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REVIEW ARTICLE

The Role of Biofactors in Diabetic Microvascular Complications

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Abstract: Microvascular complications are responsible for a major proportion of the burden associated with diabetes contributing to substantial morbidity, mortality, and healthcare burden in people with diabetes. Retinopathy, nephropathy, and neuropathy constitute the leading causes of blindness, end-stage renal disease, and lower-extremity amputations, respectively. Since the efficacy of causal therapies of diabetic microvascular complications is limited, especially in type 2 diabetes, there is an unmet need for adjunct treatments which should be effective despite ongoing hyperglycemia. Experimental studies have indicated that diabetic microvascular complications can be prevented or ameliorated by various biofactors in animal models by interfering with the pathophysiology of the underlying condition. Some of the findings related to biofactors, like α-lipoic acid and benfotiamine, could be translated into the clinical arena and confirmed in clinical trials, especially in those focusing on diabetic polyneuropathy. Given the micronutrient nature of these compounds, their safety profile is excellent. Thus, they have the potential to favorably modify the natural history of the underlying complication, but long-term clinical trials are required to confirm this notion. Ultimately, biofactors should expand our therapeutic armamentarium against these common, debilitating, and even life-threatening sequelae of diabetes.

Keywords: Biofactors, diabetic microvascular complications, diabetic sensorimotor polyneuropathy, cardiovascular autonomic neuropathy, diabetic retinopathy, diabetic nephropathy.

1. INTRODUCTION

Diabetes contributes to substantial morbidity, mortality, and healthcare burden worldwide. The global prevalence estimate of diagnosed and undiagnosed diabetes in people aged 20-79 years reached 463 million (9.3%) in 2019 and is predicted to increase to 578 million (10.2%) by 2030 [1]. This is alarming since such a rise in diabetes prevalence will presumably foster an increase in the incidence of the complications and comorbidities associated with diabetes, which have a high impact on quality of life, prognosis, and demand forhealth services [2]. Apart from diabetic macrovascular complications such as coronary heart disease, stroke, and peripheral vascular disease, diabetic microvascular complications including retinopathy, nephropathy, and neuropathy, which are the leading causes of blindness, end-stage renal disease (ESRD), and lower-extremity amputations, respectively, are responsible for a major proportion of the burden associated with diabetes. The prevalence of diabetic retinopathy has been estimated at around 30%, while diabetic kidney disease affects around 40% of all individuals with diabetes [3]. The prevalence of the various manifestations of diabetic neuropathy is difficult to estimate, but the most frequent forms are diabetic sensorimotor polyneuropathy (DSPN), affecting approximately 30% [4], and cardiovascular autonomic neuropathy (CAN), affecting around 20% of all individuals with diabetes [5] as well as erectile dysfunction which affects 38 and 66% of men with type 1 or type 2 diabetes (T1D, T2D), respectively [6]. Apart from these three classical microvascular target tissues, diabetes has also been shown to exert adverse effects on the microvasculature of

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skin, brain, adipose tissue, and cardiac and skeletal muscle [7].

The Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study and other smaller trials demonstrated that intensive insulin therapy may prevent, albeit not completely, or delay the progression of microvascular complications in patients with T1D [8]. In contrast, insufficient evidence suggests the benefit of near-normal glycemic control in patients with T2D [9]. Moreover, very intensive diabetes therapy aimed at almost normalizing glycemia was associated with an increased risk of mortality in the ACCORD trial, suggesting that such a strategy may not be applicable to patients with longer-term T2D who are at high risk of diabetic complications [10]. This notion was particularly appropriate for patients with a history of neuropathy at baseline, which was a predictor of increased mortality in participants receiving very intensive diabetes therapy [11]. To optimize diabetes management, adjunct therapies have been designed and developed to effectively target the natural history of diabetic microvascular complications by addressing their putative underlying mechanisms, among which biofactors have attracted particular attention [12, 13].

Biofactors are compounds required by the body for normal physiological functioning and may exert health-beneficial or disease-preventive biological activities. Essential biofactors are those factors that the organism either cannot produce or produce in insufficient quantity, which therefore need to be supplied from external sources. Such essential biofactors are vitamins (B1, B6, B9, B12, C, A, D, E, K), minerals (selenium, magnesium, zinc), fatty acids (α-lipoic acid (ALA), omega-3 polyunsaturated fatty acids (omega-3 PUFAs), and amino acids (acetyl-L-carnitine). Dietary supplementation with certain biofactors could be useful as a complement to established therapies for preventing and treating diabetic complications, since on the one hand diabetes is associated with systemic deficits in a number of biofactors, but on the other hand beneficial effects have also been reported in individuals without such deficiencies [12, 14]. Given that the efficacy of causal treatments of diabetic microvascular complications is limited and there is an unmet need for adjunct treatments, this review aims at discussing the role of selected biofactors in the prevention and treatment of diabetic microvascular complications, also considering new trends and areas for future research.

2. PATHOPHYSIOLOGY OF DIABETIC MICROVASCULAR COMPLICATIONS

Hyperglycemia is generally considered the major systemic risk factor for diabetic microvascular complications [7]. However, it has been emphasized that hyperglycemia cannot be considered as the sole factor responsible for the development of diabetic complications, particularly in patients with T2D. Instead, an interplay of multiple factors that have an impact on the adipose tissue fatty acid metabolism could underlie the onset and progression of diabetic microvascular complications [3]. Apart from the duration and severity of diabetes and altered lipid metabolism, other risk factors include higher age, obesity, hypertension, and genetic factors.

Several lines of evidence have linked multiple biochemical pathways resulting from the adverse effects of hyperglycemia to diabetic microvascular complications. Cellular mechanisms include the following: nonenzymatic glycation and the formation of AGEs, enhanced reactive oxygen species (ROS) production and actions, endoplasmic reticulum (ER) stress, and the activation of the polyol pathway, the diacylglycerol (DAG)-protein kinase C (PKC) pathway, Src homology-2 domain-containing phosphatase-1 (SHP-1), and the renin-angiotensin system (RAS), and kallikrein-bradykinin (BK) systems. It is likely that hyperglycemia-induced intracellular and extracellular changes alter signal transduction pathways, thereby affecting gene expression and protein function to cause cellular dysfunction and damage [7].

The potential unifying mechanism by which hyperglycemia activates four pathways of hyperglycemic damage is the induction of mitochondrial superoxide overproduction. Excess superoxide produced by the mitochondrial electron-transport chain partially inhibits the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), thereby diverting upstream metabolites from glycolysis into pathways of glucose overutilization. This results in increased flux of dihydroxyacetone phosphate (DHAP) to DAG, a PKC activator, and triose phosphates to methylglyoxal, the main intracellular advanced glycation endproducts (AGEs) precursor. Increased flux of fructose-6-phosphate to UDP-N-acetylglucosamine increases modification of proteins by O-linked N-acetylglucosamine (GlcNAc), and increased glucose flux through the polyol pathway consumes NADPH and depletes glutathione [15]. Experimental studies have shown that the lipid-soluble thiamine derivative benfotiamine can inhibit these four pathways, as well as hyperglycemia-associated NF-κB activation, by activating the pentose phosphate pathway enzyme transketolase, which converts glyceraldehyde-3-phosphate and fructose-6-phosphate into pentose-5-phosphates and other sugars. In retinas of diabetic animals, benfotiamine treatment inhibited these three pathways and NF-κB activation by activating transketolase and also prevented experimental diabetic retinopathy [16, 17]. Furthermore, as an antioxidant, ALA can block the production of ROS such as superoxide. Apart from ample evidence from experimental and clinical studies suggesting that ALA exerts favorable effects in diabetic neuropathy *via* its antioxidative properties, clinical trials have indicated that ALA may also offer protection in diabetic cardiomyopathy through inhibition of NF-kB activation and reduction of fas-ligand, decreasing matrix metalloproteinase-2 as well as diabetic nephropathy and retinopathy [18]. Actovegin, a deproteinized hemoderivative produced from calf blood by ultrafiltration that contains low-molecular weight compounds of up to 5,000 Da, has been shown to suppress the activation of poly (ADP-ribose) polymerase (PARP) [8, 19].

3. DIABETIC NEUROPATHY

Diabetic neuropathy refers to disorders of the somatosensory and/or autonomic parts of the peripheral nervous system in a person with diabetes, in whom no other cause is apparent. However, non-diabetic neuropathies may be present in patients with diabetes and may be treatable by specific measures. Diabetic neuropathy can be classified according to the type of neurologic involvement into diffuse neuropathy, mononeuropathy, and radiculopathy or polyradiculopathy [20].

The manifestations of diabetic autonomic neuropathy cause multiple symptoms and involve: 1) the cardiovascular system: resting tachycardia, reduced heart rate variability, painless myocardial ischemia/infarction, orthostatic hypotension, exercise intolerance, perioperative instability, sudden death; 2) the gastrointestinal tract: esophageal motor dysfunction, diabetic gastroparesis, gallbladder atony, diabetic enteropathy (diarrhea), colonic hypomotility (constipation), anorectal dysfunction (fecal incontinence); 3) the genitourinary tract: diabetic cytopathy, sexual dysfunction; 4) dysfunction in the neuroendocrine, sudomotor, vasomotor, pupillary motor, and respiratory systems [20].

3.1. Diabetic Sensorimotor Polyneuropathy

3.1.1. Epidemiology and Clinical Impact

DSPN represents the most relevant clinical manifestation affecting approximately 30% of diabetic patients, while the incidence of DSPN is approximately 2% per year [4]. DSPN and its measures predict the development of neuropathic foot ulceration, one of the most common causes for hospital admission and lower-limb amputations among diabetic patients [21], all-cause mortality [22, 23], and cardiovascular morbidity and mortality [24, 25]. Unfortunately, despite this major clinical impact, DSPN still remains underdiagnosed and undertreated [26]. DSPN is a symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvessel alterations as a result of chronic hyperglycemia exposure (diabetes) and cardiovascular risk covariates [27]. A simple definition of DSPN for clinical practice is the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes [20].

DSPN is commonly associated with autonomic involvement. Its onset is insidious, and, in the absence of intervention, the course is chronic and progressive. It seems that the longer axons to the lower limbs are more vulnerable to the nerve lesions induced by diabetes (length-related distribution). Up to 50% of diabetic peripheral neuropathies may be asymptomatic [20]. Chronic painful DSPN is present in up to 26% of diabetic patients [4]. The most important etiological factors that have been associated with DSPN are poor glycemic control, visceral obesity, diabetes duration, height, hypertension, age, smoking, hypoinsulinemia, and dyslipidemia [4, 27]. There is emerging evidence to suggest that prediabetes is associated with an increased risk of polyneuropathy. In the general population (region of Augsburg, Southern Germany), the prevalence of polyneuropathy was 28.0% in diabetic subjects, 13.0% in those with impaired glucose tolerance (IGT), 11.3% in those with impaired fasting glucose (IFG), and 7.4% in those with normal glucose tolerance (NGT) [28].

