T.C.
PAMUKKALE UNIVERSITY
HEALTH SCIENCES INSTITUTE

MEDICAL PHARMACOLOGY DEPARTMENT
MEDICAL PHARMACOLOGY MASTER PROGRAM
MASTER THESIS

THE EFFECT OF VITAMIN D3 ON SUBTYPES OF S100 IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RAT MODEL

Manar MUSTAFA AMIN

January 2024
DENİZLİ

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Supervisor: Prof. Dr. Funda F. BÖLÜKBAŞI HATİP
Second Supervisor: Prof. Dr. Yavuz DODURGA

Denizli, 2024

I declare that this thesis has been designed, prepared, conducted, and researched in accordance with scientific ethics and academic rules, and that due care has been taken in the analysis of its findings. I also declare that non-primary results, data, and materials used in this study have been appropriately cited and attributed to the original sources.

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Signature :

ÖZET

VİTAMİN D3 STREPTOZOTOSİN (STZ) İLE İNDÜKLENEN DİYABETİK SIÇAN MODELİNDE S100 PROTEİN ALT TİPLERİNE ETKİSİ

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Ocak 2024, 103 Sayfa

Uluslararası Diyabet Federasyonu (IDF) Diyabet Atlası (2021), 20-79 yaş aralığındaki yetişkin nüfusunda küresel olarak %10,5 diyabet prevalansını ortaya koymaktadır. Yüksek kan şeker seviyeleri ile karakterize edilen diyabet, karmaşık genetik ve çevresel faktörlerden kaynaklanan yaygın bir metabolik bozukluktur. Diyabet, enzimatik olmayan glikasyonun arttığı, toksik glikoz türevleri olarak bilinen ileri glikasyon son ürünlerinin (AGE'ler) birikimine yol açar. S100 proteinleri, çeşitli organlarda ifade edilen, çeşitli fizyolojik ve patolojik rolleri olan hasar ile ilişkili moleküler model proteinleridir. AGE'ler ve S100 proteinleri, ileri glikasyon son ürünlerin reseptörüne (RAGE) bağlandığında, oksidatif stres ve inflamasyona katkıda bulunarak, bunları diyabetle ilişkilendiren bilişsel bozulmaya neden olurlar. Vitamin D3 (Vit-D3), genomik düzeyde etki eden bir sekosteroid hormondur ve vitamin D reseptörüne (VDR) bağlanarak çeşitli genlerin aktivitesini etkiler. Ayrıca, Vit-D3'ün hücre membranındaki farklı reseptörler aracılığıyla, hızlı yanıtlar ile nörokoruyucu ve anti-inflamatuar tepkilere yol açtığı bulunmuştur. Bu çalışmada, tek doz (65 mg/kg intraperitoneal enjeksiyon ile) streptozotocin (STZ) kullanılarak oluşturulan sıçan diyabet modelinde, serum ve hipokampus örneklerinde S100 protein alt tipleri olan S100B ve S100A8/A9 (kalprotektin) üzerindeki değişikliklere odaklanılmış ve Vit-D3'ün etkisi değerlendirilmiştir. Ayrıca, sekiz kollu radyal labirent testi (RAM) aracılığıyla bilişsel değişiklikler gözlemlenmiştir. Çalışmanın sonuçlarına göre, hem STZ hem de Vit-D3 gruplarında hippokampal RAGE, kalprotektin, S100B ve insülinde ve serum kalprotektin seviyesinde kontrol grubuna göre anlamlı bir artış gözlenmiş; ancak, serum RAGE seviyelerinde anlamlı bir artış sadece STZ grubunda saptanmıştır. Kalprotektin seviyelerinde bir değişiklik olmamasına rağmen, Vit-D3 uygulamasının serumdaki artan RAGE seviyelerini azalttığı görülmüştür. Rotarod testi sonuçlarında, STZ grubundaki sıçanların dönen mil üzerinde kalma süresin azaldığı, RAM testi sonuçlarında ise STZ grubunda doğru kol seçimlerin azaldığı ve latens süresinin uzadığı görülürken, Vit-D3 uygulamasının bu motor performans ve bellek üzerindeki olumsuz etkileri düzeltici bir etkisinin olduğu gözlemlenmiştir.

Anahtar Kelimeleri: Diyabet, RAGE, Streptozotosin, S100, Vitamin D3.

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ABSTRACT

THE EFFECT OF VITAMIN D3 ON SUBTYPES OF S100 IN STREPTOZOTOCIN (STZ)-INDUCED DIABETIC RAT MODEL

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The International Diabetes Federation (IDF) Diabetes Atlas (2021) reveals a global diabetes prevalence of 10.5% among the adult population (20-79 years). Diabetes Mellitus (DM), characterized by elevated blood sugar levels, is a prevalent metabolic disorder stemming from intricate genetic and environmental factors and involves increased non-enzymatic glycation, leading to toxic glucose derivatives known as advanced glycation end products (AGEs). S100 proteins, damage-associated molecular pattern proteins expressed in various organs, play diverse physiological and pathological roles. AGEs and S100 proteins interaction with receptor for advanced glycation end products (RAGE), contribute to oxidative stress and inflammation, linking them to diabetes-related cognitive impairment. Vitamin D3 (Vit-D3), a secosteroid hormone, exerts genomic influences through vitamin D receptor (VDR) and rapid effects via cell membrane receptors like membrane-associated rapid response steroid-binding protein (MARRSBP), triggering neuroprotective and anti-inflammatory responses. In this study, in the diabetic rat model induced by a single dose of streptozotocin (STZ) (intraperitoneal injection of 65 mg/kg), the changes in S100 protein subtypes, S100B, and S100A8/A9 (calprotectin) in serum and hippocampus samples were assessed using ELISA, focusing on Vit-D3's impact. Also, cognitive changes were assessed through the eight-arm radial maze test (RAM). The results indicate that the hippocampal RAGE, calprotectin, S100B, and insulin, along with the serum level of calprotectin, significantly increased in both the STZ and Vit-D3 groups compared to the control group; however, a significant increase in serum RAGE levels was detected only in the STZ group. The rotarod test results indicated a significant decrease in the time spent on the spindle by the STZ group, and the RAM test revealed reduced correct arm choices and prolonged latency in the STZ group. The administration of Vit-D3 was observed to reduce the increased serum RAGE levels and reverse these negative effects on motor performance and memory.

Keywords: Diabetes Mellitus, RAGE, Streptozotocin, S100, Vitamin D3.

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CONTENTS

Page

ÖZET.....v
ABSTRACT..... vi
ACKNOWLEDGEMENT..... vii
FIGURES DIRECTORY x
TABLES DIRECTORY xi
SYMBOLS AND ABBREVIATIONS DIRECTORY xii
1. INTRODUCTION 1
1.1. Aim.....2
2. THEORATICAL INFORMATION AND LITERATURE REVIEW.....3
2.1. Diabetes Mellitus.....3
2.1.1. Epidemiology 3
2.1.2. Etiology 5
2.1.3. Clinical 7
2.1.4. Diabetes management and therapy (pharmacological and non-pharmacological interventions).....8
2.1.5. Diabetes complications 9
2.2. Diabetes Miletus Interplaying with Declining of Cognitive Function 10
2.2.1. Advanced glycation end products 12
2.2.2. AGEs receptors and AGEs-RAGE axis 14
2.3. S100 Protein Family 18
2.3.1. The physiological and pathological functions of the S100 proteins 19
2.3.2. Utilization of S100 proteins as biomarkers and their prospective therapeutic implications in various diseases.....21
2.3.3. S100B physiological and pathological rules 21
2.3.4. S100A8/A9 physiological and pathological rules.....23
2.4. Vitamin D3 24
2.4.1. Vitamin D3 effect on diabetes.....26
2.4.2. Vitamin D3 effect on cognitive functions and DACD.....27
2.5. Animal Models of Diabetes Mellitus 29
2.5.1. Streptozotocin-induced diabetes 30
3. MATERIALS AND METHODS.....32
3.1. Research Design 32
3.2. Chemicals 33
3.2.1. Chemicals used in vivo. 33
3.2.2. Chemicals used in vitro. 33
3.3. Experimental Animal Groups and Experimental Procedures.....33
3.4. Study Protocol 35
3.5. Streptozotocin-Induced Experimental Animal Diabetic Model 35
3.5.1. Fasting Blood glucose measurement.....36
3.5.2. Evaluation of diabetes model 38
3.6. Vitamin D3 Treatment.....39
3.7. Motor Performance.....40
3.7.1. Rotarod apparatus 40
3.7.2. Motor performance evaluation protocol.....41

3.8. Behavioral Test – Eight-Arm Radial Maze 42
3.8.1. General concept 42
3.8.2. Eight-arm radial maze: 44
3.8.3. The procedure 45
3.8.4. The evaluation criteria 46
3.9. Decapitation Scarification and Collection of Serum and Hippocampus Samples 47
3.10. Enzyme-Linked Immunosorbent Assay 49
3.11. Statistical Analysis 51
4. RESULTS 52
4.1. Streptozotocin-Induced Diabetic Model 52
4.1.1. Weight of rats 52
4.1.2. Fasting blood glucose (FBG) 54
4.2. Rotarod Motor Performance Results 56
4.3. Learning and Memory Results 58
4.3.1. The number of the correct arm choices 59
4.3.2. Latency: 62
4.4. Enzyme-Linked Immunosorbent Assay Results 65
4.4.1. Insulin measurement 65
4.4.2. RAGE measurement 66
4.4.3. sRAGE measurement 67
4.4.4. S100B protein measurement 68
4.4.5. Calprotectin measurement 69
5. DISCUSSION 71
6. CONCLUSION 80
7. REFERENCES 81
8. RESUME 100

APPENDICES

Appendix-1 Ethics committee approval.

FIGURES DIRECTORY

page

Figure 2.1. Estimations and forecasts for the worldwide incidence of diabetes among individuals aged 20–79, presented in millions. (IDF Diabetes Atlas editions 1st to 10th).....4
Figure 2.2. T1DM and T2DM.....6
Figure 2.3. Insulin neurotrophic effects. 11
Figure 2.4. Disturbance of BBB integrity is implicated to neurodegeneration in diabetes..12
Figure 2.5. AGEs formation.....13
Figure 2.6. AGEs-RAGE axis.....16
Figure 2.7. The interaction of S100 proteins with calcium ions (Ca2+) (B. Sun & Kekenes-Huskey, 2020).....19
Figure 2.8. The effect of high and low concentration of S100B protein centrally. 23
Figure 2.9. Cholecalciferol chemical structure. 25
Figure 2.10. Vitamin D3 genomic and non-genomic central effects. 29
Figure 3.1. Study protocol.....35
Figure 3.2. Intraperitoneal STZ injection.....36
Figure 3.3. Rat inside the restrainer with clarified lateral vein. 37
Figure 3.4. Accu-Chek® Performa Nano glucometer.....37
Figure 3.5. Steps of fasting blood glucose measurement. 38
Figure 3.6. Vitamin D3 administration using100ul pipette.....39
Figure 3.7. Motor performance measurement using rotarod apparatus. 41
Figure 3.8. Schemes representing eight-arm radial maze with the presence of bait in each arm and the concept of correct arm choice. 43
Figure 3.9. Eight-arm radial maze (RAM).....45
Figure 3.10. Preparations for decapitation. 47
Figure 3.11. Intracardiac blood samples collection.....48
Figure 3.12. Left and right hippocampus homogenization. 49
Figure 4.1. % Change in body weight in rats of different groups. 54
Figure 4.2. Fasting blood glucose levels. 55
Figure 4.3. Fasting blood glucose levels. 56
Figure 4.4. Rotarod motor performance. 58
Figure 4.5. Number of correct arm choices (RAM test). 61
Figure 4.6. Number of correct arm choices (RAM test). 62
Figure 4.7. The time required to finish the RAM test (Latency). 64
Figure 4.8. The time required to finish the RAM test (Latency). 65
Figure 4.9. Measurement of Insulin levels in serum and hippocampus. 66
Figure 4.10. Measurement of RAGE levels in serum and hippocampus. 67
Figure 4.11. Measurement of sRAGE levels in serum. 68
Figure 4.12. Measurement of S100B protein levels in serum and hippocampus. 69
Figure 4.13. Measurement of calprotectin levels in serum and hippocampus. 70

TABLES DIRECTORY

Page

Table 4.1. Measurement of the average body weight of rats.....53
Table 4.2. Fasting blood glucose.....55
Table 4.3. Rotarod motor performance. 57
Table 4.4. The number of correct arm choices (RAM test).....60
Table 4.5. The latency. 63

SYMBOLS AND ABBREVIATIONS DIRECTORY

$1,25(OH)_2$Vit-D3	1,25-dihydroxy vitamin D3
25(OH)Vit-D3	25-hydroxyvitamin D3
7-DHC	7-dehydrocholesterol
AD	Alzheimer's disease
AGEs	Advanced glycation end products
ATP	Adenosine triphosphate
Aβ	Amyloid beta peptide
BBB	Blood brain barrier
bFGF	Basic fibroblast growth factor
Ca2+	Calcium ions
CAPS2	Ca2+-dependent activator protein for secretion 2
CAT	Choline acetyltransferase
COX-2	Cyclooxygenase-2
CYP	Cytochrome P450
DACD	Diabetes associated cognitive decline
DAG	Diacylglycerol
DAMP	Damage-associated molecular pattern
DBD	DNA binding domain
DBP	Vitamin D-binding protein
DKA	Diabetic ketoacidosis
DM	Diabetes Mellitus
EAR	Estimated average requirement
ELISA	Enzyme-Linked Immunosorbent Assay
ENDO-AGEs	Endogenous AGEs
ER	Endoplasmic reticulum
ERp57/ERp60	Endoplasmic reticulum protein 57/60
EXO-AGEs	Exogenous AGEs
FBG	Fasting blood glucose
fl-RAGE	Full length receptor of advanced glycation end products
HbA1c	Hemoglobin A1c
i.p.	Intraperitoneal
IDDM	Insulin-dependent diabetes mellitus
IDF	International Diabetes Federation
IFN-gamma	Interferon-gamma
IGT	Impaired glucose tolerance
IL-1	Interleukin-1
IL-1β	Interleukin-1β
IL-23	Interleukin-23
IL-27	Interleukin-27
IL-6	Interleukin-6
IP3	Inositol trisphosphate
IR	Insulin receptor
IRS-1	Insulin receptor substrate
JAK/STAT	Janus kinase signal transducer and activator of transcription
LBD	Ligand binding domain

LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
MARRSBP	Membrane-associated rapid response steroid binding protein
MRP14	Myeloid-related protein-14
MRP8	Myeloid-related protein-8
NAD	Nicotinamide adenine dinucleotide
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate oxidase
NFTs	Neurofibrillary tangles
NF-κB	Nuclear factor-kappa B
NGF	Nerve growth factor
NIDDM	Non-insulin-dependent diabetes mellitus
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
OGTT	Oral glucose tolerance test
PBS	Phosphate buffered saline
PI3K/Akt	Phosphatidylinositol 3-kinase-protein kinase B
PIP2	Phosphatidyl inositol bisphosphate
PPI	Protein-protein interaction
PRR	Pattern-recognition receptor
RAGE	Receptor of advanced glycation end products
RAM	Eight-Arm Radial Maze
RDA	Recommended Dietary Allowance
ROS	Reactive oxygen species
RXR	Retinoid X receptor
SAPK/JNK	Stress-activated protein kinase/c-Jun N-terminal kinase
SGCI	Sodium-glucose cotransporter inhibitors
SOD	Superoxide dismutase
sRAGE	Soluble RAGE receptor
STZ	Streptozotocin
T1DM	Type 1 DM
T2DM	Type 2 DM
TLR 4	Toll-like receptor 4
TNFα	Tumour necrosis factor alpha
VDR	Vitamin D receptor
VDREs	Vitamin D response elements
VEGF	Vascular endothelial growth factor
Vit-D3	Vitamin D3
WHO	World Health Organization
α7nAChR	α7 Nicotinic acetylcholine receptor

1. INTRODUCTION

Diabetes Mellitus (DM) is characterized by elevated blood sugar levels, leading to the buildup of advanced glycation end products (AGEs), which are toxic glucose derivatives. These AGEs are formed when proteins or lipids become glycated non-enzymatically in a pro-oxidant environment when exposed to sugars.

S100 proteins are symmetrical dimer calcium-binding damage-associated molecular pattern (DAMP) proteins that are expressed in various organs and tissues playing different physiological functions including the neuronal maintenance and plasticity that affect cognitive processes in a healthy brain.

The interaction of AGEs and S100 proteins with the receptor called receptor for advanced glycation end products (RAGE) triggers oxidative stress and inflammation. This interaction is suggested to contribute to diabetes associated cognitive decline (DACD). However, a soluble form of the RAGE receptor, referred to sRAGE acts as a protective trapping molecule by binding to AGEs and reducing their harmful effects.

Vitamin D3 (Vit-D3) is a secosteroid hormone that has various skeletal and extra-skeletal effects in the body. Functioning on a genomic level, it modulates the activity of diverse genes by interacting with its nuclear steroid hormone receptor—the vitamin D receptor (VDR). Additionally, apart from its genomic actions through the VDR, Vit-D3 has also been found to have rapid effects mediated by different receptors on the cell membrane such as membrane-associated rapid response steroid binding protein (MARRSBP) triggering various signaling molecules and pathways which has a neuroprotective, and anti-inflammatory effect.

The thesis project will investigate the levels of specific S100 protein subtypes (S100B protein and calprotectin), identified as targeted biomarkers for diabetes diagnosis, in Wistar albino male rats (3-4 months old) with streptozotocin (STZ)-induced diabetes and assess the impact of Vit-D3 on these S100 protein subtypes. The study will also assess the motor performance using the rotarod test, and the changes in learning and cognitive functions in this model using the eight-arm radial maze (RAM) to elucidate the effect of Vit-D3, reported to have neuroprotective effects. In biochemical analyses, fasting blood glucose (FBG) will be measured using the Accu-Chek® Performa Nano glucometer. Furthermore, the levels of common biomarkers implicated in both decreased cognitive function and diabetes (insulin, RAGE, sRAGE, S100B, calprotectin) in serum and/or hippocampus samples will be analyzed through the Enzyme-Linked Immunosorbent Assay (ELISA) method using appropriate kits and following protocols provided by the kit manufacturer.

1.1. Aim

The aim of this thesis project is to assess S100 protein subtypes as potential biomarkers and investigating cognitive changes in STZ-induced diabetic rat model, examining the neuroprotective effects of Vit-D3.

2. THEORATICAL INFORMATION AND LITERATURE REVIEW

2.1. Diabetes Mellitus

2.1.1. Epidemiology

Diabetes Mellitus (DM) is an important chronic metabolic disorder characterized by chronic hyperglycemia caused by relative or absolute insulin deficiency i.e., production of insufficient amount of insulin from pancreatic β-cells, with or without concurrent impairment of insulin action (resistance). Among the different causes and types of DM, the main 2 types are insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) which are commonly known as Type 1 DM (T1DM) and Type 2 DM (T2DM) respectively.

DM is a long-term pathological condition that can result in serious acute and chronic complications, causing damage to the various systems in the body therefore, diabetes has been ranked among the top 10 global causes of mortality (IDF News, n.d.). According to the annual reports published by the International Diabetes Federation (IDF) Diabetes Atlas, in collaboration with the World Health Organization (WHO), and with the aim of providing continuous 'real-world, real-time' data about the local and global occurrence of diabetes, it has been revealed that the global incidence of diabetes mellitus is consistently on the rise. It is projected to reach 11.3% of the population (643 million) by 2030 and is expected to escalate further to an astonishing 783 million (12.2%) by 2045 (Figure 2.1).

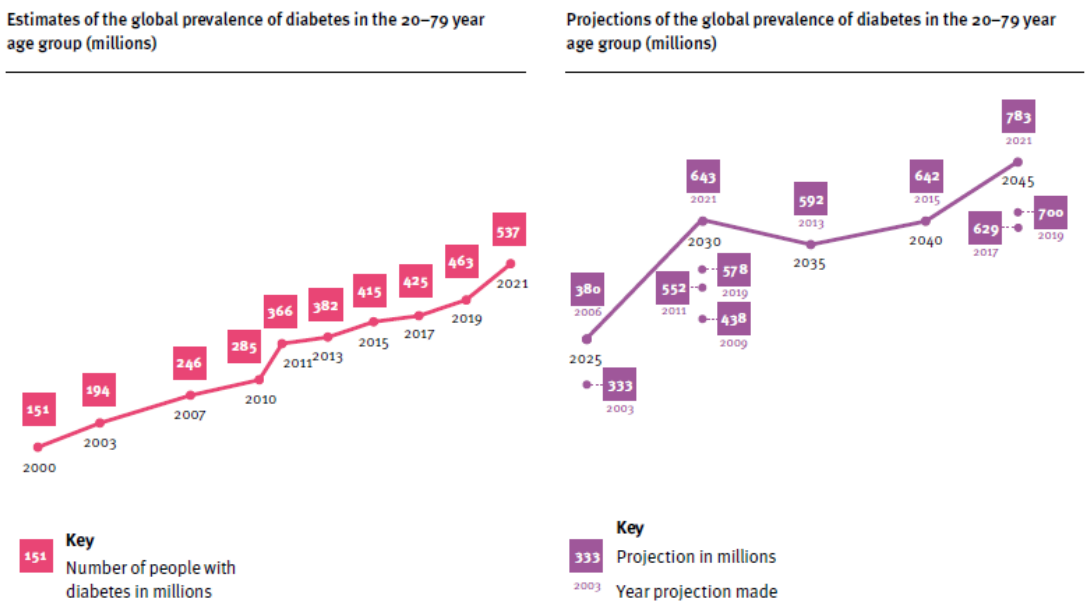

Figure 2.1. Estimations and forecasts for the worldwide incidence of diabetes among individuals aged 20–79, presented in millions. (IDF Diabetes Atlas editions 1st to 10th).

Additionally, the annual rise in diabetes prevalence is notable among children and adolescents (up to 19 years old). While aging is recognized as a risk factor for T2DM, it was reported that in 2021, the highest diabetes prevalence is observed among elderly individuals aged 75–79 years (24,0%), compared to adults aged 20–24 years (2,2% in 2021). Furthermore, in terms of impaired glucose tolerance (IGT), is defined as an elevation in blood glucose levels exceeding the normal range but not reaching the criteria for a diabetes diagnosis. It was reported that about 541 million people have impaired glucose tolerance in 2021 (International Diabetes Federation, 2021).

For diabetes distribution, according to the data provided in 10th edition of the IDF Diabetes Atlas in 2021 the highest comparative prevalence of diabetes (18,1%) was found to be in the Middle East and North Africa which was expected to increase to 20,4% by 2045 while Africa had the lowest comparative prevalence (5,3%), attributed to the low obese population, which was also expected to increase to be 5,6% by 2045. Regarding country distribution, the number of adults aged 20–79 years with diabetes in 2021 was found to be highest in Asian countries, including China, India, and Pakistan (H. Sun et al., 2022).

2.1.2. Etiology

The increase in diabetes prevalence is alarming and represents a major global health threat therefore, understanding its causes and risk factors is of concern to reduce the incidence and limit this escalating epidemic of diabetes mellitus.

The main clinical indicator for all types of diabetes is high levels of blood glucose (hyperglycemia) (Organization & Federation, n.d.). The physiological control of blood glucose level is of great importance and is achieved by many hormones including insulin (the main regulatory hormone), and its counter-regulatory hormones glucagon, glucocorticoids, and growth hormone. Insulin is an essential anabolic hormone promoting fuel conservation by enhancing the uptake and storage of glucose, amino acids, and fats after a meal. Structurally Insulin, a small protein weighing 5808 Daltons in humans, consists of 51 amino acids organized into two peptide chains—a 21-residue acidic A chain and a 30-residue basic B chain which are cross-linked by two disulfide bridges (Weiss, 2009). Pancreatic β-cells are responsible for the production and secretion of insulin, triggered by elevated blood glucose levels. Insulin binds to its receptor (kinase-linked) on the surface of target cells, facilitating the uptake of glucose from the bloodstream into the body's cells for use as energy or storage. Thus, the net results of insulin action are decreasing in blood glucose levels through an increase in the uptake and utilization of glucose, an increased formation of glycogen in the liver and muscle and decreasing glycogen breakdown and gluconeogenesis (Norton et al., 2022). Therefore, the absence of insulin or the cells' inability to respond to it results in a rise in blood glucose levels.

T1DM, also known as Juvenile-onset diabetes, as its incidence occurs in young ages (Tuomilehto et al., 2020) (children and adolescents) is the type of diabetes that is mediated through the development of an autoimmune defect i.e., the destruction of pancreatic β-cells due to the attack by the body’s immune system (Figure 2.2) results in a severe or absolute deficiency of insulin (Immune-mediated diabetes or Type1A) or genetically (Idiopathic diabetes or Type1B). T1DM only accounts for 5-10% of DM cases (Atkinson et al., 2014; Goyal & Jialal, 2022).

The most common type and the most important to be controlled is T2DM which is also known as Maturity-onset diabetes is initially marked by the body's incapacity to utilize the generated insulin, known as insulin resistance, combined with further relatively insufficient insulin production because of the incapacity of pancreatic beta cells to cope with the rising demand (Figure 2.2). Patients of this type of diabetes are usually obese and of adult age. T2DM is relatively asymptomatic with a slow onset over 5-10 years, and it is account for 90-95% of DM cases (Goyal & Jialal, 2022). Although genetic predisposition participates in the determination of the susceptibility of the individual to T2DM, lifestyle also plays a major role. Therefore, the key point to limit the current global epidemic of T2DM is the controlling of the risk factors including unhealthy diets like processed meat, the low-fiber diet with a high glycemic index, and saturated fat intake which affect insulin resistance (Aschner, 2017). Also, being overweight and obese, having a sedentary lifestyle, smoking, and alcohol intake are strongly related to the risk of developing T2DM (Y. Wu et al., 2014).

