase, myo-inositol transporter 1, taurine transporter, etc.) [53,72]. Additional target genes of NFAT5 in various cell systems are heat shock protein 70-2, tumor necrosis factor- α , and water channels AQP1 and AQP2 [53,72].

In RPE cells, the expression of NFAT5 is directly related to the level of extracellular osmolality; that is, high osmolality increases and low osmolality decreases the gene and protein expression of NFAT5 (Figure 3C) [57]. High osmolality also induces DNA binding of the NFAT5 protein [57]. Hypoxia has no effect on the expression of NFAT5 (Figure 3C) [57]. Knockdown of NFAT5 expression with siRNA reduces salt-induced transcription of the *VEGF*, *PIGF*, *bFGF*, and *AQP5 genes*, and salt-induced secretion of VEGF and bFGF from RPE cells [57,138,139]. The data suggest that the transcriptional activity of NFAT5 is critical for salt-induced induction of VEGF and other angiogenic factors in RPE cells. Whether in addition to these transcription factors, additional mechanisms of gene transcription contribute to the effects of high salt in RPE cells (e.g., transcription induced by the increase in the intracellular sodium concentration [147]) remains to be determined.

Aldose reductase, which produces sorbitol from glucose, is a prototypical NFAT5 target gene [148]. Intracellular accumulation of sorbitol is a main pathogenic factor of diabetic retinopathy [149]. In the retinas of diabetic mice and in RPE cells stimulated with high glucose, NFAT5 activity induces (directly or indirectly) the expression of various genes, including *aldose reductase* and *protein kinase C δ* , and the proapoptotic protein Bax, while NFAT5 decreases the expression of the antiapoptotic protein Bcl2; these data suggest that NFAT5 activity is involved in retinal degeneration under hyperglycemic conditions [150]. However, under high-salt conditions, production of sorbitol protects RPE cells from death [151], suggesting that intracellular sorbitol accumulation may also have protective effects. The contradictory effects of sorbitol production on cell viability in dependence on the strength or duration of osmotic imbalances may explain the limited benefits of aldose reductase inhibitors in the treatment of diabetic retinopathy [152].

High salt: Priming of the NLRP3 inflammasome: Chronic inflammation is a key factor that contributes to the development of AMD [13,15,16]. High salt was shown to increase the production of inflammatory factors, such as interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1) by RPE cells [153]. Generally, inflammatory processes are activated by cytosolic protein-signaling complexes, termed inflammasomes, which drive proteolytic activation of caspase-1 and maturation of the inflammatory cytokines IL-1 β and IL-18 [154]. Activation of the NLRP3 inflammasome in RPE cells is implicated in mediating RPE cell degeneration in geographic atrophy [155] and may also promote neovascular AMD pathologies, such as RPE barrier breakdown and CNV [156]. In RPE cells, salt-induced osmotic stress induces

priming and transient activation of the NLRP3 inflammasome, as well as expression and activation of inflammatory enzymes, such as phospholipases A₂ and cyclooxygenases [157] (data not shown). Thus, high-salt consumption may aggravate the development of AMD by stimulation of local inflammation. Salt-induced priming of the NLRP3 inflammasome in RPE cells is, in part, mediated by the activities of HIF-1 and NFAT5 [157]. Full salt-induced expression of NFAT5 is dependent on the release of ATP and autocrine and paracrine activation of purinergic receptors, such as P2Y₁ and P2X₇, (data not shown). The latter may contribute to the degeneration of the RPE in geographic atrophy [155]. RPE-derived ATP may also promote photoreceptor degeneration [158], a characteristic of AMD, as well as microvascular cell death [159], a hallmark of diabetic retinopathy.

Summary and conclusions: Various studies showed an association between systemic hypertension and the risk of AMD. Hypertension aggravates the age-dependent degeneration of the choroidal vasculature, which reduces choroidal blood flow [99]. Choroidal ischemia and the resulting outer retinal hypoxia promote angiogenic factor production in the RPE and the development of CNV. However, it was shown that the use of antihypertensive medications does not confer a decreased risk of AMD [41,89]; this suggests that additional factors associated with hypertension are also relevant for the pathogenesis of AMD. The main factor that induces acute hypertension is the elevation of the plasma salt level that results from intake of dietary salt and impaired renal salt excretion [45,46,51,54]. Because high salt causes cellular perturbations independently of blood pressure, in part via increasing extracellular osmolality [71,72], direct effects of salt and high osmolality on choroidal and RPE cells may contribute to the pathological processes involved in the development of AMD.