3.1.2. Diagnosis

The basic neurological assessment comprises the general medical and neurological history, inspection of the feet, and neurological examination of sensation using simple semi-quantitative bed-side instruments such as the 10g Semmes-Weinstein monofilament (touch, pressure), Tiptherm (temperature), calibrated Rydel-Seiffer tuning fork (vibration), pin-prick (pain), and tendon reflexes (knee and ankle). Sensory fiber involvement causes “positive“ sensory symptoms such as burning, stabbing, shooting, or lancinating pain, paresthesias, dysesthesias, and sensory ataxia (atactic gait) as well as “negative“ symptoms such as reduced sensation to temperature, pain, touch/pressure, and vibration stimuli and less frequently hypersensitivity to tactile (allodynia) and painful stimuli (hyperalgesia) [4]. Clinical assessment should be standardized using validated scores for both the severity of symptoms and the degree of neuropathic deficits such as the Michigan Neuropathy Screening Instrument (MNSI) [29], Neuropathy Symptom Score (NSS) [30], or Total Symptom Score (TSS) [31] for neuropathic symptoms and Neuropathy Disability (NDS) for neuropathic deficits (signs, impairments [30].

The intensity (severity) of neuropathic pain and its course should be assessed using an 11-Point numerical rating scale (Likert scale) or a visual analogue scale. Various screening tools (with or without limited bedside testing) have been developed to identify neuropathic pain, such as the PainDetect, LANSS, NPQ, DN-4, ID-Pain). These questionnaires use verbal descriptors and pain qualities as a basis for distinguishing neuropathic pain from other types of chronic pain, such as nociceptive pain [32].

The following findings should alert the physician to consider causes for DSPN other than diabetes and referral for a detailed neurological work-up: 1) pronounced asymmetry of the neurological deficits, 2) predominant motor deficits, 3) mononeuropathy, 4) cranial nerve involvement, rapid development or progression of the neuropathic impairments, 5) progression of the neuropathy despite optimal glycemic control, 6) development of symptoms and deficits only in the upper limbs, 7) family history of non-diabetic neuropathy, and 8) diagnosis of DSPN cannot be ascertained by clinical examination [4].

The most important differential diagnoses from the general medicine perspective include neuropathies caused by alcohol abuse, uremia, hypothyroidism, monoclonal gammopathy, vitamin B12 deficiency, peripheral arterial disease, cancer, inflammatory and infectious diseases, and neurotoxic drugs. The process of diagnostic exclusion should be based on the following minimally required standard battery of laboratory tests: complete blood count, creatinine, CRP, TSH, vitamin B12, folic acid, liver enzymes, and immunoelectrophoresis [4].

3.1.3. Treatment

The management of DSPN includes three cornerstones: 1) causal treatment including lifestyle modification, intensive diabetes therapy aimed at near-normoglycemia and multifactorial cardiovascular risk intervention, 2) pathogenesis-derived pharmacotherapy, and 3) symptomatic treatment of neuropathic pain.

3.1.3.1. Causal Treatment

Several clinical trials have shown that reducing obesity as a risk factor for both DSPN and CAN by various interventions such as lifestyle intervention may exert favorable effects on nerve function and pathology. In the lifestyle arm of the Diabetes Prevention Program, people with prediabetes had a lower prevalence of DSPN than those who had diabetes [33].

Intensive insulin therapy aimed at achieving near-normal glycemia is essential to prevent or delay the progression of DSPN in patients with T1D, but there is no convincing evidence for a favorable effect of intensive diabetes therapy on the development or progression of DSPN in T2D patients. The Steno 2 Study assessed the effect of multifactorial cardiovascular risk intervention (CVRI) on diabetes complications but could not demonstrate a favorable effect on DSPN [34, 35].

3.1.3.2. Treatment with Biofactors

Fig. (**1**) illustrates the cellular pathways implicated in the pathogenesis of diabetic neuropathy and options for pathogenetically oriented treatments with biofactors. Hyperglycemia and dyslipidemia result in a substrate excess in mitochondria leading to mitochondrial dysfunction and oxidative and carbonyl stress. Oxidative and carbonyl stress-mediated nuclear DNA damage activates poly (ADP-ribose) polymerase (PARP) 1, which in turn leads to depletion of NAD+/ATP and inhibition of GAPDH. Inactivation of GAPDH activates key pathways implicated in the development of diabetic neuropathy (polyol pathway, hexosamine pathway, PKC activity, and the formation of AGEs. Furthermore, hyperinsulinemia and inflammation cause impaired insulin signaling. All aforementioned risk factors and pathways converge in the activation of stress and inflammatory pathways (IKK/NF-κB, JNK, AMPK, COX2) [8, 15, 20].

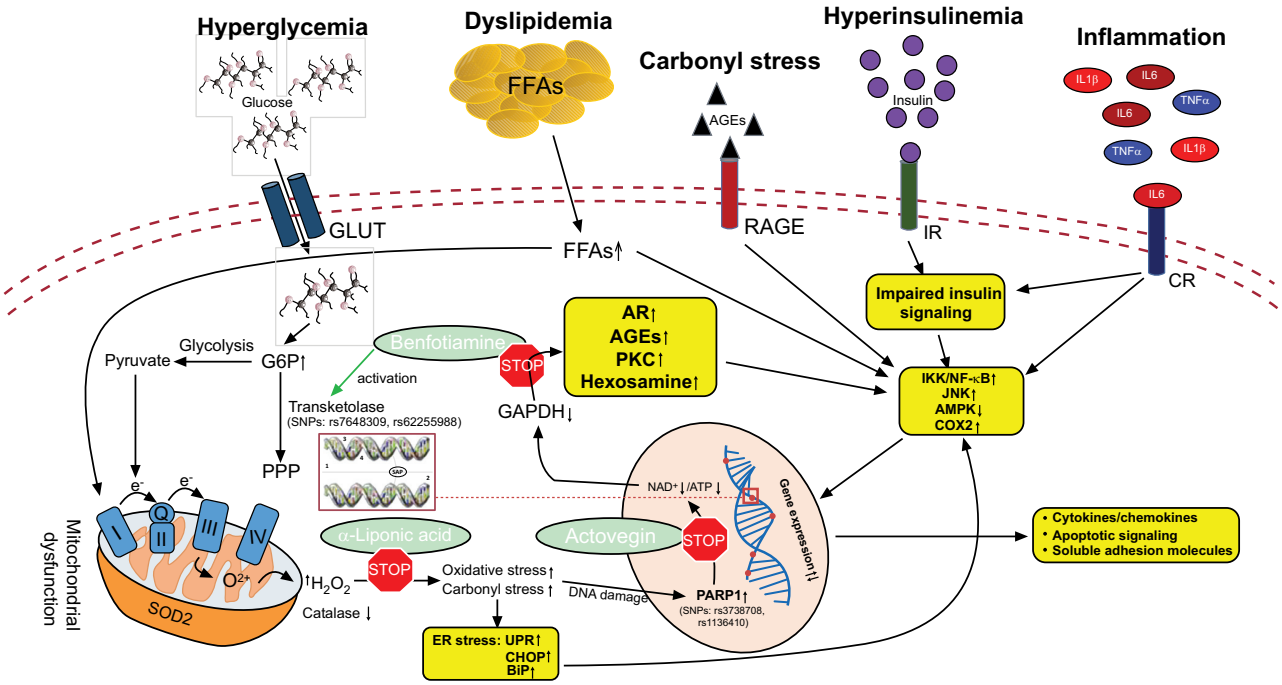

Fig. (1). Cellular pathways implicated in the pathogenesis of diabetic neuropathy and mechanisms of action of specific biofactors (highlighted in green) that are linked to the various maladaptive processes with STOP signs indicating experimentally verified blocking of the corresponding pathways. Adapted from [8].

Abbreviations: AGEs, Advanced Glycation End products; AMPK, 5' Adenosine Monophosphate-activated Protein Kinase; AR, Aldose Reductase; ATP, Adenosine Triphosphate; BiP, Binding immunoglobulin Protein; CHOP, CCAAT/enhancer-binding Protein Homologous Protein; COX2, Cyclooxygenase 2; CR, Cytokine Receptor; ER, Endoplasmic Reticulum; FFAs, Free Fatty Acids; G6P, Glucose-6-Phosphate; GAPDH, Glyceraldehyde 3-Phosphate Dehydrogenase; GLUT, Glucose Transporter; IKK, IκB Kinase; IR, Insulin Receptor; JNK, c-Jun *N*-terminal Kinase; NAD^+, oxidized Nicotinamide adenine Dinucleotide; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; PKC, Protein Kinase C; PPP, Pentose Phosphate Pathway; PARP1, Poly (ADP-ribose) Polymerase 1; RAGE, Receptor of AGEs; SNPs, Single-Nucleotide Polymorphisms; UPR, Unfolded Protein Response (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Randomized, double-blind, placebo-controlled trials using α-lipoic acid in diabetic polyneuropathy.

Study (Refs.)	*n T1D/T2D*	Daily Dose (mg)	Duration	Effects	Adverse Events
ALADIN [31]	0/328	100/600/1200/placebo	3 weeks i.v.	TSS+ NDS+ HPAL+	None
ALADIN II [53]	65*	600/1200/placebo	2 years p.o.	Sural SNCV+ Sural SNAP+ Tibial MNCV+	None
ALADIN III [54]	0/508	600 i.v./1800 p.o./placebo	3 weeks i.v./ 6 months p.o.	TSS(+)/- NIS+/(+) NIS-LL(+)/(+)	None
ORPIL [55]	0/24	1800/placebo	3 weeks p.o.	TSS+ HPAL(+) NDS+	None
SYDNEY [56]	30/90	600/placebo	3 weeks i.v.	TSS+ NSC+ NIS+	None
SYDNEY 2 [57]	30/151	600/1200/1800/placebo	5 weeks p.o.	TSS+ NSC+ NIS+	Dose-dependent GI symptoms
NATHAN 1 [45]	110/344	600/placebo	4 years p.o.	NIS+, NIS-LL+ NSC+ NCV-	SAEs increased *vs.* placebo
Mansoura [58]	0/200	1200/placebo	6 months	NSS+ NDS VPT	Mild nausea
Meta-analysis [59]	103/1155	600/placebo	3 weeks i.v.	TSS+ NIS+ NIS-LL+	None
Meta-analysis [60]	60/1100	Trials: ALADIN, ALADIN III, SYDNEY SYDNEY 2, ORPIL	See individual trials above	See individual trials above	See individual trials above
Meta-analysis [61]	60/593	Trials: ALADIN, SYDNEY, SYDNEY 2, ORPIL,			
Meta-analysis [62]	60/593	Trials: ALADIN, SYDNEY, SYDNEY 2, ORPIL			
Meta-analysis [63]	170/1445	Trials: ALADIN, ALADIN III, SYDNEY SYDNEY 2, ORPIL, NATHAN 1			
Meta-analysis [64]	170/937	Trials: ALADIN, SYDNEY, SYDNEY 2, ORPIL, NATHAN 1	-	-	-
Meta-analysis [65]	170/585	Trials: SYDNEY, SYDNEY 2, NATHAN 1	-	-	-

DML: Distal Motor Latency; GIU: Gastrointestinal; HPAL: Hamburg Pain-Adjective List; i.v.: intravenous administration; MNCV: Motor Nerve Conduction Velocity; NDS: Neuropathy Disability Score; NIS-LL: Neuropathy Impairment Score - lower limbs; NSC: Neuropathy Symptoms and Changes; p.o.: oral administration; SAEs: Severe Adverse Events; SNAP: Sensory Nerve Action Potential; SNCV: Sensory Nerve Conduction Velocity; TSS: Total Symptom Score; T1D: Type 1 Diabetes; T2D: Type 2 Diabetes; VPT Vibration Perception Threshold. *Diabetes type not available; + = Improvement compared to placebo; (+) = Trend towards improvement compared to placebo; - = No difference compared to placebo.

