

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

Investigation of changes in oxidative stress and advanced glycation
end products (AGEs) in diabetic neuropathy and Hashimoto's
thyroiditis

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The PhD Defense takes place at the Lecture Hall of Bldg. A,
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Introduction

Advanced glycation end products (AGEs) are a heterogeneous group of irreversible molecules formed during non-enzymatic reactions of proteins, lipids, or nucleic acids. AGEs play a key role in aging, accumulating in the body under physiological conditions, however, their formation is further enhanced by persistent hyperglycaemia, oxidative stress, and inflammatory conditions. Additionally, they can enter the body exogenously through food and tobacco products. Pathological AGE accumulation can accelerate aging and, over the long term, contribute to the development and progression of chronic diseases and their complications. AGEs can modify the structure and function of proteins, leading to vascular and tissue damage, and, through activation of the receptor for advanced glycation end products (RAGE), initiate various inflammatory signalling pathways. In autoimmune thyroid diseases, such as Hashimoto's thyroiditis, chronic inflammation and increased oxidative stress promote the formation and accumulation of AGEs in thyroid tissue, potentially contributing to tissue damage and amplified autoimmune mechanisms. Similarly, in different types of diabetes, particularly type 2 diabetes, persistently elevated blood glucose levels significantly enhance AGE production and accumulation. Through these processes, AGEs play a key role in the development of macrovascular and microvascular complications, including retinopathy, nephropathy, neuropathy, and cardiovascular abnormalities.

Oxidative stress induced by glycation end products plays a major role in the development of both diabetic neuropathy and Hashimoto's thyroiditis. From a therapeutic perspective, it is crucial to develop new strategies aimed primarily at reducing AGE levels, decreasing oxidative stress, and thereby inhibiting the release of reactive free radicals. Among such strategies, alpha-lipoic acid, an antioxidant and

mitochondrial coenzyme, has been shown to reduce AGE-induced oxidative damage, improve peripheral nerve metabolism, and alleviate neuropathic symptoms. Overall, AGEs and their tissue-specific effects play a central role in both Hashimoto's thyroiditis and various types of complications associated with diabetes, particularly in the development of neuropathic alterations.

Literature Review

Oxidative Stress

Oxidative stress is a condition characterized by the disruption of the cellular redox balance, leading to excessive production of reactive oxygen and nitrogen species (ROS and RNS) that exceed the organism's antioxidant capacity. This process results in cellular damage, including lipid peroxidation, protein modification, and deoxyribonucleic acid (DNA) damage. Ultimately, it contributes to the development of numerous diseases and their complications, including cardiovascular, neurodegenerative, and autoimmune disorders, malignant diseases, the onset of diabetes, and plays a key role in the process of aging.

In Hashimoto's thyroiditis (HT), chronic thyroid inflammation and autoimmune processes increase free radical production, which may contribute to tissue damage and hormonal imbalances. Similarly, in type 2 diabetes mellitus, persistent hyperglycemia enhances the formation of AGEs and further increases oxidative stress levels, promoting the development of diabetic peripheral polyneuropathy (DSPN). Investigating molecular markers of oxidative stress in both patient groups provides an opportunity for a more detailed understanding of disease pathophysiology and for assessing the effects of therapeutic interventions such as levothyroxine or alpha-lipoic acid (ALA) treatment. Today, oxidative stress receives intense research attention worldwide, as the incidence of associated diseases continues to rise, continually expanding our understanding of its pleiotropic pathogenic role.

Advanced Glycation End Products (AGEs)

AGEs constitute a heterogeneous group of irreversible adducts formed during the nonenzymatic glycation and oxidation of proteins, nucleic acids, and lipids. They originate from both exogenous and endogenous sources and are slowly cleared from the bloodstream, enabling lifelong accumulation. They play a crucial role in aging and in the development of various diseases and their complications. Exogenous AGEs are present in high amounts in processed foods prepared at high temperatures, such as grilled, high-fat, and high-protein dishes. Cigarette smoking is also considered a significant external source of AGEs. AGEs are key contributors to the aging process and to the development of numerous diseases and complications, including metabolic syndrome, type 2 diabetes mellitus, malignant diseases, atherosclerosis, and autoimmune thyroid disorders.

AGEs are formed through the classical Maillard reaction, in which reducing sugars bind non-enzymatically to the amino groups of proteins, lipids, or nucleic acids. The process begins with the formation of a Schiff base, followed by an Amadori rearrangement and subsequent oxidative modifications (glycoxidation), ultimately leading to AGE formation. Under normal physiological conditions, AGEs form only in moderate amounts; however, under persistent hyperglycemia, increased glucose availability significantly accelerates their production. Consequently, AGE levels are markedly elevated in individuals with diabetes, further enhanced by diminishing elimination following kidney function impairment.

AGEs interact with several membrane-bound, cell surface receptors that, upon activation, initiate various signaling pathways. Some receptors, such as advanced glycation end product receptor 1 (AGER-1), contribute to AGE clearance, while others, such as RAGE, activate several signaling pathways, including MAPK/ERK, TGF- β , c-Jun N-terminal kinases (JNK), and the nuclear factor kappa B (NF- κ B) pathway. Among these, activation of the NF- κ B pathway increases receptor expression, promotes

the release of inflammatory cytokines, enhances oxidative stress, and stimulates the formation of reactive oxygen species (ROS). The resulting increase in oxidative stress may further accelerate endogenous AGE formation, creating a self-amplifying cycle that contributes to the development of chronic diseases and their complications. Additionally, AGEs can form cross-links between proteins, altering their structure and function, which also plays an essential role in the pathogenesis of a wide range of AGE-associated disorders. Alongside the full-length RAGE receptor, several naturally occurring RAGE variants have been identified, such as soluble RAGE (sRAGE). sRAGE can competitively inhibit the AGE–RAGE interaction, thereby reducing the production of inflammatory cytokines and ROS. Decreased serum levels of sRAGE have been demonstrated in several conditions, including essential hypertension, coronary artery disease, chronic obstructive pulmonary disease, hyperthyroidism, rheumatoid arthritis, and Alzheimer’s disease. Recent studies have summarized diseases in which an increased AGE/sRAGE ratio is a reliable biomarker, including non–ST–elevation myocardial infarction, hyperthyroidism, thoracic aortic aneurysm, and hypercholesterolemia.