Recent studies showed that high salt and high extracellular osmolality have direct effects on RPE cells, including activation of MAPK pathways, activation of transcription factors, such as HIF-1, NF- κ B, and NFAT5, transcriptional activation and secretion of angiogenic and inflammatory factors, such as VEGF, PIGF, bFGF, IL-6, and MCP-1, priming of the NLRP3 inflammasome, and upregulation of AQP5 (Figure 4) [57,138,139,153,157]. Salt-induced production of inflammatory factors may contribute to the development of local inflammation, a characteristic of AMD [13,15,16]. Salt-induced production of angiogenic factors may promote the development of CNV and edema, two hallmarks of neovascular AMD. Salt-induced production of TGF- β may contribute to choroidal fibrosis. Because hypoxia and high salt induce expression of the *VEGF* gene in a non-additive fashion (Figure 3A), high salt consumption may facilitate the

development of CNV and edema under normoxic conditions. The data also suggest that dietary salt may act via three mechanisms: an increase in blood pressure, an increase in plasma osmolality, and alteration of the transmembrane sodium chloride gradient. These mechanisms may independently and additively stimulate the production of VEGF in the RPE (Figure 5). However, whether the increased plasma salt level and high extracellular osmolality are risk factors of AMD, independently of hypertension, remains to be determined in clinical studies.

NFAT5 is a transcription factor that may contribute to the aggravating effects of high salt on the retinal disease processes in AMD. The expression of *VEGF*, *PIGF*, and *AQP5* genes in RPE cells is directly related to the level of extracellular osmolality, likely because of the osmolality-dependent activation of NFAT5 (Figure 3C) [57,138]. However, the roles of NFAT5 activity in the regulation of retinal cell responses to osmotic stress and the development of age-related retinal diseases remain to be further investigated. In various cell systems, NFAT5 can be also activated in an osmolality-independent manner by different stimuli, such as cytokines, growth factors, receptor and integrin activation, ions, and reactive oxygen species [160]. It is unclear whether inhibition of NFAT5 may represent an approach to prevent the effects of high salt and osmotic stress on the RPE. Because NFAT5 is a transcription factor that improves cell survival in osmotic stress [71,72,151], it is conceivable that inhibition of NFAT5 may facilitate disease progression. However, downregulation of proapoptotic proteins and upregulation of antiapoptotic proteins induced by knockdown of NFAT5 in the retina of diabetic mice [150] may indicate that inhibition of NFAT5 will decelerate disease progression.

Osmotic conditions at the basal side of the RPE regulate the tightness of the outer blood-retinal barrier; high osmolality increases and low osmolality decreases the permeability of the barrier [121]. Up- and downregulation of VEGF under high- and low-osmolality conditions [57] may represent one mechanism by which osmotic alterations regulate the tightness of the barrier. Alterations in the expression of AQP5 in dependence on the osmotic conditions [57] may be implicated in the resolution of osmotic gradients across the RPE. High plasma osmolality may facilitate AQP5-mediated water transport from the subretinal space through the RPE. This may support the resolution of subretinal edema under conditions of osmotic opening of the outer blood-retinal barrier. Under conditions of low plasma osmolality, downregulation of AQP5 may reduce the water transport through the RPE and thus may diminish the osmotic water flux from the blood to the retinal tissue. However, whether the water permeability

of the RPE is regulated by alterations in AQP5 expression remains to be confirmed by further studies. Hypoxic and serum-induced downregulation of AQP5 [57] may counteract the upregulation of AQP5 induced by high plasma osmolality. This will impair the fluid clearance across the RPE under hypoxic conditions and in the case of hemorrhage. Triamcinolone acetonide inhibits the expression and secretion of VEGF induced by high osmolality but does not prevent the upregulation of AQP5 [57]. The inhibitory effect on the production of VEGF and the ineffectiveness on the expression of AQP5 may support the resolution of retinal edema in situ.