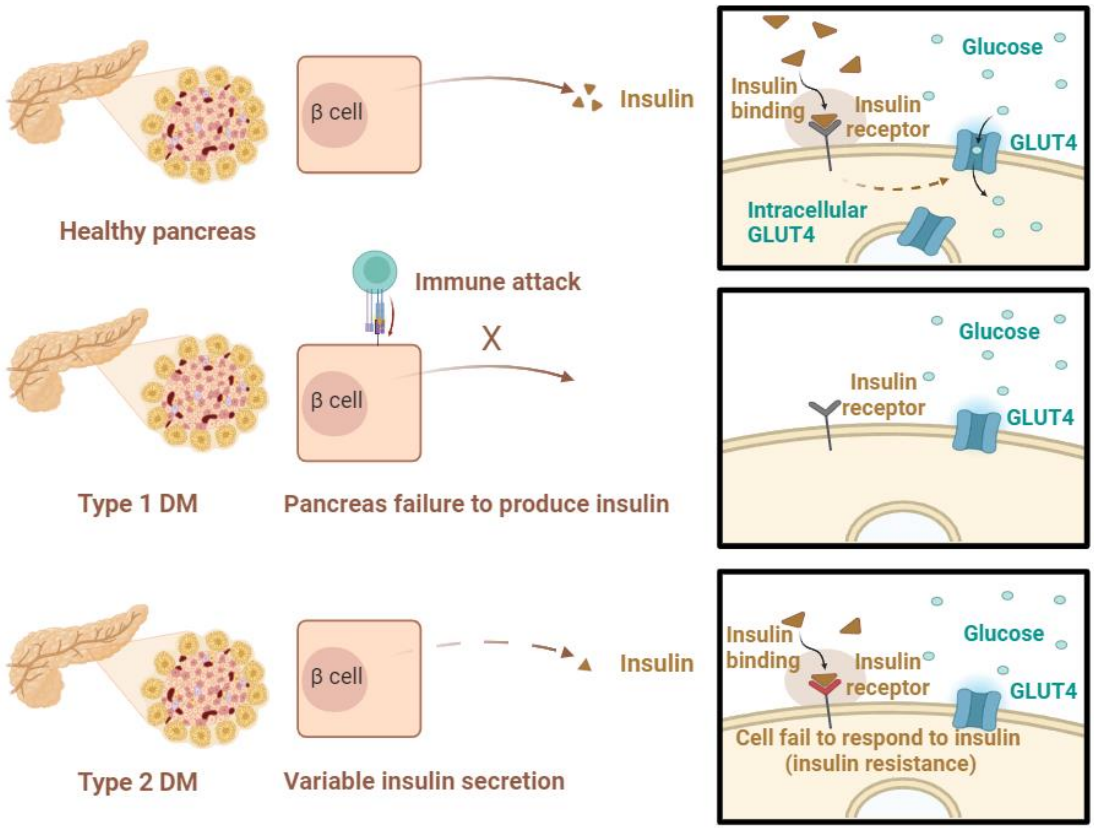

Figure 2.2. T1DM and T2DM.

2.1.3. Clinical

One of the limitations in the data related to the prevalence of diabetes is the fact that a considerable portion of diabetes cases remains unidentified, with statistics revealing that almost half (44.7%; 239.7 million) of individuals in the age range of 20 to 24 years were living with undiagnosed diabetes in 2021 (International Diabetes Federation, 2021). Undiagnosed cases are more widespread in countries with lower and moderate incomes, but also the diagnosis of diabetes may encounter delays even in countries with higher economic means (Dall et al., 2014) and this is related to the fact that despite the association of both T1DM and T2DM with some clinical symptoms such as excessive thirst (polydipsia), continual hunger, energy depletion or fatigue, frequent urination (polyuria) and reduction in weight (Harreiter & Roden, 2019), diagnosis of T1DM may be delayed until the first Diabetic Ketoacidosis (DKA) hospitalization and T2DM is either symptomless or with the less clear clinical picture.

So, in order to prevent delaying or entirely missing the diagnosis along with improving the quality of life and avoiding subsequent complications of diabetes, the rates of clinical diagnosis have to be increased by raising the awareness of the importance of early diagnosis and improving the quality of care after diagnosis. Various diagnostic methods and criteria are used to diagnose diabetes. Most guidelines follow the guidelines specified by IDF and WHO which recommend for diagnosis of T2DM be made based on, in the absence of symptoms, the utilization of an oral glucose tolerance test (OGTT), with a plasma glucose level exceeding 11.1 mmol/L or 200 mg/dL two hours after the administration of 75-grams of anhydrous glucose, is considered. OGTT is regarded as more sensitive and specific compared to the fasting blood glucose test (FBG), where a plasma glucose level surpassing 7.0 mmol/L or 126 mg/dL following an overnight fasting period indicates diabetes. Additionally, a random plasma glucose concentration exceeding 11.1 mmol/L or 200 mg/dL, coupled with the manifestation of diabetes symptoms, is deemed diagnostic for T2DM (Goyal & Jialal, 2022; Organization & Federation, n.d.).

Furthermore, it is advised to conduct assessments for both OGTT and FPG to identify prediabetes, encompassing IGT with an elevated OGTT range of 140-200 mg/dL, or impaired fasting glucose (IFG) with an FBG falling between 100-126 mg/dL. This approach aims to proactively address the risk of progression to diabetes through lifestyle modifications (Tabák et al., 2012). WHO advocates for the adoption of Hemoglobin A1c (HbA1c) measurement for diabetes diagnosis (HbA1c of 6.5% or more) but not for prediabetes (Organization & Federation, n.d.).

In concerning to T1DM, although its diagnosis may be difficult or may require testing the presence of autoantibodies against pancreatic β-cells or insulin to be distinguished from T2DM, the diagnosis is relying on the detection of hyperglycemia and the presence of some or all the symptoms (Primavera et al., 2020).

2.1.4. Diabetes management and therapy (pharmacological and non-pharmacological interventions)

Despite the variety of drug classes that are currently available for the treatment of diabetes, the cornerstone of the management for prediabetes and diabetes is lifestyle modification and managing risk factors involves refraining from smoking and alcohol consumption, maintaining a healthy body weight through a well-balanced diet, and engaging in regular exercises (Cefalu et al., 2016; Howells et al., 2016). Additionally, as the role of nutrition (Harreiter & Roden, 2019) and vitamins are of great importance for health, promising approaches focusing on the use of prebiotics and probiotics due to the relation between gut microbiota and obesity and thereby the decreased glucose tolerance and insulin resistance (Iatcu et al., 2021), also due to association of diabetes with chronic inflammation, production of reactive oxygen species (ROS) and reduced antioxidant status, research is underway on antioxidant compounds, including vitamins like A and D3, to explore their potential in aiding the management of diabetes and its related complications. (Lips et al., 2017a).

Pharmacologically, the approach to treating T1DM primarily revolves around administering daily insulin injections. Various types with distinct onset and duration of action

are utilized, accompanied by regular blood glucose monitoring to sustain their blood glucose levels within the recommended range (Ahmad, 2014).

For T2DM, in some cases the change in lifestyle can achieve the required glycemic control. However, if it is insufficient oral hypoglycemic drugs are used as monotherapy or in combination (Aschner, 2017). Various classes of hypoglycemic agents are employed to manage T2DM. These include insulin secretagogues like sulfonylureas and meglitinides, euglycemic agents such as biguanides and thiazolidinediones, alpha-glucosidase inhibitors, incretin-based therapies mimicking incretin effects, amylin analogs, sodium-glucose cotransporter inhibitors (SGCI), and bile acid sequestrants. Each class operates through distinct mechanisms, influencing insulin release, normalizing glucose levels, inhibiting digestion, or impacting signaling pathways related to glucose metabolism (Taylor et al., 2021). Furthermore, in some cases, if glycemic control in T2DM is not achieved with hypoglycemic drugs, insulin injections may be added to the treatment regimen.

2.1.5. Diabetes complications

The delay in the diagnosis of diabetes or the failure to control the blood glucose level within the recommended levels cause damage to various organs in the body and the development of different acute and chronic complications. The most important acute complication is DKA which is an emergency case characterized by significant hyperglycemia, dehydration, and accumulation of ketone bodies as acetone from the fatty acid metabolism leading to ketoacidosis (Muneer & Akbar, 2021). Long-term complications are the main the major factors contributing to morbidity and mortality among individuals with DM including vascular complications which can be microvascular including visual impairment due to retinopathy, nephropathy, and loss of sensation due to neuropathy or macrovascular including cardiovascular (various heart diseases), cerebrovascular (risk of stroke) and peripheral vascular diseases (increased risk of poorly healing lower-limb ulcers) (Cole & Florez, 2020).

2.2. Diabetes Miletus Interplaying with Declining of Cognitive Function

The increased prevalence of DM is of public concern as well as intriguing for researchers, especially concerning acute and chronic complications. One of them is that DM predisposes to a gradual deterioration in cognitive functions, and this is found to occur in both T1DM and T2DM and in all age groups (Pugazhenthi et al., 2017). Several studies have demonstrated the correlation of higher values of HbA1c with dementia and cognitive dysfunction in uncontrolled T2DM. Also, compared to patients who achieved glycemic control, those with HbA1c levels > 8.8% exhibited moderate declines in cognition and motor speed (Dove et al., 2021).

In 2006, Mijnhout et al. initiated the development of the diabetes-associated cognitive decline (DACD) concept. Based on the degree of cognitive dysfunction in diabetic patients, three stages are identified: diabetes-associated cognitive decline, mild cognitive impairment, and dementia. Cognitive dysfunction and the likelihood of dementia are roughly two-fold higher in the elderly diabetic population compared to older individuals without diabetes. Furthermore, diabetes is identified as a contributing factor to Alzheimer's disease (AD) as increased cerebral amyloid beta peptide (Aβ) formation and aggregation and increased hyperphosphorylation of Tau protein were found in non-AD diabetic rat models induced chemically by streptozotocin (STZ) administration or using of high-fat diet (Biessels & Despa, 2018b).

There are different non-AD mechanisms that have been supposed for how diabetes causes the decline in cognitive function including, elevated blood glucose adversely affects neurons and synapses, ultimately altering the plasticity of synapses in the hippocampus and reducing long-term potentiation (LTP). Also, insulin receptors (IR) expressed in neuronal soma and synaptic terminals are essential for maintaining normal mitochondrial function and neuronal synaptic plasticity, thus influencing cognitive processes in the brain. Additionally, insulin promotes the expression of N-methyl-D-aspartate (NMDA) receptors, facilitating LTP in the hippocampus, thereby improving functions related to learning and memory.

Insulin also influences the concentration of different neurotransmitters including acetylcholine (ACh), noradrenaline (NA) and adrenaline that have a role in memory (Figure 2.3). Insulin signaling is vital for memory maintenance in the hippocampus; therefore, alterations in insulin metabolism and action may contribute to the progression of cognitive impairment related to diabetes. The neurotrophic activity of insulin is supported by research on the administration of intranasal insulin that has been found to enhance cognitive performance in both healthy individuals and those with cognitive impairment (Pugazhenthi et al., 2017).

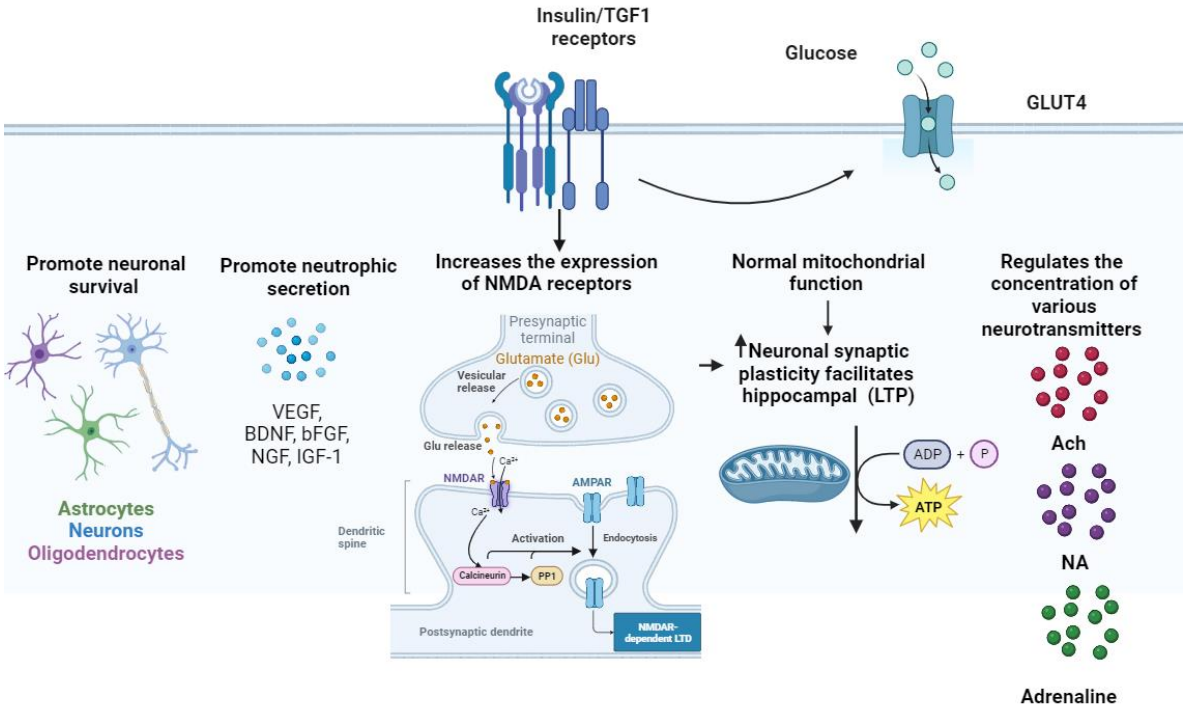

Figure 2.3. Insulin neurotrophic effects.

*bFGF, basic fibroblast growth factor; NGF, nerve growth factor; PPI, protein-protein interaction; VEGF, vascular endothelial growth factor.

On another side, diabetes causes endothelial dysfunction and vascular damage, resulting in reduced cerebral blood flow. This reduction can cause hypoxic neuronal injury, coupled with the upregulation of inflammatory mediators and ROS associated with vascular

endothelial dysfunction. These factors may contribute to the disruption of the blood-brain barrier (BBB) (Figure 2.4) (Bogush et al., 2017).

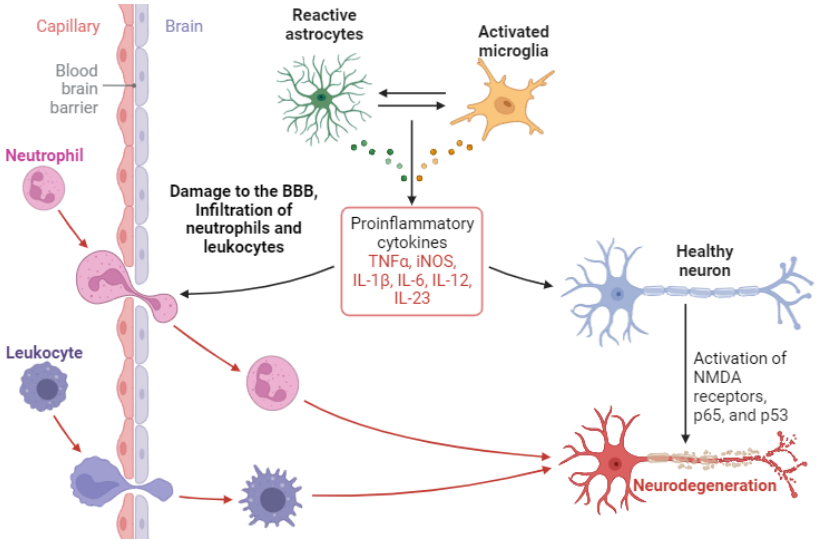

Figure 2.4. Disturbance of BBB integrity is implicated to neurodegeneration in diabetes.

Most importantly, prolonged hyperglycemia is correlated with an enhanced generation of advanced glycation end products (AGEs), commonly referred to as mycotoxins. These AGEs induce sustained central inflammation and oxidative damage, contributing to neurodegeneration (Nowotny et al., 2015a).

2.2.1. Advanced glycation end products

Chronic uncontrolled hyperglycemia is the primary contributor to diabetic complications and is one of the factors that initiate and accelerate the glycation reaction (Khalid et al., 2022). Glycation is a simple Maillard reaction, when the carbonyl group of reducing sugars, glucose (usually) or other saccharides like fructose or pentose, react non-enzymatically through an addition reaction in a pro-oxidant environment to macromolecules including proteins (addition to the amino group side chain of the lysine, histidine, or arginine residues), fatty acids, and nucleic acids. This forms a reversible Schiff base with subsequent

rearrangement to Amadori products, which are relatively unstable and undergo subsequent oxidative modifications (glycoxidation) and dehydration, ultimately generating a diverse set of harmful glucose derivatives known as advanced glycation end products (AGEs) (Figure 2.5) (Reddy & Beyaz, 2006).

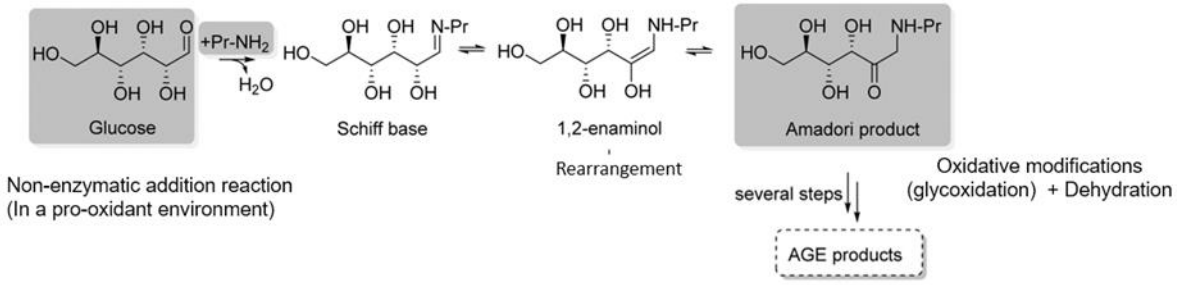

Figure 2.5. AGEs formation.

The source of AGEs can be endogenous through the glycation reaction that occurs intracellularly and extracellularly, physiologically the glycation reaction is slow and was considered only to affect proteins like collagen and elastin which are long-lived proteins that result in moderate production of AGEs (Goh & Cooper, 2008). However, in diabetes, the chronic elevation in the concentration of circulating glucose increases the availability of the glucose to the reaction as well as the exposure of macromolecules to elevated glucose levels results in interactions with rapidly degradable proteins, circulating plasma proteins and lipids resulting in markedly elevated levels of AGEs in diabetic patients (Ahmed, 2005). Also, aging accelerates the production of AGEs and their accumulation in plasma as well as in different tissues as insoluble and non-degradable aggregations which contribute to age-related diseases including DM, and progressive impairments like that occur in cognitive function (Chaudhuri et al., 2018).

Interestingly, the source of AGEs can be exogenous as they are found in cigarette smoke, and they are formed during the thermal processing of food using techniques involving elevated temperatures, and also during various processes made to improve the conservation and flavor of the food (Gill et al., 2019; Snelson et al., 2019). Therefore, the body's AGEs

pool is augmented by dietary AGEs increasing the serum level of AGEs that are suggested to be sufficient even to trigger the progression of T2DM as diets abundant in AGEs were noted to negatively impact insulin secretion and induce the death of β-cells in rats (Garay-Sevilla et al., 2021; research & 2006, 2006).

AGEs can disrupt normal body functions in various ways, including direct interference through the cross-linking of proteins. This can result in the impairment of structural integrity of intracellular proteins or damage to proteins in the extracellular matrix, leading to cell death, hindered cell adhesion, and impaired cell movement. On the other hand, AGEs can indirectly impact cellular processes by interacting with various cell surface receptors. This binding initiates the activation of several signaling pathways associated with pathological responses, often involving inflammation, oxidative stress, and disruptions in cellular functions (Goh & Cooper, 2008).

2.2.2. AGEs receptors and AGEs-RAGE axis

Cellular processes can be influenced by AGEs as they bind and interact with a range of cell surface receptors, including scavenger receptors, toll-like receptors, G-protein-coupled receptors, and pattern recognition receptors. Among these, the receptor of advanced glycation end products (RAGE) stands out as an extensively studied receptor. Functioning as a pattern-recognition receptor (PRR), RAGE is a transmembrane receptor belonging to the immunoglobulin superfamily. Its unique classification lies in its ability to recognize three-dimensional structures rather than specific amino acid sequences (Teissier & Boulanger, 2019). Consequently, RAGE can bind to various ligands beyond AGEs, including the S100 protein family and Aβ (Bierhaus et al., 2005; Chuah et al., 2013).

RAGE, alternatively referred to as full-length RAGE (fl-RAGE), consists of three distinct regions. The first region is the extracellular region, which contains a V-type domain at the N-terminal. This domain includes two N-glycosylation sites that are responsible for interacting with potential ligands extracellularly. Additionally, there are two C-type domains, C1 and C2, which are immunoglobulin domains. The second region is the transmembrane domain, which is hydrophobic in nature. It connects the extracellular region to the cytosolic

domain. The third region is the cytosolic domain, located on the inside of the cell. This domain consists of highly charged amino acids and serves as a scaffolding structure. It functions as a critical component in initiating intracellular signal transduction when the RAGE receptor is activated (E. J. Lee & Park, 2013).

When the transcribed RAGE (fl-RAGE) undergoes certain modifications like alternative splicing and proteolytic cleavage, it gives rise to truncated isoforms of RAGE. One example is soluble RAGE (sRAGE), formed through C-terminal truncation (Hudson et al., 2008; Sterenczak et al., 2013). sRAGE is devoid of the transmembrane domain, enabling its release into the extracellular space for general circulation. It functions as a decoy molecule by binding to surplus AGEs, effectively impeding cell signaling between fl-RAGE and its ligands. Furthermore, sRAGE can impact the production of RAGE ligands, thereby assuming an antagonistic mechanism against RAGE-mediated harmful effects (K. C. Tan et al., 2006). Thereby, the high level of sRAGE is suggested to be protective. This result is supported by researchers who concluded that maintaining elevated levels of sRAGE can help protect against atherosclerosis and improves vascular permeability. However, it has been observed that sRAGE concentrations decrease in hypertensive, obese, and patients with cardiovascular issues (Colhoun et al., 2011; Prasad, 2014; Yamagishi & Matsui, 2010). Also, macrophage scavenger receptors are crucial for the acceleration of the uptake, degradation, and clearance of AGEs once they bind to these receptors. These receptors have contrasting functions to RAGE and help maintain a balance in AGEs homeostasis by counteracting the oxidative stress induced by the AGEs-RAGE interaction (Shen et al., 2020; Torreggiani et al., 2009).

Typically, the expression of fl-RAGE is low in various cell types across different body tissues. Nevertheless, when there is an upsurge in the generation and buildup of AGEs, a positive feedback loop is triggered, leading to an upregulation of fl-RAGE expression. This suggests that the RAGE receptor, when stimulated by its ligands, contributes to the propagation and perpetuation of cellular responses (Bierhaus et al., 2005). When RAGE engages with its ligands, it initiates signaling through diverse downstream effectors, including mitogen-activated protein kinase (MAPK), Janus kinase signal transducer and activator of transcription (JAK/STAT), stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), and phosphatidylinositol 3-kinase-protein kinase B (PI3K/Akt) (Sanajou et al., 2018). These signaling pathways result in the sustained activation of nuclear

factor kappa B (NF-κB) yielding several outcomes. Initially, it binds to the proximal promoter region of RAGE, positively regulating RAGE expression. Secondly, NF-κB translocation to the nucleus and activates the NLRP3 inflammasome, fostering the expression of inflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) contributing to persistent inflammation (Kelley et al., 2019; Rheinheimer et al., 2017). Additionally, NF-κB activation leads to the stimulation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), increasing ROS production (Figure 2.5). This disrupts antioxidant mechanisms involving superoxide dismutase (SOD), catalase, and glutathione, inducing oxidative stress within the endoplasmic reticulum (ER), culminating in mitochondrial dysfunction (J. H. Lim et al., 2009).

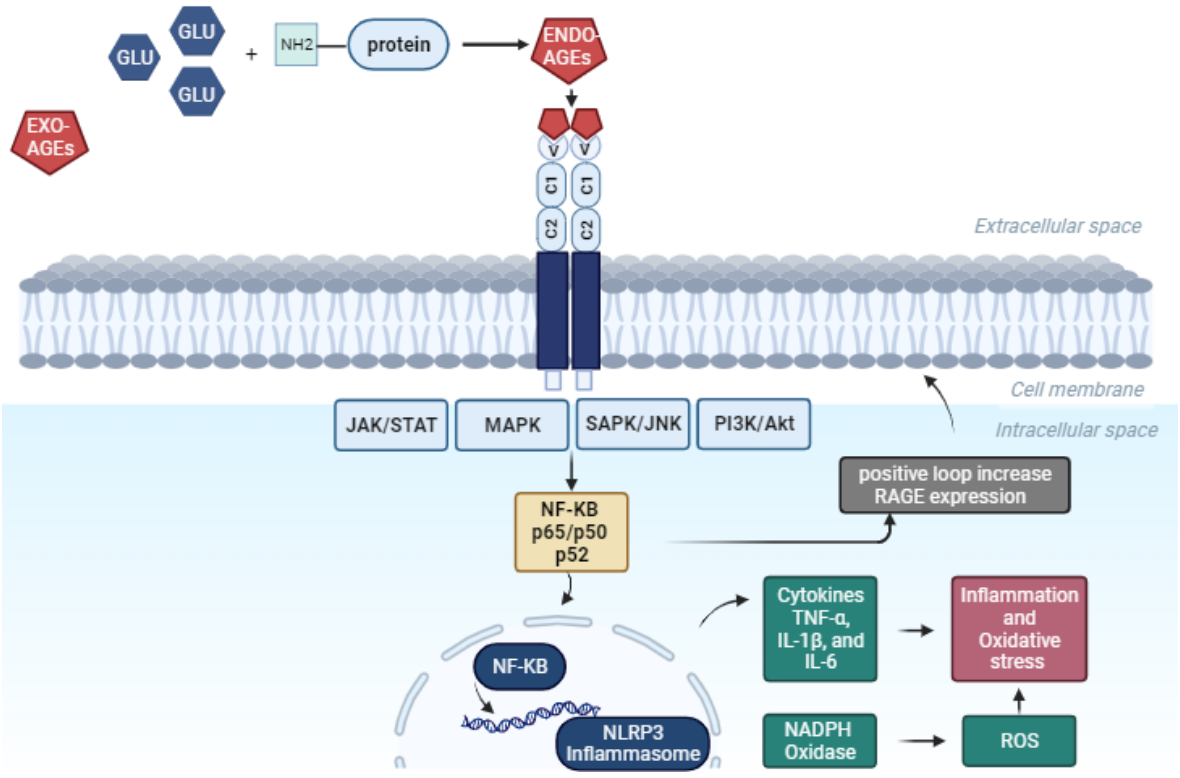

Figure 2.6. AGEs-RAGE axis.