3.1.3.2.1. Lipoic Acid

ALA is one of the most important cellular antioxidants besides glutathione, vitamins A, C, and E, acetyl-L-carnitine, taurine, and melatonin [36]. ALA is biosynthesized in plants and animals, where it is metabolized to dihydrolipoic acid (DHLA) upon uptake into cells. Both ALA and DHLA are potent free radical scavengers with the ability to raise intracellular glutathione and regenerate vitamins C and E [37-39]. In addition, ALA can also indirectly act as an antioxidant by chelating divalent transient metal ions [40]. Given its unique amphiphilic character, ALA exerts its effects both in the plasma membrane and cytosol, in contrast to other antioxidants, which are either water or lipid-soluble agents [40]. Experimental studies have shown that ALA blocks the generation of ROS, caspase-3 invigoration, nuclear DNA degeneration, and stimulation of the receptor for AGEs [41-43].

Since oxidative stress plays a major role in the pathogenesis of diabetic microvascular complications, including neuropathy, the rationale for treatment using antioxidants such as ALA to diminish enhanced oxidative stress and thereby favorably influence of DSPN is obvious [44]. Among the biofactors reviewed herein, ALA has the best evidence favoring its use as a pathogenetically oriented treatment option in DSPN. The results of the randomized controlled trials (RCTs) using ALA for the treatment of DSPN and the corresponding meta-analyses are summarized in Table **1**. Collectively, these trials demonstrated that infusions of ALA (600 mg i.v./day) ameliorate neuropathic symptoms and deficits (signs, impairments) after 3 weeks. Moreover, treatment for

5 weeks using 600 mg QD of ALA orally reduces the chief symptoms of diabetic polyneuropathy, including pain, paresthesia, and numbness to a clinically meaningful degree. In the NATHAN 1 trial, including 460 diabetic patients with mild to moderate largely asymptomatic DSPN, after 4 years of ALA treatment using 600 mg QD, neuropathic deficits (signs, impairments) were improved, suggesting a potential to favorably influence the underlying neuropathy. Furthermore, the drug was well tolerated throughout the trial [45]. A post-hoc response analysis suggested that a better outcome in neuropathic impairments following 4-year treatment with ALA was predicted by normal BMI and blood pressure albeit higher burden due to cardiovascular disease, diabetes, and DSPN, while improvement in cardiac autonomic function was predicted by angiotensin-converting enzyme (ACE) inhibitor treatment. Thus, optimal control of cardiovascular disease (CVD) risk factors could contribute to improved efficacy of ALA in patients with higher disease burden [46]. Clinical and post-marketing surveillance studies have revealed a highly favorable safety profile [44, 47].

Moreover, recent meta-analyses suggest that, apart from its favorable effects on diabetic neuropathy, ALA supplementation improves endothelial function and glycemic control and reduces weight, inflammatory biomarkers, triglycerides, total cholesterol, and LDL cholesterol [48-52].

3.1.3.2.2. Benfotiamine

Thiamine (vitamin B1) is a water-soluble vitamin that constitutes an essential cofactor of several enzymes involved in carbohydrate metabolism. Benfotiamine (S-benzoylthiamine O-monophosphate), a synthetic S-acyl derivative (prodrug) of thiamine, is a lipid-soluble allithiamine homolog that is converted into the active coenzyme form thiamine diphosphate (TDP) within the body. Unlike thiamine, benfotiamine is absorbed passively and traverses the intestinal barrier faster and more readily, leading to markedly higher plasma, blood, and erythrocyte concentrations [66]. Experimental data suggest that benfotiamine is a transketolase activator that directs the elevated levels of hexose and triose phosphates to the pentose phosphate pathway, thereby acting as an AGE inhibitor that has the potential to favorably influence hyperglycemia-induced diabetic complications [16]. Low plasma thiamine concentration appears to be prevalent in patients with T1D and T2D, associated with increased thiamine clearance [67, 68]. Four RCTs conducted over 3-12 weeks assessed the efficacy and safety of various daily doses of benfotiamine in diabetic patients with DSPN using different neuropathic endpoints (Table **2**). The BENDIP study showed that neuropathic symptoms, with NSS as the primary endpoint, were improved after 6 weeks of treatment using a benfotiamine dose of 300 mg BID but not 300 mg QD [69], while the BEDIP study showed an improvement in a score combining neuropathic symptoms and signs after 3 weeks of treatment with benfotiamine 100 QID [70], as did an early trial using a combination of benfotiamine, pyridoxine, and cyanocobalamin [71]. Another trial, using the latter combination therapy, demonstrated an improvement in peroneal motor NCV, but not vibration perception threshold (VPT) after 12 weeks [72]. In a 2-year study, treatment with benfotiamine (300 mg/day) did not exert an effect on peripheral nerve function superior to placebo in T1D patients [73], but since the majority of the participants did not have DSPN, the design and clinical relevance of this trial were questioned [74]. All trials documented that the incidence of adverse events did not differ between active and placebo treatment. Collectively, these trials indicate that benfotiamine treatment is safe and improves neuropathic symptoms and, in combination with vitamins B6 and B12, also neuropathic deficits and nerve conduction. A dose of 300 mg BID appears appropriate, at least in the short-term setting.

Table 2. Randomized, double-blind controlled clinical trials of benfotiamine in diabetic patients with polyneuropathy.

Refs.	Compound n/dose (mg)	Duration (Weeks)	Primary Endpoint	Secondary Endpoints	Efficacy: Primary Endpoint	Efficacy: Secondary Endpoints	Adverse Events
Stracke *et al.*, 2008; BENDIP study [69]	B: 47/300 BID B: 45/300 QD P: 41	6	NSS	TSS NDS VPT	B300 BID: NSS: PP+ ITT(+)	TSS↔ NDS↔ VPT↔	↔
Haupt *et al.*, 2005; BEDIP study [70]	B: 20/100 QID P: 20	3	Score: Muscle strength, pain, sensory function, coordination, reflexes	Pain VPT PGIC	Score+	Pain+ PGIC(+)	↔
Stracke *et al.*, 1996 [72]	B: 80*-40 QID+ B6: 180*-90 QID+ B12: 11/0.5*-0.25 QID P: 13	12	MNCV VPT		Peroneal MNCV+ Median MNCV↔ VPT↔		↔
Ledermann & Wiedey, 1989 [71]	B: 80 QID+ B6: 180 QID+ B12: 10/0.5 QID P: 10	3	Score: Muscle strength, pain, sensory function, coordination, reflexes; VPT, pin-prick, pain		Score+ Pain+ Pin-prick+ VPT+		↔

B: Benfotiamine, P: Placebo, B6: vitamin B6, B12: vitamin B12, QD: once daily, BID: twice daily, QID: four times daily, NSS: Neuropathy Symptom Score, NDS: Neuropathy Disability Score, TSS: Total Symptom Score, VPT: Vibration Perception Threshold, PGIC: Patient Global Impression of Change, MNCV: Motor Nerve Conduction Velocity, PP: Per Protocol, ITT: Intention To Treat.

+ improvement, (+) borderline improvement (P<0.06), ↔ no difference between active and placebo treatment.

*Dose during the first 2 weeks of study.

Combination treatment was evaluated in an open-label pilot study in T1D patients in whom benfotiamine (300 mg BID) plus ALA (600 mg BID) completely normalized increased AGE formation, reduced elevated monocyte hexosamine-modified proteins by 40%, and normalized a 70% decrease in prostacyclin synthase activity after 4 weeks [75], suggesting that the pathways involved in the pathogenesis of diabetic complications in rodents may also be relevant in humans. Results from another 8-week pilot study are also supportive of combination treatment with benfotiamine (300 mg/d) and ALA (600 mg/d), indicating a reduction of neuropathic symptoms when compared to either monotherapy [76]. However, RCTs are needed to confirm these preliminary findings.

A randomized, double-blind, placebo-controlled parallel-group 1-year study (BOND Study) to assess the effects of treatment with benfotiamine on morphometric, neurophysiological, and clinical measures in T2D patients with mild to moderate symptomatic DSPN is currently underway. Several transketolase single nucleotide polymorphisms (SNPs) were recently found to be associated with measures of DSPN in patients recently diagnosed with diabetes [77]. Consequently, it has been suggested to focus on the therapeutic attempt to target thiamine and transketolase [78]. Genetic variations in the transketolase enzyme could be useful for the identification of responders/non-responders to benfotiamine treatment and might open up new perspectives in the pharmacogenomics of this drug in the future.

3.1.3.2.3. Vitamin B12 and Vitamin B Combinations

Vitamin B12 deficiency can have hematological or neurological consequences, including polyneuropathies, and thus is an important differential diagnosis of DSPN. While metformin constitutes the first-line pharmacotherapy in T2D, this compound may interfere with vitamin B12 absorption, exposing patients to an increased risk of vitamin B12 deficiency. A recent meta-analysis showed that patients treated with metformin had a two-fold higher risk of vitamin B12 deficiency and lower serum vitamin B12 concentrations, depending on the dose and treatment duration, compared to those not treated with metformin [79]. Due to the increased risk of a vitamin B12 deficiency associated with metformin treatment, the American Diabetes Association (ADA) recommended that a periodic measurement of vitamin B12 levels should be considered in metformin-treated patients [80]. A recent 12-month RCT assessed the effects of oral vitamin B12 supplementation of 1000 μg per day in 90 metformin-treated T2D patients with both DSPN and CAN who had low vitamin B12 levels (<400 pmol/L). Oral vitamin B12 treatment increased plasma B12 levels and improved neurophysiological parameters, pain score, sudomotor function, and quality of life but not the MNSI and cardiovascular autonomic reflex tests [81].