Clinical implications: Although hypertension is associated with a higher risk of AMD, the use of antihypertensive medications is associated with an unaltered or even increased risk [41,89-92]. This may suggest that lifestyle modifications are more relevant than antihypertensive medications to decelerate progression of AMD. The effects of salt-induced high osmolality in RPE cells (Figure 4) suggest that a decrease in plasma osmolality may have protective effects. A decrease in plasma osmolality can be achieved by reducing the intake of dietary salt. Many meta-analyses suggest that most people will benefit from a reduction in salt consumption in terms of

the risk of stroke and cardiovascular disease (e.g., Li et al. [161] and Aburto et al. [162]). Daily salt intake of <4 or <6 g is recommended by the American Heart Association and the Institute of Medicine, respectively [163]. Reduced dietary salt intake may also improve plasma cholesterol and triglyceride levels [164], known risk factors of AMD [29,32]. Therefore, it might be helpful to monitor dietary salt consumption in patients with AMD. However, there is an ongoing discussion regarding the beneficial effects of dietary salt restriction for cardiovascular disease [54]. There is a wide range of salt consumption (5–8 g/d) that has little effect on the risk of cardiovascular disease; higher (>10 g/d) and lower (<4 g/d) levels of salt intake are associated with increased risks of cardiovascular disease [54]. It is unlikely that dietary salt restriction has beneficial effects in populations with moderate salt consumption, such as in North America, Great Britain, and Germany; here, salt restriction may even be harmful [54]. In populations with high salt consumption, such as in Finland and Japan, dietary salt restriction may have beneficial effects [54].

Another approach to decrease the plasma salt level and osmolality is increased water intake. To obtain adequate

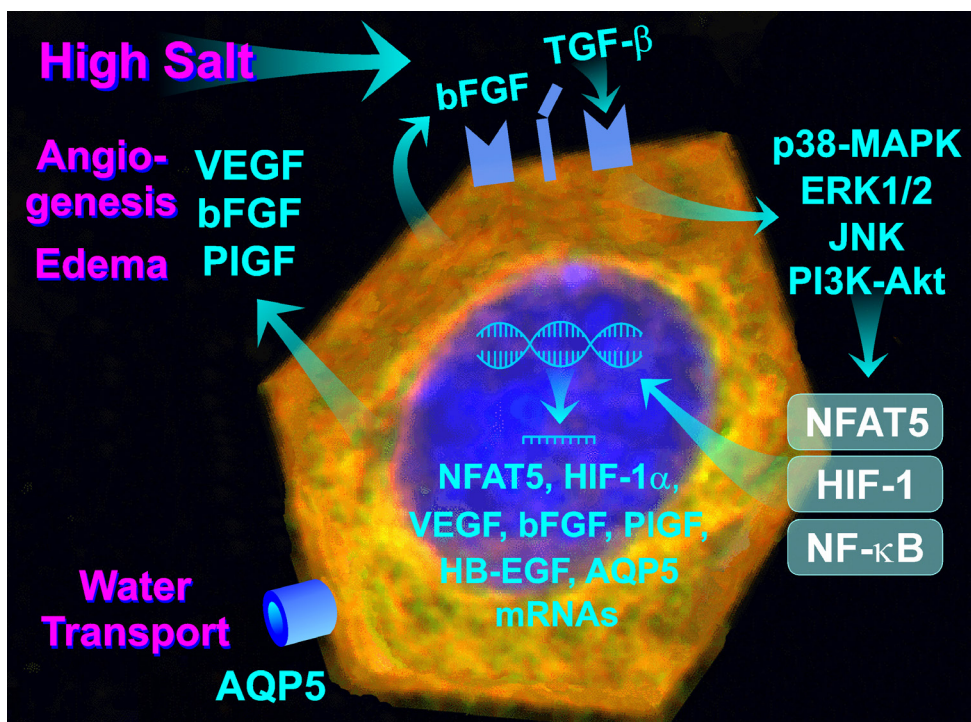


Figure 4. High salt consumption may aggravate neovascular retinal diseases by stimulation of angiogenic factor production in RPE cells. Excess salt induces the release of growth factors, such as basic fibroblast growth factor (bFGF) and transforming growth factor-β (TGF-β) from RPE cells. Autocrine and paracrine TGF-β and FGF receptor signaling induces activation of the intracellular signal transduction pathways, including p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) 1/2, c-Jun NH₂-terminal kinase (JNK), and phosphatidylinositol-3 kinase (PI3K)-Akt pathways, resulting in activation of the transcription factors nuclear factor of activated T cell 5 (NFAT5), hypoxia-inducible factor-1 (HIF-1), nuclear factor