Hashimoto’s Thyroiditis

Hashimoto’s thyroiditis (HT), also known as chronic autoimmune or chronic lymphocytic thyroiditis, is an autoimmune disorder in which immune-mediated processes lead to the production of anti-thyroid antibodies, anti-thyroid peroxidase (anti-TPO), and anti-thyroglobulin (anti-TG), and to T-cell activation, resulting in the gradual destruction of thyroid follicular cells. HT is the most common autoimmune thyroid disease, with an incidence of 0.3–0.5 per 1,000 individuals. In iodine-rich regions, HT is the leading cause of hypothyroidism. The pathogenesis of the disease is fundamentally influenced by genetic predisposition and environmental factors. At the same time, advanced age and female sex are also major risk factors, the latter associated with an estimated 8–10-fold increased risk. The clinical signs of hypothyroidism

associated with Hashimoto's thyroiditis are the consequences of hormone deficiency and affect multiple organ systems. Common symptoms of HT-induced hypothyroidism include dry skin, hair loss, cold intolerance, weight gain, hoarseness, and bradycardia. The diagnosis of Hashimoto's thyroiditis is based on the clinical signs of hypothyroidism and the presence of anti-thyroid antibodies, although a seronegative form occurs in 5–10% of cases. Ultrasound imaging can assist in differential diagnosis, especially in anti-TPO-negative patients; characteristic ultrasonographic features of HT include reduced echogenicity, heterogeneity, increased vascularization, and the presence of small cysts.

Pathomechanism and Complications

Hashimoto's thyroiditis patients may exhibit various stages of thyroid function, ranging from euthyroidism to subclinical and overt hypothyroidism; in rarer cases, transient hyperthyroidism may also occur. The autoimmune process in Hashimoto's thyroiditis develops as a result of interactions between genetic and environmental factors, leading to thyroid cell damage and the presentation of autoantigens. The infiltration of autoreactive immune cells into the thyroid triggers cellular and humoral immune responses, stimulates cytokine production, and results in cytotoxic effects and apoptosis. At the cellular level, the loss of self-tolerance is primarily caused by autoreactive CD4⁺ T-helper cells, especially the Th1 and Th17 subsets, which infiltrate the thyroid and secrete pro-inflammatory cytokines such as IFN- γ , IL-17, and TNF- α . These cytokines activate cytotoxic CD8⁺ T-cells and local macrophages, leading to the gradual destruction of thyroid cells. Impaired function of regulatory T-cells (Tregs) further exacerbates the imbalance of the immune response. At the molecular level, polymorphisms in the CTLA4, CD40, and PTPN22 genes modify co-stimulatory signaling and antigen presentation, thereby amplifying autoimmune processes. Additionally, abnormal activation of the JAK/STAT signaling pathway enhances the transcription of inflammatory mediators, sustaining chronic autoimmune inflammation.

Previous studies have observed increased oxidative stress and reduced antioxidant levels in the pathogenesis of Hashimoto's thyroiditis. Over time, the disrupted redox balance promotes the formation of endogenously produced advanced glycation end products (AGEs). In an earlier study of 50 newly diagnosed, euthyroid HT patients, lower sRAGE levels and higher AGE concentrations were found; however, this relationship has not yet been investigated in HT patients receiving levothyroxine treatment. In another study, Giannakou and colleagues found that oxidative parameters were higher in patients receiving levothyroxine (LT4) replacement than in those who did not require hormone therapy. However, AGE and sRAGE values were not assessed.

Therapeutic Options

According to therapeutic guidelines, levothyroxine monotherapy is the recommended treatment for hypothyroidism resulting from Hashimoto's thyroiditis. Levothyroxine is the precursor (prohormone) of triiodothyronine (T3), which functions as the physiologically active thyroid hormone in the body. However, levothyroxine alone does not always provide adequate metabolic regulation for a small subset of hypothyroid patients. According to some international guidelines, in such cases, it may be appropriate to consider combination therapy with levothyroxine and liothyronine (T4–T3). This approach is based on the assumption that in some patients, the efficiency of T4-to-T3 conversion may be reduced, or if thyroid function is severely impaired, the body may not be able to produce sufficient T3 for normal physiological function. Earlier clinical trials of combined LT4+T3 replacement have not demonstrated clear advantages and have shown at most modest clinical improvements. Its use is primarily considered in cases of low fT3 levels or in the presence of deiodinase enzyme polymorphisms, indicating insufficient T3 availability in target tissues.

Type 2 Diabetes Mellitus

The incidence and prevalence of type 2 diabetes mellitus (T2DM) are increasing worldwide due to sedentary lifestyles and unhealthy dietary habits. According to the NCD Risk Factor Collaboration, in 2022, approximately 828 million adults globally were living with diagnosed type 2 diabetes. The World Health Organization (WHO) predicts that by 2030, diabetes will rank as the seventh leading cause of death. Diabetes is a complex chronic disease associated with metabolic disturbances and multiple complications. The persistent hyperglycemia characteristic of T2DM is driven by insulin resistance and impaired β -cell function. Chronically elevated blood glucose contributes to the development of microvascular and macrovascular complications, which are major causes of morbidity and mortality. Neuropathy, estimated to develop in about half of patients with type 2 diabetes, reduces quality of life, contributes to decreased or lost work capacity, and significantly increases healthcare costs. Of its four main types (autonomic neuropathy, proximal neuropathy, distal peripheral neuropathy, and focal neuropathy), distal symmetric polyneuropathy (DSPN) is the most common. The pathophysiology of diabetic complications is complex; advanced glycation end-products (AGEs) are known to play a key role in the development and progression of neuropathy, nephropathy, retinopathy, and atherosclerosis.

Pathomechanism and Complications of T2DM

Although the pathomechanism of T2DM and its complications is extremely complex, it is assumed that AGEs and the AGE/RAGE interaction play a crucial role in disease development. AGE formation and accumulation in the pancreas due to chronic hyperglycemia contribute to β -cell toxicity, primarily by activating and amplifying inflammatory signaling cascades and inducing oxidative stress. Elevated AGE levels increase RAGE expression in the islets of Langerhans. The AGE/RAGE axis activates NF- κ B signaling, leading to chronic inflammation, mitochondrial dysfunction, β -cell

damage, and apoptosis, while AGE accumulation causes irreversible structural change in the arteries. The developing hyperglycemic memory driven by the AGE/RAGE axis is associated with diabetic complications and cardiovascular morbidity and mortality. AGE/RAGE signaling enhances oxidative stress and inflammation in pancreatic β -cells, promoting the formation and aggregation of toxic islet amyloid polypeptide (IAPP) species. By binding to these intermediates, RAGE contributes to amyloid plaque formation, leading to β -cell toxicity further promoting pancreatic β -cell damage.

Studies have shown that activation of the AGE/RAGE axis, as well as the MAPK and NF- κ B signaling pathways, increases intracellular ROS production, leading to enhanced expression of inflammatory and profibrotic mediators such as VCAM-1, ICAM-1, PAI-1, MCP-1, and MMP-2. These mediators contribute to arterial stiffness, atherosclerosis, and plaque accumulation in vessels susceptible to atherosclerosis. Activation of the AGE/RAGE axis further increases oxidative stress and promotes LDL oxidation, both of which are crucial to the pathogenesis of cardiovascular diseases. Oxidized LDL binds to RAGE as a ligand, activating multiple intracellular signaling pathways (e.g., NF- κ B, p38, JNK, and MAPK), which enhance the expression of TGF- β , C-reactive protein (CRP), inflammatory cytokines, and protein kinase C (PKC). This process accelerates atherosclerosis and the stiffening of the vascular intima-media layer, ultimately contributing to the development of cardiovascular disease.