Low folate levels and vitamin B6 deficiency were also reported to be prevalent among patients with diabetes [82, 83], which was in line with low thiamine levels [67, 68]. In addition, patients with diabetes may have an increased risk of polyneuropathies due to multiple vitamin B deficiencies [84].

In diabetic fatty Zucker rats, Metanx (a specific combination of vitamin B6, vitamin B12, and L-methylfolate) improved intraepidermal nerve fiber density in the tibial nerve, hind-limb digital sensory nerve conduction as well as thermal and mechanical hypoalgesia [85]. In a 24-week trial, there was no improvement in VPT (primary endpoint) with Metanx compared to placebo. However, neuropathic symptoms (secondary endpoint) were ameliorated with Metanx compared to placebo at week 16 and week 24. This was accompanied by an improvement in neuropathic deficits at week 16 but not at week 24 [86].

Several meta-analyses reported beneficial effects of vitamin B12 alone or in combination with other B vitamins, Prostaglandin E1 or ALA, on neuropathic symptoms and nerve conduction studies in patients with DSPN [87-90]. However, the quality studies included were limited, and the need for additional RCTs was emphasized [87, 88, 90]. In contrast, a Cochrane review analyzing data from randomized or quasi-randomized trials concluded that there is insufficient evidence to determine if vitamin B combinations, in general, are effective for treating diabetic or alcoholic neuropathy [91].

Altogether, it has recently been concluded that the evidence for favorable effects of B vitamins in diabetic neuropathy and neuropathic pain shows substantive heterogeneity with respect to study designs and reported outcomes, highlighting the need for larger, placebo-controlled studies [92].

3.1.3.2.4. Actovegin

Actovegin is a deproteinized ultrafiltrate of calf blood composed of more than 200 active biological substances. In a multicenter trial, 567 patients with symptomatic DSPN were randomized to receive 20 daily *i.v.* infusions of Actovegin (2000 mg/day) followed by three Actovegin tablets daily (1800 mg/day) or placebo for 140 days. Both neuropathic symptoms and VPT improved with Actovegin treatment, and the drug was well tolerated, with an adverse event profile similar to placebo [93]. The response to Actovegin treatment for neuropathic symptoms and/or impairments was clinically meaningful, but in contrast to the NATHAN 1 study with ALA [45], no specific predictors of this response could be identified [94]. There is also evidence suggesting a beneficial effect of Actovegin treatment after 6 months on cognitive outcomes in patients with poststroke cognitive impairment [95].

3.1.3.2.5. Curcumin

Curcumin is a polyphenol isolated from the roots of *Curcuma longa* with antioxidative, anti-inflammatory, and anti-amyloid properties [96]. Since curcumin is a rapidly metabolized lipophilic molecule with low bioavailability, new formulations such as emulsions or nanoparticles to improve systemic bioavailability have been developed [97]. In an RCT using 80 mg Nano-curcumin (72% curcumin, 25% demethoxycurcumin, 3% bisdesmethoxycurcumin) per day in 80 T2D patients with DSPN, HbA1c, fasting plasma glucose, and the Toronto Clinical Neuropathy Score (TCNS)

were improved compared to placebo administration after 8 weeks [98]. Further studies are needed to confirm these preliminary findings. The role of curcumin in the treatment of neuropathic pain is also being elucidated [99].

3.1.3.2.6. Acetyl-L-Carnitine

Acetyl-L-carnitine is a naturally occurring amino acid that is sometimes used as a dietary supplement. In humans, the metabolic pool of carnitine comprises nonesterified levocarnitine (L-carnitine) and acyl carnitine esters. Of these, acetyl-L-carnitine forms the greatest component. A Cochrane review analyzed four studies involving 907 participants with DSPN, which were reported in three publications. The evidence was sparse and of low certainty as to whether acetyl-L-carnitine causes a reduction in pain after 6 to 12 months' treatment in people with DPN compared to placebo. Data on functional and sensory impairment and symptoms were lacking or of very low certainty [100].

3.1.3.2.7. Combination of Superoxide Dismutase, ALA, Vitamin B12, and Acetyl-L-Carnitine

In a prospective, double-blind, placebo-controlled study, 85 T2D patients with DSPN were randomized to receive a combination of 10 mg superoxide dismutase (SOD), 570 mg ALA, 300 mg acetyl-L-carnitine (ALC), and 250 µg vitamin B12 daily or placebo for 12 months. This combination was associated with an improvement in neurophysiological parameters, neuropathic pain, and quality of life [101].

3.1.3.2.8. Omega-3 PUFAs

Pre-clinical studies have provided evidence that treating diabetic rodents with eicosapentaenoic and docosahexaenoic acids (omega-3 20:5 and 22:6, respectively) as well as menhaden oil, a natural source of long-chain fatty acids, improve vascular and neural deficits [102, 103]. An open-label 1-year pilot trial showed an increase in corneal nerve fiber length (CNFL) following omega-3 PUFA supplementation in T1D patients with or without DSPN, whereas no change was observed in nerve conduction or sensory function [104]. A 12-week multicenter, parallel-group, double-blind, double-dummy, randomized, noninferiority trial compared the efficacy of gamma-linolenic acid (GLA, 320 mg/day) and ALA (600 mg/day) in 100 T2D patients with painful DSPN. Both neuropathic symptoms and pain improved after 12 weeks, and GLA was found to be non-inferior to ALA in reducing pain intensity [105].

3.1.3.2.9. Magnesium

Magnesium is the second most abundant intracellular divalent cation involved in hundreds of metabolic reactions where it mainly serves as a cofactor [106] and plays an important role in carbohydrate metabolism and cellular bioenergetics. For example, magnesium is a crucial cofactor for several carbohydrate metabolism enzymes, including transketolase [107]. Epidemiological studies have suggested that reduced magnesium intake and systemic magnesium levels are associated with both prediabetes and diabetes [108, 109]. Moreover, a recent cross-sectional study has demonstrated that low serum magnesium levels are associated with DSPN in T2D individuals [110]. In an experimental study on rats, oral magnesium administration prevented diabetes-induced thermal hyperalgesia [111].

In an open RCT over 5 years, magnesium supplementation (Group A) was compared to no supplementation (Group B) in patients with T1D. Magnesium in red blood cells (R-BC) increased to normal levels in Group A but remained low in group B. Staging for DSPN in Group A *vs.* Group B after 5 years showed an improvement in 39 *vs.* 8%, no change was observed in 49 *vs.* 31%, and worsened condition was observed in 12 *vs.* 61%, respectively. Logistic regression analysis showed that a longer duration of diabetes and low RBC magnesium concentrations were the major determinants of the development of DSPN [112].

3.1.3.2.10. Vitamin D

Vitamin D deficiency, for which obesity, prediabetes, and T2D constitute important risk factors, is widespread worldwide [113]. There is also accumulating evidence suggesting a link between low systemic vitamin D levels and DSPN. A meta-analysis including six cohort and cross-sectional studies, respectively, comprising 1,484 T2D patients, reported decreased serum vitamin D levels in patients with DSPN compared to those without DSPN and identified vitamin D deficiency as an independent risk factor for the development of DSPN in patients with T2D [114]. Reduced vitamin D levels were also shown in patients with painful DSPN compared to those with non-painful DSPN [115, 116]. Moreover, two meta-analyses showed an association between vitamin D status and the risk of developing a diabetic foot ulcer [117, 118].

There is no evidence available from RCTs on the efficacy of vitamin D in DSPN. A meta-analysis of four non-randomized studies including 364 patients with DSPN showed a reduction of neuropathic pain scores using variable doses of vitamin D [119]. In a randomized open-label study including 67 patients with T2D and DSPN, the majority of whom was vitamin D deficient, improvements in neuropathic symptoms and deficits were observed after 24 weeks of high-dose vitamin D treatment (40,000 IU/week) compared to the control group supplemented with 5,000 IU Vitamin D per week [113]. These studies suggest that vitamin D deficiency should be considered in the management of DSPN and raise the possibility that vitamin D supplementation may be an effective adjuvant therapy for alleviating neuropathic pain and even deficits [120], but these findings should be confirmed by RCTs.

3.1.3.2.11. Vitamin E

In a recent large clinical trial, vitamin E (200 mg of mixed tocotrienols BID) did not improve neuropathic symptoms over 1 year in diabetes patients with DSPN. However, in post hoc subgroup analyses, tocotrienols reduced lancinating pain among patients with HbA1c levels >8% and normal homocysteinemia after 1 year [121]. By contrast, in a recent

study, supplementation with tocotrienol-rich vitamin E (Tocovid; 200 mg BID) for 8 weeks resulted in improved motor and sensory nerve conduction velocity (NCV) and increased nerve growth factor (NGF) levels in T2D patients, but neither the criteria for the definition of DSPN nor the proportion of participants with DSPN was reported [122]. Thus, further studies are needed to clarify whether vitamin E is useful for the treatment of DSPN.

3.1.3.3. Symptomatic treatment of painful DSPN

Analgesic treatment of painful DSPN is beyond the scope of this review. In brief, multimodal pain management should consider the individual risk profile, pharmacotherapy, and non-pharmacological options. The response rate to analgesic monotherapy in painful DSPN is only around 50%. Thus, inadequate response to analgesic drug treatments constitutes a substantial unmet need in patients with neuropathic pain [123]. Thus, combination pharmacotherapy is required in patients who have only a partial response or in whom the drug cannot be further titrated due to intolerable side effects. Effective pain treatment considers a favorable balance between pain relief and side effects without implying a maximum effect. Since the strength of evidence derived from systematic reviews, on which recommendations for pharmacotherapy of painful DSPN are based, is highly variable and inconclusive, efforts should be made toward harmonizing these guidelines to prevent the physician from making wrong decisions [124, 125].

3.2. Cardiovascular Autonomic Neuropathy

3.2.1. Epidemiology and Clinical Impact

CAN is a relatively frequent complication of diabetes. Interestingly, impairment of cardiovascular autonomic control may occur even in prediabetes [126]. Due to damage of autonomic nerve fibers that innervate the heart and peripheral blood vessels, both the physiological heart rate control and vascular tone/dynamics may be altered, resulting in signs and symptoms of CAN. The prevalence of CAN depends on the method used for its detection. Generally, CAN affects at least 20% of unselected patients with diabetes, but the prevalence is higher among older patients and with a longer duration of diabetes [5]. Importantly, CAN is associated with an increased risk of mortality which was documented in two meta-analyses [127, 128].

In patients with T1D, CAN develops predominantly in association with poor glycemic control some years after the manifestation of diabetes. In contrast, CAN may occur early in patients with T2D, but recent studies have documented that CAN may also be present in prediabetes [129]. Besides long-term glycemic control, clinical correlates such as age, diabetes duration, and cardiovascular risk factors (hypertension, lipid abnormalities, smoking, overweight, and obesity) as well as low-grade inflammation and oxidative stress may play a role in the pathogenesis of CAN [130]. Therefore, all modifiable factors should be addressed in the prevention and treatment of CAN.