*EXO-AGEs, exogenous AGEs; ENDO-AGEs, endogenous AGEs.

In the context of diabetes, the AGEs-RAGE axis is considered a contributing factor for insulin resistance (Ozaki et al., 2016). Various activated protein kinases, including

MAPK, JNK, p38, and PKC, directly downregulate insulin receptor expression (Sidarala & Kowluru, 2016; Sutherland et al., 2004) and also induce the phosphorylation of serine residues on insulin receptor substrate (IRS-1). This leads to impaired insulin signal transduction, consequently diminishing insulin sensitivity in target cells (Copps & White, 2012; Ramasamy et al., 2011). Moreover, studies have shown that AGEs can glycate proteins, increasing levels of glycated albumin. This, in turn, triggers the expression of TNFα, which suppresses insulin signaling (Nandipati et al., 2017).

AGEs, through the generation of ROS, particularly nitric oxide (NO), disrupt pancreatic insulin secretion. This disruption involves direct damage to pancreatic beta cells through the ROS-mediated mitochondrial dysfunction and release of cytochrome c resulting in caspase activation along with decreased expression of the anti-apoptotic protein Bcl2, ultimately causing dysfunction and apoptosis of the pancreatic beta cells (M. Lim et al., 2008; Lin et al., 2012). Most significantly, AGEs-mediated oxidative stress disturbs the normal functioning of the respiratory chain and the cytochrome c oxidase, leading to reduced ATP levels. Consequently, it impairs the closure of ATP-sensitive potassium channels thereby hindering the calcium influx, which is crucial for triggering insulin granule exocytosis and subsequent insulin secretion (Zhao et al., 2009).

The buildup of AGEs has been related with the deterioration of cognitive and the onset of AD (D'Cunha et al., 2022). In the brain, RAGE is expressed in neurons, microglia, astrocytes, monocytes, and macrophages (Byun et al., 2017). Astrocytes are one of the main cells that contribute to the maintenance of a microenvironment to support and protect neurons through monitoring synaptic remodeling, controlling the extracellular ion concentrations, and maintaining the health of the protective barriers as the BBB (Sofroniew & Vinters, 2010). On the other side microglia which are the main active immune defense act as resident macrophage cells functioning in the maintenance of neuronal networks, and injury repair (Colonna & Butovsky, 2017). Activation of RAGE in these cells prompts their activation, what is known as astrogliosis and microgliosis, leading to a loss of their supportive functions and triggering the production of ROS and inflammatory cytokines (Tobon-Velasco et al., 2014). The combination of astrogliosis and microgliosis contribute to neuroinflammation and synaptic damage which are related to impairments in learning and cognitive functions associated with DACD (Kubis-Kubiak et al., 2020; Li et al., 2019).

For therapeutic strategies, it is crucial to emphasize that even if blood glucose level is being controlled, the interplay between AGEs accumulation, RAGE overexpression, sustained activation of NFκB, chronic inflammation, impaired antioxidant systems along with ROS overproduction and mitochondrial dysfunction result in long term phenomena which is known as “metabolic memory”. This refers to the persistent effects of this axis implicated in β-cell damage, insulin resistance, and diabetes-associated complications (Ceriello, 2012). Therefore, prevention of endogenous AGEs formation, restriction of dietary AGEs uptake (Vlassara et al., 2011), and use of antioxidants or antiglycation agents such as pyridoxamine may all be considered as part of a therapy to target the harmful consequences of the AGEs-RAGE pathway (Howells et al., 2016; Zhu et al., 2011).

2.3. S100 Protein Family

The S100 protein family encompasses 25 identified members characterized by their acidic nature, low molecular weight (approximately 10-12 kDa), and calcium (Ca2+)-binding properties. Predominantly, they undergo posttranslational modifications to form functional oligomers, including homodimers (such as tetramers, hexamers, and octamers) (Botelho et al., 2012). Some S100 proteins can also form heterodimers, as observed in a few members such as S100A8/A9 and S100A6/B (Teigelkamp et al., 1991; Yang et al., 1999). Each S100 monomer comprises two EF-hand domains separated by a flexible hinge region. Each EF-hand is a helix-loop-helix structure with two calcium binding sites (Sastry et al., 1998). The binding of Ca2+ induces conformational changes, including up to a 90-degree rotation, revealing a hydrophobic cleft essential for interacting with specific target molecules (Figure2.7) (Smith & Shaw, 1998). S100 proteins exist in a dynamic equilibrium between Ca2+-free and Ca2+-bound states. They can also form complexes with target peptides (Blanchard et al., 1997). S100 proteins can also bind to other metals such as zinc (Zn+2) and copper (Cu+2) (Cunden et al., 2017). Their presence is typical in various organs and tissues, playing diverse roles in physiological functions and being associated with various pathological conditions (Heizmann et al., 2002).

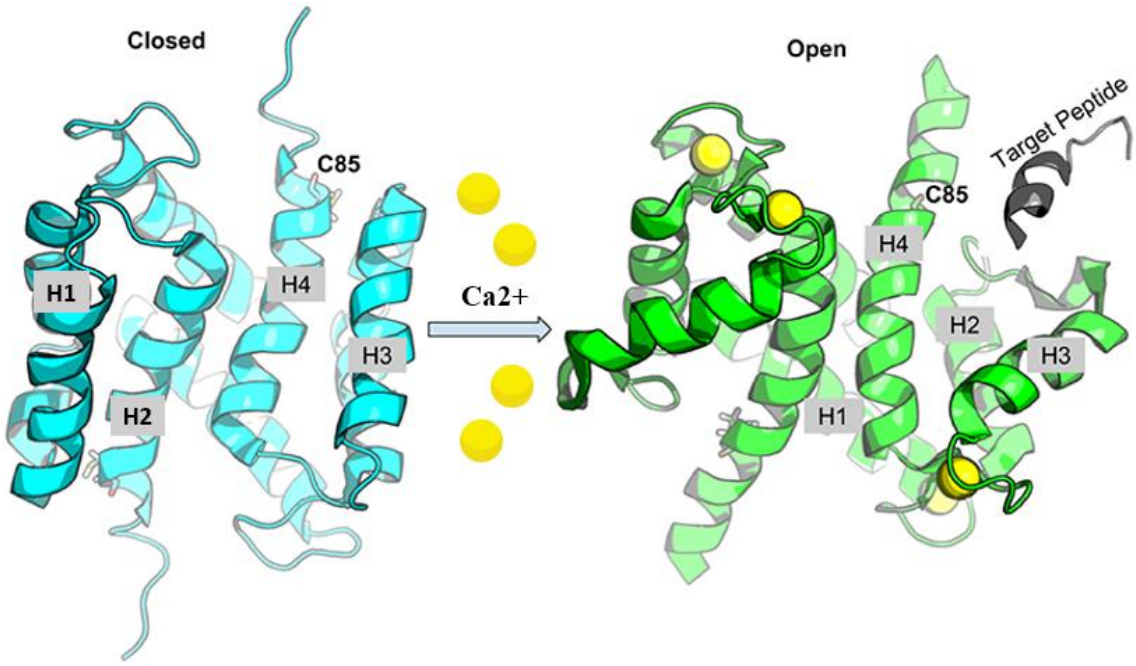

Figure 2.7. The interaction of S100 proteins with calcium ions (Ca2+) (B. Sun & Kekenes-Huskey, 2020).

2.3.1. The physiological and pathological functions of the S100 proteins

The physiological roles of S100 proteins depend on many crucial factors including their ability to bind to divalent cations, their oligomerized form, and the specific concentration required for their specific effects (low or high). Some of the S100 protein family members have intracellular regulatory effects, acting as intracellular mediators that transfer the signal from the second messenger to various types of receptors, enzymes, transcription factors, and nucleic acids. Their intracellular functions are involved in signal transduction, protein phosphorylation, transcription, maintaining intracellular calcium balance and energy metabolism, cell proliferation and differentiation, cell cycle progression, controlling of cell movement, inflammation and apoptosis (Donato et al., 2013). Meanwhile, other S100 proteins predominantly exert regulatory effects in the extracellular environment; are released extracellularly and modulate the activities of target cells in both paracrine and autocrine manners through binding to various cell surface receptors, including G-protein-coupled receptors and scavenger receptors. These proteins contribute to the regulation of cell

survival, growth, specialization, and movement under both normal and pathological circumstances. They also participate in tissue repair and inflammation. Additionally, some S100 members have both intracellular and extracellular functions, combining their roles in both compartments (Heizmann et al., 2002).

In certain pathological conditions, S100 proteins can be produced by cell types that don't normally express them. This induction of S100 gene expression and subsequent release occurs in a specific manner, triggered by factors like toll-like receptor ligands, cytokines, or growth factors and is functionally related to the cell's response to an interposing condition, particularly stress, leading to their secretion as extracellular alarmins or damage-associated molecular pattern (DAMP) proteins (Foell et al., 2007). They exert regulatory effects on different immune cells, resulting in the modulation of the immune responses.

For instance, the induction of S100B when cardiomyocytes experience an infarct is a protective mechanism to avoid an excessive hypertrophic response. Additionally, S100B overexpression is related to neurodegenerative disorders and is implicated in cognitive dysfunction, AD, and dementia (Cristóvaõ & Gomes, 2019). Other examples include S100A1, pivotal in heart function regulation, experiencing reduced levels in advanced heart failure patients, impacting calcium balance and contraction-related proteins, ultimately influencing overall cardiac performance (Most et al., 2006). S100A4 acts as a prognostic indicator with selective cancer expression and functions as an angiogenic factor promoting new blood vessel formation implicated in metastasis; blocking its extracellular function inhibits angiogenesis (Ambartsumian et al., 2001). S100A2 differential expression in normal versus tumorigenic bronchial cells suggests diagnostic potential for early-stage lung cancer (Feng et al., 2001). S100A8/S100A9 and S100A12, with chemotaxis functions as cytokine-like molecules, exhibit upregulated expression during oxidative stress and inflammation; S100A8/S100A9 are implicated in rheumatoid arthritis, while S100A12, activating RAGE and NF-κB, contributes to atherosclerosis pathophysiology by influencing endothelial cell adhesion molecules, cytokine production, and monocyte recruitment (Gonzalez et al., 2020).

2.3.2. Utilization of S100 proteins as biomarkers and their prospective therapeutic implications in various diseases

The overexpression of S100 proteins in biological fluids during pathological conditions has sparked increased research into their potential use as biomarkers for clinical diagnosis and therapeutic targets in various diseases. For example, S100A4 serves as a potential biomarker for metastatic cancers and lupus erythematosus. S100A6 shows elevated expression in gastric cancer patients. S100A12 is elevated in diabetic patients and inflammatory conditions like juvenile idiopathic arthritis. The S100A8/A9 complex is associated with Crohn's disease, obesity, and coronary artery disease. Assessing levels of S100B is useful indicator for individuals with cerebral ischemia caused by stroke, AD, Parkinson's disease, and Down syndrome (Heizmann, 2019; Heizmann et al., 2002).

Overall, the identification of specific S100 proteins as biomarkers holds promise for diagnosing and monitoring various diseases. Additionally, targeting these proteins therapeutically may offer new avenues for treatment in the future.

2.3.3. S100B physiological and pathological rules

S100B protein, a homodimer S100 protein with two beta subunits each weighing 9-14 kDa, functions both intracellularly and extracellularly. Mainly produced by astrocytes in the nervous system, it operates in a paracrine and autocrine manner within diverse glial cell types and neurons (Rothermundt et al., 2003). The influence of S100B protein on nervous cells depends on various factors, such as its concentration, RAGE expression, duration, and intensity of RAGE stimulation. It also relies on how much S100B affects the increase in RAGE expression in neurons, astrocytes, and microglia. At low concentrations (normal nanomolar levels), S100B demonstrates neurotrophic activity by interacting with RAGE, activating the Ras-MEK-ERK1/2-NF-κB pathway, resulting in enhanced expression of the anti-apoptotic factor Bcl-2 that supports neuron development, maintenance, and

differentiation (Figure 2.8). This aids in neuronal survival, cognitive recovery, and mitigating the negative impacts of neurotoxic substances (Bui et al., 2001). Additionally, it facilitates neurite extension, the propagation of hippocampal progenitor cells and influence synaptic plasticity leading to enhanced LTP (Baecker et al., 2020; Nishiyama et al., 2002; Yu et al., 2020a).

In situations marked by inflammation, such as those observed in neurodegenerative disorders and DACD resulting from overactivation of the AGEs-RAGE interaction, there is an elevation in the expression of both the S100B protein and its receptor, RAGE, in neural and inflammatory cells. The continuous stimulation of RAGE by high levels of S100B triggers signaling cascades that promote NF-kB and excess ERK1/2 activation, resulting in elevated ROS generation and upregulated inducible nitric oxide synthase (iNOS) (Mattson et al., 2000). Astrocytes, the primary contributors to extracellular S100B, exhibit distinct responses to varying doses of S100B. Low doses stimulate astrocyte proliferation, whereas high doses induce their activation. Astrocytes also have a S100B mediated RAGE-dependent mechanism for reup-taking S100B itself in vesicles (Lasič et al., 2016). Similarly, at low concentrations, S100B attenuates STAT3-mediated microglia activation, while at high concentrations, it stimulates NF-κB- and AP-1-mediated increasing in cyclooxygenase-2 (COX-2), chemokine production, secretion, and the upregulation of chemokine receptors (Bianchi et al., 2010). These reactions collectively lead to neuro-inflammation, astrogliosis and microgliosis, contributes to neuronal toxicity, a decrease in neurogenesis, and ultimately, the decline in cognitive function (Figure 2.8) (Cristóvaõ & Gomes, 2019).

During the early stages of neurodegenerative conditions and cellular damage in the central nervous system, S100B is found in high concentrations in various biological fluids. This includes conditions such as AD, Parkinson's disease, malignant melanoma, ischemic stroke, and acute brain injury (e.g., traumatic injury), as well as psychiatric disorders like mood disorders, major depression, and schizophrenia (Heizmann, 2019). The cerebrospinal and blood levels of S100B are often correlated with the progression of these diseases. Therefore, the measurement of S100B levels in biological fluids is of great importance as a potential biomarker for indicating neural distress, aiding in prognosis and clinical diagnosis (Rothermundt et al., 2003; Winocur et al., 2001).

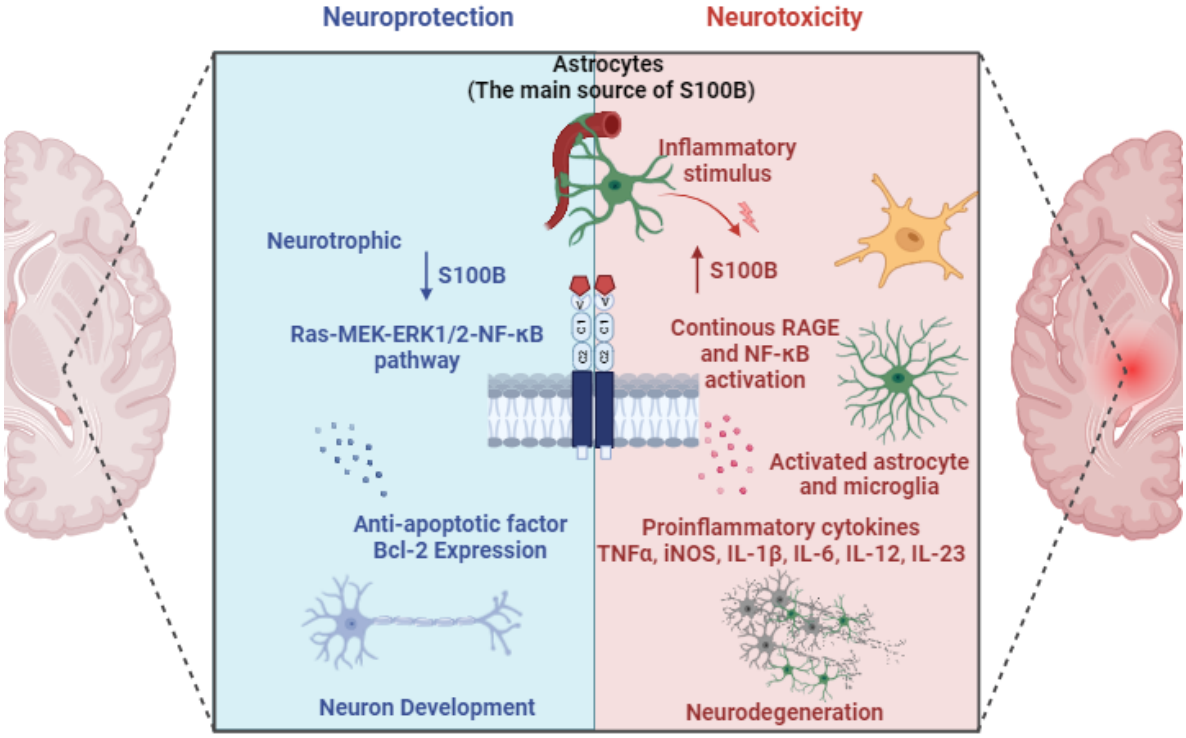

Figure 2.8. The effect of high and low concentration of S100B protein centrally.

2.3.4. S100A8/A9 physiological and pathological rules

S100A8, alternatively called myeloid-related protein-8 (MRP8), and S100A9, alternatively identified as myeloid-related protein-14 (MRP14), are natural molecules in our body that have both dependent and independent functions when they come together (heterocomplex formation) (Pruenster et al., 2016). Physiologically, the heterodimerization of S100A9 with S100A8 leads to the stabilization of S100A9, elongating its C-terminal α-helix and exposing important functional sites at the interface. This results in the formation of the heterodimeric complex S100A8/A9 (also known as calprotectin or MRP8/114), characterized by two high-affinity Zn2+-binding sites in the two proteins. This complex is involved in zinc sequestration, regulating crucial processes in the body by inhibiting certain

enzymes related to inflammation and angiogenesis, such as zinc-dependent enzymes and matrix metalloproteinases (Vogl et al., 2012).

Expressed predominantly within immune system cells like neutrophils, monocytes, and early macrophages, S100A8/A9 holds a crucial role in the differentiation of cells within the myeloid lineage (Zwadlo et al., 1988). Calprotectin can be released extracellularly either passively or actively, the latter induced by IFN-gamma, IL-1, and TNF-α (Suryono et al., 2005). Once released, calprotectin interacts with multiple cell membrane receptors, such as toll-like receptor 4 (TLR4), RAGE, and scavenger receptor CD36, activating NF-κB and p38 MAPK signaling pathways, thereby contributing to the inflammatory response (Vogl et al., 2007). Additionally, it can activate NADPH oxidase, leading to ROS generation in phagocytes (Catalán et al., 2011). At elevated concentrations, S100A8/A9 100A8/A9 has demonstrated the ability to inhibit the growth of normal cells and induce apoptotic effect in various tumor cell lines (Jukic et al., 2021).

Calprotectin serves as a recognized biomarker for both acute and chronic inflammation, with elevated levels observed in various inflammatory conditions, including obesity, insulin resistance, arthritis, and atherosclerosis (Bouma et al., 2004; Mortensen et al., 2009). Centrally, Mrp8 and Mrp14 have been observed to be expressed in microglia and neurons and identified as extracellular DAMP proteins (M. Wu et al., 2018). The S100A9/S100A8 complex's activation of microglia expressed TLR4 contributes to inflammation propagation, suggesting a significant role for calprotectin in both short-term and long-term neuroinflammatory conditions (Zervides et al., 2022).

2.4. Vitamin D3

The inactive form of vitamin D, Vitamin D3 (Vit-D3), or cholecalciferol (Figure 2.9), is obtained from sources like dairy products and synthesized through sunlight exposure from 7-dehydrocholesterol (7-DHC). Once acquired, it forms a complex with vitamin D-binding protein (DBP) in the circulation, transported to the liver, where it undergoes hydroxylation to produce the main circulating form, 25-hydroxyvitamin D3 (25(OH)Vit-D3) (D. Bikle et

al., 2013). Further hydroxylation in the proximal renal tubule by 25(OH)Vit-D31α hydroxylase generates the active form of Vit-D3, 1,25-dihydroxy vitamin D3 (1,25$(OH)_2$Vit-D3), also known as calcitriol (Jones et al., 2018).

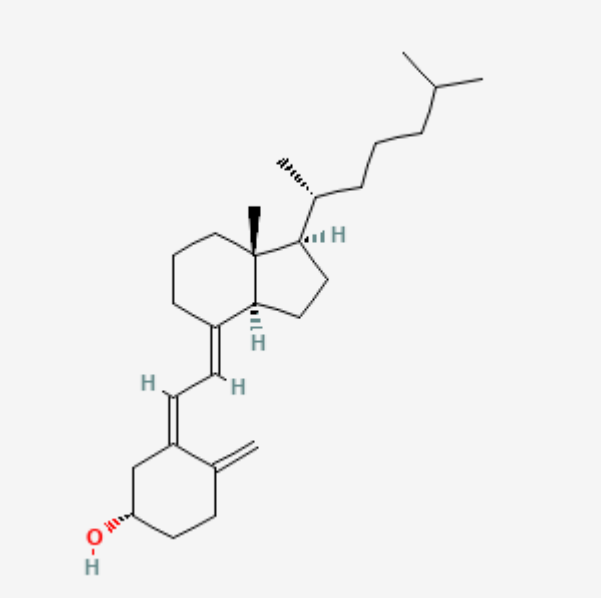

Figure 2.9. Cholecalciferol chemical structure.

The measurement of the blood level of 25(OH)Vit-D3, which serves as a reliable indicator of Vit-D3 level, has allowed researchers to identify optimal levels for various health outcomes A serum level of 50nmol/L is considered sufficient for the general population's needs. However, a level of 40nmol/L is the median concentration necessary for optimal calcium absorption and overall bone health, while a minimum level of 30nmol/L is recommended to stay within the healthy range (Calcium et al., 2011). To attain these optimal 25(OH)Vit-D3 levels, the estimated average requirement (EAR) is set at 400 International Units (IU) or 10 micrograms for all age groupings, while the recommended dietary allowance (RDA) ranges from 600 to 800 IU or 15 to 20 micrograms, with higher values recommended for older adults (Bresson et al., 2016).

Calcitriol, a secosteroid hormone, exerts various effects on the body. It operates on a genomic level through a nuclear steroid receptor known as the vitamin D receptor (VDR). The VDR comprises two significant functional domains: a DNA binding domain (DBD) at

the N-terminal and a ligand binding domain (LBD) at the C-terminal, connected by a hinge region. Interaction of 1,25$(OH)_2$Vit-D3 with the VDR triggers a conformational change enabling heterodimerization of 1,25$(OH)_2$Vit-D3-VDR with the retinoid X receptor (RXR) to increase the affinity for binding to specific regions in the DNA known as vitamin D response elements (VDREs), resulting in the selective recruitment of transcriptional coregulatory proteins which can either enhance gene activity (coactivators) or suppress it (corepressors), depending on the targeted genes (D. D. Bikle, 2021). Apart from its genomic actions through the VDR, 1,25$(OH)_2$Vit-D3 has also been found to exert rapid effects mediated by different receptors on the cell membrane, such as membrane-associated rapid response steroid binding protein (MARRSBP), also known as endoplasmic reticulum protein 57/60 (ERp57/ERp60). It is a G protein-coupled receptor that activates phospholipase C (PLC), leading to the release of molecules such as phosphatidyl inositol bisphosphate (PIP2), inositol trisphosphate (IP3), and diacylglycerol (DAG). This cascade of events could increase calcium levels by activating calcium channels (figure 2.10) (Nemere et al., 2004).

In addition to its well-known effects on mineral balance and normal bone development, Vit-D3 has been associated with several extra-skeletal effects. It may inhibit cellular proliferation and stimulate differentiation, making it a potential treatment for proliferative conditions like psoriasis (Brożyna et al., 2022). Adequate Vit-D3 levels have also been linked to decreased cancer incidence and improved cancer outcomes (Muñoz & Grant, 2022). Furthermore, Vit-D3 is involved in immune function regulation (Charoenngam & Holick, 2020; Hahn et al., 2022). Interestingly, Vit-D3 contributes to the regulation of hormone secretion, it has been found to reduce parathyroid hormone release (Cantley et al., 1985) and increase insulin secretion (S. Lee et al., 1994).

2.4.1. Vitamin D3 effect on diabetes

In relation to diabetes, Vit-D3 is essential for the propagation and differentiation of pancreatic β-cells, influencing the synthesis of insulin. Both animal studies and in vitro experiments with pancreatic beta cell cultures have demonstrated that 1,25$(OH)_2$Vit-D3 stimulates insulin secretion from these cells, possibly through an increase in intracellular

calcium levels (Lips et al., 2017a). Additionally, Vit-D3 may contribute to the improvement of insulin sensitivity through its anti-inflammatory properties, as suggested by increased markers of inflammation in those with Vit-D3 deficiency (Kolb Mandrup-Poulsen, 2005).

Insufficient Vit-D3 levels are consistently linked to reduced insulin release, insulin resistance, and higher fasting glucose levels. Epidemiological studies further associate lower 25(OH)Vit-D3 levels with an elevated probability of developing metabolic disorders and T2DM. Moreover, genetic variations in vitamin D-related genes may also contribute to susceptibility to poor glycemic control and T2DM (Oosterwerff et al., 2011).

Several clinical trials have explored the efficacy of Vit-D3 in improving glycemic control in T2DM patients or preventing diabetes progression from prediabetic conditions. Combining calcium and Vit-D3 supplements has shown promising results, leading to reduced serum insulin, HbA1c, and insulin resistance (Lips et al., 2017b).