Additionally, AGE accumulation in long-lived matrix proteins such as collagen and elastin leads to cross-link formation, resulting in increased arterial stiffness, endothelial dysfunction, and disruption of extracellular matrix (ECM)–cell interactions. Non-enzymatic collagen modification contributes not only to cardiovascular disease development but also significantly to nephropathy, neuropathy, and retinopathy. Arterial stiffness associated with systemic inflammation may cause pressure fluctuations in the microvasculature of various organs, particularly the kidneys, thereby increasing the risk of renal failure. Furthermore, reduced kidney function enhances AGE accumulation.

Diabetic Peripheral Neuropathy (DSPN)

Microvascular complications of diabetes are common and can affect any part of the body. Among the four primary forms of diabetic neuropathy, peripheral neuropathy is the most common. DSPN is one of the most frequent complications of T2DM, leading to impaired function of the peripheral nervous system. Major symptoms include tingling, numbness, neuropathic pain, burning sensations, and paresthesia, most commonly in the toes or feet. Approximately 50% of patients diagnosed with diabetes are affected, and it is estimated that by 2045, there will be around 350 million people living with DSPN worldwide.

Dorsal root ganglion (DRG) neurons are susceptible to oxidative stress–induced damage because they contain large mitochondria and exhibit intense oxidative metabolism. This increased vulnerability is mainly a consequence of hyperglycemia-induced oxidative stress, which activates the polyol, hexosamine, PKC, and AGE pathways, ultimately causing endothelial and neuronal cell damage or apoptosis. Activation of NF- κ B and NADPH oxidase increases oxidative stress, leading to reduced endoneurial blood flow, impaired nerve tissue integrity, and compromised regenerative mechanisms in diabetic neuropathy. Increased AGE formation further disrupts endoneurial nitric oxide (NO) metabolism, resulting in vasoconstriction, activation of cellular adhesion molecules, and increased coagulation activity. Together, these processes further impair the blood supply to nerve fibers.

Therapeutic Options

The essential pillars of DSPN treatment include adequate blood glucose control, lifestyle management, and alleviation of neuropathic pain. The optimal therapy also involves a balanced diet, regular physical activity, and optimal regulation of lipid levels and blood pressure.

Oxidative stress plays a key role in the pathophysiology of diabetic peripheral neuropathy, and numerous studies have shown that antioxidant-based interventions may

have beneficial effects in both animal models and patients with DSPN. Supplementation with exogenous antioxidants and regular exercise is a fundamental therapeutic tool, as they can reduce ROS-induced harmful processes. Although most antioxidant supplements have not yet been tested in randomized controlled trials, preclinical findings are promising and, if confirmed, may become valuable adjuncts to future prevention and treatment strategies for DSPN. Regular physical exercise improves metabolic processes and mitigates diabetic complications by enhancing mitochondrial oxidative capacity in skeletal muscles and increasing nitric oxide bioavailability

Alpha-lipoic acid (ALA)

Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid, ALA) is a short-chain fatty acid with numerous pharmacological benefits, including anti-inflammatory, antidiabetic, antioxidant, antitumor, cognitive, and neuroprotective effects. ALA, also known as thioctic acid, is a natural antioxidant synthesized by mitochondria and is widely found in both animal and plant tissues. In the human, ALA originates from two main sources: endogenous synthesis and exogenous sources. ALA from exogenous sources is found in foods of both animal origin (red meats, liver) and plant origin (spinach, tomatoes, potatoes, beets, carrots, Brussels sprouts, yeast). ALA is an essential cofactor for the functioning of four mitochondrial enzyme complexes involved in energy metabolism: the glycine-cleaving system (GCS), the 2-ketoglutarate dehydrogenase complex (2-KGDH), the pyruvate dehydrogenase complex (PDHC), and the branched-chain 2-ketoacid dehydrogenase (BCKDH). In vitro and in vivo animal studies have shown that ALA treatment can effectively reduce AGE levels, thereby mitigating oxidative stress, which may favorably affect the pathogenesis of neuropathy. As proved in clinical trials, ALA is an effective and well-tolerated treatment option for diabetic neuropathy, resulting in significant improvements in

clinical symptoms. When taken exogenously, ALA is easily absorbed from the gastrointestinal tract and is widely used in Europe to treat diabetic polyneuropathy.

Previous in vitro studies have shown that ALA significantly inhibited the activation of the AGE-induced NF- κ B signaling pathway in vascular endothelial cell lines and retinal endothelial cells. Another study found that ALA reduced AGE-induced VCAM-1 expression in endothelial cells and monocyte adhesion to the endothelium. Additionally, ALA decreased the expression of inducible nitric oxide synthase (iNOS) induced by AGEs, as well as NO production in mouse microglia cells. Other studies have noted that ALA reduced the formation of lipid peroxidation products mediated by AGEs in neurons. ALA has been shown to prevent or mitigate cell damage caused by hyperglycemia, thereby improving nerve function and reducing pain. As an effective antioxidant, it neutralizes free radicals generated in peripheral nerves and enhances nerve growth factor (NGF) production, which promotes nerve fiber regeneration. Clinical studies have confirmed that ALA supplementation has significant analgesic effects and improves the quality of life for diabetics. However, the impact of ALA on AGEs has not yet been studied in human patient groups.

Our research team has previously investigated the effects of ALA treatment on neuropathy associated with type 2 diabetes, particularly focusing on levels of asymmetric dimethylarginine (ADMA) and progranulin (PGRN). The previous results of F. Sztanek and B. Nádró showed a decrease in ADMA levels, a known risk factor for atherosclerosis due to the inhibition of NO synthase, as well as an increase in progranulin levels, which contribute to neuronal survival, maintenance of function, and protection of the endothelium from inflammatory processes and atherosclerosis.

Objectives

In our research, we sought to answer the following questions:

1. Determining the AGE and sRAGE levels, as well as the AGE/sRAGE ratio in patients suffering from Hashimoto's thyroiditis who are undergoing levothyroxine treatment, and age-, gender-, and BMI-matched controls, along with examining their associations with thyroid function and metabolic parameters.
2. Investigating the changes in AGE and sRAGE levels and the development of the AGE/sRAGE ratio in patients with peripheral neuropathy suffering from type 2 diabetes, with particular emphasis on the effect of alpha-lipoic acid (ALA) treatment.
3. Evaluating the effect of ALA treatment on the serum levels of AGEs, sRAGE, oxidative stress markers, and vascular parameters, as well as the severity of neuropathy based on the current perception threshold (CPT).
4. Exploring the relationships between AGEs, sRAGE, and vascular parameters related to atherosclerosis.