Resting tachycardia may be due to an earlier onset of parasympathetic impairment resulting in relative sympathetic predominance. Postural hypotension may cause dizziness, weakness, or even syncope, but orthostatic symptoms are relatively infrequent. Silent myocardial ischemia or infarction are well-known clinical manifestations of atherosclerotic cardiovascular disease in patients with diabetes; CAN may be a contributing factor to the asymptomatic nature of these events. Prolongation of QT-interval due to CAN may cause arrhythmias and even sudden death. The non-dipper phenomenon of arterial blood pressure in diabetes may be due to CAN (sympathetic-vagal imbalance and relative nocturnal sympathetic excess). The perioperative and intraoperative risk may be higher in patients with diabetes in the presence of CAN [131].

3.2.2. Diagnosis

For clinical practice, five cardiovascular autonomic reflex tests (deep breathing test for assessing beat-to-beat variation, Valsalva maneuver, lying-to-standing changes in heart rate for assessing the 30:15 ratio, lying-to-standing changes in systolic blood pressure, changes in diastolic blood pressure during sustained handgrip) were introduced in the early eighties; these historical, "gold standard," Ewing's tests remained very popular for decades [132]. Computerized analysis of these tests became available later. Nevertheless, the clinical usefulness of the sustained handgrip test has been challenged and, therefore, was omitted in recent guidelines [133, 134]. As the QT-interval may be prolonged in patients with diabetes and CAN, measurement of the corrected QT interval or QT dispersion may be considered a useful but less specific sign of CAN [135].

In the last two decades, measures of heart rate variability (HRV) (time domain and frequency domain analyses) were developed. This method needs ECG recordings and dedicated computer software. Reduced HRV is the hallmark and earliest sign of CAN. Assessing arterial baroreflex sensitivity is another approach for detecting CAN. Finally, radionuclide scanning of myocardial adrenergic neurons with radiolabeled MIBG (meta-iodobenzyl-guanidine) may also be useful. Due to the relatively high costs and time-depending nature of these investigations, the latter two techniques are used primarily in clinical research [128].

3.2.3. Treatment

Due to the multifactorial pathomechanisms of CAN, an effective preventive and treatment strategy should include appropriate glycemic control, lifestyle intervention including weight management and physical activity, as well as smoking cessation. Other cardiovascular risk factors such as hypertension and dyslipidemia should also be addressed [130]. As hyperglycemia is an essential determinant of the prevalence and progression of CAN in diabetes, achieving and maintaining appropriate glycemic control is a prerequisite to preventing and alleviating signs and symptoms of CAN. The beneficial effect of near-normoglycemia on CAN in patients with T1D was documented in the DCCT

(Diabetes Control and Complications Trial) [136]. Nevertheless, the beneficial effects of intensive *versus* conventional insulin therapy on CAN persisted after the closeout of DCCT, indicating that the concept of the "metabolic memory" appeared to apply to CAN measures in EDIC (Epidemiology of Diabetes Interventions and Complications) during follow-up of 13 years to 14 years [136]. In the STENO-2 study, the multifactorial intensive treatment approach (*vs.* standard care) resulted in beneficial effects on CAN (assessed by variation in the RR interval on ECG with paced breathing and by an orthostatic hypotension test) in patients with T2D and microalbuminuria [137].

Pathogenetically oriented treatment of CAN may include ALA, omega-3 PUFAs, vasodilators, lipid-soluble vitamin B1, and therapy of concomitant diseases.

Symptomatic postural hypotension is an uncommon but bothersome symptom of CAN, the initial treatment of which includes non-pharmacological approaches (fluid intake, avoidance of sudden changes in posture, elevating the headrest of the bed, elastic stockings, reconsidering all hypotensive drugs such as diuretics, vasodilators, antidepressants, *etc.*). Pharmacotherapy should be introduced in severe cases only, and first-line agents include fludrocortisone and midodrine [138].

3.2.3.1. Treatment with Biofactors

3.2.3.1.1. Lipoic Acid

The first clinical study to demonstrate a beneficial effect of ALA on CAN was the DEKAN (Deutsche Kardiale Autonome Neuropathie) study, where 73 patients with diabetes and CAN were randomized to take 800 mg ALA or placebo for 16 weeks [139]. CAN measured as root mean square of successive differences increased, *i.e.,* improved, in the ALA treated *vs.* placebo group [139]. In a randomized, open-label study, 46 T1D patients with various manifestations of autonomic neuropathy were administered ALA (600 mg *i.v.* daily for 10 days, followed by 600 mg/d orally for 50 days, while 29 patients who did not receive ALA served as a control group. Improvements in the cardiovascular autonomic score, Valsalva maneuver, deep breathing test, and orthostatic blood pressure change were observed in the ALA group but not in the control group [140]. In a recent RCT, 75 T2D patients with CAN were randomized to placebo or ALA treatment (600 mg/d for 12 weeks followed by 1200 mg/d for another 12 weeks). CAN assessed by HRV indices showed a trend toward improvement after 24 weeks [141]. The results of these trials indicate that ALA could be useful in the treatment of CAN, but further RCTs are required to confirm these findings.

3.2.3.1.2. Benfotiamine

Thiamine is an important co-factor of intermediary (both catabolic and anabolic) metabolism, such as the intracellular glucose metabolism (glycolysis, Krebs cycle, pentose-phosphate cycle), and is also a modulator of neuronal and neuromuscular transmission, probably through its activation of ionic chloride channel [142].

Data of clinical studies on the efficacy of benfotiamine in CAN are limited. In a small pilot study, 16 T2D patients were treated for 10 days with ALA (600 mg/d i.v.) and a B vitamin combination (100mg thiamine chloride, 100 mg pyridoxine chloride, and 0,1 mg cyanocobalamin) followed by treatment with ALA (600 mg/d oral) and benfotiamine + pyridoxine combination (100 mg + 100 mg, TID) until week 12. Ten subjects without diabetes served as controls. After 12 weeks of supplementation, HRV (measured by frequency-domain indices) shifted towards vagal activity and hence improved sympathovagal balance [143]. These preliminary data should be confirmed by RCTs.

3.2.3.1.3. Vitamin B12

Vitamin B12 is necessary for the development and initial myelination of the central nervous system as well as for the maintenance of its normal function [144]. Metformin-treated T2D patients frequently develop vitamin B12 deficiency [145]. Patients with vitamin B12 deficiency may also suffer from autonomic nervous dysfunction [146].

Hansen *et al.*, assessed the association between serum levels of vitamin B12 and CAN in 469 T2D patients [147]. Patients treated with metformin and/or proton pump inhibitors had lower serum levels of vitamin B12 than patients not treated with these drugs. Vitamin B12 was inversely associated with CAN and resting heart rate and positively associated with the deep breathing test (E/I ratio) and the ratio of low-frequency and high-frequency power (LF/HF ratio) of frequency domain outcomes. These findings could merit the initiation of RCTs assessing the efficacy of vitamin B12 supplementation in diabetes patients with CAN.

3.2.3.1.4. Vitamin D

Limited data are available on the relationship between CAN and vitamin D deficiency. Vitamin D receptors are present in the smooth vascular cells, endothelium, and cardiomyocytes of the cardiovascular system [148]. Several studies have reported that vitamin D deficiency may be associated with cardiovascular disease, tumors, autoimmune conditions, and overall mortality and may play a role in the development of diabetes and neurodegenerative diseases [149, 150]. One study has indicated that vitamin D supplementation may improve measures of CAN in people without diabetes [151]. CAN and HRV have been studied according to the vitamin D status in 163 T2D patients [152]. Patients were divided into three groups: sufficient (25(OH)D - 20 ng/ml), insufficient (10 ng/ml - 25(OH)D <20 ng/ml), and deficient (25(OH)D <10 ng/ml) groups. Vitamin D deficiency correlated with HRV parameters but not with the presence of CAN [152]. More recently, a pilot study has suggested for the first time a strong association between a high dose of vitamin D supplementation and improved CAN in T1D patients, which has been observed without any variation in HbA1c, blood pressure levels, blood lipids, and insulin dose [153].

3.2.3.1.5. Omega-3 PUFAs

Both nervous and heart tissues have a high content of omega-3 PUFAs [154]. Pharmacological doses of omega-3

Table 3. Clinical staging of diabetic retinopathy based on the lesions observed.

Retinal Lesions	Clinical Stage
None	No DR (diabetic retinopathy)
Rare microaneurysms and blot hemorrhages	Mild Non-Proliferative DR (NPDR)
More microaneurysms and blot hemorrhages Hard exudates, cotton wool spots	Moderate NPDR
More cotton wool spots and flame-shaped hemorrhages Venous Beading Intraretinal Microvascular Abnormalities	Severe (pre-proliferative) NPDR
New vessels Pre-retinal hemorrhages Fibro-glial membranes	Proliferative DR (PDR)
Retinal detachment Rubeosis iridis Neo-vascular glaucoma	Advanced diabetic eye disease

PUFAs affect serum lipids, particularly by reducing TG and very low-density lipoprotein (VLDL) concentrations.

There is limited evidence from small short-term uncontrolled studies suggesting that the administration of omega-3 PUFAs to T2D patients with CAN may improve several time and frequency domain measures of HRV as well as arterial stiffness [155-157], but longer-term controlled studies are required to confirm these findings.

4. DIABETIC RETINOPATHY

4.1. Epidemiology and Clinical Impact

Diabetic Retinopathy (DR) was the sixth leading cause of blindness and moderate to severe visual impairment (MSVI) worldwide in 2015 [158] and the only one that has been increasing in terms of prevalence since the 1990s. Projections estimate that near 4 million people suffered from blindness or MSVI due to DR in 2020 [158]. While the burden of DR is growing in some regions, like East Asia, North Africa, and the Middle East, its prevalence is decreasing in high-income countries, attesting to the efficacy of newly displayed prevention programs [159]. In a pooled analysis involving 22,896 individuals with diabetes worldwide, the overall prevalence of any DR was 34.6%, with 6.96% for proliferative DR (PDR), 6.81% for diabetic macular edema (DME), and 10.2% for sight-threatening DR from either or both. Major risk factors are diabetes duration, HbA1c levels, blood pressure levels, and T1D. Projecting this analysis on the global population, approximately 93 million people suffered from any form of DR in 2010 [160].

Although visual loss is the final clinical outcome, severe DR can progress to its late stages (Table **3**) without producing symptoms [161]. Microaneurysms and microhemorrhages, caused by capillary occlusions and increased permeability of the vessel wall, are common features of non-proliferative DR (NPDR). Plasma and lipoprotein leakage causes retinal edema, detectable as hard exudates, while focal ischemia is manifested as “cotton-wool spots,” white-grayish areas with blurred margins. Progressive damage by ischemia leads to two major complications at high risk of sight loss: DME and PDR [162]. In DME, microvascular lesions involve the macula, the portion of the retina responsible for the vision of colors and details, causing severe functional impairment. In PDR, progressive retinal ischemia leads to the growth of new vessels, which may extend to the vitreous and iris, causing intraocular hemorrhages, fibrovascular proliferation, tractional retinal detachment, and rubeosis iridis, with terminal evolution in neo-vascular glaucoma.