2.4.2. Vitamin D3 effect on cognitive functions and DACD

Both 25(OH)Vit-D3 and 1,25$(OH)_2$Vit-D3 can traverse the BBB, and the central nervous system can locally activate and inactivate Vit-D3 (Sultan et al., 2020). Various roles for Vit-D3 in brain function have been identified. Deficiency during early life stages can lead to structural changes in the brain and behavioral issues (Cui et al., 2007). Vit-D3 deficiency is associated with an increased risk of cognitive impairment and is implicated in the pathogenesis and development of numerous neurological diseases, including cognitive impairment, dementia, and Alzheimer's (Herrmann et al., 2017). Additionally, patients with Alzheimer's or cognitive impairment tend to exhibit decreased levels of 25(OH)Vit-D3 (Anastasiou et al., 2014; Ouma et al., 2018). Research has also indicated that polymorphisms in the VDR gene are linked to cognitive dysfunction and diseases such as Alzheimer's, Parkinson's, and multiple sclerosis (Moretti et al., 2018).

Vit-D3 receptors are widely distributed in neurons and glial cells in different regions of the brain, suggesting that Vit-D3 has a neurodevelopmental role through its contribution to neuronal proliferation, stem-cell differentiation, and normal brain functioning and most

importantly in the hippocampus which is severely impacted by neurodegenerative disorders (Eyles et al., 2005). 1,25$(OH)_2$Vit-D3, through VDR, stimulates PI3K/AKT signaling in neurons, resulting in reduced gene expression of markers associated with AD, including the precursor of Aβ protein, GSK-3β, and Tau protein (Patel & Shah, 2017; Zaulkffali et al., 2019). Moreover, it has neuroprotective effects and can increase the generation of neurotrophic factors that support neuronal viability, neurite growth, differentiation, and synaptic plasticity (Quialheiro et al., 2023) with protection against apoptosis (Figure 2.10) (Latimer et al., 2014).

Furthermore, 1,25$(OH)_2$Vit-D3 activates the Wnt/β-catenin signaling pathway, enhancing cholinergic transmission, and ameliorating learning and memory deficits. This is achieved through the upregulation of gene expression for the α7 nicotinic acetylcholine receptor (α7nAChR) and increased levels of the choline acetyltransferase (CAT) enzyme (Kumar et al., 2011). The activation of Wnt/β-catenin signaling impacts the expression of cytokines. It elevates the levels of the anti-inflammatory cytokine IL-27 while reducing the levels of the proinflammatory cytokine IL-23. This shift in cytokine balance helps protect the integrity of the BBB and mitigates cognitive dysfunction (Muneeb et al., 2022a). On the other hand, Vit-D3 has a notable impact on regulating inflammation, apoptosis, and oxidative stress via the activation of MARRS. It serves as an anti-inflammatory agent, diminishing the synthesis of inflammatory substances such as TNF-α, IL-6, and NO. Also, its antioxidative characteristics aid in managing ROS by lowering their concentrations and enhancing the ROS scavenger, glutathione. Additionally, it prevents lipid peroxidation and apoptosis and repairs mitochondrial dysfunction (Gáll & Székely, 2021).

Therefore, understanding the importance of vitamin D in brain health can provide insights into potential therapeutic strategies for neuroinflammatory conditions.

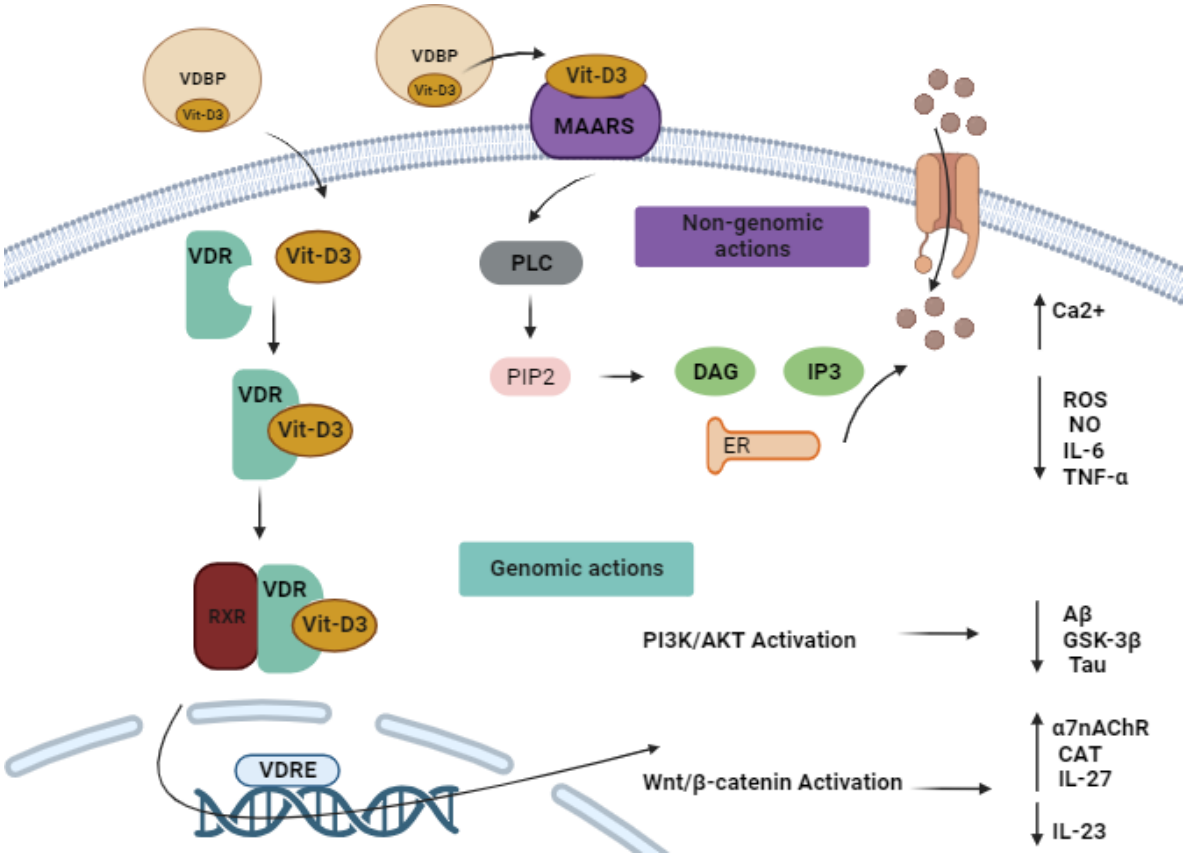

Figure 2.10. Vitamin D3 genomic and non-genomic central effects.

2.5. Animal Models of Diabetes Mellitus

Animal models are extensively used in experimental studies in laboratories to gain insights into human biological functions. Diabetes induction in these models involves two main mechanisms: genetic manipulation or the administration of specific chemical compounds to mimic patterns observed in humans, including hyperglycemia, a key clinical manifestation of the disease, along with associated complications. Various animal models are available for diabetes research, depending on the type of diabetes under investigation. For T1DM, characterized by insulin deficiency, chemical agents damaging pancreatic β-cells or breeding autoimmune diabetic rats can induce the condition. T2DM, marked by fluctuating insulin levels, insulin resistance, and obesity, is studied using diverse models that include

both obese and non-obese animals with varying levels of insulin resistance and β-cells dysfunction (Kottaisamy et al., 2021; P. Sharma et al., 2016).

2.5.1. Streptozotocin-induced diabetes

Streptozotocin (STZ), a metabolite derived from *Streptomyces Achromogenes*, a soil microorganism, was initially recognized in 1960 for its antibiotic, antitumor, and carcinogenic properties. Later, in 1963, it was identified as a diabetogenic substance in dogs and rats (J. Wu & Yan, 2015). STZ, in its solid state, has α and β isomers, but it is unstable and should be stored frozen and shielded from light. In aqueous solutions, it quickly decomposes, with optimal stability at pH 4 using a citrate buffer (Ogbonnaya Eleazu et al., 2013).

STZ causes toxic effects on pancreatic β-cells through various mechanisms. As a glucose derivative, it binds to glucose receptors on the cell membrane, inhibiting insulin secretion triggered by glucose. Inside the cell, streptozotocin decomposes, producing reactive carbonyl ions that alkylate DNA bases, causing DNA damage and initiating a repair process that depletes cellular substrate Nicotinamide adenine dinucleotide (NAD), leading to cell death. Additionally, STZ's oxidizing properties reduce levels of cellular antioxidants like SOD and glutathione. Its selective toxicity to the pancreas is due to entering β-cells through a glucose-mediated mechanism. However, STZ also has nephrotoxic and hepatotoxic effects and can cause local tissue ulceration and necrosis if extravasation occurs (Lenzen, 2008).

The diabetogenic dose of STZ is around 65 mg/kg, administered either intraperitoneally or intravenously. STZ-induced diabetes is an effective model in various experimental animals, including rodents, rabbits, guinea pigs, dogs, cats, and monkeys. Repeated dosing in mice, at 40 mg/kg per day over five consecutive days, mimics aspects of T1DM, leading to pancreatic changes and a cellular immune response similar to human T1DM (Kottaisamy et al., 2021).

STZ is preferred over alloxan for inducing experimental diabetes due to its longer half-life and greater stability. STZ-induced hyperglycemia lasts for several months, compared to the shorter duration of hyperglycemia caused by alloxan, which is less than a

month. STZ is also more selective in targeting islet β-cells, causing less damage to other cell types and resulting in lower animal mortality compared to alloxan, which is associated with higher mortality due to ROS production and ketosis (Ighodaro et al., 2017). Additionally, STZ-induced diabetes provides a suitable model for studying diabetes-associated complications (Lenzen, 2008).

Diabetic animal models are pivotal in advancing our understanding of the pathophysiology and molecular mechanisms of diabetes and associated complications, as well as in investigating novel biomarkers and new therapeutic interventions. A comprehensive analysis of published literature on selected models indicates that the STZ diabetic rat has received the highest number of publications on PubMed over the past decade (Pandey & Dvorakova, 2019).

3. MATERIALS AND METHODS

3.1. Research Design

The behavioural and experimental components of the study were carried out in various departments of Pamukkale University, Faculty of Medicine. The behavioural part was carried out in the Laboratories of the Anatomy Department. The rotarod test was performed in the Behavioural Pharmacology Laboratory of the Medical Pharmacology department, while the experimental part took place in the Experimental Research Laboratories of the Experimental Surgery Practice and Research Centre. Finally, the biochemical measurements were performed in the Physiology Department Laboratories.

Before starting, the study received approval from the Pamukkale University Animal Experiments Ethics Committee (PAUHDEK-2022/29), and all ethical guidelines were strictly adhered to throughout the study. The relevant ethics committee document is included in Appendix-1.

The experimental animals were housed in plastic top-wired cages (30x35x17 cm) filled with sawdust, with a wire top, in groups of two in a controlled environment in the experimental animal's unit with a temperature of 22±1°C and humidity of 50±5%, and a 12-hour light/dark cycle. The cages were cleaned three times per week, and the rats were provided with a standard diet and water, except during the restriction period, which was 6-8 hours prior to STZ injection, FBG measurements, and during food restriction for the RAM test. All procedures performed on the animals were supervised by a veterinarian and were in accordance with considerations for hygiene.

3.2. Chemicals

3.2.1. Chemicals used in vivo.

Phosphate buffered saline (PBS) 1xPBS 500 ml (MULTICELL Cat#.311-010-CL, LOT NO. 311010126), Streptozotocin (Santa Cruz Biotechnology (U-9889) Cat#.SC-200719, LOT NO. H1413 store at -20°C 1g), Devit-3 (Vitamin D3 50,000 I.U./15ml oral drop) (Deva Holding LOT NO. A104840 store at room temperature below 25°C), Ketalar (Pfizer, Ketamine 50 mg/ml), and Ksilazol (Provet, Xylazine 20 mg/ml).

3.2.2. Chemicals used in vitro.

Phosphate buffered saline (PBS) 1xPBS 500 ml (MULTICELL Cat#.311-010-CL, LOT NO. 311010126), BioVision EZBlockt™ Protease Inhibitor Cocktail VI (Cat#. K291-1, LOT NO. 3F19K02910, store at -20°C.

3.3. Experimental Animal Groups and Experimental Procedures

The study involved 4-5 month 35 Wistar albino male rats, weighing 300-450 grams, obtained from Pamukkale University, Faculty of Medicine, Experimental Surgery Practice and Research Center. The rats were randomly placed in their cages and their weights were measured. In the preliminary study, 3 rats were tested for diabetes using STZ with a single intraperitoneal dose of 65 mg/kg for 4 weeks.

The remaining 32 experimental rats were divided into three groups:

Group (1): the control group (n=8): rats in this group were injected intraperitoneally (i.p.) with 0,5 ml of the STZ solvent (Phosphate Buffered Saline (PBS) PH: 4,5-5,5 adjusted with Hydrochloric acid) on the 4th day of the study.

Group (2): Streptozotocin (STZ)-induced diabetic group (n=12, considering that there may be an animal loss, the number (n) was kept as 12): rats in this group were injected with a single dose of 65 mg/kg STZ i.p. on the 4th day of the study.

Group (3): Vitamin D3 (Vit-D3) group (n=12): same as group (2) firstly rats in this group were injected with a single dose of 65 mg/kg STZ i.p. on the 4th day of the study. After that Vit-D3 was administered orally using a pipette at a dose of 12,5 µg/kg/day (500 IU/kg/day) 1 week after STZ injection for 4 weeks (From the 12th day to the 39th day of the study).

The learning process in the eight-arm radial maze (RAM) test is driven by the food-seeking instinct, therefore, all rats were subjected to a food restriction procedure 2 weeks before and during the RAM test allowing the rats to lose 10-15% of their normal weight during the test. In this procedure, the daily amount of feed consumed by each rat was calculated. This amount was reduced by 10-15%, and weekly weight monitoring of the rats was carried out. This practice is below the 20% feed restriction limit specified in the ethical rules. No water restriction was applied throughout the experiment.

After 1 week of food restriction rats were pre-trained for three days and their motor performance was tested using the rotarod apparatus prior to RAM pre-training, after 3 weeks from diabetes induction, and before decapitation.

3.4. Study Protocol

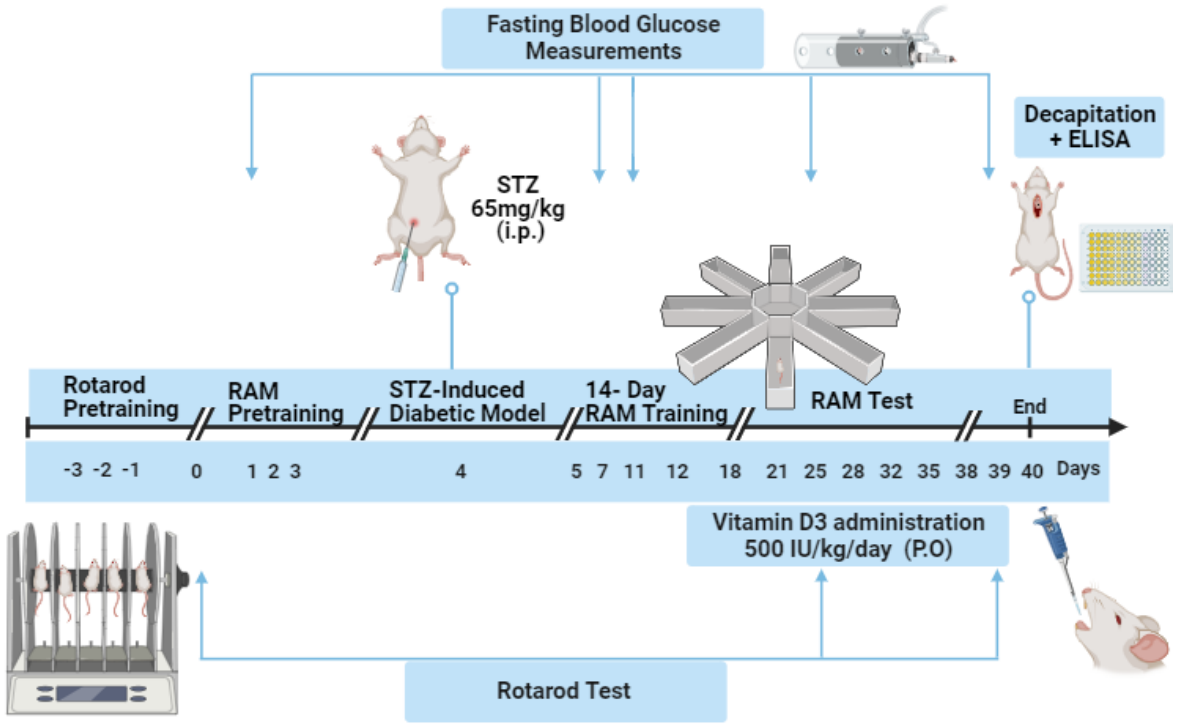

Figure 3.1. Study protocol.

3.5. Streptozotocin-Induced Experimental Animal Diabetic Model

The Streptozotocin-induced diabetic model is a widely used experimental animal model to study diabetes. In this model, the dose and protocol of STZ administration vary for different experimental animals. However, the most used dose for diabetes induction in rats is administering a single intraperitoneal injection of 65mg/kg (Furman, 2021).

For each rat in groups 2 and 3 in our study, according to the weight of the rat the dose of STZ was calculated and prepared by weighing the required amount of STZ, which was

stored in 1g vials at 4°C, using a precision scale. The calculated amount was then dissolved in 0,5 ml of PBS of PH 4,5 adjusted using hydrochloric acid and injected i.p. (Figure 3.2). An equivalent amount (0,5 ml) of PBS was injected i.p. into the control group rats.

To prevent animal loss due to hyperinsulinemia and hypoglycaemic shock, which are expected within the first 24 hours after STZ injection, 5% glucose solution was placed in the cages of the rats that received STZ (groups 2 and 3) within 24 hours after injection (Calgaroto et al., 2014; Muneeb et al., 2022b).

Figure 3.2. Intraperitoneal STZ injection

3.5.1. Fasting Blood glucose measurement

The measurement of fasting blood glucose (FBG) levels was conducted using the Accu-Chek® Performa Nano CAT/TYP 59105061195 brand glucometer (Figure 3.4), with strips of the same brand utilized for accurate measurement. In each measurement rats were

fasted for 8 hrs; blood samples were obtained using an insulin needle from the tail vein of the rats after inducing vasodilation through warm water (Figures 3.3 and 3.5).

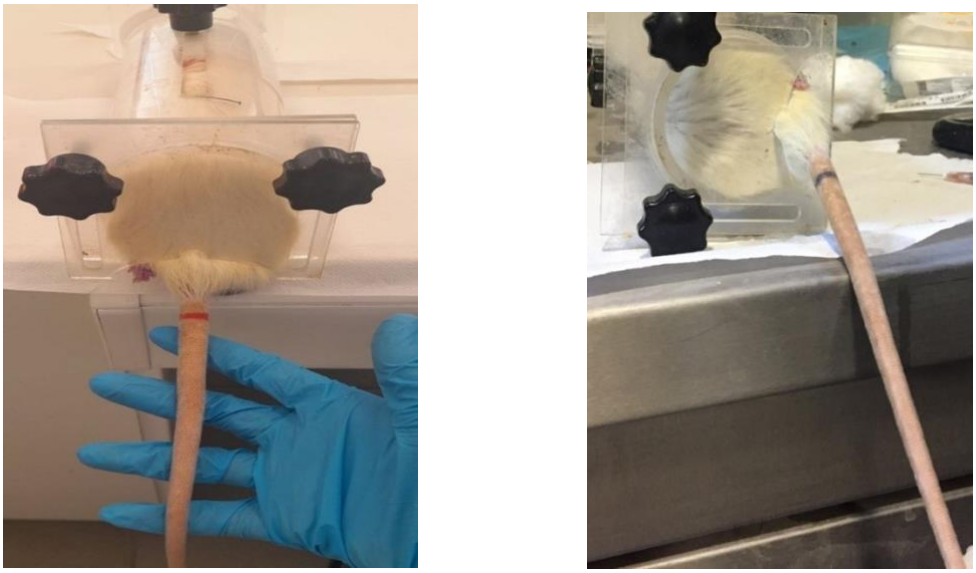

Figure 3.3. Rat inside the restrainer with clarified lateral vein.

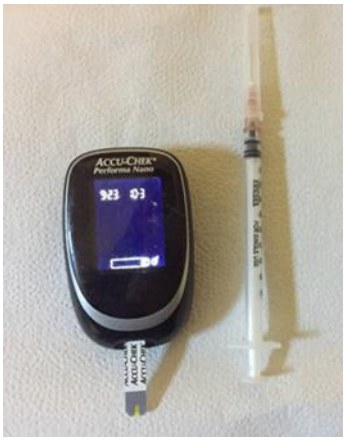

Figure 3.4. Accu-Chek® Performa Nano glucometer.

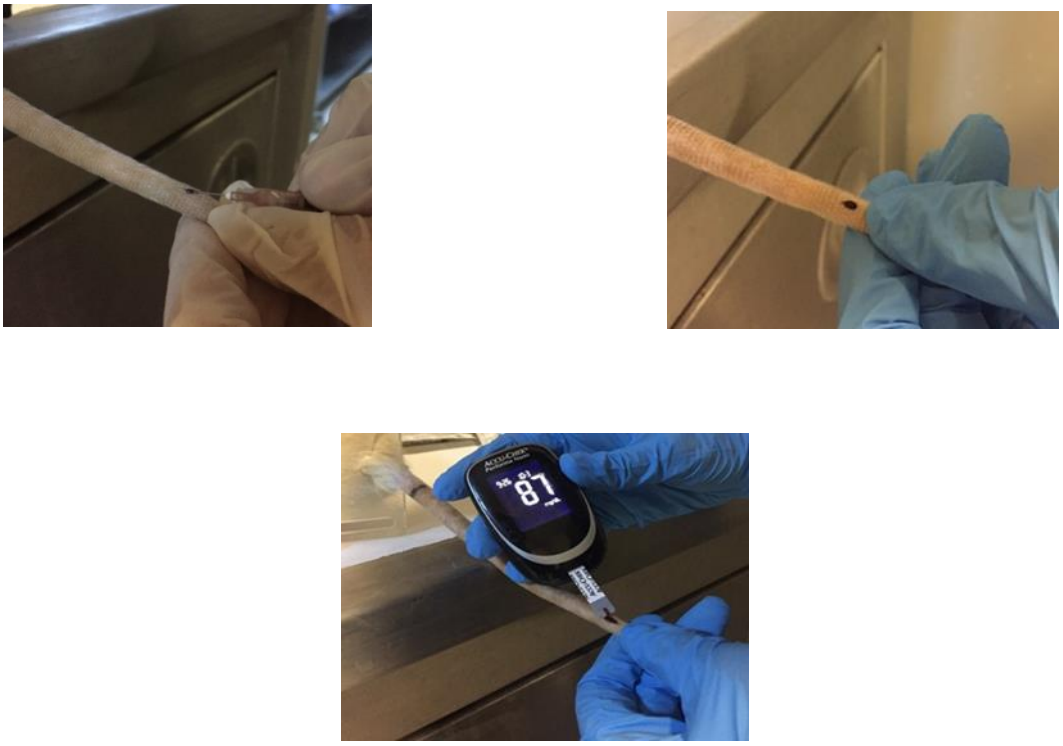

Figure 3.5. Steps of fasting blood glucose measurement.

3.5.2. Evaluation of diabetes model

Our diabetes model was evaluated by monitoring the rats daily and measuring their body weights weekly using an analytical balance. The Accu-Chek® Performa Nano was used to record the FBG level of the rats, which was measured on the 1st day of the study, the 3rd day, the 7th day, and the 21st day after STZ administration, and before decapitation.

Rats with FBG levels exceeding 250 mg/dL measured on the 7th day after STZ injection, in comparison to their levels on the 1st and 3rd day, were classified as diabetic, in group 2, 10 rats out of 12, and in group 3, all rats developed diabetes.

Also, the presence of polyuria and polydipsia symptoms were investigated.

3.6. Vitamin D3 Treatment

For group 3, Vit-D3 administration commenced after diabetes was established, from the 12th day of the study, lasting for 28 consecutive days (Kumar et al., 2011; Mitrašinović-Brulić et al., 2021) i.e., until the end of the study. A dose of 12,5 µg/kg/day (500 IU/kg/day) (Muneeb et al., 2022b) of Vit-D3 (Devit-3) was calculated for each rat and administered orally via pipette (Figure 3.6).

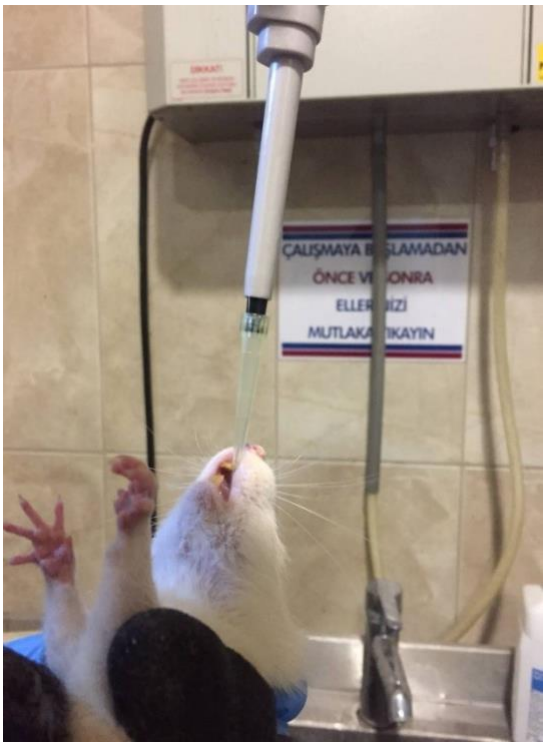

Figure 3.6. Vitamin D3 administration using100ul pipette.

Decapitation was carried out on all rats from the different groups on day 40, following the completion of the memory studies.