Materials and Methods

Patient and Control Groups

Patient Group with Hashimoto's Thyroiditis and Control Group

Our study aimed to explore the relationships among serum AGE levels, sRAGE levels, and thyroid function parameters in patients undergoing levothyroxine substitution therapy for hypothyroidism due to Hashimoto's thyroiditis. The study involved 73 patients (69 women and four men; average age: 47 ± 14 years; average body mass index: 27.4 ± 5.6 kg/m²), with an average disease duration of 6.6 ± 4.4 years. The control group consisted of 82 individuals without thyroid disease (76 women and six men; average age: 46 ± 14 years; average body mass index: 27.2 ± 6.1 kg/m²), matched to the patient group on age, gender, and BMI. In the control participants, TSH, fT4, fT3, and anti-TPO levels were within the reference range. Pregnant individuals, as well as those with diabetes mellitus, cancer, or other concomitant autoimmune diseases, were excluded from the study. The research was approved by the Regional and Institutional Ethics Committee of the University of Debrecen (RKEB.IKEB 6275-2022/DE), and all participants, including both patients and members of the control group, provided written consent to participate in the study. We hypothesized that in HT patients, the AGE level would be higher, while the sRAGE level would be lower than in the healthy control group. Furthermore, we anticipated associations between AGE levels and thyroid and metabolic parameters.

Patient and Control Group with Type 2 Diabetes Mellitus and Diabetic Neuropathy

The study included a total of 54 patients with type 2 diabetes and neuropathy (22 men and 32 women; average age: 64.15 ± 8.66 years). The average duration of diabetes at the start of the study was 12.4 years (interquartile range: 4.1–14.7 years),

while the average duration of neuropathy was 3.2 ± 1.4 years. Additionally, the control group comprised 24 individuals, age- and gender-matched, who did not have neuropathy and had a known duration of diabetes of 12.1 years (interquartile range: 4.0–14.6 years).

Participants in the study were recruited from the Internal Medicine Department of the University of Debrecen and the Debrecen Diabetic Neuropathy Center. Participants were provided with prior information about the research, and all gave written consent to participate. The research protocol was approved by the Regional and Institutional Ethics Committee of the University of Debrecen Clinical Center (DE RKEB/IKEB; 4775-2017) and the National Scientific and Ethics Committee of the National Medical Research Council (5287-2/2019/EÜIG). The study complied with the principles of the Helsinki Declaration.

The research was not registered in a clinical trial database, as the use of alpha-lipoic acid (ALA) is a common and accepted therapeutic option for neuropathy treatment. Members of the intervention group received 600 mg of ALA (WÖRWAG Pharma GmbH, Böblingen, Germany) orally, daily, for 6 months. Before the start of the study, neither the members of the intervention group nor the control group had undergone treatment for neuropathy.

All participants received oral antidiabetic treatment according to the current therapeutic guidelines (metformin, sulfonylureas, and/or DPP-4 inhibitors), and the treatment remained unchanged during the follow-up period. Both groups also received lifestyle counseling. Patients who were treated with insulin, SGLT-2 inhibitors, or GLP-1 receptor agonists were excluded from the study. Additionally, patients with diabetic nephropathy (eGFR <60 ml/min/1.73 m² or persistent albuminuria), type 1 diabetes, diabetic proliferative retinopathy, known liver disease, autoimmune disease, or those

with hematologic or neurological diseases affecting peripheral nerve function were excluded.

We also excluded pregnant patients and patients with previous cardiovascular event (e.g., myocardial infarction, stroke, peripheral arterial disease), who had known coronary artery disease, or suffered from advanced heart failure (NYHA stage II–IV), as well as those affected by alcohol issues, malignant tumors, or who were active smokers.

Sample collection and measurement of laboratory parameters

In both the Hashimoto's thyroiditis (HT) patient group and the corresponding control group, blood samples were collected in accordance with the clinic's local guidelines. Plasma and serum were separated by centrifugation at 3500 g for 10 minutes, then aliquoted and stored at -80°C until further analyses. Serum levels of advanced glycation end products (AGEs) were determined by autofluorescence-based quantification using a multifunctional microplate reader (Biotek Synergy H1, Agilent Technologies, Santa Clara, CA, USA). Soluble RAGE (sRAGE) levels were measured by enzyme-linked immunosorbent assay (ELISA) using the Human RAGE Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Thyroid hormone concentrations, free thyroxine (fT4), free triiodothyronine (fT3), and thyroid-stimulating hormone (TSH), were determined using electrochemiluminescence immunoassays (fT4 G2, fT3, TSH Elecsys; Roche Diagnostics GmbH, Mannheim, Germany). The laboratory reference ranges were as follows: fT4: 12–22 pmol/L; fT3: 2.4–6.3 pmol/L; TSH: 0.3–4.2 mU/L. Serum concentrations of anti-thyroid peroxidase (anti-TPO) autoantibodies were measured using a chemiluminescent immunoassay (LIAISON® Anti-TPO, DiaSorin S.p.A., Saluggia, Italy).

Serum creatinine concentration was determined using the Jaffé colorimetric method, while C-reactive protein (CRP) levels were measured by immunoturbidimetric

assay. The lipid profile (triglycerides, total cholesterol, LDL-C, HDL-C) was determined using enzyme-based colorimetric methods, and serum glucose levels were measured by a hexokinase-based kinetic method using a Cobas c6000 automated analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

In patients with type 2 diabetes mellitus (T2DM) associated neuropathy, blood sampling was performed before initiation of alpha-lipoic acid (ALA) treatment and again after 6 months. In T2DM control subjects without neuropathy, blood samples were collected at enrollment. In both groups, samples were obtained from fasting venous blood. Sample processing and storage were identical to those used for the HT group (centrifugation: 3500×g, 10 minutes; storage: −80 °C).

Laboratory parameters—HDL-C, LDL-C, triglycerides, total cholesterol, glucose, HbA1c, high-sensitivity CRP (hsCRP), uric acid, and creatinine were measured at the Department of Laboratory Medicine, University of Debrecen, using a Cobas c6000 analyzer and fresh serum samples according to the manufacturer's protocols. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol.

Measurement of anthropometric parameters

Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). Waist circumference was measured at a standardized anatomical site in the horizontal plane, midway between the lower costal margin and the iliac crest.

Determination of serum AGE and sRAGE levels

Serum AGE levels were determined by autofluorescence-based quantification (Biotek Synergy H1, Agilent Technologies, Santa Clara, CA, USA), and results were expressed in AU/ μ g protein, based on the method previously described by Münch et al. The interassay coefficient of variation was 8.9% (based on three parallel measurements

performed on the same day, $n = 35$). The day-to-day variation (over five consecutive days) was 10.5% ($n = 35$).

Soluble RAGE (sRAGE) concentrations were determined using a sandwich ELISA method (Human sRAGE Quantikine ELISA Kit, R&D Systems Europe Ltd., Abingdon, United Kingdom) according to the manufacturer's instructions. Serum samples were analyzed after threefold dilution, and sRAGE results were expressed in pg/mL. Intraassay coefficients of variation ranged from 2.6% to 5.3%, while interassay precision ranged from 5.5% to 8.8%. The AGE/sRAGE ratio was also calculated and analyzed.