4.2. Diagnosis

Early diagnosis of DR is a priority to safeguard sight and quality of life. Unfortunately, only a minority of patients are regularly screened due to poor information or logistical problems [163]. Today, screening includes fundus examination, preferably in mydriasis and with digital retinography, and the staging or grading is done according to the lesions observed (Table **3**). Optical Coherence Tomography (OCT) is a non-invasive diagnostic tool that offers quasi-histological *in vivo* images of the retinal layers and the vitreoretinal interface. It is important in particular to assess DME [164]. In a recent development, OCT Angiography (OCTA) allows the study of retinal microcirculation without contrast media, including deep retinal layers that are not visible otherwise with fluorescein angiography [165].

4.3. Treatment

Treatment of DR includes optimization of glucose [166-169] and blood pressure control by lifestyle and pharmacological means, as well as laser photocoagulation, intravitreal drugs (anti-VEGF agents and corticosteroids), and vitreoretinal surgery. Current guidelines have recommended maintaining HbA1c below 7.0% (53 mmol/mol) and blood pressure below 130/80 mmHg [170, 171]. Although in real life, many patients do not attain recommended targets, among individuals with T1D, those with similar duration of disease but more recent diagnosis, a lower incidence of PDR appears to be in association with historical improvements in Hb1Ac, blood pressure, and proteinuria [172]. On the other hand, stricter glycemic control leads to a greater risk of severe hypoglycemia [173]. Possible positive effects on DR were described for RAS inhibitors (ACE inhibitors and AT2-receptor blockers) [174-177] and fenofibrate [178,

179], but these drugs are not registered for this specific indication. Aspirin may be protective only in very mild DR [180, 181] and does not increase the risk of bleeding in PDR [182].

4.3.1. Treatment with Biofactors

At a cellular level, DR is characterized by early loss of pericytes from retinal capillaries and thickening of the basement membrane. Pericytes and their interactions with endothelial cells play a key role in the regulation of vascular formation and stabilization and vascular permeability [183]. Endothelial cells are first exposed to the complex signals deriving from the blood flow and therefore influence pericytes, while pericytes are in contact with the surrounding tissues, such as the neuroretina. Loss of pericytes leads to acellular capillaries and abnormal endothelial cells proliferation with subsequent abnormal angiogenesis and neovascularization [184]. The primum movens of these abnormalities is hyperglycemia; excess glucose causes accumulation of highly toxic glycolytic metabolites in the cytoplasm and overproduction of ROS inside the mitochondria, which, in turn, inhibit GAPDH, re-directing the flux towards the four pathways of hyperglycemic damage: hexosamine pathway, polyol pathway, DAG/PKC, and AGE formation [185].

To address these damaging metabolic effects, two strategies can be of help: enhancing the activity of transketolase, a glycolytic enzyme able to shift toxic intermediates to the pentose-phosphate shunt [186, 187], and using antioxidants [188]. Fig. (**2**) provides an overview on how biofactors can interfere with the pathogenesis of diabetic retinopathy.

4.3.1.1. α-Lipoic Acid

ALA is a natural antioxidant found in vegetables and meat, which acts as a cofactor for enzymes involved in glucose metabolism and energy production, similarly to TDP. It has been shown to reduce ROS accumulation inside the cell by scavenging several free radicals [189]. While other antioxidants have failed to protect retinal capillaries from metabolic damage [190-192], several studies have demonstrated the beneficial role of ALA in counteracting retinal damage in experimental models of DR. Treatment of streptozotocin-induced diabetic rats with ALA reduced oxidative stress, NF-κB activation, angiopoietin-2 and vascular endothelial growth factor (VEGF), and prevented pericyte loss from retinal capillaries [188]. Markers of metabolic damage, such as dysregulation of retinal mitochondria biogenesis and decreased number of mitochondria, and the increased number of acellular capillaries characteristic of DR, were also prevented by ALA supplementation in rats with poor glycemic control [193]. More recently, a study on mouse retina has shown that ALA is able to reduce VEGF levels, protect ganglion cells, and preserve the inner and outer retinal layer thickness [194]. In another study, ALA was shown to reduce oxidative stress, increase glutathione peroxidase, and inhibit retinal cell death in streptozotocin-induced diabetic mice [195]. However, clinical studies on T2D diabetic patients have shown discordant results. The administration of ALA alone in an RCT did not prevent the development of diabetic macular edema [196], while another study demonstrated that ALA administration in combination with other antioxidants improves electroretinogram in diabetic patients [197]. In a further clinical study, it was shown that oral ALA supplementation of 300 mg per day for 3 months stabilized contrast sensitivity in T1D patients and improved it in T2D patients [198].

4.3.1.2. Thiamine and Benfotiamine

The active form of thiamine, TDP, acts as a coenzyme for transketolase and other enzymes of the Krebs' cycle. TDP and its lipophilic derivative benfotiamine have been shown to prevent cell damage and apoptosis caused by hyperglycemia in retinal microvascular cells [186, 199, 200] by enhancing transketolase activity, and thus shifting excess toxic glucose to alternative, less dangerous, pathways [201, 202]. In addition, they also have an important antioxidant action since they are able to normalize the four pathways of metabolic damage, thus reducing excess ROS production in microvascular cells and diabetic rat retinas [16, 201]. Hammes' group also demonstrated that benfotiamine is able to inhibit the formation of acellular capillaries in rats with 6-month induced diabetes, thus preventing experimental DR [16]. In addition, thiamine and benfotiamine prevent glycation of basement membrane proteins due to highly-glycating glucose intermediates, leading to a reduced detachment of pericytes from retinal capillaries [17, 203].

More recently, it has been demonstrated that two SNPs located in the SLC19A3 gene encoding for thiamine transporter-2 (THTR2) are associated with absent or minimal DR and nephropathy despite long-term type-1 diabetes [204]. A subsequent study aimed at investigating the role of THTR2 in great glucose-induced damage and altered thiamine availability in cell models of experimental DR showed that high glucose conditions concur with reduced thiamine availability in determining impairment in thiamine transport inside retinal cells and through the inner blood-retinal barrier by modulation of THTR2 expression [202]. In addition, a cross-sectional study compared TDP blood concentrations of 80 patients with T2D and different stages of DR with those in 20 healthy control subjects. The study revealed that mean TDP concentration decreased with increasing stages of DR. A negative correlation between the TDP level and presence of DR was found, suggesting that thiamine might contribute to the pathophysiology and progression of DR [205]. This reinforces the hypothesis that thiamine or benfotiamine supplementation to diabetic patients may be a strategy to prevent or treat diabetic microvascular complications [202]. Unfortunately, clinical trials addressing thiamine/benfotiamine potential to prevent DR in human diabetic subjects are still lacking.

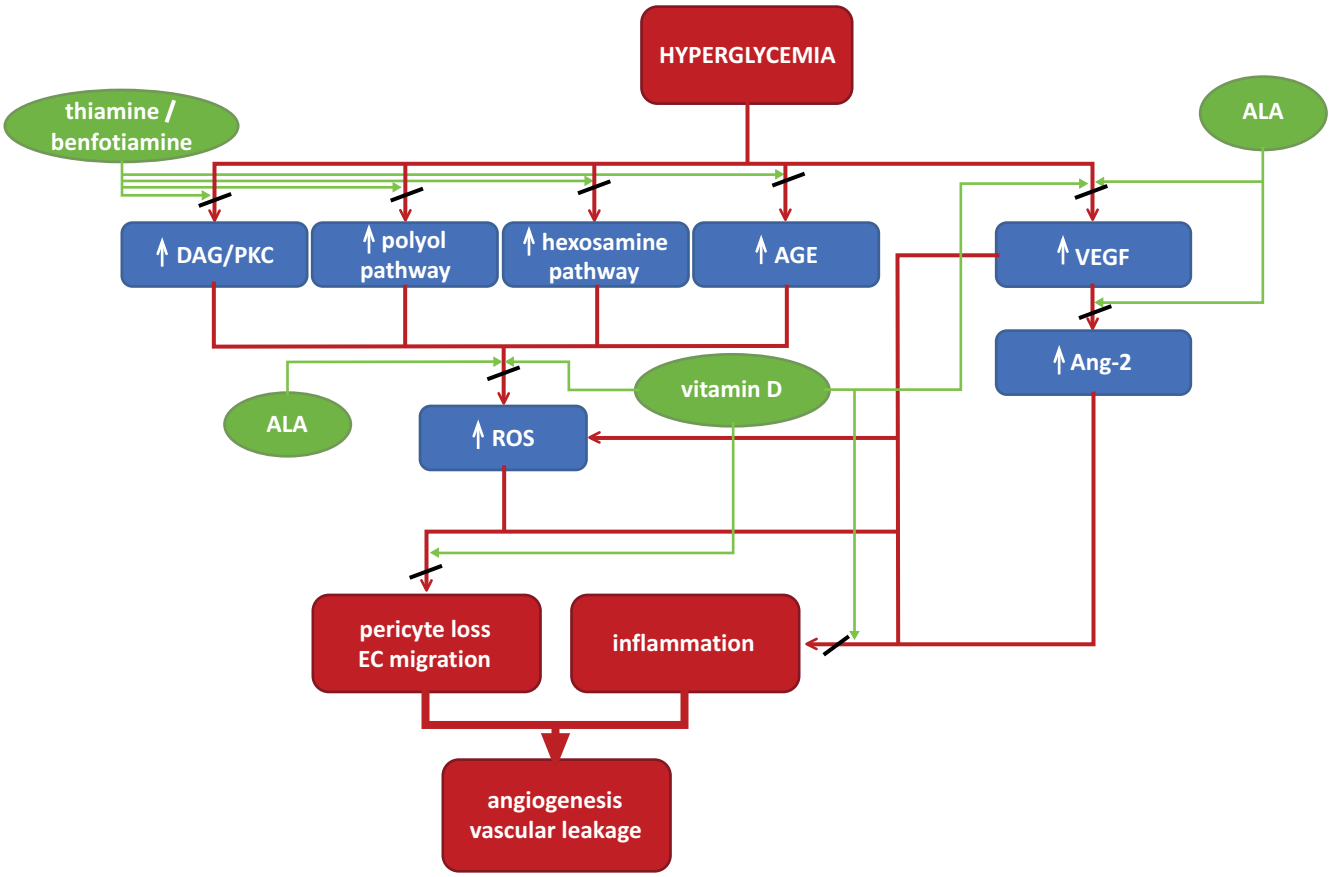

Fig. (2). Pathogenesis of diabetic retinopathy and mechanisms of action of various biofactors.
Abbreviations: AGEs, Advanced Glycation End products; ALA, α-Lipoic Acid; Ang-2, Angiopoietin 2; DAG, Diacylglycerol; EC, Endothelial Cells; PKC, Protein Kinase C; ROS, Reactive Oxygen Species; VEGF, Vascular Endothelial Growth Factor (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