kappa beta (NF-κB). NFAT5 and HIF-1 induce the transcription of the *VEGF* gene, while NFAT5 and NF-κB induce the transcription of the *AQP5* gene. NFAT5 also induces expression of the *bFGF* gene. The salt-induced secretion of VEGF, bFGF, and placental growth factor (PIGF) may stimulate the development of the central nervous system (CNV) and edema. Increased expression of AQP5 may improve water transport through the RPE, which may facilitate the development and/or resolution of edema, in dependence on the osmotic and hydrostatic gradients across the RPE.

body hydration and plasma osmolality, it was recommended to consume 1.5 l/d drinking water for women and 1.9 l/d for men [165]. These values are achieved in European countries (1.5–1.9 l/d) and are exceeded in North America (2.4 l/d) [166–168]. To avoid hyponatremia, lower daily water consumption is recommended for patients with distinct diseases, for example, severe congestive heart failure that requires high-dose diuretics [169]. Excess water intake may also cause hypertension and congestion of the cardiovascular system resulting from the increased blood volume.

Although the beneficial effects of reducing salt consumption and increasing water intake in the whole population are uncertain, there are two exceptions: salt-sensitive individuals and individuals who show signs of body dehydration. Because salt-resistant individuals show minimal changes in plasma osmolality and blood pressure after salt intake [47], dietary salt restriction does not have a benefit. However, salt-sensitive (normotensive and hypertensive) individuals display abnormal changes in plasma osmolality and blood pressure after salt intake [60]. The salt-induced upregulation

of angiogenic factors in RPE cells is transient (Figure 2C) [57,139]. This suggests that repetitive salt-induced increases in plasma osmolality during postprandial phases will have greater effects than a persistent elevation of the plasma salt level. The fact that high extracellular osmolality and alteration of the transmembrane sodium chloride gradient induce upregulation of VEGF in RPE cells [57] could, at least in part, explain why the use of antihypertensive medications is not associated with a decreased risk of AMD [41,89]. This may suggest that, in salt-sensitive subjects, approaches to reduce plasma salt levels with dietary salt restriction may be more beneficial than antihypertensive medications. However, it should be kept in mind that the salt sensitivity of blood pressure is induced less by high salt consumption and more by low intake of blood pressure–lowering minerals (potassium, calcium, magnesium, phosphorus, bicarbonate) [64]. Therefore, salt sensitivity can also be attenuated by other dietary modifications, including the DASH diet and adequate dietary mineral intake [50,51,61,64]. Increased consumption of calcium-, magnesium-, potassium- (milk and dairy products), and bicarbonate-rich foods (fruits and vegetables)