Determination of serum thyroid hormones and anti-TPO levels

Serum levels of free thyroxine (fT4), free triiodothyronine (fT3), and thyroid-stimulating hormone (TSH) were determined using electrochemiluminescence immunoassays (FT4 G2 Elecsys, FT3 Elecsys, TSH Elecsys; Roche Diagnostics GmbH, Mannheim, Germany). The reference ranges were as follows: TSH: 0.3–4.2 mU/L, fT4: 12–22 pmol/L, and fT3: 2.4–6.3 pmol/L. Serum concentrations of anti-thyroid peroxidase (anti-TPO) autoantibodies were measured using a chemiluminescence immunoassay (LIAISON® Anti-TPO; DiaSorin S.p.A., Saluggia, Italy).

Determination of routine laboratory parameters

Serum creatinine was measured using the Jaffé colorimetric method, while an immunoturbidimetric assay determined C-reactive protein (CRP) levels. Triglycerides, total cholesterol, LDL-C, and HDL-C were measured using enzymatic colorimetric tests, whereas glucose concentration was determined by a hexokinase-based kinetic method using a Cobas c600 automated analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Measurement of serum progranulin, ADMA, sICAM-1, sVCAM-1, TNF α , oxLDL, and VEGF levels

Serum progranulin levels were determined using a competitive ELISA kit (BioVendor, Brno, Czech Republic) according to the manufacturer's instructions. The intra-assay coefficients of variation (CVs) ranged from 3.4% to 4.4%, and the inter-assay CVs ranged from 6.4% to 7.9%. Results were expressed in ng/mL.

Serum asymmetric dimethylarginine (ADMA) concentrations were measured using a commercially available competitive ELISA (ADMA-ELISA; DLD Diagnostika GmbH, Hamburg, Germany) according to the manufacturer's recommendations. Intra-assay CVs ranged from 5.7% to 6.4%, and inter-assay CVs ranged from 8.3% to 10.3%. Results were expressed in μ mol/L.

Serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were determined using sandwich ELISA kits (Human Soluble ICAM-1 and VCAM-1 ELISA; R&D Systems Europe Ltd., Abingdon, United Kingdom), following the manufacturer's instructions. Intra-assay and inter-assay CVs ranged from 3.7% to 5.2% and from 4.4% to 6.7% for sICAM-1, and from 2.3% to 3.6% and from 5.5% to 7.8% for sVCAM-1, respectively. Results were expressed in ng/mL.

Serum tumor necrosis factor-alpha (TNF α) concentrations were measured using a TNF α ELISA kit (R&D Systems Europe Ltd., Abingdon, United Kingdom) according to the manufacturer's instructions. Intra-assay CVs ranged from 1.9% to 2.2%, while inter-assay CVs ranged from 6.2% to 6.7%. Results were expressed in pg/mL.

Serum oxidized LDL (oxLDL) levels were determined using a sandwich ELISA method (Mercodia AB, Uppsala, Sweden). Intra-assay CVs ranged from 5.5% to 7.3%, and inter-assay CVs ranged from 4.0% to 6.2%. The assay sensitivity was 1 mU/L.

Serum vascular endothelial growth factor (VEGF) concentrations were measured using an ELISA kit (R&D Systems Europe Ltd., Abingdon, United Kingdom) according to the manufacturer's protocol. Intra-assay CVs ranged from 3.5% to 6.5%, and inter-assay CVs ranged from 6.7% to 8.5%. Results were expressed in pg/mL.

Determination of nitrite concentration

Serum nitrite levels, as an indicator of nitric oxide (NO) production, were determined using the Griess reaction. Optical density was measured spectrophotometrically at 550 nm. Nitrite concentrations were calculated using a sodium nitrite standard curve (10–100 $\mu\text{mol/L}$) and expressed in $\mu\text{mol/L}$.

Assessment of autonomic and peripheral nerve function

Peripheral neuropathy was assessed using the Douleur Neuropathique en 4 Questions (DN4) and the Neuropathy Total Symptom Score-6 (NTSS-6) questionnaires, as well as current perception threshold (CPT) testing and quantitative sensory testing. Patients with retinopathy were identified with the involvement of an ophthalmologist using in vivo corneal confocal microscopy, and those with confirmed retinopathy were excluded from the study. Peripheral sensory nerve function was evaluated using a Neurometer® (Neurotron Inc., Baltimore, MD, USA) to determine CPT values, while autonomic function was assessed using Ewing's five standard cardiovascular reflex tests: heart rate variability during deep inspiration and expiration, heart rate response to standing (30/15 ratio), the Valsalva maneuver, systolic blood pressure response to standing, and diastolic blood pressure response to sustained handgrip. The severity of autonomic neuropathy was expressed as a composite autonomic score derived from the test results (normal = 0, borderline = 1, abnormal = 2), ranging from 0 to 10. Scores of 0–1 were considered normal, 2–3 mild, 4–6 moderate, and 7–10 severe autonomic neuropathy.

The DN4 questionnaire is a 10-item clinical assessment tool suitable for screening the presence and severity of neuropathic pain. The total score of 10 consists of sensory descriptors and examination items (pain description, associated paresthesia/dysesthesia, sensory deficits, evoked pain, sensitivity to touch and pinprick, thermal sensation testing, and tactile and pressure allodynia). The diagnostic cutoff for neuropathic pain is a total score of 4/10. The instrument assesses sensory symptoms and evoked responses (e.g., paresthesia, pain sensitivity, thermal and tactile sensation), and scores above 4/10 indicate the presence of neuropathic pain. The NTSS-6 scale also takes symptom frequency into account; its scores range from 0 to 21.96, with values above 6 indicating clinically relevant neuropathic symptoms.

Statistical methods

Statistical analyses were performed using STATISTICA® version 13.5.0.17 (TIBCO Software Inc., Palo Alto, CA, USA), and graphs were generated using GraphPad Prism version 6.01 (GraphPad Software, San Diego, CA, USA). Normality of data distribution was assessed using the Kolmogorov–Smirnov test. Data are presented as mean $\pm$ standard deviation for normally distributed variables, or as median (interquartile range) for non-normally distributed variables, expressed as the 25th and 75th percentiles (IQR).

For comparisons between groups of continuous variables, Student's t-test was used for normally distributed data, whereas the Mann–Whitney U test was applied for non-normally distributed data. Associations between categorical variables were analyzed using the chi-square test. Relationships between continuous variables were assessed using Pearson's correlation analysis. Multiple linear regression analyses were performed to examine associations between AGE or sRAGE levels (dependent variables) and multiple independent variables.