4.3.1.3. Vitamin D

Vitamin D is a lipid-soluble vitamin produced by the skin following UV-B exposure or obtained from aliments. It is then converted in the liver and kidney to its active, hormonal form $1,25(OH)_2D$ [206, 207]. The vast majority of the world population presents nowadays with vitamin D deficiency due to a lack of direct sun exposure [208]. Even though the primary role of vitamin D is the maintenance of calcium homeostasis and bone metabolism, and its supplementation is used for treating rickets, osteoporosis, and hypocalcemia. Recent studies have demonstrated that vitamin D also plays an important role in immune function and has anti-inflammatory and anti-angiogenetic properties [209-211]. The use of vitamin D for the prevention and treatment of various cancers and chronic diseases, such as diabetes and multiple sclerosis, is currently under evaluation [211-213]. With regards to the eye, it was reported that vitamin D treatment reduced retinal inflammation in aging mice [210] and inhibited retinal neovascularization in mice with oxygen-induced ischemic retinopathy [214]. The capability of vitamin D to inhibit angiogenesis and neovascularization has suggested a role in the prevention of DR. Ren *et al.*, found that diabetic rats treated with vitamin D showed decreased retinal expression of VEGF and transformed growth factor-β1 (TGF-β1) [215]. Vitamin D_3 was also shown to downregulate intracellular ROS and inhibit inflammasome pathway activation, while reducing VEGF expression, retinal vascular permeability and retinal capillary cell apoptosis in diabetic rats and retinal endothelial cells cultured in high glucose conditions [216]. However, Jamali *et al.*, found increased production of VEGF in retinal pericytes following exposure to vitamin D [211]. With regards to clinical studies, serum vitamin D concentrations were found to be inversely related to the severity of retinopathy in diabetic patients [217, 218], while vitamin D deficiency was associated with increased risk of DR in type 1 adolescent diabetic subjects [219].

5. DIABETIC NEPHROPATHY

5.1. Epidemiology and Clinical Impact

Diabetic Nephropathy (DN) or Kimmelstiel-Wilson syndrome is a component of diabetic chronic kidney disease (D-

CKD), which is a severe chronic complication of diabetes and represents the leading cause of chronic kidney disease (CKD) in industrialized countries. It accounts for 45% of patients receiving renal replacement therapy and is a rapidly growing problem worldwide. CKD is defined by the repeated presence of increased urinary albumin excretion (urinary albumin/creatinine ratio, from spontaneous urine-ACR ≥30mg/g) and/or estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m^2 or other manifestations related to kidney injury. The natural history of DN was first described in T1D because, in these patients, the onset of the disease can be set with great precision. DN in T1D is characterized by an initial period of hyperfiltration, then the onset of proteinuria followed by the progressive decline of eGFR. In the absence of preventive measures, CKD may progress to ESRD, which may be overcome by dialysis or kidney transplantation [80, 220-222]. DN can be detected concurrently with the diagnosis of T2D, but in T1D, it usually manifests only after ≥10 years following diabetes onset, depending on the degree of metabolic balance, and is encountered in 20-40% of diabetes patients. The presence of DN increases the risk of hypoglycemia by reducing glucose production, the elimination and catabolization of the antidiabetic drugs, lowering food intake, *etc.* The type of antidiabetic drugs, prohibition of certain drugs, and their doses will be established according to the stage of DN. In the presence of anemia, hemoglobin carbamylation can lead to false glucose and HbA1c readings and wrong therapeutic decisions [80, 223].

Unfortunately, CKD is still underdiagnosed and undertreated. Clinical practice guidelines of the National Kidney Foundation define CKD, classify its stages, evaluate laboratory measurements for the clinical assessment of CKD, and associate the level of kidney function with complications of CKD and cardiovascular disease [220]. Stages 1-2 CKD have been defined by eGFR >60 mL/min/1.73 m^2 (normal - mildly decreased) and evidence of albuminuria (ACR moderately or severely increased: 30-299mg/g or ≥ 300mg/g, respectively). Stages 3-5 CKD have been defined by progressively lower ranges of eGFR: 59-45 (mildly to moderately decreased)-stage 3a; 30-44 (moderately to severely decreased)- stage 3b; 15-29 (severely decreased)- stage 4; < 15 mL/min/1.73m^2 (severely decreased)- stage 5. At stages 3-5 of CKD, albuminuria may be normal to mildly increased (A1-ACR<30mg / g) or moderately (A2) or severely increased (A3) [220]. In the initial stages of DN, the patients can be asymptomatic or present with high blood pressure, dyselectrolytemia. When eGFR< 30mL/min/1.73m^2, the clinical condition worsens, and patients might present with symptoms such as nausea, vomiting, metallic taste, itching, osteomuscular pain, dyspnea, lower limb edema, *etc.* The initial stages may be reversible by lifestyle optimization, control of glycaemia, hypertension, and other risk factors. The degree of albuminuria correlates with the risk of cardiovascular disease, CKD progression, mortality, regardless of eGFR [223]. The degree of both albuminuria and eGFR are important for selecting antidiabetic and antihypertensive drugs and determining their doses as well as their indications or contraindications [80].

5.2. Diagnosis

Diagnosis of DN is based on history (*e.g.,* patients with long duration of diabetes, the concomitant presence of retinopathy or other microvascular complications), clinical investigation, and biological data (albuminuria, worsening of renal function). In patients with undiagnosed T2D, the prevalence of nephropathy is around 30% [224]. On the other hand, even in patients diagnosed with T2D, DN frequently remains undiagnosed [225]. Therefore, screening for T2D and DN is required. In T1D patients, after 5 years of disease evolution and in all patients with T2D, starting with diagnosis, eGFR and ACR should be determined in a random spot urine collection at least once a year. In both T2D and T1D, the prevalence of an isolated decrease in GFR without albuminuria has increased in the recent decades, which is in parallel to the epidemic increase of diabetes, possibly due to the frequent use of RAS blockade agents to treat hypertension in diabetes patients [80, 226]. When eGFR falls below 60mL/min/1.73m^2, further investigations to assess CKD complications such as hypertension, fluid overload, pathological changes of electrolytes, metabolic acidosis, anemia, and metabolic bone disease are required [80].

5.3. Treatment

Prevention of DN is accomplished by a healthy lifestyle as well as adequate glycemic and blood pressure control [80, 220].

5.3.1. Dietary Changes

In advanced CKD (the last two stages), liquid intake should be reduced. Intake of sodium, potassium, and phosphorus should be adapted to the following factors: laboratory data, CKD stage, comorbidities, use of drugs (the RAAS blockers, diuretics). In the event of hypocalcemia, calcium supplementation is needed. Moreover, protein intake should be reduced to 0.8 g/kg body weight/day, while dialyzed patients need higher levels (1.0-1.3 g/kg body weight/day) due to increased catabolism, dialysate amino acid losses, protein-energy wasting [80, 227, 228].

5.3.2. Glycemic Control

To slow CKD progression, stringent glycemic control is required, but with the avoidance of hypoglycemia. There are now two classes of antihyperglycemic agents (sodium-glucose cotransporter 2 inhibitors (SGLT-2i) and glucagon-like peptide 1 receptor agonists (GLP-1RAs)), which can reduce the risk of CKD. Not only do these new agents improve glycemic control, but they also exert beneficial actions on the kidney and CKD risk factors. In T2D, metformin remains the first-line glucose-lowering drug until eGFR is reduced to below 30 mL/min/1.73m^2. The FDA has approved the use of SGLT-2i in patients with eGFR≥45 mL/min/1.73 m^2, while some of them can be administered at eGFR <45 mL/min/1.73 m^2, but with reduced doses. When DN progresses to ESRD, usually dialysis is required, and most patients need insulin treatment [80, 229].

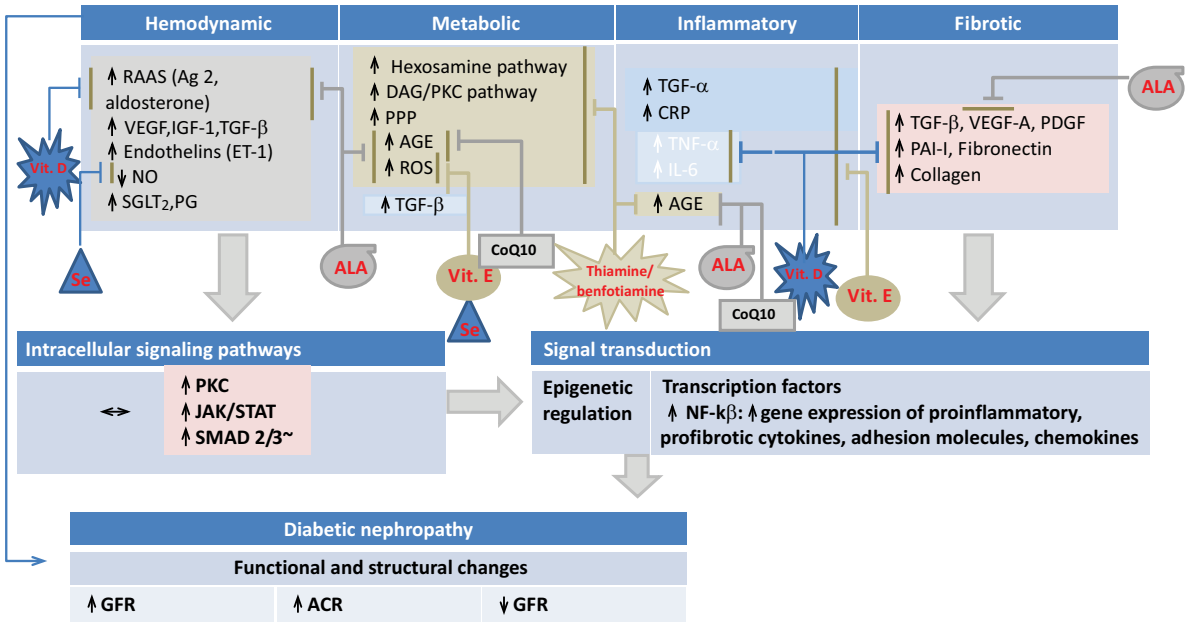

Fig. (3). Pathogenesis of diabetic nephropathy and mechanisms of action of various biofactors. Adapted from [232].
Abbreviations: ACR, Albumin-to-Creatinine Ratio; AGE, Advanced Glycation end products; ALA, α-Lipoic Acid; Ang-2, Angiopoietin 2; CRP, C-Reactive Protein; DAG, Diacylglycerol; GFR, Glomerular Filtration Rate; IGF-1, Insulin-like Growth Factor 1; IL-6, Interleukin-6; JAK-STAT, Janus kinase/signal transducer and activator of transcription; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; NO, Nitric Oxide; PAI-1, Plasminogen activator inhibitor-1; PDGF, Platelet-Derived Growth Factor; PG, Plasma Glucose; PKC, Protein Kinase C; PPP, Pentose Phosphate Pathway; RAAS, Renin-Angiotensin-Aldosterone System; ROS, Reactive Oxygen Species; $SGLT_2$, Sodium dependent glucose co-transporter 2; TGF-α, Transforming Growth factor-alpha; TGF-β, Transforming Growth Factor-beta; TNF-α, Tumor Necrosis Factor-alpha; VEGF, Vascular Endothelial Growth Factor; VEGF-A, Vascular endothelial Growth factor-A (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

5.3.3. Blood Pressure Control

Treatment of hypertension is also important. ACE inhibitors or angiotensin type 1 (AT1) receptor antagonists are first-line drugs. Patients should be referred to the nephrologist when there is uncertainty about the etiology of CKD, rapidly decreasing eGFR or increasing albuminuria, and when there are difficulties in the treatment of secondary hyperparathyroidism, anemia, hypertension, electrolytic disorders, *etc.* [80, 230].