3.7. Motor Performance

In the field of behavioural neuroscience, it is crucial to ascertain the presence of motor abnormalities prior to conducting evaluations of cognitive functions such as memory. It has been demonstrated that performance in behavioural tests that require strength and coordination, such as the Morris water maze and the eight-arm radial maze, may be negatively impacted by motor impairments rather than cognitive deficits in rats. The inclusion of animals with motor impairments in behavioural experiments may lead to inaccurate results, as alterations in motor performance have the potential to significantly affect maze performance. Previous research has also indicated that sensorimotor disorders can significantly impact maze performance (Deacon, 2013). Therefore, in this study, the motor coordination and performance of experimental animals were assessed using the rotarod performance test prior to and during the eight-arm radial maze. Specifically, all rats underwent training and testing by the rotarod performance test prior to initiation of the study and were retested during the maze on the 25th and the 39th day of the experiment.

3.7.1. Rotarod apparatus

The rotarod apparatus operates on the principle that animals can maintain a certain walking speed and height on a rotating shaft powered by electricity for a specified duration, without losing balance or falling. The device consists of four adjacent compartments, each measuring 15 cm in width, 30 cm in depth, and 50 cm in wall height (Figure 3.7). To prevent falling, the animal strives to walk in the opposite direction to the rotation of the shaft.

Figure 3.7. Motor performance measurement using rotarod apparatus.

3.7.2. Motor performance evaluation protocol

3.7.2.1. Pre-training

Prior to the study initiation, pre-training was carried out on rats in each group. The rats were allowed to familiarize themselves with the rotarod apparatus without rotation for a period of two minutes (Shabani & Mirshekar, 2018). Subsequently, the pre-training was conducted for three consecutive days, three times daily at 20-minute intervals at a speed of 5 rpm for 300 seconds, in order to facilitate walking and training (Monville et al., 2006). Inclusion in the study required rats to be able to remain on the rotarod system for a minimum of 120 seconds at the commencement of the study (Coelho et al., 2016).

3.7.2.2. Motor performance measurement

The motor performance of the rats in each group was assessed at the beginning of the study (day 0), 21 days after STZ administration (day 25), and following Vit-D3 treatment

(day 39). This was done by utilizing the rotarod apparatus at a speed of 10 rpm for 300 seconds, three times a day at 20-minute intervals. The duration of time that the rat was able to remain on the spindle was recorded as the rotarod motor performance value (R. Tan et al., 2018).

3.8. Behavioral Test – Eight-Arm Radial Maze

3.8.1. General concept

The cognitive function of all rats involved in the study was assessed using the eight-arm radial maze (RAM) test, which is commonly utilized to evaluate spatial learning and memory (Stanojevic et al., 2022; Xu et al., 2019). Preceding the learning stage, the rats underwent a three-day pre-training section with a one-day interval. During the learning phase, the rats were trained for 14 days with three sessions per day, spaced one hour apart. Each session was terminated when the rats visited all arms or after 10 minutes. Rats that made no or only one mistake in the last three days of the learning period were selected for the study. Performance evaluation is based on the placement of bait in the arms.

Correct arm choices are defined as one entry per arm when the bait was present in all arms, and this is considered a reference (long-term) memory indicator. Re-entry into the previously visited arm is regarded as an incorrect choice and reflects working (immediate or short-term) memory impairment. Entry into an arm without bait, even when the bait was present in certain arms, indicated reference memory impairment. A single entry into the bait arm indicated intact reference memory, while re-entry indicated impairment of short-term memory (Figure 3.8).

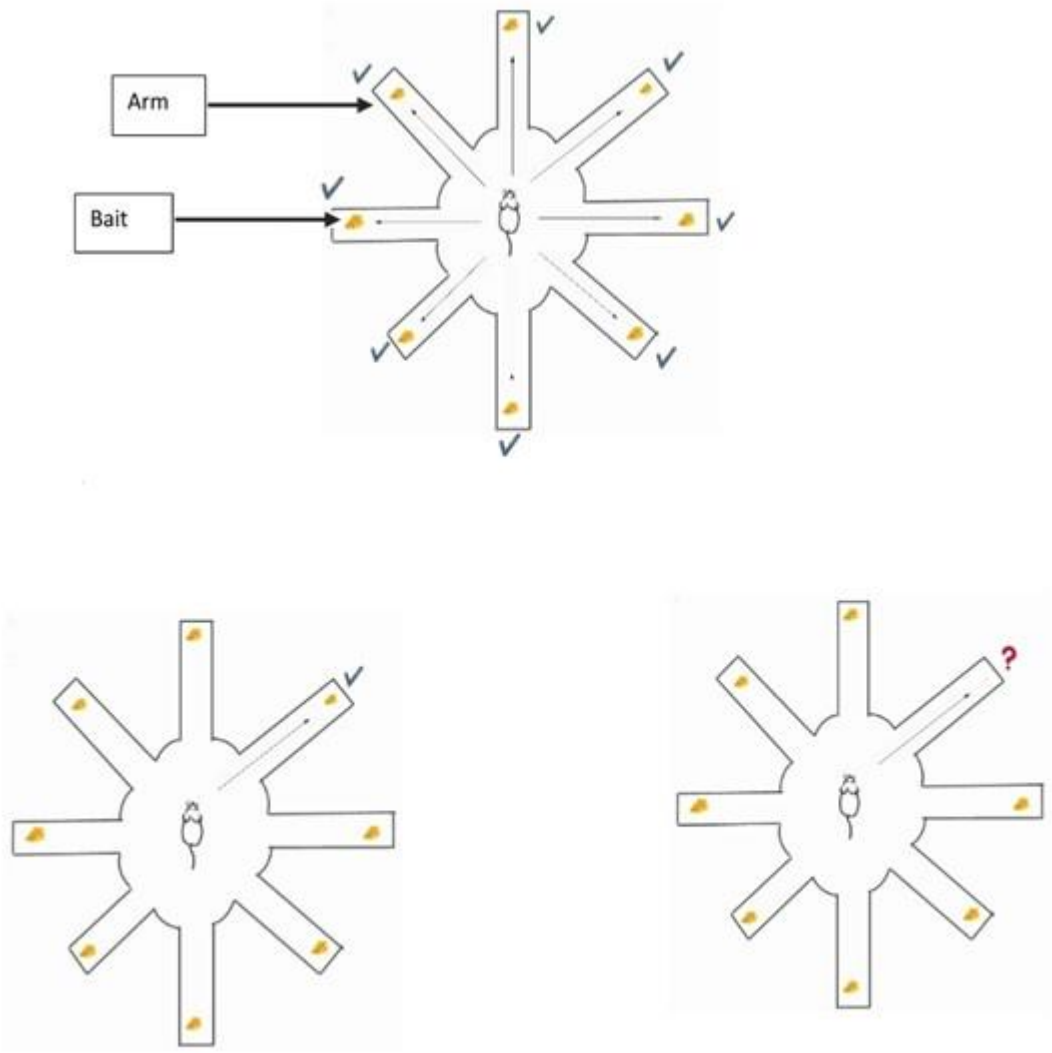

Figure 3.8. Schemes representing eight-arm radial maze with the presence of bait in each arm and the concept of correct arm choice.

3.8.2. Eight-arm radial maze:

The RAM device utilized in the test consisted of eight arms attached to all sides of an octagonal central platform with a diameter of 24 cm to control releasing of animals from this central area and their exposure to the different arms. Each arm was 10 cm wide and 50 cm long and was enclosed by a 50 cm high plexiglass wall, with feed containers of 3 cm diameter and 1 cm depth at the end of each arm. The device was placed 50 cm above the ground, and each arm was numbered 1-8 in order (Figure 3.9). Brown sugar, weighing 50-60 mg, was left in the feed containers at the end of each arm. Fixed visual cues with different colours were placed at least 50 cm away from the edges of the test device on the four walls of the testing room so that the rats could see them to help the rats find their way. The arms and feed containers were cleaned with alcohol after each animal finished testing so that the rat subjected to the test to not be affected by the urine and faeces of the previously tested rat and be able to smell the food more easily.

The room where the experiment was carried out was a quiet and bright room. All test images were transferred to a computer via a web camera placed 2m above the device, and data were recorded using Amonra Stopwatch software that recorded the rats' visits to each arm as triplet "time-selection number-arm number" (Egashira et al., 2018).

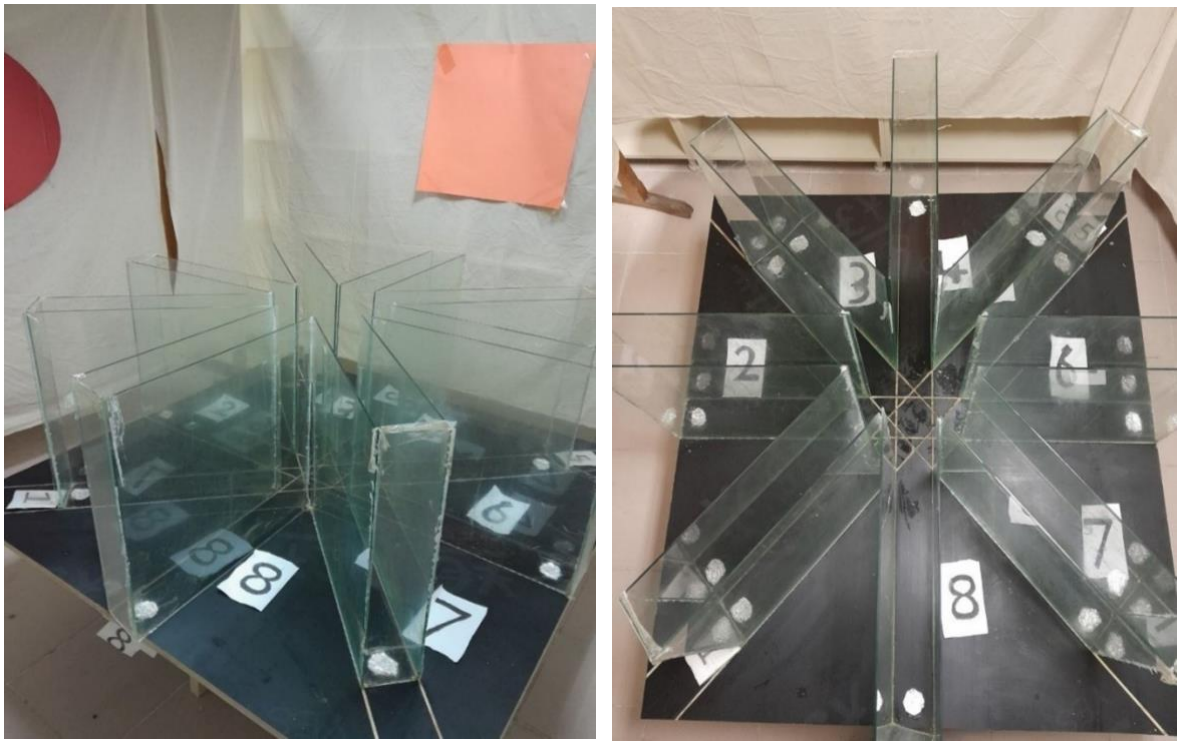

Figure 3.9. Eight-arm radial maze (RAM)

3.8.3. The procedure

The RAM test was divided into three sections: the pretraining section, the learning (14-day training) section, and the post-learning evaluation or test section.

The pretraining section aimed to acclimatize the rats to the test device and the environment. Rats were placed in the device in groups of 4 at one-hour intervals, three times a day, for three consecutive days (from day 1 to 3 of the study), with each round limited to 10 minutes. Following the pretraining section, there was a one-day break for PBS injection for the group (1) or STZ injection for groups (2 & 3), after which the training section commenced.

The training section aimed to teach rats how to navigate the RAM test. The test was applied to each rat individually, three times a day, at one-hour intervals, for 14 consecutive days (from day 5 to 18 of the study), with each test round limited to 10 minutes. If a rat visited all eight arms, the test was terminated without waiting for 10 minutes. Rats that made 7 or 8

correct selections within the first eight selections in any round on day 14 were considered learned and were selected for the experiment. Rats that could not meet this criterion were excluded from the study. The evaluation was carried out by comparing the election-day data with the post-learning data.

In the post-learning section, rats were subjected to a memory assessment test based on the same parameters as those used in the training section. This test was conducted on the 3rd, 7th, 10th, 14th, 17th, and 20th days following the final day of the learning section, corresponding to the 21st, 25th, 28th, 32nd, 35th, and 38th days of the study, respectively. The rats' memory was evaluated based on their first eight choices.

3.8.4. The evaluation criteria

3.8.4.1. Number of correct arm choices

In our study, all arms of the maze contained bait, and it was expected that the rat would enter each arm once without making any errors. The correct arm choice was defined as the number of arms entered once by the rat. Specifically, if the rat correctly entered seven arms but failed to enter the eighth arm, the correct arm choice was scored as seven. On the other hand, if the rat did not enter any of the arms during the 10-minute period, it was scored as zero. For instance, if the bait was placed in all eight arms of the maze, and the rat entered the third arm once, the fifth and sixth arms twice each, and the seventh arm once, without entering the first, second, fourth, and eighth arms, the number of correct arm choice would be two.

3.8.4.2. Latency

Response delay or latency, defined as the total time spent in the maze, is calculated as follows: if the animal entered all eight arms in less than 10 minutes, the time spent entering

all arms is measured in seconds. However, if the animal did not enter all arms within 10 minutes, the experiment is terminated, and the latency is recorded as 10 minutes (600 seconds).

3.9. Decapitation Scarification and Collection of Serum and Hippocampus Samples

On the final day of the study protocol (day 40), after cognitive function and motor performance tests were conducted on rats, the rats in each group were anesthetized using a mixture of ketamine hydrochloride (80 mg/kg) and xylazine hydrochloride (10 mg/kg) i.p., in accordance with their measured body weights (Demircan et al., 2020; Derakhshanian et al., 2019). To ensure deep anaesthesia, rats were decapitated only after non-responsiveness to claw compression with forceps was confirmed.

Figure 3.10. Preparations for decapitation.

After decapitation, an anterior thoracotomy was performed, and intracardiac blood samples were taken by intracardiac puncture using a 10 ml syringe (Figure 3.11).

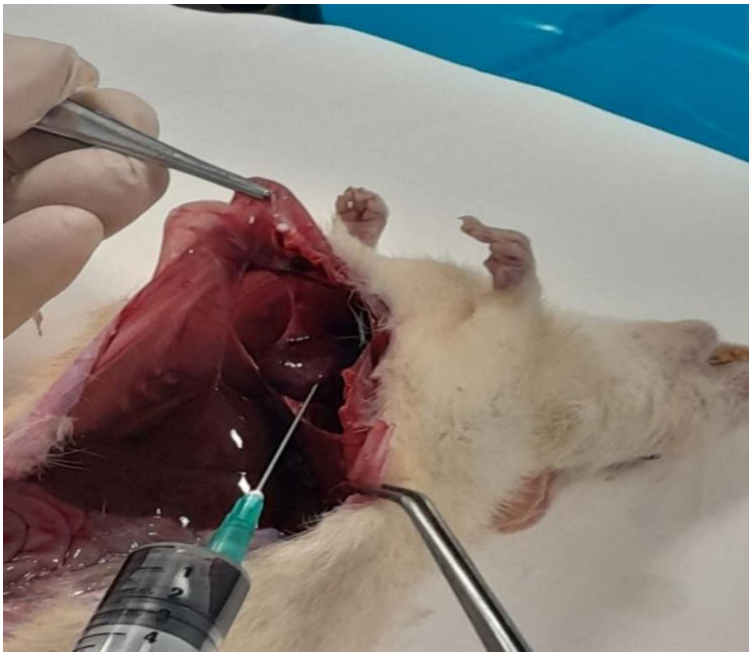

Figure 3.11. Intracardiac blood samples collection.

The collected blood samples were centrifuged at 7000 rpm for 5 minutes to separate the serum. The serum was then frozen at -80°C until further analysis for insulin, RAGE, sRAGE, S100B, and calprotectin (S100A8/S100A9) using Enzyme-Linked Immunosorbent Assay (ELISA) method as per the manufacturer's instructions.

Left hippocampus dissection from the brain was performed rapidly after decapitation without any loss and the samples were weighed and transferred to 1,5 ml microcentrifuge tubes to be dissected into small pieces and rinsed in 1 tablet protease inhibitor-containing cold PBS (0,01M, pH=7,4) (tissue weight (g): PBS (ml) volume=1:9) to remove any excess blood and then were homogenized using a glass homogenizer on ice (Figure 3.12).

Figure 3.12. Left and right hippocampus homogenization.

The homogenates were then centrifuged for 15 minutes at 12000 rpm at 4°C to obtain the supernatant. Supernatant and serum samples were stored in a deep freezer at -80°C until further molecular analysis for insulin, RAGE, S100B, and calprotectin (S100A8/S100A9), and levels using ELISA kits according to the manufacturer's instructions.

3.10. Enzyme-Linked Immunosorbent Assay

In this study, various biomarkers were measured in both serum and hippocampus samples using commercially available ELISA kits. Specifically, Rat Insulin ELISA kit (INS) (Bioassay Technology Laboratory, China. Cat# E0707Ra), Rat Receptor for Advanced Glycation End Products ELISA kit (RAGE) (Bioassay Technology Laboratory, China. Cat# E0928Ra), Rat Soluble Receptor for Advanced Glycation End Products ELISA kit (sRAGE) (Bioassay Technology Laboratory, China. Cat# E1234Ra), Rat S100-B Protein ELISA kit (S100B) (Bioassay Technology Laboratory, China. Cat# E1360Ra), Rat Calprotectin ELISA

kit (Calp) (Bioassay Technology Laboratory, China Cat#E1210Ra), each kit came with its own manual, which was followed precisely.

To prepare the standards, different standard concentrations were used for each biomarker with its own kit. For INS, standard concentrations of 24, 12, 6, 3, 1,5 mIU/L were prepared from the 48 mIU/L standard in the INS kit, using the standard dilution, respectively. For RAGE, standard concentrations of 3200, 1600, 800, 400, 200 ng/L were prepared from the 6400 ng/L standard in the RAGE kit, using the standard dilution, respectively. For sRAGE, standard concentrations of 8, 4, 2, 1, and 0,5 ng/mL were prepared from the 16ng/mL standard in the sRAGE kit, using the standard dilution, respectively. For S100B protein, the standard concentrations of 800, 400, 200, 100, and 50 pg/ml were prepared from the 1600 pg/ml standard in the kit, using the standard dilution, respectively. For CALP protein, standard concentrations of 160, 80, 40, 20, 10 ng/mL were prepared from the 320 ng/mL standard in the Calp kit, using the standard dilution, respectively.

To carry out the measurements, 96-well plates were used, and 50 µL of standards were added in duplicate. Since there is a biotinylated antibody in the standard solution, no additional biotinylated antibody was added. Then, both hippocampus (in triplicate manner) and serum samples (in duplicate manner) were added in 40 µL volume to each well, followed by adding 10 µL of the biomarker antibody. The plate was then incubated at 37°C for 60 minutes after closing with a sealer. Subsequently, 50 µL of streptavidin-HRP was added to the wells containing the standards and samples (however, the blank was not added to the well).

After mixing well, the plate was incubated at 37°C for 60 minutes, then the sealer was removed, and the wells were washed 5 times with kit wash buffer. In each wash, the wells were soaked with 300 µL – 350 µL wash buffer and were kept for 30 seconds to 1 minute. The wash buffer was then poured, and the plate was blotted onto absorbent paper. For each well, 50 µL of substrate solution A was added first, then 50 µL of substrate solution B. The plate was then covered with a new sealer and incubated in the dark at 37°C for 10 minutes.

At the end of the incubation period, 50 µL of stop solution was added to each well. This caused the blue colour to change immediately to yellow. The optical densities of the plates were measured using a microplate reader (Thermo Fisher Scientific, UK) at 450nm

wavelength within 10 minutes, and the standard curve was used to calculate the concentrations of the samples with the assistance of SkanIt Software 4.1 for statistical analysis.

3.11. Statistical Analysis

For the statistical analysis of the data obtained in the study, SPSS 25.0 (IBM SPSS Statistics 25 Software, Armonk, NY: IBM Corp.) was utilized. The data for each group were expressed in the table as mean ± standard error and presented graphically. Except for ELISA and rotarod data, One-Way Analysis of Variance (ANOVA) was employed for data that followed a normal distribution to compare independent group differences. However, for results that did not pass the normality test, the Kruskal–Wallis's test was used. t-test was employed for ELISA and rotarod results. Values with $p<0,05$ in the statistical analysis were considered statistically significant.

4. RESULTS

4.1. Streptozotocin-Induced Diabetic Model

4.1.1. Weight of rats

The weights of the rats in each group were measured using a digital balance initially and weekly throughout the 7 weeks of the experiments: 1st week (before the start of Rotarod training), 2nd week (before the commencement of the 14-day training section of the RAM test), 3rd week (10th day of the 14-day training section of the RAM test), 4th week (the end of the 14-day training section of the RAM test), 5th week (the 25th test day of the RAM test), 6th week (the 32nd test day of the RAM test), and the 7th week, with pre-decapitation weights.

The initial weights of rats in the control, STZ, and Vit-D3 groups were 379,88 ± 11,89 g, 377,5 ± 6,40 g, and 429,45 ± 10,64 g, respectively. After 7 weeks, the weights of rats were 354,13 ± 11,10 g for the control group, 336,92 ± 13,76 g for the STZ group, and 309,45 ± 8,19 g for the Vit-D3 group. The percentage of weight loss was -6,69 ± 1,66, -10,81 ± 3,03, and -27,82 ± 1,45 for the control, STZ, and Vit-D3 groups, respectively.

Rats in the STZ and Vit-D3 groups significantly showed weight loss at the end of the experiment compared to the control group ($p<0,05$, $p<0,0005$ respectively, ANOVA). There was also a significant difference in body weight loss between the STZ and Vit-D3 groups at the end of the experiment ($p<0,005$, ANOVA). These data are shown in (Table 4.1) and represented graphically (Figure 4.1).

Table 4.1. Measurement of the average body weight of rats.

Weeks / Groups	Control	STZ	Vit-D3
Initial weight	379,88 ± 11,89	377,5 ± 6,40	429,45 ± 10,64
Week 1	371,13 ± 13,83	364 ± 6,32	410,55 ± 9,25
Week 2	363,38 ± 13,98	351,92 ± 5,34	386,36 ± 10,20
Week 3	363,13 ± 13,94	342,5 ± 6,79	347 ± 8,18
Week 4	356,75 ± 10,74	337,25 ± 8,68	324,64 ± 8,84
Week 5	354 ± 11,61	333,17 ± 10,29	305,36 ± 9,25
Week 6	348,63 ± 11,00	320,08 ± 11,71	293,64 ± 8,23
Week 7	354,13 ± 11,10	336,92 ± 13,76	309,45 ± 8,19
% Weight loss	-6,69 ± 1,66	-10,81 ± 3,03*	-27,82 ± 1,45$^{***, \#\#}$

($^{*}p<0,05$, $^{***}p<0,0005$ significant difference compared to the control group).
($^{\#\#}p<0,005$ significant difference between STZ and Vit-D3 groups).

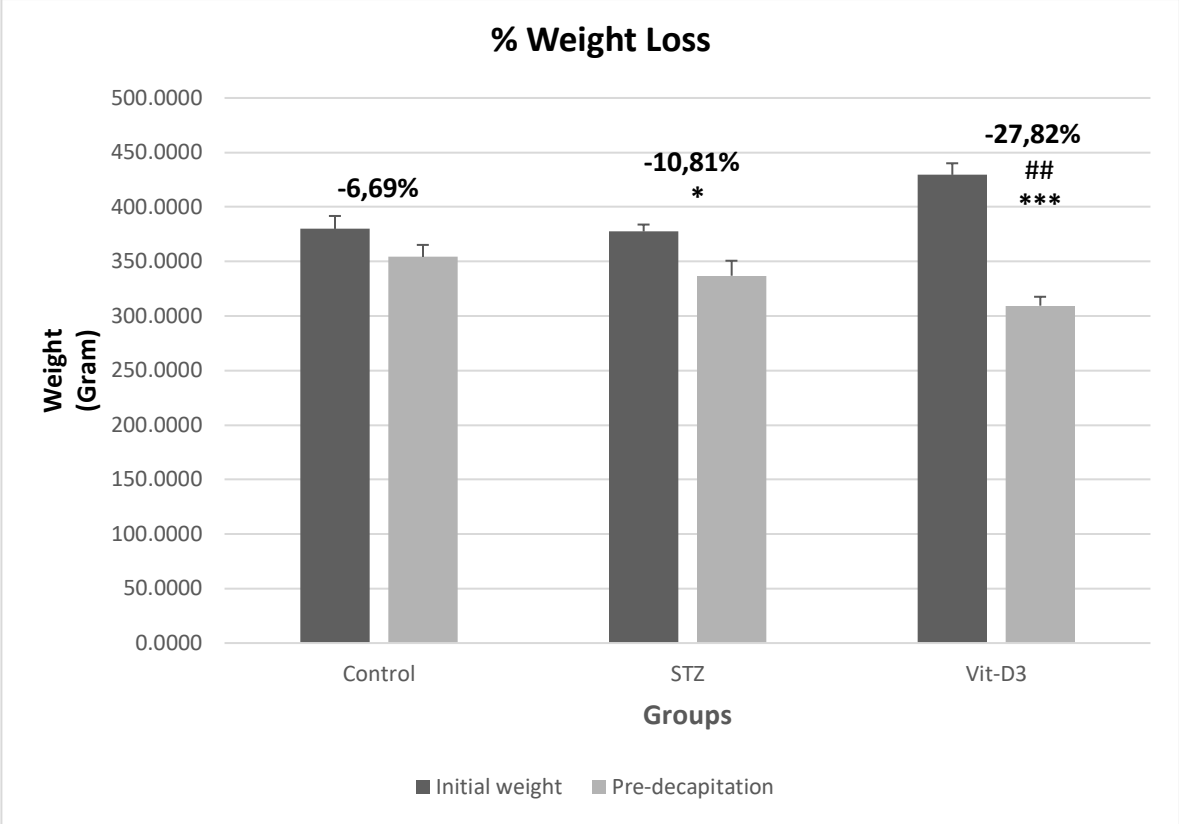

Figure 4.1. % Change in body weight in rats of different groups.