Differences in anthropometric and laboratory parameters between the control group and neuropathic patients before alpha-lipoic acid (ALA) treatment were analyzed using

unpaired t-tests for normally distributed data (parametric tests) or Mann–Whitney U tests for non-normally distributed data (nonparametric tests). Within the neuropathic group, differences before and after ALA treatment were assessed using paired t-tests for normally distributed data or the Wilcoxon signed-rank test for non-normally distributed data.

Sample size calculation for AGE values in neuropathic patients was performed using the sample size calculator of the Department of Statistics and Operations Research, University of Vienna (version 1.062). The minimum required sample size for the two-sample Wilcoxon Mann–Whitney U test was $n = 8$, with a statistical power of 80% (0.8). The actual statistical power was 99.97% (0.9997), with $P(X > Y) = 0.9303$, a two-sided alpha level of 0.05, and $n = 54$. Pearson's correlation coefficient was used to assess relationships between variables. The null hypothesis was rejected at two-sided p-values < 0.05 . Statistical significance was defined as $p < 0.05$.

Results

Findings in the Hashimoto's thyroiditis patient group and the control group

All patients with Hashimoto's thyroiditis (HT) were receiving levothyroxine substitution therapy. The median daily dose was $1.15 \mu\text{g/kg}$ body weight (interquartile range [IQR]: 0.83–1.41; min–max: 0.32–3.28 $\mu\text{g/kg/day}$). At the time of assessment, 64% of patients were euthyroid, while subclinical hypothyroidism and hyperthyroidism were present in 22% and 6% of cases, respectively. The prevalence of overt hypo- and hyperthyroidism was 4% each. Regarding thyroid function parameters, serum TSH and fT4 levels were significantly higher in the HT group compared with controls (TSH: 2.47 vs. 1.77 mU/L, $p = 0.024$; fT4: 17.6 vs. 15.5 pmol/L, $p < 0.0001$), whereas fT3 levels were lower (4.6 vs. 5.0 pmol/L, $p < 0.0001$).

Significant differences were also observed in the levels of advanced glycation end products (AGEs) and their receptor. Serum AGE concentrations were lower in HT patients (10.71 vs. 11.45 AU/ μg protein, $p = 0.023$), while sRAGE levels were higher

(923 vs. 755 pg/mL, $p < 0.0005$) compared with the control group. Furthermore, AGE levels showed a positive correlation with age and serum creatinine levels, whereas sRAGE levels were negatively correlated with BMI both in the entire study population and when controls and HT patients were analyzed separately.

No significant association was found between AGE and sRAGE levels ($r = 0.191$, $p = 0.086$ in controls; $r = 0.205$, $p = 0.083$ in HT patients; and $r = 0.140$, $p = 0.082$ in the total population). In HT patients, negative correlations were observed between serum AGE concentrations and fT3 levels, as well as between sRAGE and TSH levels, whereas in the control group neither AGE nor sRAGE correlated with thyroid function parameters.

In addition, a positive correlation was observed between anti-TPO antibody (TPOAb) levels and AGE concentrations in HT patients. In this group, fT3 levels were also correlated with age and serum creatinine. Since serum AGE concentrations were associated with several factors (age, creatinine, TPOAb, fT3), multiple linear regression analysis was performed. According to the model, serum creatinine and TPOAb levels were significant predictors of AGE concentrations.

For sRAGE, similar analyses were performed in relation to BMI and TSH, and both parameters were found to have a significant effect in the multiple regression analysis. The median AGE/sRAGE ratio was lower in HT patients than in controls (2.4, IQR 1.9–3.1 vs. 3.3, IQR 2.3–4.1 AU/pg; $p < 0.0001$). The AGE/sRAGE ratio showed a positive correlation with age in controls ($r = 0.286$, $p < 0.01$), whereas in HT patients it correlated positively with BMI ($r = 0.275$, $p = 0.02$) and TSH ($r = 0.255$, $p = 0.03$), and negatively with fT3 levels ($r = -0.239$, $p = 0.04$).

Findings in patients with type 2 diabetes mellitus and diabetic neuropathy and their controls

In our analyses, no significant differences were observed in glucose, hemoglobin A1c (HbA1c), creatinine, hsCRP, lipid parameters, sVCAM-1, sICAM-1,

VEGF, or oxLDL between neuropathic and non-neuropathic patients, nor within the neuropathic group before and after alpha-lipoic acid (ALA) treatment. In contrast, serum ADMA and TNF α levels were significantly higher in the neuropathic group compared with controls. Similarly, the current perception threshold (CPT) and the composite autonomic score (CAS) were also elevated in patients with neuropathy. After 6 months of daily oral ALA treatment, plasma levels of TNF α and ADMA decreased significantly, whereas NO and progranulin (PGRN) levels increased significantly. Furthermore, CPT and CAS values were significantly reduced following ALA treatment, indicating improvements in peripheral and autonomic nerve function. Significant improvements were also observed in both DN4 and NTSS-6 scores after ALA treatment, reflecting a reduction in pain and neuropathic symptoms.

At baseline, serum AGE concentrations in patients with type 2 diabetes and neuropathy were 11.89 AU/ μ g protein (IQR: 9.44–12.88), which was significantly higher than in the control group of diabetic patients without neuropathy (9.80 AU/ μ g protein; IQR: 8.56–10.97; $p = 0.016$). After six months of ALA treatment, AGE levels showed a significant decrease to 10.95 AU/ μ g protein (IQR: 9.81–12.82; $p = 0.017$). In contrast, sRAGE levels did not change significantly in response to ALA treatment: baseline values were 814.20 pg/mL (IQR: 651.70–1094.10), and post-treatment values were 806.55 pg/mL (IQR: 637.20–1074.60; $p = 0.660$). sRAGE levels in the neuropathic group did not differ significantly from those in the control group (685.60 pg/mL; IQR: 596.70–978.90; $p = 0.438$). The AGE/sRAGE ratio in neuropathic patients (2.87 AU/pg; IQR: 2.42–3.54) was similar to that of the non-neuropathic control group (2.70 AU/pg; IQR: 2.11–3.27) and did not change significantly after ALA treatment (2.68 AU/pg; IQR: 2.12–3.18).

Before ALA treatment, a negative correlation was observed between AGE and progranulin (PGRN) levels, while AGE levels showed a positive association with sVCAM-1 concentrations. After treatment, a negative correlation was found between AGE and oxidized LDL (oxLDL). For sRAGE, associations with VEGF and NO levels

were observed before ALA treatment, whereas after treatment, sRAGE levels were associated with sVCAM-1, sICAM-1, VEGF, and NO. For the AGE/sRAGE ratio, negative correlations with sVCAM-1 and VEGF levels were observed before ALA treatment.

No significant associations were found between AGE or sRAGE levels and major anthropometric or routine laboratory parameters in neuropathic patients, either before or after treatment. Changes in CPT values and ADMA levels showed negative correlations with changes in AGE levels, whereas increases in PGRN levels showed a positive correlation with changes in AGE levels.