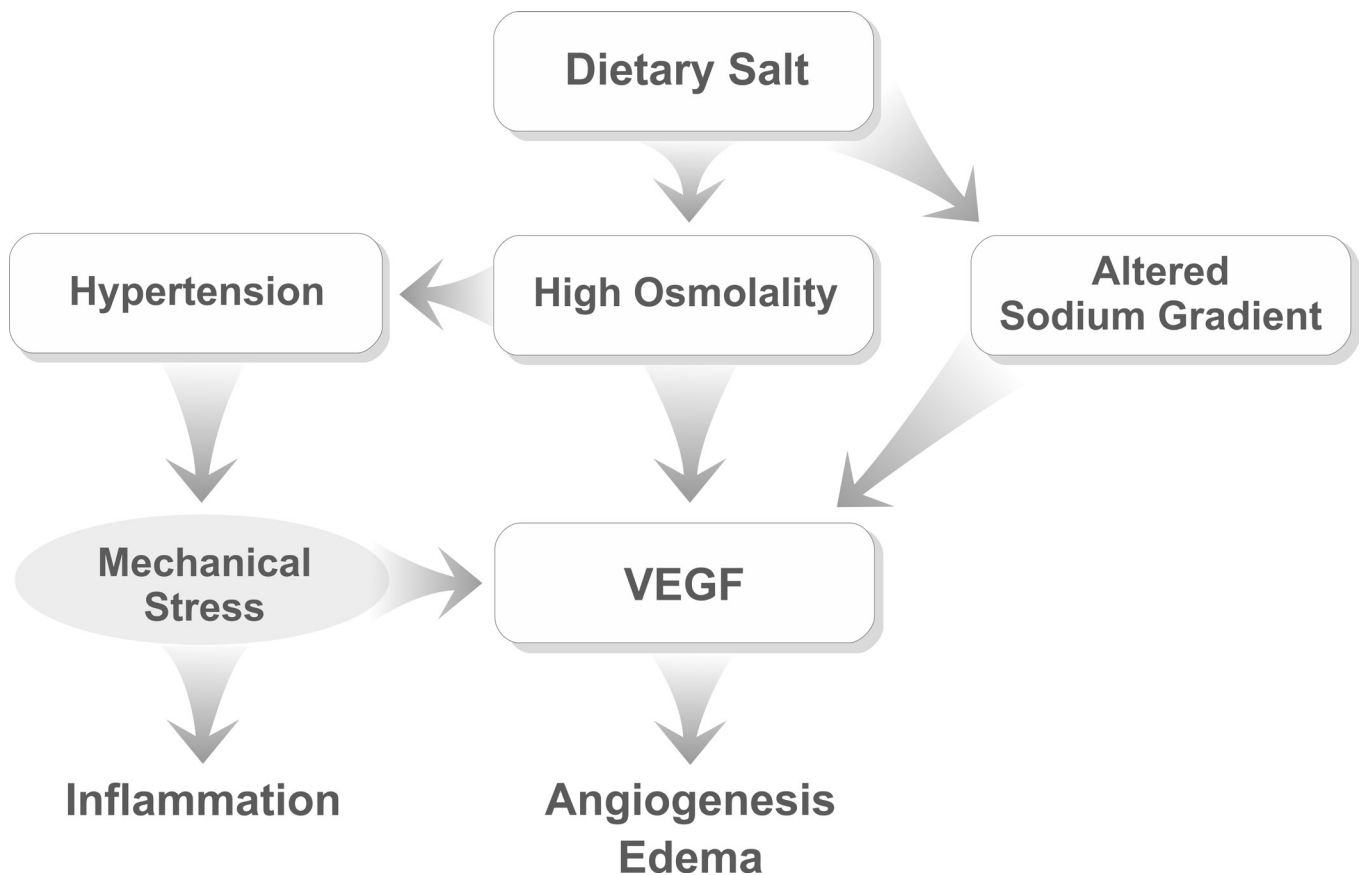


Figure 5. Dietary salt intake may stimulate the production of VEGF in RPE cells via three independent mechanisms. High salt increases plasma osmolality resulting in raised blood pressure and membrane stretch, and it alters the transmembrane sodium gradient.

promotes renal salt excretion and blunts the pressor effect of dietary salt [65,170,171].

Inadequate water intake is common in elderly individuals. Therefore, the water consumption of aged patients should be monitored, and attention should be paid to signs of body dehydration (e.g., decreased skin turgor and increased urine osmolality as indicated by the yellow color of the urine). It is important that dehydrated elderly individuals drink more plain water and less of other fluids, such as fruit juice and soft drinks. The latter have higher osmolalities (550–840 mOsm/kg) than plasma (285–295 mOsm/kg) [172] and thus cause net movement of water from the vascular system into the intestinal lumen; this leads to impaired water intake and increased plasma osmolality. At least, beverages should be diluted with plain water. Dilution of high-energy beverages is also beneficial in terms of another risk factor of AMD, obesity [33,34].

ACKNOWLEDGMENTS

Some of the work presented in this review was conducted with grants from the Deutsche Forschungsgemeinschaft (KO 1547/7–1 to L.K.) and the Geschwister Freter Stiftung (Hannover, Germany, to P.W.).

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 22 December 2016. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.