(*p<0,05, ***p<0,0005 significant difference compared to the control group).
($^{\#\#}$p<0,005 significant difference between STZ and Vit-D3 groups).

4.1.2. Fasting blood glucose (FBG)

The FBG levels of rats in each group were measured on the 1st, 7th, 11th, and 25th day of the study and before decapitation. There was no significant difference in the initial FBG levels among rats of different groups at the start of the experiment (p>0,05, ANOVA). Rats in the STZ and Vit-D3 groups showed significantly higher FBG levels compared to the control group on the 7th, 11th, and 25th day of the study (p<0,0005, ANOVA) and before decapitation (p<0,0005, Kruskal–Wallis). These data are shown in (Table 4.2) and represented graphically (Figures 4.2 and 4.3).

Regarding the STZ group, 8 out of 12 rats, however, all rats in the Vit-D3 group, had FBG levels above 250 mg/dl on all measurements after the induction of diabetes.

Table 4.2. Fasting blood glucose

Days/Groups	Control	STZ	Vit-D3
1.Day	89,25 ± 2,12	88,75 ± 2,24	93,09 ± 1,97
7.Day	89,25 ± 1,84	407,92 ± 56,18***	516,55 ± 21,69***
11.Day	91,13 ± 2,14	390,17 ± 53,65***	491,27 ± 22,36***
25.Day	88,75 ± 3,46	391,42 ± 48,30***	481,73 ± 19,26***
39.Day (Before the decapitation)	96,25 ± 2,87	346,42 ± 53,50***	505,64 ± 20,56***

(***p<0,0005 significant difference compared to the control group).

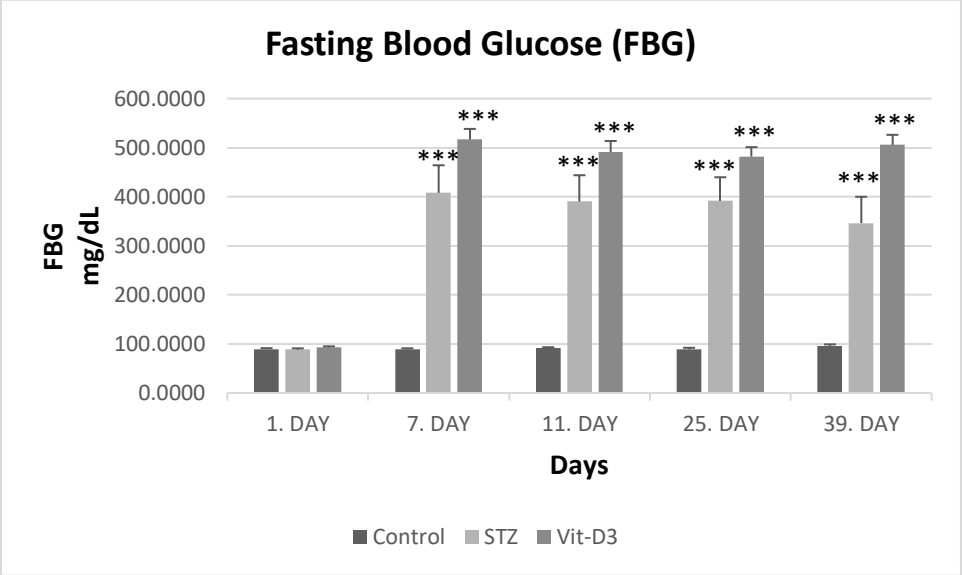

Figure 4.2. Fasting blood glucose levels.

(***p<0,0005 significant difference compared to the control group).

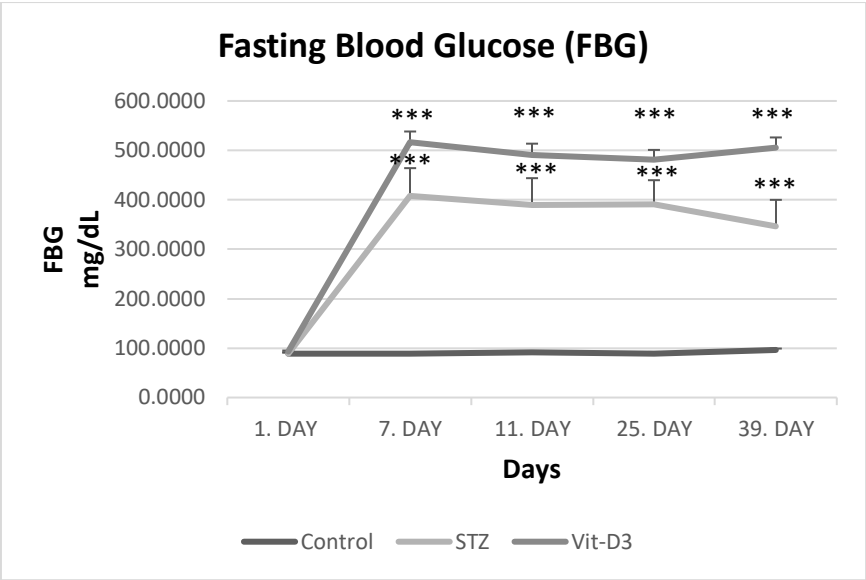

Figure 4.3. Fasting blood glucose levels.
(***p<0,0005 significant difference compared to the control group).

Additionally, the frequency of cage cleaning and the daily number of water bottle consumptions were monitored, and symptoms of polyuria and polydipsia were observed in diabetic rats (8 out of 12 rats in the STZ group and all rats in the Vit-D3 group).

One rat in the Vit-D3 group died 1 day after the STZ injection.

More severe diabetes symptoms were observed in the Vit-D3 group, with higher blood glucose levels and balanoposthitis (inflammation of the penis).

4.2. Rotarod Motor Performance Results

The locomotor activity of the rats in each group was assessed using the rotarod test before the induction of diabetes (at day 0 of the study), after the establishment of the STZ-induced diabetes model (21 days after STZ administration), and before decapitation. The time the rat could maintain its position on the spindle was noted as the rotarod motor performance value. Statistical analysis of the rotarod performance test revealed that there was no

significant difference in the initial motor performance among rats in different groups at the start of the experiment ($p>0,05$, t-test). After 3 weeks (25^{th} day of the study), the duration of time that rats in the STZ and Vit-D3 groups were able to remain on the spindle was significantly reduced compared to the control group ($p<0,005$, $p<0,05$, respectively, t-test). However, the Vit-D3 group still maintained significantly higher motor performance compared to the STZ group ($p<0.05$, t-test). Before decapitation, the motor performance of the STZ group continued to decrease compared to the control ($p<0,0005$, t-test). On the other hand, despite the administration of STZ, the Vit-D3 group exhibited significantly higher motor performance, with a longer duration of time that the rats were able to remain on the spindle compared to the STZ group ($p<0,005$, t-test). These data are shown in (Table 4.3) and represented graphically (Figure 4.4).

Table 4.3. Rotarod motor performance.

Days/Groups	Control	STZ	Vit-D3
0.Day	273,79 ± 7,59	235,56 ± 34,12	267,21 ± 22,48
25.Day	238,63 ± 14,37	134,72 ± 33,38**	164,88 ± 9,54*, #
39.Day	258,50 ± 8,59	44,88 ± 24,23***	204,00 ± 39,91##

(*p<0,05, **p<0,005, ***p<0,0005 significant difference compared to the control group).
(#p<0,05, ##p<0,005 significant difference between STZ and Vit-D3 groups).

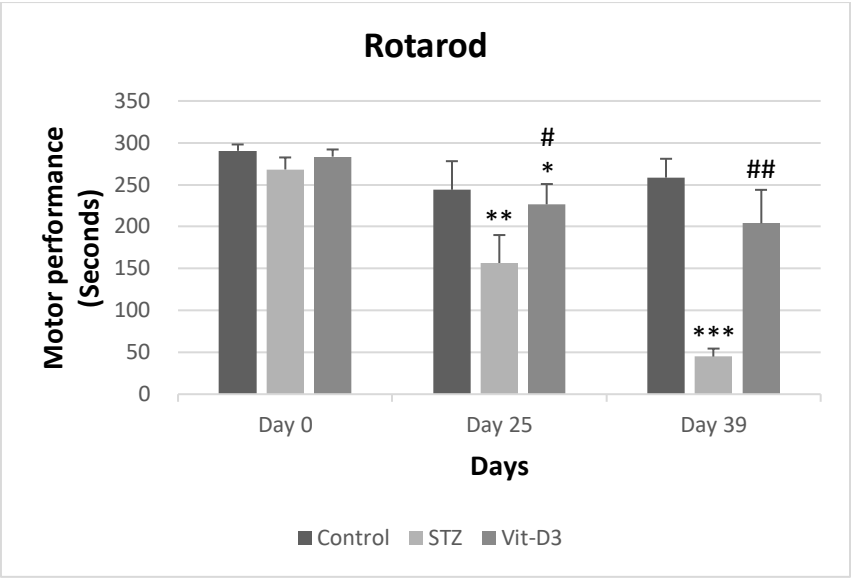

Figure 4.4. Rotarod motor performance.

(*p<0,05, **p<0,005, ***p<0.0005 significant difference compared to the control group).
($^{\#}$p<0,05, $^{\#\#}$p<0,005 significant difference between STZ and Vit-D3 groups).

4.3. Learning and Memory Results

The assessment of learning and cognitive functions was conducted through the eight-arm radial maze (RAM) test, a sensitive evaluation measuring behavioral changes in accordance with cellular changes in the hippocampus. In this test, a total of 32 rats across all groups underwent a 14-day training section for the radial maze test. The number of correct arm choices and the duration of time spent in the maze to finish the test (latency) were recorded throughout the 14-day training section and during test days using Amonra Stopwatch software. The obtained results were then evaluated and statistically analyzed using SPSS 25.0 software.

4.3.1. The number of the correct arm choices

The arrangement of the arms and the distribution of the baits are retained in memory throughout the experiment. The number of correct arm choices reflect reference memory which is referred to the memory required for the knowledge of the unchanged aspects i.e., a task that remains constant between trials which is being represented here in the retention of the arrangement of arms and the distribution of the baits throughout the experiment.

The number of correct arm choices increased between the first and last day of the 14-day training period. By the end of the training period, the Vit-D3 group showed a significant increase in the number of correct arm choices compared to the STZ group on the 14^{th} day ($p<0,05$, Kruskal–Wallis, $p=0,017$).

Regarding the test days, the number of correct arm choices made by the STZ group significantly decreased compared to the control group on the 38^{th} day ($p<0,005$, ANOVA). On the other hand, despite the administration of STZ, the Vit-D3 group demonstrated a significant increase ($p<0,05$, Kruskal–Wallis, $p=0,015$, $p=0,046$, $p=0,003$, and $p=0,001$) in the number of correct arm choices on the 25^{th}, 32^{nd}, 35^{th}, and 38^{th} days respectively, compared to the STZ group. These data are shown in (Table 4.4) and represented graphically (Figures 4.5 and 4.6).

Table 4.4. The number of correct arm choices (RAM test).

Days/Groups	Control	STZ	Vit-D3
Day 1	5,25 ± 0,72	5,22 ± 0,22	4 ± 0,07
Day 7	6,08 ± 0,80	6,22 ± 0,24	5,67 ± 0,07
Day 14	7,17 ± 0,36	7,03 ± 0,11$^{\#}$	7,73 ± 0,03
Day 21	7,38 ± 0,29	7,17 ± 0,09	7,55 ± 0,03
Day 25	6,92 ± 0,46	6,64 ± 0,14	7,76 ± 0,04$^{*, \#}$
Day 28	6,83 ± 0,70	6,33 ± 0,21	7,58 ± 0,06
Day 32	7,04 ± 0,48	6,56 ± 0,15	7,88 ± 0,04$^{\#}$
Day 35	6,92 ± 0,56	6,64 ± 0,17	7,94 ± 0,05$^{*, \#\#}$
Day 38	7,54 ± 0,34	6,28 ± 0,10**	7,73 ± 0,03$^{\#\#}$

(*p<0,05, **p<0,005 significant difference compared to the control group).
($^{\#}$p<0,05, $^{\#\#}$p<0,005 significant difference between STZ and Vit-D3 groups).

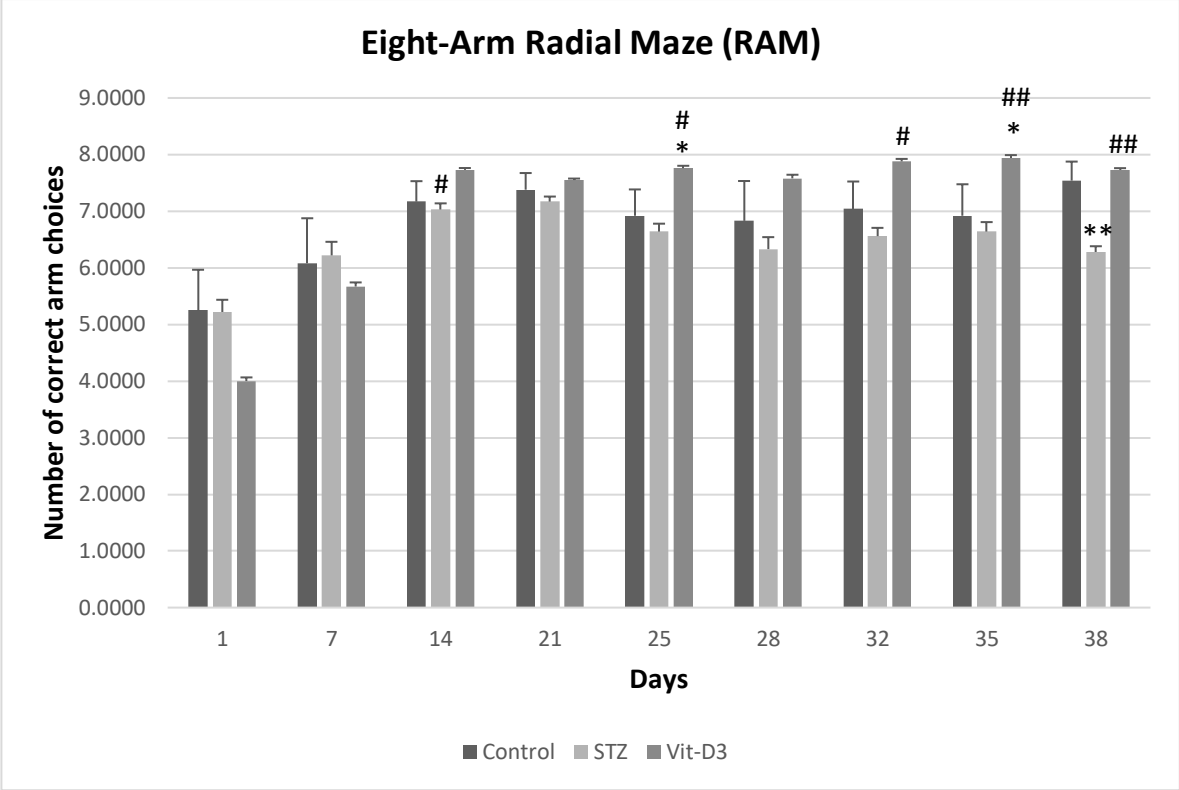

Figure 4.5. Number of correct arm choices (RAM test).

(*p<0,05, **p<0,005 significant difference compared to the control group).
(#p<0,05, ##p<0,005 significant difference between STZ and Vit-D3 groups).

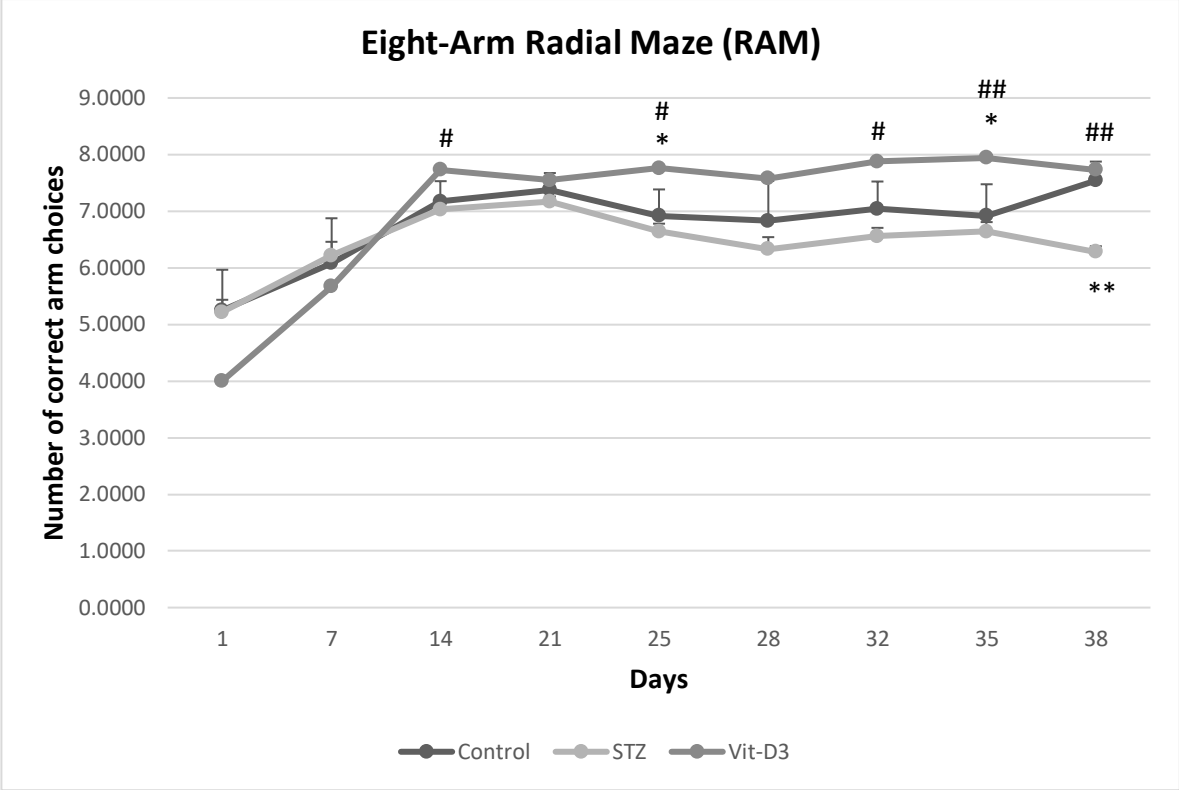

Figure 4.6. Number of correct arm choices (RAM test).

(*p<0,05, **p<0,005 significant difference compared to the control group).
(#p<0,05, ##p<0,005 significant difference between STZ and Vit-D3 groups).

4.3.2. Latency:

The time spent to finish RAM test (latency) is related to working memory which is the memory that is involved in retaining and manipulating information extensively in goal-directed behaviors. The time to complete the test decreased between the first and last day of the 14-day training period. By the end of the training period, the Vit-D3 group showed a significant decrease in the time required to finish the test compared to the STZ group on the 14th day (p<0,005, Kruskal–Wallis, p=0,001). In terms of the test days, despite the administration of STZ, the Vit-D3 group demonstrated significantly shortened latency on the 28th, 32nd, and 38th days (p<0,05, Kruskal–Wallis, p=0,026, p=0,009, and p=0,011, respectively), compared to the STZ group. Also, there was a statistically significant increase

in the time spent in the maze to finish the test by the STZ group on the 38th day compared to the control group ($p<0,05$, ANOVA, $p=0,027$).

Therefore, in conclusion, both the reference memory and the working memory impaired due to diabetes have been significantly improved by the administration of Vit-D3.

These data are shown in (Table 4.5) and represented in (Figures 4.7 and 4.8).

Table 4.5. The latency.

Days/Groups	Control	STZ	Vit-D3
Day 1	373,08 ± 76,86	382,67 ± 23,17	428,73 ± 6,99
Day 7	342,96 ± 95,99	282,28 ± 28,94	282,45 ± 8,73
Day 14	147,21 ± 40,34	224,72 ± 12,16	86,15 ± 3,67$^{\#\#}$
Day 21	85,13 ± 19,23	168,89 ± 5,80	100,33 ± 1,75
Day 25	123,04 ± 50,36	181,14 ± 15,18	76,03 ± 4,58
Day 28	109,75 ± 28,31	275,94 ± 8,54	102,39 ± 2,57$^{\#}$
Day 32	146,17 ± 39,82	293,78 ± 12,01	100,97 ± 3,62$^{\#}$
Day 35	115,21 ± 38,51	188,92 ± 11,61	111,79 ± 3,50
Day 38	96,33 ± 15,04	262,36 ± 4,53*	109,76 ± 1,37$^{\#}$

($^{*}p<0,05$ significant difference compared to the control group).
($^{\#}p<0,05$, $^{\#\#}p<0,005$ significant difference between STZ and Vit-D3 groups).

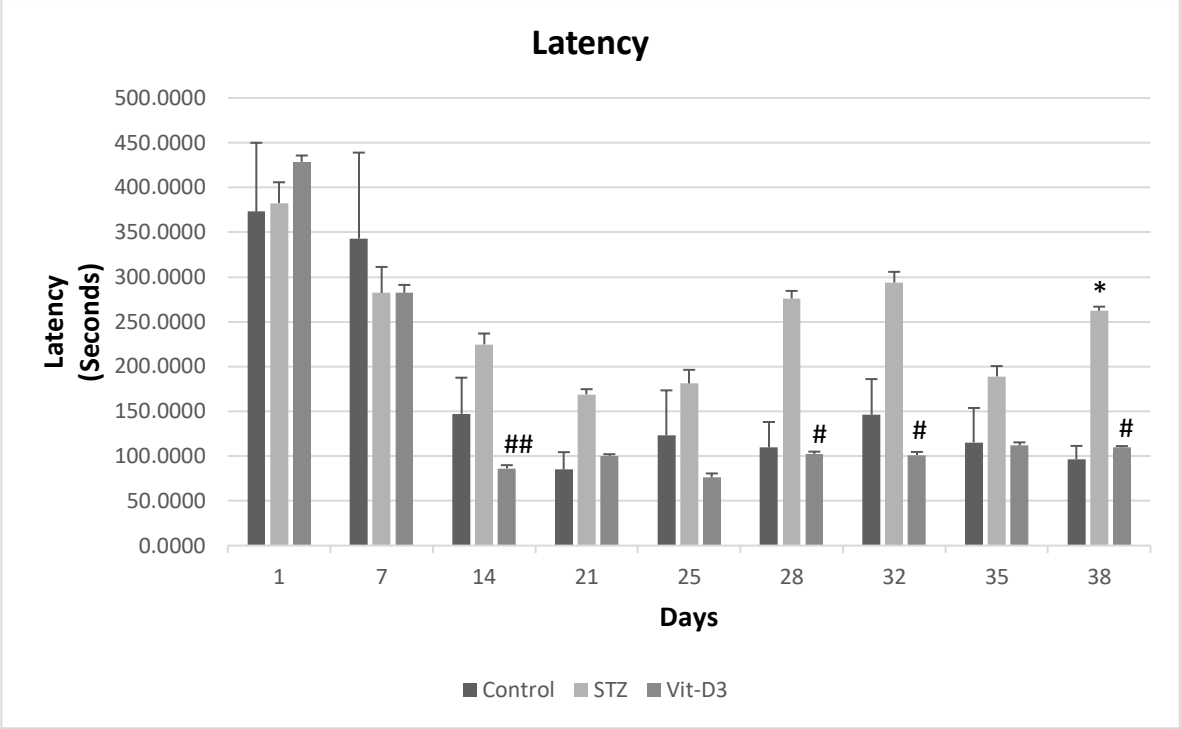

Figure 4.7. The time required to finish the RAM test (Latency).

(*p<0,05 significant difference compared to the control group).
(#p<0,05, ##p<0,005 significant difference between STZ and Vit-D3 groups).

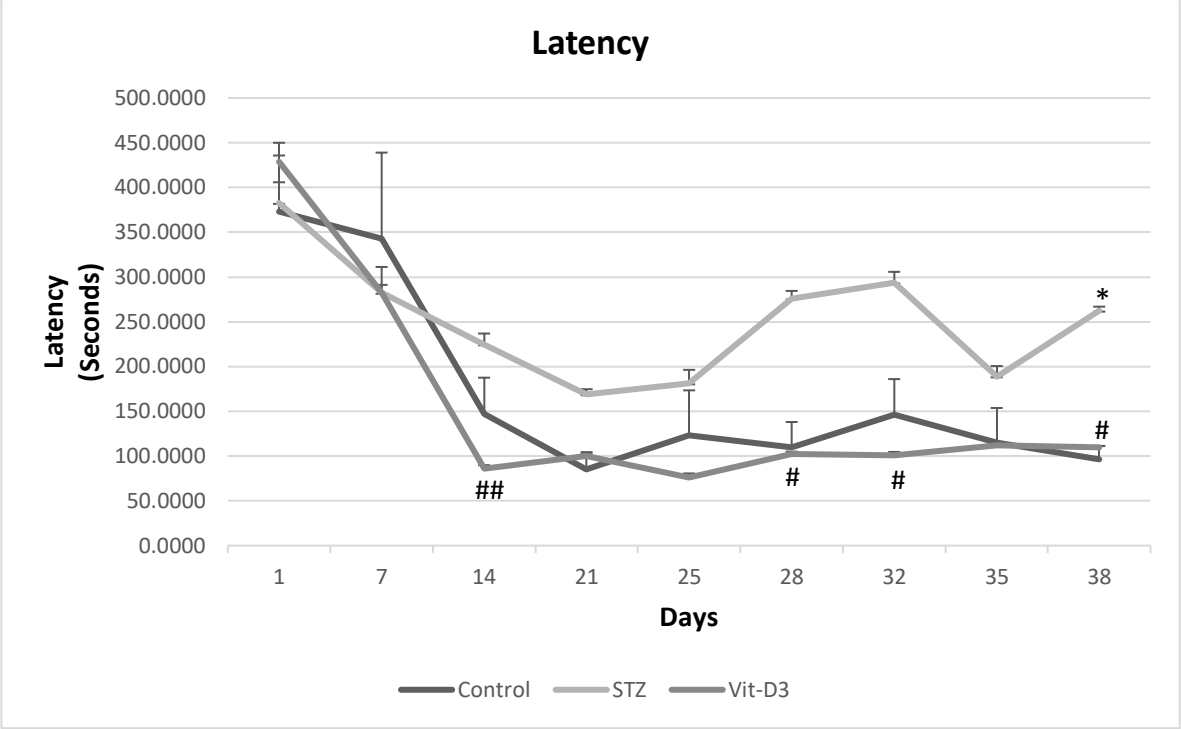

Figure 4.8. The time required to finish the RAM test (Latency).