Novel scientific findings

1. In euthyroid patients with Hashimoto's thyroiditis receiving levothyroxine therapy, we were the first to demonstrate that AGE levels are lower. At the same time, sRAGE concentrations are higher, compared with those in healthy control subjects.
2. We were the first to show that the AGE/sRAGE ratio is significantly lower in levothyroxine-treated patients with Hashimoto's thyroiditis than in healthy controls.
3. We identified previously unreported associations between the AGE–RAGE axis and thyroid status in Hashimoto's thyroiditis:
 - a negative correlation between AGE and fT3 levels,
 - a positive correlation between sRAGE and TSH levels.Using multiple regression analysis, we were the first to demonstrate that TPOAb and creatinine are significant predictors of AGE levels, whereas BMI and TSH are significant predictors of sRAGE levels.
4. We demonstrated that BMI shows a significant negative correlation with sRAGE levels across all study groups, independent of thyroid status and disease category.
5. Our clinical study was the first to provide evidence that six months of alpha-lipoic acid (ALA) treatment significantly reduces AGE levels in patients with type 2 diabetes mellitus.
6. In patients with type 2 diabetes mellitus, we found no difference in sRAGE levels according to the presence or absence of neuropathy, in contrast to the results of several previous studies.
7. We identified a novel association with vascular markers: AGE levels showed a significant positive correlation with sVCAM-1 values in type 2 diabetes mellitus.

After ALA treatment, a moderate negative correlation emerged between

sRAGE and sVCAM-1, suggesting previously unrecognized dynamics of the AGE–RAGE–adhesion molecule axis.

Discussion

Patients with Hashimoto's thyroiditis and the control group

Hypothyroidism is one of the most common endocrine disorders and represents a risk factor for cardiovascular diseases, obesity, and the development of type 2 diabetes mellitus. When disturbances in glucose metabolism are also present, hypothyroidism may further aggravate the severity of clinical complications, particularly through complex underlying pathophysiological mechanisms. In these processes, advanced glycation end products (AGEs) and the associated inflammatory responses play a central role. Increased arterial stiffness—further exacerbated by hypothyroidism-related hyperlipidemia, enhanced coagulation tendency, and endothelial dysfunction—may potentiate the deleterious effects of traditional cardiovascular risk factors on the vascular wall.

AGEs contribute to increased vascular stiffness at both structural and functional levels. Structurally, the irreversible binding (cross-linking) of AGEs to collagen and elastin fibers significantly reduces vascular elasticity. This effect is further aggravated by increased collagen synthesis and reduced elastin production associated with glycation. At the functional or dynamic level, impaired endothelial function results from reduced nitric oxide (NO) production, increased endothelin-1 synthesis, and altered neuroendocrine regulation, all of which contribute substantially to the development of vascular dysfunction.

According to a recent systematic review encompassing 22 studies, obesity significantly increases the prevalence of hypothyroidism and is also closely associated with the development of Hashimoto's thyroiditis. In obesity, transmembrane transport of thyroid hormones is impaired by low-grade inflammation, leading to thyroid hormone resistance. In our study, we observed a negative correlation between body mass index (BMI) and soluble RAGE (sRAGE) levels in both patients and controls. This finding suggests that overweight or obesity alone may represent a predisposing factor for inflammation- and oxidative stress-related pathological processes,

independent of the presence of Hashimoto's thyroiditis (HT). The observed correlations were present across all body weight categories. In both the patient and control groups, AGE levels showed a positive correlation with age and serum creatinine levels. The age-related increase in AGEs is well documented in the literature, reflecting lifelong AGE accumulation and declining renal function. In our HT patients, fT3 levels also showed positive correlations with age and serum creatinine, suggesting a complex interaction between thyroid and renal function.

Further studies are required to clarify whether the altered AGE and sRAGE patterns observed in our treated HT patients are consequences of chronic low-grade inflammation associated with autoimmune thyroid disease or rather reflect changes in the hormonal milieu induced by levothyroxine therapy compared with physiological conditions. Most of the studied HT patients were in euthyroid state with normal TSH levels, while their fT4 and fT3 concentrations reflected the characteristic hormonal profile of levothyroxine-substituted hypothyroidism.

Overall, alterations in thyroid hormone levels may contribute to the development of obesity, atherosclerosis, and inflammatory processes. In addition, thyroid dysfunction may enhance immune activation, potentially leading to a bidirectional interaction between obesity and thyroid autoimmunity.

Taken together, the association between sRAGE and BMI suggests that maintaining a normal body weight may protect against the metabolic and cardiovascular complications of HT. It should be noted that lifestyle factors, such as dietary habits, as well as geographical and climatic differences, may also influence the results and should be considered when concluding. The less favorable findings in our control group may differ from those reported in Italian study populations, which may be explained by local dietary habits rich in saturated fats and less healthy nutritional patterns compared with the healthier diets typical of Mediterranean countries.

In our study, we observed lower AGE levels and higher sRAGE concentrations in HT patients, suggesting a potential antioxidant effect of levothyroxine (LT4) therapy.

Furthermore, we demonstrated an association between TPOAb and AGE levels. We were the first to investigate AGE and RAGE levels in LT4-treated HT patients. Our results suggest that a shift in the AGE/RAGE axis, together with increased carotid intima–media thickness (cIMT), may contribute to atherosclerotic processes in HT. Although anti-TPO levels correlated with AGE values, overall AGE concentrations were not higher in LT4-treated HT patients than in healthy controls, which is consistent with our observations of reduced oxidative stress in this patient group. In a multiple regression analysis, serum creatinine and thyroid peroxidase autoantibody (TPOAb) levels were significant predictors of AGE concentrations, whereas BMI and TSH levels were significant predictors of sRAGE concentrations.

The limitations of our study include a relatively small sample size, a wide age range in the study population, the lack of an HT patient group not receiving levothyroxine substitution, and limited assessment of oxidative and inflammatory parameters. A larger, prospective, longitudinal study incorporating measurements of arterial stiffness and carotid intima–media thickness (cIMT) would further refine our understanding of the impact of lifelong AGE accumulation on cardiovascular risk in patients with HT.

A major strength of our study is that we were the first to demonstrate a lower AGE/sRAGE ratio in treated HT patients compared with controls, as well as negative correlations between AGE and fT3 levels and positive correlations between sRAGE and TSH levels. Based on our findings, we hypothesize that levothyroxine supplementation may contribute to a more favorable AGE/sRAGE ratio in treated HT patients; moreover, the patient-specific correlations suggest that higher fT3 levels and lower TSH levels within the reference range may exert beneficial effects on oxidative stress in treated patients. Favorable modulation of the AGE–RAGE axis may be achieved not only through pharmacological interventions but also through lifestyle modifications.

Patients with Type 2 Diabetes Mellitus and Diabetic Neuropathy and the Control Group

It is estimated that approximately half of patients living with diabetes will develop neuropathy during their lifetime, which markedly impairs quality of life and increases morbidity. The primary goals of prevention and treatment of diabetic neuropathy are the reduction of hyperglycemia and the alleviation of neuropathic pain; however, glycemic control alone has not proven to be effective in the management of pain.