5.3.4. Treatment with Biofactors

In experimental studies including streptozotocin-induced diabetes rats, treatment with several biofactors (benfotiamine, thiamine, ALA, vitamin B6, B12, D, E, K, selenium, Co Q10, *etc.*) demonstrated favorable effects on DN. However, given the complex pathophysiology of DN in humans, results must be interpreted with caution and more clinical experience is certainly warranted before their use in clinical reality [231]. Fig. (**3**) depicts how different biofactors may interfere with the pathogenesis of diabetic nephropathy.

5.3.4.1. α-Lipoic Acid

Experimental studies have shown increased oxidative stress in the renal cortex and early podocyte impairment in DN, which were prevented or improved by the administration of ALA [233-238].

Marcos *et al.*, conducted a prospective, non-randomized pilot study including 84 T1D and T2D patients with albumin concentrations <200mg/L who received ALA 600mg/day or placebo for 18 months. Plasma thrombomodulin (a marker of endothelial cell damage) decreased in the ALA-treated group and increased in the control group. Albuminuria increased in patients without ALA treatment but was unchanged with ALA [239]. Similarly, Noori *et al.*, showed that treatment with 800 mg ALA/day plus pyridoxine 80 mg/day for 3 months was associated with decreased albuminuria compared to placebo [240]. In an 8-week open-label study, Sun *et al.*, showed favorable effects of ALA treatment on albuminuria, serum creatinine, malonaldehyde, and plasma superoxide dismutase activity as well as flow-mediated vasodilation [241]. In a 12-week RCT, the supplementation of 600 mg ALA orally per day reduced asymmetric dimethylarginine (an endogenous inhibitor of NO synthase) in 50 diabetes patients with ESRD on hemodialysis [242]. A reduction of asymmetric dimethylarginine was also shown in an RCT including 30 T2D patients after intravenous ALA administration of 600 mg per day for 3 weeks [243].

5.3.4.2. Thiamine and Benfotiamine

Thiamine is a cofactor of the transketolase enzyme. Benfotiamine increases the activity of this enzyme by over 250% in endothelial cells [16] and has a 5-fold higher bioavailability than an equivalent dose of thiamine [244]. In ex-

perimental studies, both thiamine and benfotiamine have shown beneficial effects on microvascular complications, including DN [16, 187, 231, 245, 246].

A pilot study including 40 patients with T2D and early-stage DN who had persistent microalbuminuria (30-299 mg/day) reported that high doses of thiamine (100 mg TID) for 3 months induced regression of urinary albumin excretion in comparison to placebo. In a clinical study including T2D patients (*n*=73), albumin excretion (30-300mg/day) was observed [247]. Meena *et al.*, confirmed that high-dose thiamine (300 mg/day) for 3 months resulted in a significant decrease in albuminuria compared to placebo [221]. However, another clinical study reported negative findings. Alkhalaf *et al.*, found no decrease in albuminuria in 82 patients with T2D, albuminuria (15-300 mg/24 h), and eGFR >30 ml/min/1.73m^2 during 12-week treatment of benfotiamine (900 mg/day) or placebo [248]. Obviously, larger studies with longer follow-up and clearer inclusion criteria are needed to provide a definitive answer [246].

5.3.4.3. Vitamin B6

Experimental evidence points towards the beneficial effects of vitamin B6 (pyridoxine) on DN by reducing AGEs formation and oxidative stress [249]. This appears promising, given the high prevalence of vitamin B6 deficiency among DN patients [82].

The multicenter RCT, Diabetic Intervention with Vitamins to Improve Nephropathy (DIVINe), included 238 T1D and T2D patients with DN (CKD stages 1 to 3). High doses of B vitamins (2.5 mg/day of folic acid, 25 mg/day of vitamin B6, and 1 mg/day of vitamin B12) or placebo were used. Surprisingly, high-dose B vitamins resulted in a greater decrease in GFR and an increase in vascular events [250]. However, this trial has been criticized in terms of patient enrollment, small sample size, and statistical data processing [251, 252]. Indeed, two further trials showed positive results. Polizzi *et al.*, studied 61 patients with DN and reported that combined administration of vitamins B1 and B6 (but not vitamin B6 alone) decreased DNA glycation in leukocytes [253]. Cetin *et al.*, studied the evolution of AGE-peptides in T2D patients with DN compared to those without DN. At study inclusion, DN patients had higher concentrations of AGE-peptides which increased in patients without DN after 5 months, but not in those with DN receiving either vitamin B1 plus vitamin B6 (250mg/day each) or vitamin B6 (250mg/day) only [254]. In an RCT, using 80 mg vitamin B6 plus 800 mg ALA for 12 weeks resulted in a reduction in albuminuria and serum malondialdehyde as well as an increase in serum nitric oxide compared to the placebo group [213]. Pyridoxamine dihydrochloride 300 mg/day in humans has failed to improve DN after 12-month treatment [255].

5.3.4.4. Folic acid

In a clinical study including 50 T2D patients with and without DN, folic acid levels were lower among those with DN than in those without DN [256]. Thus, an investigation of its potential therapeutic use would be needed.

5.3.4.5. Vitamin B12

Bherwani *et al.*, found reduced levels of vitamin B12 in DN patients, but no intervention studies are available on DN [257].

5.3.4.6. Vitamin D

Vitamin D has numerous promising pleiotropic effects, notably suppression of the RAS, anti-inflammatory actions, and preservation of endothelial function [258]. Vitamin D receptor agonists (VDRAs), especially the selective VDRA paricalcitol, may prevent renal fibrosis and slow DN progression [259, 260]. Importantly, macro-albuminuric T2D patients had significantly lower serum 25(OH)D concentrations than both group and normoalbuminuric T2D patients [261].

An RCT including T2D patients with early DN (urinary albumin >30 mg/g of creatinine and GFR >30 mL/min) assessed the effects of high-dose vitamin D (50,000 IU intramuscular monthly for 6 months) on the cardiovascular risk profile. While high-density lipoprotein (HDL) cholesterol was increased, no changes were seen for other cardiovascular disease risk factors [262]. A further 8-week RCT included 50 T2D patients with DN and marginal status of vitamin D (defined as 25(OH)D 37.5-75 nmol/L). Weekly vitamin D3 (50,000 IU) reduced proteinuria and inflammatory markers, such as TNF-α and IL-6 [263]. In a meta-analysis of RCTs, supplementation of vitamin D3 showed a favorable effect on 24-hour proteinuria and urine albumin/creatine ratio [264].

5.3.4.7. Vitamin E

Animal studies with vitamin E have shown reduced oxidative stress [265, 266]. In humans, vitamin E also reduced oxidative stress and thromboxane A2 production, especially when diabetes was well controlled (HbA1c <7%) and DN stage was early [267-269]. In an RCT with 60 DN patients in the intervention group, an oral high-dose vitamin E supplementation for 12 weeks (1200IU/ day) exerted significantly favorable effects on biomarkers of kidney injury (ACR reduction), inflammation, and oxidative stress [270]. In a prospective, multicenter, double-blinded, placebo-controlled clinical trial including 54 patients with DN, Tan *et al.*, reported that 200 mg/day Tocovid (tocotrienol-rich vitamin E) for 12 weeks resulted in improved albuminuria [271].

5.3.4.8. Magnesium

Magnesium deficiency is usually associated with poor glycemic control due to insulin resistance. More than 30% of T2D patients have hypomagnesemia, mainly caused by enhanced renal excretion. Magnesium deficiency induces endothelial cell dysfunction, inflammation, and oxidative stress [272-275]. Thus, hypomagnesemia may be a modifiable risk factor of DN. However, in a 12-week RCT including 80 hypomagnesemic T2D patients with early-stage nephropathy, oral magnesium (250 mg per day) had no effect on microalbuminuria and renal function [276]. Thus, fur-

ther clinical studies are needed to determine its potential utility.

5.3.4.9. Selenium

Selenium nanoparticles have the potential to prevent DN by lowering oxidative stress and activating the beneficial cytoprotective proteins HSP70 and SIRT1 [277]. In DN, selenium administration had favorable effects on plasma glutathione peroxidase, serum insulin levels, HOMA-IR and HOMA-B, serum matrix metalloproteinase-2 (MMP-2), nitric oxide, total antioxidant capacity (TAC), and glutathione [278], but its long-term effects on renal function have not been studied.

5.3.4.10. Coenzyme Q10

A deficiency in mitochondrial oxidized CoQ10 (ubiquinone) may be a contributing factor to DN. Thus, CoQ10 administration could play a protective role in T2D by preserving mitochondrial function [279]. Administration of CoQ10 (100 mg/day) for 12 weeks in patients with DN had favorable effects on glucose metabolism, plasma malondialdehyde, and AGEs but did not influence fasting plasma glucose, lipid profiles, and MMP-2 levels [280]. Whether CoQ10 may favorably influence, DN has to be established in long-term controlled clinical trials [281, 282].

CONCLUSION

Diabetic microvascular complications are the leading causes of blindness, end-stage renal disease, and lower-extremity amputations contributing to substantial morbidity, mortality, and healthcare burden in people with diabetes. There is an unmet need for adjunct treatments of diabetic microvascular complications since the efficacy of causal therapies may be limited. Diabetic microvascular complications share common pathogenetic mechanisms resulting from gluco-/lipotoxicity leading to oxidative stress and inflammation with activation of various metabolic pathways, which ultimately foster microvascular damage. Experimental studies have demonstrated that each of these complications can be prevented or ameliorated by various biofactors by targeting the underlying mechanisms. Some of these findings have been successfully replicated in controlled clinical trials, particularly in diabetic polyneuropathy. Thus, they have the potential to favorably modify the natural history of the underlying complications, but longer-term clinical trials with a larger sample size are required to confirm these encouraging findings.

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