(*p<0,05 significant difference compared to the control group).
(#p<0,05, ##p<0,005 significant difference between STZ and Vit-D3 groups).

4.4. Enzyme-Linked Immunosorbent Assay Results

4.4.1. Insulin measurement

Insulin levels were measured in serum and hippocampus samples using an appropriate ELISA kit. In the STZ group, the serum insulin level significantly increased compared to the control group (p<0,05, t-test). In the hippocampus, there was a statistically significant increase in insulin level in both the STZ group (p<0,05, t-test) and the Vit-D3 group (p<0,005, t-test) compared to the control group (Figure 4.9).

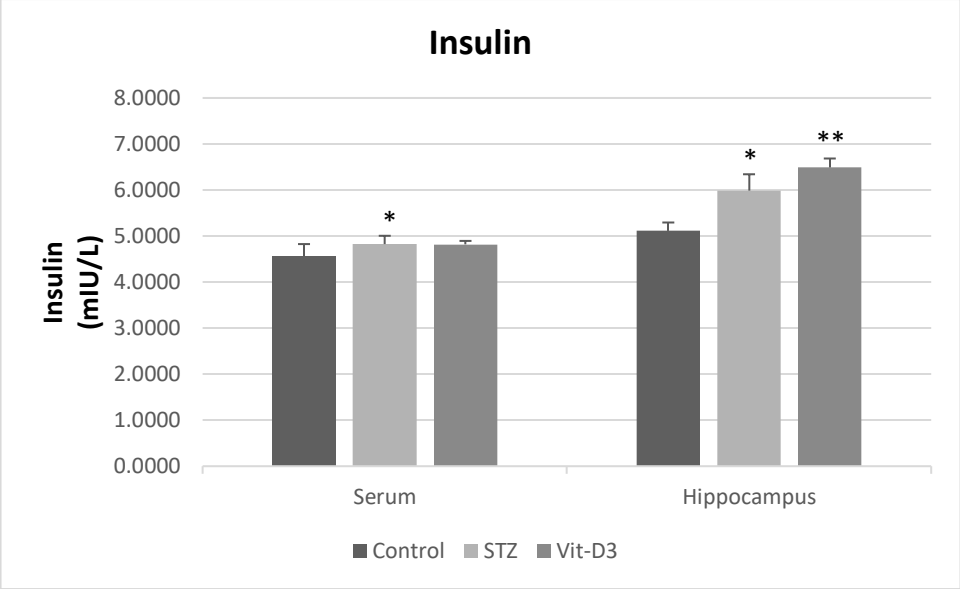

Figure 4.9. Measurement of Insulin levels in serum and hippocampus.
(*p<0,05, **p<0,005 significant difference compared to the control group).

4.4.2. RAGE measurement

RAGE levels were measured in serum and hippocampus samples using an appropriate ELISA kit. There was a significant increase in the serum level of RAGE in the STZ group compared to the control group (p<0,005, t-test). However, this increase in RAGE, despite STZ administration, was significantly decreased in the Vit-D3 group (p<0,05, t-test). On the other hand, there was a significant increase in hippocampal RAGE level in the STZ-administered groups (both STZ and Vit-D3 groups) compared to the control group (p<0,005, t-test). Although this increase was reduced by Vit-D3 administration, it was nonsignificant (p>0,05, t-test) (Figure 4.10).

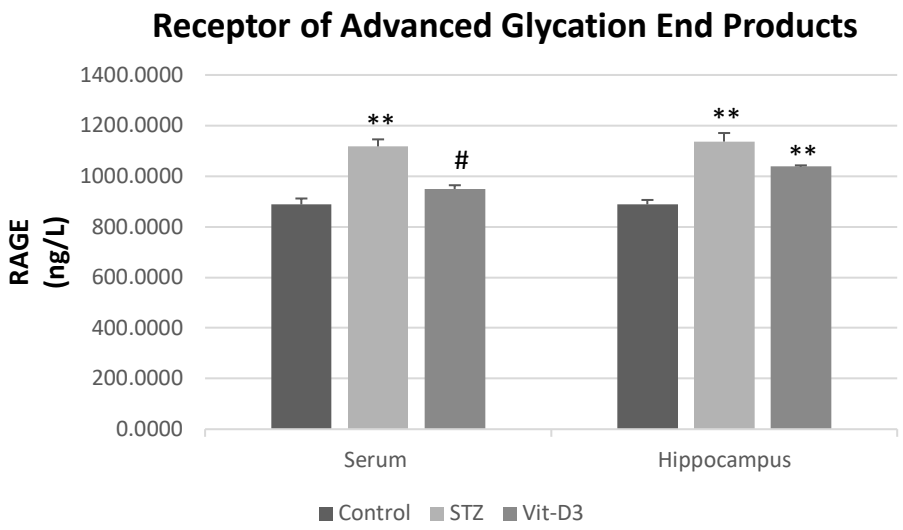

Figure 4.10. Measurement of RAGE levels in serum and hippocampus.

(**p<0,005 significant difference compared to the control group).
(#p<0,05 significant difference between STZ and Vit-D3 groups).

4.4.3. sRAGE measurement

The level of sRAGE was measured in serum samples using an appropriate ELISA kit. Although the serum level of sRAGE in the Vit-D3 group increased compared to the control and STZ groups, this increase is not statistically significant (p>0,05, t-test) (figure 4.11).

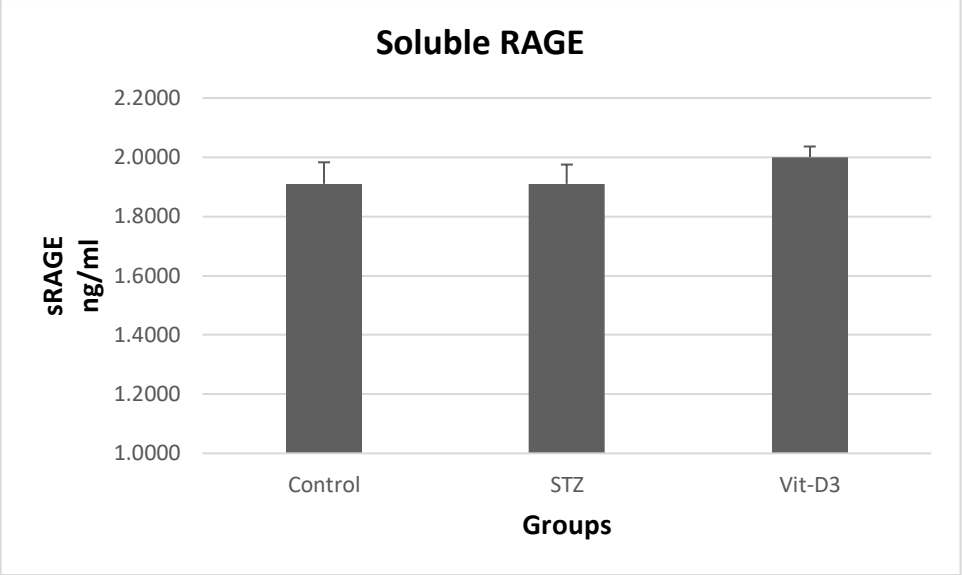

Figure 4.11. Measurement of sRAGE levels in serum.

4.4.4. S100B protein measurement

S100B levels were measured in serum and hippocampus samples using an appropriate ELISA kit. No significant difference was observed in the serum S100B levels between groups ($p>0,05$, t-test). However, in the hippocampus, there was an increase in S100B level in the STZ-administered groups (both STZ and Vit-D3 groups) compared to the control group ($p<0,05$, t-test). While Vit-D3 administration decreased this increase, the difference was nonsignificant (Figure 4.12).

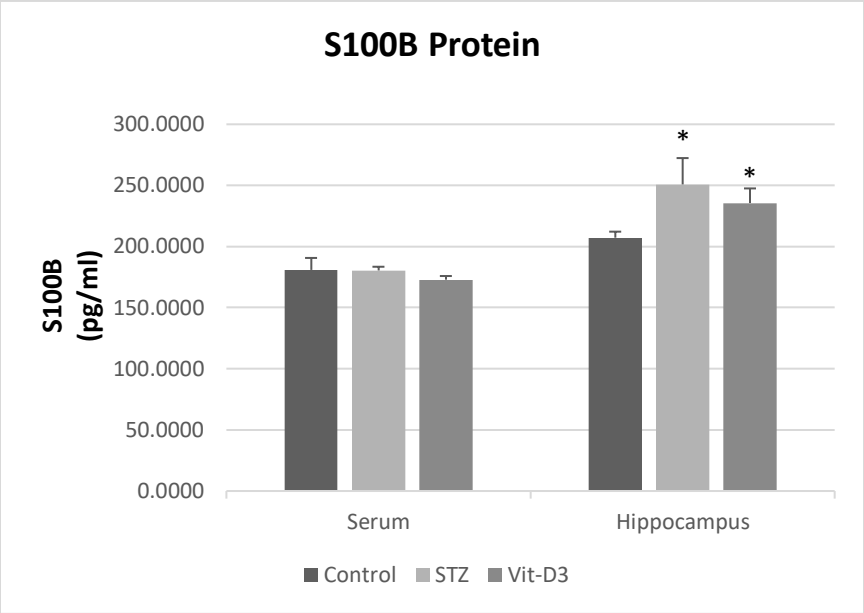

Figure 4.12. Measurement of S100B protein levels in serum and hippocampus.
(*p<0,05 significant difference compared to the control group).

4.4.5. Calprotectin measurement

Calprotectin levels were measured in serum and hippocampus samples using an appropriate ELISA kit. A statistically significant increase was observed in the serum level of calprotectin in the STZ-administered groups (both STZ and Vit-D3 groups) compared to the control group (p<0,05, t-test). Similarly, there was a statistically significant increase in hippocampal calprotectin level in the STZ-administered groups (both STZ and Vit-D3 groups) compared to the control group (p<0,05, t-test) (Figure 4.13).

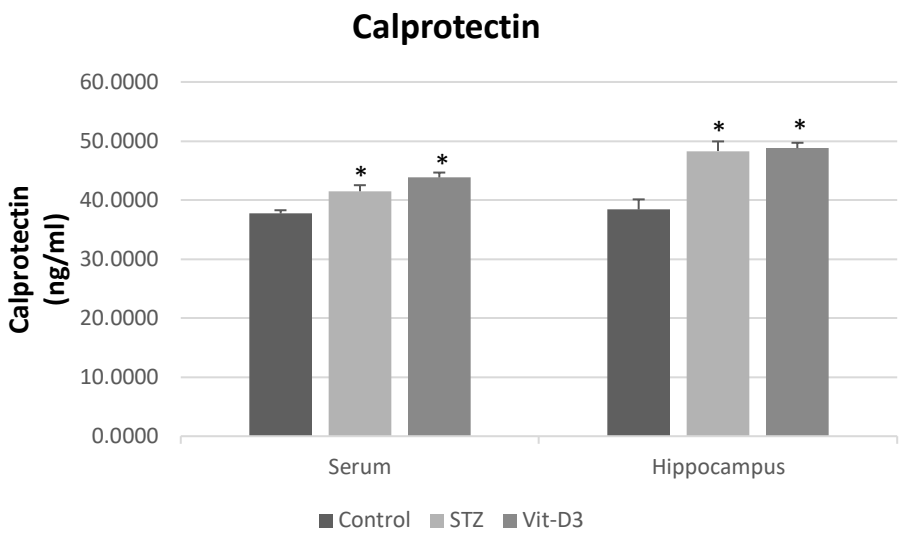

Figure 4.13. Measurement of calprotectin levels in serum and hippocampus.

(*p<0,05 significant difference compared to the control group).

5. DISCUSSION

Diabetes, a prevalent metabolic disorder characterized by abnormal blood sugar levels, has reached epidemic proportions globally, with its origins in complex genetic and environmental factors; its consequences extend beyond hyperglycemia, leading to severe complications affecting various organ systems. Among the various factors linked to metabolic syndrome, high blood sugar stands out as the most strongly connected to the development of cognitive problems (Zilliox et al., 2016). Research consistently indicates a correlation between diabetes and cognitive decline, potentially elevating the likelihood of developing dementia in individuals with both T1DM and T2DM (Biessels & Despa, 2018a; Pugazhenthi et al., 2017).

The STZ-induced diabetic rat model is a widely utilized experimental approach to simulate diabetes mellitus, particularly resembling T1DM. STZ, an antibiotic used experimentally as a diabetogenic agent, is administered to induce pancreatic islet β-cell damage, resulting in an insufficient production of insulin and subsequent hyperglycemia in rats. This model mirrors key features of human diabetes, including polydipsia and polyuria. The most commonly employed protocol includes administering a single i.p. injection of STZ, typically at a dosage of approximately 65 mg/kg (Furman, 2021). This method is a simple, reproducible, and efficient approach with a relatively rapid onset for the induction of diabetes, providing a valuable model that allows researchers to investigate the pathogenesis of diabetes, diabetes-associated complications, and to evaluate potential antidiabetic agents and other therapeutic interventions (Pandey & Dvorakova, 2019).

Vit-D3, or cholecalciferol, is a fat-soluble vitamin that exerts its genomic actions through the VDR or non-genomic actions, mediated through membrane receptors, contribute to diverse effects on cellular signaling pathways. These aspects have become a subject of

increasing research interest for potential clinical applications in preventing and treating a spectrum of non-skeletal disorders, including diabetes and neurodegenerative conditions (Anastasiou et al., 2014). Understanding the intricate molecular mechanisms underlying the beneficial effect of Vit-D3 centrally provides valuable insights for potential therapeutic interventions mitigating cognitive dysfunction, particularly in the context of neuroinflammatory conditions, including DACD.

Throughout the study, it was determined that rats administered STZ, including both the STZ group and the group that received Vit-D3, showed significant weight loss compared to their initial weights at the beginning of the experiment, as measured before decapitation. Studies consistently show that STZ administration in rats induces significant weight loss (Georgy et al., 2013; J. Q. Wang et al., 2014). The weight loss observed in these studies is often considered a distinctive trait of the diabetic condition induced by STZ. It reflects the metabolic alterations, energy imbalance, and catabolic processes associated with diabetes development in these animal models.

Additionally, symptoms such as polydipsia and polyuria were observed in both groups. Elevated blood glucose levels caused by STZ lead to glucose spilling into the urine. Glucose in the urine attracts water, causing osmotic diuresis and resulting in increased urine production (polyuria). This excessive urination further contributes to the state of dehydration and electrolyte imbalance, stimulating excessive thirst (polydipsia) in an attempt to compensate for fluid loss. Weight loss, polyuria, and polydipsia, as measurable indicators, are considered consistent signs of the effectiveness of STZ in inducing diabetes in experimental settings (Furman, 2021).

STZ administration induces hyperglycemia, a hallmark feature of the resulting diabetic phenotype, by selectively damaging pancreatic islet β-cells and disrupting insulin production and glucose regulation. According to the literature, FBG levels exceeding 250 mg/dl 72 hours after STZ administration are considered indicative of successful STZ-induced diabetes (Furman, 2021; Lenzen, 2008). The FBG of the STZ group and Vit-D3 group was significantly increased compared to the control group. Although many studies have shown that Vit-D3 supplementation is associated with improvements in glycemic control (Mitrašinović-Brulić et al., 2021; Muneeb et al., 2022b), our study found that Vit-D3 had no

effect on glucose levels. The lack of impact of Vit-D3 on glucose levels in diabetic rats in our study might be attributed to the severity of STZ-induced diabetes (FBG levels of many rats in the STZ and Vit-D3 group were >600 mg/dl). This severity can lead to significant damage to tissues and organs, potentially rendering them less responsive to treatments aimed at reversing or improving the hyperglycemic condition. To better understand our hypothesis, it's essential to consider that STZ specifically damages the pancreatic β-cells. Since Vit-D3 doesn't seem to affect the changes in the mass of these β-cells or their microanatomy, the remaining damaged β-cells following STZ-induced damage are unable to release insulin. Consequently, without the action of insulin to reduce glucose levels, there is no observable impact on hyperglycemia. Research by Del Pino-Montes et al. has indicated that when β-cells remain unaffected, Vit-D3 can indirectly influence blood sugar levels by converting to 1.25-$(OH)_2$Vit-D3, which acts on the pancreatic β-cells through vitamin D receptors, promoting increased insulin synthesis and secretion. In our study, we observed an increase in insulin levels in STZ group compared to the control group. Typically, the STZ-induced diabetes model is accompanied with reduced insulin level, resembling T1DM. However, recent research suggests that this model may also be linked to insulin resistance. In response to developed insulin resistance, the body might compensate for the loss of functional β-cells by increasing insulin production in the remaining cells (Rad et al., 2022; M. Sharma et al., 2023). Interestingly, the serum insulin level in the Vit-D3 group surpassed that of the STZ group, suggesting a potentially favorable impact of Vit-D3 on insulin secretion. Studies have revealed that Vit-D3 has the potential to induce the expression of autophagic markers, including LC3 and Beclin 1, acting as a compensatory mechanism and the expression of the anti-apoptotic protein Bcl-2 suggests that Vit-D3 may help in the protection and suppression of apoptosis in pancreatic β-cells, ultimately preventing insulitis and consequently increasing insulin secretion (Y. Wang et al., 2016). Also, this effect could be associated with the protective regulatory role of Vit-D3 against cytokine expression and inflammation, reducing the levels of inflammatory cytokines such as IL-6, and empowering the antioxidant mechanisms, including glutathione and SOD (Fathi et al., 2022). In addition, experimental studies including our study have highlighted Vit-D3's ability to decrease the harmful effect of the AGEs-RAGE axis on β-cells (Contreras-Bolívar et al., 2021).

Several studies have consistently highlighted the pivotal functions of insulin encompassing neuroprotection, neuronal glucose uptake, appetite regulation, and the control of growth hormone secretion. Traditionally, insulin was thought to originate exclusively from pancreatic β-cells, traversing from the general circulation into the cerebrospinal fluid through receptor-mediated transcytosis (Baura et al., 1993). However, recent investigations have unveiled the presence of insulin, its mRNA, and C-peptide centrally in different regions and prominently in the hippocampus (Mehran et al., 2012). Subsequent research has demonstrated that insulin not only traverses the BBB but is also expressed in neurons in response to glucose stimulation. Its actions occur in an autocrine and/or paracrine manner within the brain, contributing to neural growth and differentiation through the MAPK pathway. It remains unclear which actions can be attributed to brain-produced insulin versus pancreatic insulin, and the extent to which their functions overlap in the brain. But the association between impaired insulin expression and signaling and memory decline, particularly in AD, underscores the significance of brain-produced insulin in maintain in cognition (Sędzikowska & Szablewski, 2021). Another study highlighted the involvement of proinsulin in reducing the levels of neuroinflammation markers by activating the protein kinase B (Akt) pathways. Consequently, a reduction in the concentrations of TNF-α and IL-1β was observed in the hippocampus of mice, aligning with enhanced cognitive performance. Also, it was revealed that hippocampal neuron insulin secretion is controlled by the Ca2+-dependent activator protein for secretion 2 (CAPS2) (Dakic et al., 2023). In the course of our investigation, we observed a significant increase in hippocampal insulin levels within the Vit-D3 group. This noteworthy finding prompts the necessity for further research to explore the mechanism of Vit-D3's impact on brain-produced insulin expression and its potential interaction with CAPS2.

The motor performance of the rats in each group was evaluated using a rotarod apparatus. STZ group showed a significant decrease in motor performance indicated by the decrease in the time to fall from the rotating rod in compared to the control and Vit-D3 groups. Previous studies have consistently reported that STZ-induced diabetic rats showed impairment in various motor coordination and balance tests, such as the rotarod test, indicating that diabetes induced by STZ adversely affects the rats' ability to perform coordinated motor tasks (Muramatsu, 2020). The compromised motor performance observed

in rats with STZ-induced diabetes is likely a result of various factors. These factors include neurodegeneration and central changes accompanying diabetes that extend beyond regions linked to cognitive function, such as the hippocampus. They also impact the central systems responsible for motor and sensory functions in the cortex, cerebellum, and basal ganglia disturbing mesolimbic dopaminergic and muscarinic systems, which are essential for motor coordination (Chen et al., 2017; Lv et al., 2022; Peeyush et al., 2010). Also, peripheral neuropathy, where sensory nerve damage in the extremities can lead to disruptions in motor function and coordination. The accumulation of AGEs in neural tissues may contribute to structural alterations and functional impairments in motor neurons, mediated through neuroinflammation and increased oxidative stress. Additionally, diabetes-related mitochondrial dysfunction could result in inadequate energy production in neurons, impacting their support for motor functions (Yagihashi et al., 1124). Vit-D3 supplementation exhibited a mitigative impact on diabetes-induced motor dysfunction in rats, as evidenced by enhanced coordination and improved rotarod test performance in different studies including our study. The suggested mechanisms underlying this beneficial effect encompass the restoration of neurotransmission alterations and rebalancing of glucose utilization in the cerebellum associated with diabetes (Peeyush et al., 2010). Also, its anti-inflammatory properties may help mitigate the impact of chronic inflammation and AGEs-RAGE axis overactivation associated with diabetes on nerves contributes to nerve health, potentially addressing neuropathy linked to diabetes (Nimitphong & Holick, 2011). Additionally, improved insulin sensitivity, influenced by adequate Vit-D3 levels, can positively affect glucose metabolism, aiding energy production during physical activity. Moreover, Vit-D3 supports muscle strength and function by regulating calcium and promoting muscle protein synthesis along with improved bone health collectively enhancing balance and coordination.

The eight-arm radial maze test was chosen as a behavioral assessment, a test widely used in various STZ-induced Alzheimer's models (Egashira et al., 2018; Kosaraju et al., 2014). In our maze test results, the significant increase in the time spent to complete the test as well as the significant decrease in the number of correct arm choices made by STZ group compared to the control and Vit-D3 groups demonstrating impairment in short and long-term memories due to STZ which was effectively improved by Vit-3, a finding consistent with previous studies (Alrefaie & Alhayani, 2015; Calgaroto et al., 2014; Kumar et al., 2011;

Muneeb et al., 2022b). The exact mechanisms linking diabetes to cognitive dysfunction are complex and involve a combination of 1) Metabolic factors including chronic hyperglycemia that affects the health of neurons and synapses, as well as the disruption of insulin neurotrophic activity. 2) Inflammatory factors play a crucial role, with the AGEs- RAGE axis mediating chronic inflammation and oxidative stress that contribute to neurodegeneration. 3) Vascular factors including endothelial dysfunction and vascular damage (Biessels & Despa, 2018a). Understanding the pathological mechanisms of cognitive impairment in diabetes is crucial for developing targeted interventions to mitigate cognitive decline in this population. Vit-D3 receptors are found in the brain (Eyles et al., 2005), and Vit-D3 has been implicated in modulating neurotrophic factors, regulation of neurotransmitters and has been associated with improved learning and memory, stimulation of PI3K/AKT signaling, leading to downregulation of Alzheimer's-associated markers (Zaulkffali et al., 2019), and activation of the Wnt/β-catenin pathway, enhancing cholinergic transmission and influencing the balance between the pro- and anti-inflammatory responses (Muneeb et al., 2022b). Also, Vit-D3 also modulates inflammation, apoptosis, and oxidative stress through the MARRS receptor, acting as an anti-inflammatory and antioxidant agent reducing neuroinflammation. These mechanisms may contribute to its positive effects on cognitive function. Previous studies have highlighted Vit-D3's ability to alleviate cognitive dysfunction through various mechanisms. In our study, we aimed to focus on the impact of Vit-D3 on the harmful effects of the AGEs–RAGE system and S100 proteins as potential biomarkers for early diagnosis and prevention of diabetic complications.

AGEs are molecules produced through the non-enzymatic glycation and oxidation of various macromolecules. The AGEs-RAGE signaling pathway plays a crucial role in various diseases, including diabetes and neurodegenerative conditions. We measured the serum and hippocampal levels of RAGE using an appropriate ELISA kit. A noticeable increase in RAGE serum concentration was observed in the STZ group compared to the control group. Additionally, RAGE levels in the hippocampus were found to be significantly elevated in both the STZ and Vit-D3 groups compared to the control group. However, this increase was highest in the STZ group. Vit-D3 reduced both RAGE levels in the hippocampus and serum samples compared to the STZ group. The effect of Vit-D3 on reducing increased serum RAGE levels was significant when compared to the STZ group. In diabetes, chronic

increased glucose in the bloodstream provides more substrate for non-enzymatic glycation reactions, increasing the generation of AGEs (K. C. Tan et al., 2006) which is closely related to the neuroinflammatory response in the progression of cognitive dysfunction. Numerous studies have shown that AGEs accumulation leads to the upregulation of RAGE. The AGEs-RAGE interaction establishes a positive feedback loop, activating signaling pathways that enhance RAGE expression and initiate inflammatory responses, particularly through NF-κB. This leads to the transcription of pro-inflammatory genes, perpetuating a cycle of inflammation and oxidative stress (Chuah et al., 2013; Nowotny et al., 2015b). These responses create a microenvironment favoring increased RAGE expression. The impact of Vit-D3 on AGEs concentration is suggested to involve multiple mechanisms. Vit-D3 is known for its anti-inflammatory effects, modulating pathways contributing to the formation and accumulation of AGEs. By reducing inflammation, Vit-D3 could indirectly impact AGEs generation (Mousa et al., 2018). Additionally, by mitigating oxidative damage caused by AGEs-RAGE interaction, Vit-D3 may help control AGEs production (Talmor et al., 2008). Despite conflicting findings in existing studies on the influence of Vit-D3 supplementation on RAGE expression in various tissues and cells, our study reveals a promising effect of Vit-D3 both on serum and hippocampal RAGE levels, possibly as a result of reduced AGEs. Moreover, the antioxidant property of Vit-D3 mitigates mitochondrial dysfunction and reduces oxidative stress, both associated with RAGE overexpression induced by hyperglycemia (Hussien et al., 2019). Another suggested mechanism involves the positive effect of Vit-D3 on sRAGE (Jung et al., 2013). Acting as a binder to AGEs inhibiting AGEs–RAGE interaction. Although our results showed an increased serum level of sRAGE in the Vit-D3 group compared to the control and STZ groups, this increase is not statistically significant, possibly attributed to the severity of diabetes in this group or to the study's small sample size.