AGEs interact with several membrane-bound cell surface receptors, thereby activating various intracellular signaling pathways. The receptor for advanced glycation end products (RAGE) induces signaling cascades, such as the NF- κ B pathway, leading to upregulation of receptor expression, increased inflammatory cytokine release, generation of reactive oxygen species (ROS), and induction of oxidative stress through activation of NADPH oxidase. Persistent oxidative stress further stimulates endogenous AGE formation, creating a self-perpetuating “vicious cycle” that ultimately plays a decisive role in the development of numerous chronic diseases and their complications. In our study, sRAGE levels were similar in neuropathic and non-neuropathic patients with type 2 diabetes mellitus (T2DM), which differs from previous reports, as Aubert et al. found a positive association between sRAGE levels and the occurrence of peripheral neuropathy.

Our study was the first to demonstrate the AGE-lowering effect of alpha-lipoic acid (ALA) in patients with T2DM, in line with the well-established antioxidant properties of ALA. In the first part of this dissertation, our research showed that in non-diabetic patients with Hashimoto’s thyroiditis, the mean AGE value was 10.7 AU/ μ g and the sRAGE level was 923 pg/mL, whereas in healthy controls these values were 11.4 AU/ μ g and 755 pg/mL, respectively; these differences are well comparable to the data observed in the present diabetic population. In our previous study, the more favorable AGE levels observed in patients compared with healthy controls were

explained by reduced oxidative stress, presumably attributable to levothyroxine therapy. Numerically, AGE values in the healthy control group of the HT study were higher than those observed in both treated neuropathic (10.9 pg/mL) and non-neuropathic (9.8 pg/mL) patient groups in our T2DM study. Quade-Lyssy et al. demonstrated that statins increase sRAGE levels, which may explain their anti-inflammatory effects. In the present study, patients received metformin and statins in accordance with current guidelines, which, in itself, may have improved AGE and sRAGE levels, thereby favorably influencing the AGE/sRAGE ratio—similar to what we previously observed in non-diabetic HT patients treated with levothyroxine.

It is also plausible that different pharmacological treatments and lifestyle modifications—such as reducing the intake of exogenous dietary AGEs—may exert synergistic beneficial effects on oxidative stress. However, evaluation of ALA's independent effect on AGE levels in a diabetic population is limited, as antihyperglycemic therapy and correction of lipid parameters are fundamental and early components of treatment aimed at preventing complications. In diabetes, increased RAGE expression accompanied by low sRAGE levels has been reported, potentially altering redox status. In contrast, in our study we found no differences in sRAGE levels among the study groups.

Regarding vascular parameters, previous in vitro studies have shown that ALA treatment reduces AGE-induced endothelial VCAM-1 expression and decreases monocyte adhesion to the endothelium. In our research, sVCAM-1 levels did not differ between neuropathic and non-neuropathic patients, and no changes in sVCAM-1 concentrations were observed before and after ALA treatment. Based on the previously described favorable effects of ALA on endothelial adhesion molecule expression, we expected lower levels of inflammatory markers after 6 months of treatment. Although TNF- α levels decreased significantly, the study duration was likely insufficient to detect substantial changes in sVCAM-1, sICAM-1, and VEGF concentrations. Nevertheless, a significant correlation was observed between AGE and sVCAM-1 levels, while a

moderate, non-significant association between sRAGE and sVCAM-1 emerged after ALA treatment. In neuropathic patients, the AGE/sRAGE ratio showed a negative correlation with sVCAM-1 before ALA treatment. Our results suggest that sVCAM-1 shows a stronger correlation with AGE levels when ALA treatment is present and beneficial.

In the present study, we identified associations between sRAGE and sVCAM-1, as well as between sICAM-1 and VEGF, an inducer of vascular and blood–nerve barrier permeability. In vitro models have shown that AGEs increase, whereas sRAGE decreases, VEGF expression. In our patient groups, no association was found between VEGF and AGEs; however, a correlation was observed between VEGF and sRAGE. In the neuropathic group prior to ALA treatment, the AGE/sRAGE ratio correlated negatively with VEGF, supporting the hypothesis that in T2DM, sRAGE may represent a potential risk factor for endothelial dysfunction and vascular events. According to in vitro findings by Dedert et al., progranulin (PGRN) facilitates the cellular clearance of AGEs, which is partially supported by our observation of a correlation between progranulin and AGE levels. Our results indicate that ALA-induced increases in PGRN promote AGE clearance, as evidenced by lower serum levels after 6 months. It is conceivable that sRAGE levels were elevated during the early phase of treatment; confirmation of this hypothesis will require further investigation.

Nitric oxide (NO) exerts vasoprotective effects on the vascular wall, contributing to improved endothelial function in patients treated with ACE inhibitors and statins. In our study, increased NO levels were observed in neuropathic patients following ALA treatment. An association between NO and sRAGE was identified in the intervention group prior to ALA treatment. In addition, progranulin levels increased, and asymmetric dimethylarginine (ADMA) levels decreased after ALA therapy; both favorable changes correlated with alterations in AGE levels. In a previous study, Ren et al. demonstrated that AGEs reduce endothelial nitric oxide synthase (eNOS) expression

in human coronary artery endothelial cells. At the same time, ADMA is an endogenous inhibitor of NO production.

Collectively, these data support our findings and suggest that ALA exerts complex, beneficial effects on nitric oxide synthase through multiple converging pathways. Further studies are required to elucidate the precise role of AGEs in chronic inflammation and atherosclerosis, as well as the neuroprotective and endothelial function-improving mechanisms of ALA. Our results clearly demonstrate the synergistic neuroprotective and atheroprotective effects of ALA, with its AGE-lowering action being of fundamental importance.

Nevertheless, several limitations should be acknowledged. Despite an adequate effect size confirmed by the sample size calculation, a larger patient population would enhance the study's statistical power. Our investigation focused on a Caucasian population; therefore, extrapolation of the results to other ethnic groups is limited. Given the study's cross-sectional and prospective design, causal inferences should be interpreted with caution. Moreover, the reasons underlying unchanged sRAGE levels during ALA treatment remain partly unclear. Soluble RAGE was measured in human serum, and the exact mechanisms of its formation require further *in vitro* experiments. Additional research is also needed to determine whether ALA can prevent the development of diabetic complications. Our hypothesis should be confirmed in larger patient cohorts, and it would be advantageous to enroll patients at the time of T2DM diagnosis to more accurately evaluate the effects of ALA in combination with dietary modifications and physical activity. Finally, further *in vitro* studies are warranted to clarify the precise mechanisms by which ALA affects AGEs and endothelial function, including assessment of RAGE expression.

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List of own publications:



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Total IF of journals (all publications): 61,8

Total IF of journals (publications related to the dissertation): 7,8

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

04 November, 2025