In addition to AGEs, RAGE interacts with various endogenous proteins, such as S100 proteins, acting as ligands and contributing to inflammatory responses implicated in neurodegenerative disorders and inflammatory conditions. The study observed no significant difference in serum concentrations of the S100B protein among different groups, possibly due to the study's limited sample size, constraining the ability to conduct a comprehensive analysis. Prior studies have reported decreased serum levels of S100B protein in both diabetic

patients and STZ-induced diabetic rats (Yu et al., 2020b). The underlying cause of this decrease is not fully understood but has been linked to damaged Schwann cells, suggested as the origin of persistent S100B expression in pancreatic islets. Studies with S100B knockout mice demonstrated reduced expression of the S100B receptor RAGE and preserved β-cell function, indicating the apoptotic potential of S100B. In vitro experiments with β-cells further supported these findings, showing increased caspase-3 activity after S100B treatment (Mohammadzadeh et al., 2018). Moreover, S100B knockout in mice resulted in resistance to STZ-induced diabetes, possibly due to the downregulation of GLUT2, a glucose transporter necessary for selective STZ absorption by beta cells. S100B is believed to regulate GLUT2 expression by activating a pathway involving RAGE and AP-1 (activator protein-1) (Bianchi et al., 2011; Zhang et al., 2016). Additionally, S100B-deficient mice exhibited enhanced glucose tolerance, reduced hyperglycemia, urine volume, and lower water and food consumption (Mohammadzadeh et al., 2018).

In concern of hippocampus, the S100B protein levels were significantly increased in STZ-administered groups (both STZ and Vit-D3 groups) in compared to the control group. This increase is reduced in Vit-D3 group but not significantly. In situations characterized by inflammation, such as those occurring in neurodegenerative disorders and DACD due to AGEs-RAGE axis overactivation, there is an increase in the levels of S100B protein and its receptor, RAGE, in both neural and inflammatory cells. It is worth noting that in hippocampal slices, the activation of RAGE has been demonstrated to induce the secretion of S100B through the stimulation of pro-inflammatory cytokines (Kögel et al., 2004). These findings were consistent with ours. Because there was a decrease in S100B protein levels in the Vit-D3 group, which coincided with a decrease in hippocampal RAGE levels. S100B functions as a cytokine. When RAGE is persistently overactivated, it triggers signaling cascades that enhance NF-kB and excessive ERK1/2 activation, amplifying inflammation and oxidative damage. This contributes to astrogliosis, microgliosis, and impairment of mitochondrial function, ultimately leading to neuronal and glial apoptosis (Bianchi et al., 2010, 2011). Decrease in hippocampal S100B levels within the Vit-D3 group may be attributed to its anti-inflammatory and antioxidant effects related with RAGE.

The association between calprotectin serum levels and insulin resistance in T2DM, obesity-related chronic inflammation, and diabetic cardiovascular complications has been

extensively studied (Catalán et al., 2011; Jukic et al., 2021; Mortensen et al., 2009). However, research on the serum and hippocampal levels of calprotectin in type 1 diabetes is limited. In our study, we observed an increase in calprotectin levels in both the serum and hippocampus of STZ-induced diabetic rats, indicating heightened inflammatory responses. The neuroinflammation associated with extensive activation of microglial and astroglia cells, triggered by calprotectin, contributes to the pathogenesis of DACD. Interestingly, Vit-D3 administration did not affect the elevated calprotectin levels, suggesting that the impact of Vit-D3 on memory may not be mediated through a reduction in hippocampal calprotectin levels. Studies exploring the influence of Vit-D3 on calprotectin serum levels in individuals with T1DM have yielded conflicting results. While some studies suggest a potential decrease in calprotectin levels with Vit-D3 supplementation, indicating a favorable effect on inflammatory markers linked to T1DM (Jin et al., 2013), others report limited or inconsistent changes. Vit-D3's anti-inflammatory properties, regulation of oxidative stress, inhibition of the RAGE pathway, and interaction with inflammatory cytokines collectively contribute to its potential in modulating the formation and accumulation of AGEs, highlighting its multifaceted role in mitigating AGEs-related processes.

6. CONCLUSION

In our study, the levels of specific S100 protein subtypes, S100B, and S100A8/A9 (calprotectin), as well as changes in motor performance and cognitive function were investigated in an STZ-induced diabetic rat model, exploring the effect of Vit-D3.

1- STZ induced weight loss, high fasting blood glucose levels, and signs of polyuria and polydipsia throughout the study.
2- STZ and Vit-D3 increased insulin levels in both serum and hippocampus.
3- STZ impaired rotarod performance, whereas Vit-D3 improved it.
4- STZ impaired RAM performance, decreased correct arm choices and increased RAM completion duration. Vit-D3 reversed both effects of STZ, indicating its memory-improving property.
5- STZ increased RAGE levels in both serum and hippocampus. Vit-D3 decreased RAGE in serum, but this decrease was nonsignificant in the hippocampus. No significant difference was detected for serum sRAGE and S100B. In the hippocampus, STZ increased S100B protein expression, an effect non significantly decreased by Vit-D3.
6- An elevated calprotectin level was observed in the serum and hippocampus of STZ rats.

According to the results, calprotectin and RAGE serum levels are increased in the STZ-induced rat diabetes model, suggesting their potential use as biomarkers for monitoring the disease and following the beneficial effects of Vit-D3. Monitoring components of the AGEs-RAGE axis and S100 proteins in DM and DACD pathophysiology and supporting future studies will be beneficial.

7. REFERENCES

Ahmad, K. (2014). Insulin sources and types: a review of insulin in terms of its mode on diabetes mellitus. *Journal of Traditional Chinese Medicine = Chung i Tsa Chih Ying Wen Pan*, *34*(2), 234–237. https://doi.org/10.1016/s0254-6272(14)60084-4

Ahmed, N. (2005). Advanced glycation endproducts - Role in pathology of diabetic complications. *Diabetes Research and Clinical Practice*, *67*(1), 3–21. https://doi.org/10.1016/J.DIABRES.2004.09.004

Alrefaie, Z., & Alhayani, A. (2015). Vitamin D3 improves decline in cognitive function and cholinergic transmission in prefrontal cortex of streptozotocin-induced diabetic rats. *Behavioural Brain Research*, *287*, 156–162. https://doi.org/10.1016/J.BBR.2015.03.050

Ambartsumian, N., Klingelhöfer, J., Grigorian, M., Christensen, C., Kriajevska, M., Tulchinsky, E., Georgiev, G., Berezin, V., Bock, E., Rygaard, J., Cao, R., Cao, Y., & Lukanidin, E. (2001). The metastasis-associated Mts1(S100A4) protein could act as an angiogenic factor. *Oncogene*, *20*(34), 4685–4695. https://doi.org/10.1038/SJ.ONC.1204636

Anastasiou, C. A., Yannakoulia, M., & Scarmeas, N. (2014). Vitamin D and Cognition: An Update of the Current Evidence. *Journal of Alzheimer's Disease*, *42*, S71–S80. https://doi.org/10.3233/JAD-132636

Aschner, P. (2017). New IDF clinical practice recommendations for managing type 2 diabetes in primary care. In *Diabetes research and clinical practice* (Vol. 132, pp. 169–170). https://doi.org/10.1016/j.diabres.2017.09.002

Atkinson, M. A., Eisenbarth, G. S., & Michels, A. W. (2014). Type 1 diabetes. *Lancet (London, England)*, *383*(9911), 69–82. https://doi.org/10.1016/S0140-6736(13)60591-7

Baecker, J., Wartchow, K., Sehm, T., Ghoochani, A., Buchfelder, M., & Kleindienst, A. (2020). Treatment with the Neurotrophic Protein S100B Increases Synaptogenesis after Traumatic Brain Injury. *Journal of Neurotrauma*, *37*(8), 1097–1107. https://doi.org/10.1089/NEU.2019.6475

Baura, G. D., Foster, D. M., Porte, D., Kahn, S. E., Bergman, R. N., Cobelli, C., & Schwartz, M. W. (1993). Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. *Journal of Clinical Investigation*, *92*(4), 1824–1830. https://doi.org/10.1172/JCI116773

Bianchi, R., Giambanco, I., & Donato, R. (2010). S100B/RAGE-dependent activation of microglia via NF-kappaB and AP-1 Co-regulation of COX-2 expression by S100B, IL-1beta and TNF-alpha. *Neurobiology of Aging*, *31*(4), 665–677. https://doi.org/10.1016/J.NEUROBIOLAGING.2008.05.017

Bianchi, R., Kastrisianaki, E., Giambanco, I., & Donato, R. (2011). S100B protein stimulates microglia migration via RAGE-dependent up-regulation of chemokine expression and release. *The Journal of Biological Chemistry*, *286*(9), 7214–7226. https://doi.org/10.1074/JBC.M110.169342

Bierhaus, A., Humpert, P. M., Morcos, M., Wendt, T., Chavakis, T., Arnold, B., Stern, D. M., & Nawroth, P. P. (2005). Understanding RAGE, the receptor for advanced glycation end products. *Journal of Molecular Medicine*, *83*(11), 876–886. https://doi.org/10.1007/S00109-005-0688-7

Biessels, G. J., & Despa, F. (2018a). Cognitive decline and dementia in diabetes: mechanisms and clinical implications. *Nature Reviews. Endocrinology*, *14*(10), 591. https://doi.org/10.1038/S41574-018-0048-7

Biessels, G. J., & Despa, F. (2018b). Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. *Nature Reviews. Endocrinology*, *14*(10), 591–604. https://doi.org/10.1038/S41574-018-0048-7

Bikle, D., Adams, J. S., & Christakos, S. (2013). Vitamin D: Production, Metabolism, Mechanism of Action, and Clinical Requirements. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism: Eighth Edition*, 235–247. https://doi.org/10.1002/9781118453926.CH29

Bikle, D. D. (2021). Vitamin D: Production, Metabolism and Mechanisms of Action. *Endotext*.

Blanchard, H., Grochulski, P., Li, Y., Arthur, J. S., Davies, P. L., Elce, J. S., & Cygler, M. (1997). Structure of a calpain Ca(2+)-binding domain reveals a novel EF-hand and Ca(2+)-induced conformational changes. *Nature Structural Biology*, *4*(7), 532–538. https://doi.org/10.1038/nsb0797-532

Bogush, M., Heldt, N. A., & Persidsky, Y. (2017). Blood Brain Barrier Injury in Diabetes: Unrecognized Effects on Brain and Cognition. *Journal of Neuroimmune Pharmacology*, *12*(4), 593–601. https://doi.org/10.1007/s11481-017-9752-7

Botelho, H. M., Fritz, G., & Gomes, C. M. (2012). Analysis of S100 oligomers and amyloids. *Methods in Molecular Biology (Clifton, N.J.)*, *849*, 373–386. https://doi.org/10.1007/978-1-61779-551-0_25

Bouma, G., Lam-Tse, W. K., Wierenga-Wolf, A. F., Drexhage, H. A., & Versnel, M. A. (2004). Increased serum levels of MRP-8/14 in type 1 diabetes induce an increased expression of CD11b and an enhanced adhesion of circulating monocytes to fibronectin. *Diabetes*, *53*(8), 1979–1986. https://doi.org/10.2337/DIABETES.53.8.1979

Bresson, J. L., Burlingame, B., Dean, T., Fairweather-Tait, S., Heinonen, M., Hirsch-Ernst, K. I., Mangelsdorf, I., McArdle, H., Naska, A., Neuhäuser-Berthold, M., Nowicka, G., Pentieva, K., Sanz, Y., Siani, A., Sjödin, A., Stern, M., Tomé, D., Turck, D., Van Loveren, H., … Willatts, P. (2016). Dietary reference values for vitamin D. *EFSA Journal*, *14*(10), e04547. https://doi.org/10.2903/J.EFSA.2016.4547

Brożyna, A. A., Slominski, R. M., Nedoszytko, B., Zmijewski, M. A., & Slominski, A. T. (2022). Vitamin D Signaling in Psoriasis: Pathogenesis and Therapy. *International Journal of Molecular Sciences*, *23*(15). https://doi.org/10.3390/IJMS23158575

Bui, N. T., Livolsi, A., Peyron, J. F., & Prehn, J. H. M. (2001). Activation of Nuclear Factor κb and bcl-x Survival Gene Expression by Nerve Growth Factor Requires Tyrosine Phosphorylation of IκBα. *Journal of Cell Biology*, *152*(4), 753–764. https://doi.org/10.1083/JCB.152.4.753

Byun, K., Yoo, Y. C., Son, M., Lee, J., Jeong, G. B., Park, Y. M., Salekdeh, G. H., & Lee, B. (2017). Advanced glycation end-products produced systemically and by macrophages: A common contributor to inflammation and degenerative diseases. *Pharmacology & Therapeutics*, *177*, 44–55. https://doi.org/10.1016/J.PHARMTHERA.2017.02.030

Calcium, I. of M. (US) C. to R. D. R. I. for V. D. and, Ross, A. C., Taylor, C. L., Yaktine, A. L., & Valle, H. B. Del. (2011). *Dietary Reference Intakes for Adequacy: Calcium and Vitamin D*.

Calgaroto, N. S., Thomé, G. R., da Costa, P., Baldissareli, J., Hussein, F. A., Schmatz, R., Rubin, M. A., Signor, C., Ribeiro, D. A., Carvalho, F. B., de Oliveira, L. S., Pereira, L. B., Morsch, V. M., & Schetinger, M. R. C. (2014). Effect of vitamin D3 on behavioural and biochemical parameters in diabetes type 1-induced rats. *Cell Biochemistry and Function*, *32*(6), 502–510. https://doi.org/10.1002/CBF.3044

Cantley, L. K., Russell, J., Lettieri, D., & Sherwood, L. M. (1985). 1,25-Dihydroxyvitamin D3 Suppresses Parathyroid Hormone Secretion from Bovine Parathyroid Cells in Tissue Culture. *Endocrinology*, *117*(5), 2114–2119. https://doi.org/10.1210/ENDO-117-5-2114

Catalán, V., Gómez-Ambrosi, J., Rodrígue, A., Ramírez, B., Rotellar, F., Valentí, V., Silva, C., Gil, M. J., Fernández-Real, J. M., Salvador, J., & Frühbeck, G. (2011). Increased levels of calprotectin in obesity are related to macrophage content: impact on inflammation and effect of weight loss. *Molecular Medicine (Cambridge, Mass.)*, *17*(11–12), 1157–1167. https://doi.org/10.2119/MOLMED.2011.00144

Cefalu, W. T., Buse, J. B., Tuomilehto, J., Fleming, G. A., Ferrannini, E., Gerstein, H. C., Bennett, P. H., Ramachandran, A., Raz, I., Rosenstock, J., & Kahn, S. E. (2016). Update and Next Steps for Real-World Translation of Interventions for Type 2 Diabetes Prevention: Reflections From a Diabetes Care Editors' Expert Forum. *Diabetes Care*, *39*(7), 1186–1201. https://doi.org/10.2337/dc16-0873

Ceriello, A. (2012). The emerging challenge in diabetes: The "metabolic memory." *Vascular Pharmacology*, *57*(5–6), 133–138. https://doi.org/10.1016/J.VPH.2012.05.005

Charoenngam, N., & Holick, M. F. (2020). Immunologic Effects of Vitamin D on Human Health and Disease. *Nutrients*, *12*(7), 1–28. https://doi.org/10.3390/NU12072097

Chaudhuri, J., Bains, Y., Guha, S., Kahn, A., Hall, D., Bose, N., Gugliucci, A., & Kapahi, P. (2018). The Role of Advanced Glycation End Products in Aging and Metabolic Diseases: Bridging Association and Causality. *Cell Metabolism*, *28*(3), 337–352. https://doi.org/10.1016/J.CMET.2018.08.014

Chen, J., Zhang, J., Liu, X., Wang, X., Xu, X., Li, H., … B. C.-E., & 2017, undefined. (2017). Abnormal subcortical nuclei shapes in patients with type 2 diabetes mellitus. *SpringerJ Chen, J Zhang, X Liu, X Wang, X Xu, H Li, B Cao, Y Yang, J Lu, Z ChenEuropean Radiology, 2017•Springer*, *27*(10), 4247–4256. https://doi.org/10.1007/s00330-017-4790-3

Chuah, Y. K., Basir, R., Talib, H., Tie, T. H., & Nordin, N. (2013). Receptor for advanced glycation end products and its involvement in inflammatory diseases. *International Journal of Inflammation*, *2013*. https://doi.org/10.1155/2013/403460

Coelho, V. R., Sousa, K., Pires, T. R., Papke, D. K. M., Vieira, C. G., De Souza, L. P., Leal, M. B., Schunck, R. V. A., Picada, J. N., & Pereira, P. (2016). Genotoxic and mutagenic effects of vigabatrin, a γ-aminobutyric acid transaminase inhibitor, in Wistar rats submitted to rotarod task. *Human and Experimental Toxicology*, *35*(9), 958–965. https://doi.org/10.1177/0960327115611970/ASSET/IMAGES/LARGE/10.1177_0960327115611970-FIG2.JPEG

Cole, J. B., & Florez, J. C. (2020). Genetics of diabetes mellitus and diabetes complications. *Nature Reviews. Nephrology*, *16*(7), 377–390. https://doi.org/10.1038/S41581-020-0278-5

Colhoun, H. M., Betteridge, D. J., Durrington, P., Hitman, G., Neil, A., Livingstone, S., Charlton-Menys, V., Bao, W., DeMicco, D. A., Preston, G. M., Deshmukh, H., Tan, K., & Fuller, J. H. (2011). Total soluble and endogenous secretory receptor for advanced glycation end products as predictive biomarkers of coronary heart disease risk in patients with type 2 diabetes: an analysis from the CARDS trial. *Diabetes*, *60*(9), 2379–2385. https://doi.org/10.2337/DB11-0291

Colonna, M., & Butovsky, O. (2017). Microglia Function in the Central Nervous System During Health and Neurodegeneration. *Annual Review of Immunology*, *35*, 441. https://doi.org/10.1146/ANNUREV-IMMUNOL-051116-052358

Contreras-Bolívar, V., García-Fontana, B., García-Fontana, C., & Muñoz-Torres, M. (2021). Mechanisms involved in the relationship between vitamin D and insulin resistance: impact on clinical practice. In *Nutrients* (Vol. 13, Issue 10). MDPI. https://doi.org/10.3390/nu13103491

Copps, K. D., & White, M. F. (2012). Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. *Diabetologia*, *55*(10), 2565–2582. https://doi.org/10.1007/S00125-012-2644-8

Cristóvaõ, J. S., & Gomes, C. M. (2019). S100 Proteins in Alzheimer's Disease. *Frontiers in Neuroscience*, *13*(MAY). https://doi.org/10.3389/FNINS.2019.00463

Cui, X., McGrath, J. J., Burne, T. H. J., Mackay-Sim, A., & Eyles, D. W. (2007). Maternal vitamin D depletion alters neurogenesis in the developing rat brain. *International Journal of Developmental Neuroscience : The Official Journal of the International Society for Developmental Neuroscience*, *25*(4), 227–232. https://doi.org/10.1016/J.IJDEVNEU.2007.03.006

Cunden, L. S., Brophy, M. B., Rodriguez, G. E., Flaxman, H. A., & Nolan, E. M. (2017). Biochemical and Functional Evaluation of the Intramolecular Disulfide Bonds in the Zinc-Chelating Antimicrobial Protein Human S100A7 (Psoriasin). *Biochemistry*, *56*(43), 5726–5738. https://doi.org/10.1021/ACS.BIOCHEM.7B00781

Dakic, T., Jevdjovic, T., Lakic, I., Ruzicic, A., Jasnic, N., Djurasevic, S., Djordjevic, J., & Vujovic, P. (2023). The Expression of Insulin in the Central Nervous System: What Have We Learned So Far? *International Journal of Molecular Sciences*, *24*(7). https://doi.org/10.3390/IJMS24076586

Dall, T. M., Yang, W., Halder, P., Pang, B., Massoudi, M., Wintfeld, N., Semilla, A. P., Franz, J., & Hogan, P. F. (2014). The economic burden of elevated blood glucose levels in 2012: diagnosed and undiagnosed diabetes, gestational diabetes mellitus, and prediabetes. *Diabetes Care*, *37*(12), 3172–3179. https://doi.org/10.2337/dc14-1036

D'Cunha, N. M., Sergi, D., Lane, M. M., Naumovski, N., Gamage, E., Rajendran, A., Kouvari, M., Gauci, S., Dissanayka, T., Marx, W., & Travica, N. (2022). The Effects of Dietary Advanced Glycation End-Products on Neurocognitive and Mental Disorders. *Nutrients*, *14*(12). https://doi.org/10.3390/nu14122421

Deacon, R. M. J. (2013). Measuring motor coordination in mice. *Journal of Visualized Experiments : JoVE*, *75*. https://doi.org/10.3791/2609

Demircan, S., Önalan, E., Kuloğlu, T., Aydın, S., Yalçın, M. H., Gözel, N., & Dönder, E. (2020). Effects of vitamin D on apoptosis and betatrophin in the kidney tissue of

experimental diabetic rats. *Acta Bio Medica : Atenei Parmensis*, *91*(4), 1–10. https://doi.org/10.23750/ABM.V91I4.8944

Derakhshanian, H., Djazayery, A., Javanbakht, M. H., Eshraghian, M. R., Mirshafiey, A., Jahanabadi, S., Ghadbeigi, S., Zarei, M., Alvandi, E., & Djalali, M. (2019). Vitamin D downregulates key genes of diabetes complications in cardiomyocyte. *Journal of Cellular Physiology*, *234*(11), 21352–21358. https://doi.org/10.1002/JCP.28743

Donato, R., R. Cannon, B., Sorci, G., Riuzzi, F., Hsu, K., J. Weber, D., & L. Geczy, C. (2013). Functions of S100 Proteins. *Current Molecular Medicine*, *13*(1), 24. https://doi.org/10.2174/156652413804486214

Dove, A., Shang, Y., Xu, W., Grande, G., Laukka, E. J., Fratiglioni, L., & Marseglia, A. (2021). The impact of diabetes on cognitive impairment and its progression to dementia. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, *17*(11), 1769–1778. https://doi.org/10.1002/ALZ.12482

Egashira, N., Akiyoshi, Y., Iba, H., Arai, T., Hatip-Al-Khatib, I., Mishima, K., & Iwasaki, K. (2018). Tokishakuyakusan ameliorates spatial memory deficits induced by ovariectomy combined with β-amyloid in rats. *Journal of Pharmacological Sciences*, *136*(3), 149–154. https://doi.org/10.1016/j.jphs.2018.01.005

Eyles, D. W., Smith, S., Kinobe, R., Hewison, M., & McGrath, J. J. (2005). Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *Journal of Chemical Neuroanatomy*, *29*(1), 21–30. https://doi.org/10.1016/J.JCHEMNEU.2004.08.006

Fathi, F. E. Z. M., Sadek, K. M., Khafaga, A. F., Al senosy, A. W., Ghoniem, H. A., Fayez, S., & Zeweil, M. F. (2022). Vitamin D regulates insulin and ameliorates apoptosis and oxidative stress in pancreatic tissues of rats with streptozotocin-induced diabetes. *Environmental Science and Pollution Research*, *29*(60), 90219–90229. https://doi.org/10.1007/s11356-022-22064-2

Feng, G., Xu, X., Youssef, E. M., & Lotan, R. (2001). Diminished expression of S100A2, a putative tumor suppressor, at early stage of human lung carcinogenesis. *Cancer Research*, *61*(21), 7999–8004.

Foell, D., Wittkowski, H., Vogl, T., & Roth, J. (2007). S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. *Journal of Leukocyte Biology*, *81*(1), 28–37. https://doi.org/10.1189/JLB.0306170

Furman, B. L. (2021). Streptozotocin-Induced Diabetic Models in Mice and Rats. *Current Protocols*, *1*(4), e78. https://doi.org/10.1002/CPZ1.78

Gáll, Z., & Székely, O. (2021). Role of Vitamin D in Cognitive Dysfunction: New Molecular Concepts and Discrepancies between Animal and Human Findings. *Nutrients*, *13*(11). https://doi.org/10.3390/NU13113672

Garay-Sevilla, M., Rojas, A., Portero-Otin, M., Nutrients, J. U.-, & 2021, undefined. (2021). Dietary AGEs as Exogenous Boosters of Inflammation. *Mdpi.Com*, *13*(8). https://doi.org/10.3390/nu13082802

Georgy, G. S., Nassar, N. N., Mansour, H. A., & Abdallah, D. M. (2013). Cerebrolysin Ameloriates Cognitive Deficits in Type III Diabetic Rats. *PloS One*, *8*(6). https://doi.org/10.1371/JOURNAL.PONE.0064847

Gill, V., Kumar, V., Singh, K., Kumar, A., Biomolecules, J. K.-, & 2019, undefined. (2019). Advanced glycation end products (AGEs) may be a striking link between modern diet and health. *Mdpi.Com*, *9*(12). https://doi.org/10.3390/biom9120888

Goh, S. Y., & Cooper, M. E. (2008). Clinical review: The role of advanced glycation end products in progression and complications of diabetes. *The Journal of Clinical Endocrinology and Metabolism*, *93*(4), 1143–1152. https://doi.org/10.1210/JC.2007-1817

Gonzalez, L. L., Garrie, K., & Turner, M. D. (2020). Role of S100 proteins in health and disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1867*(6), 118677. https://doi.org/10.1016/J.BBAMCR.2020.118677

Goyal, R., & Jialal, I. (2022). *Diabetes Mellitus Type 2*.

Hahn, J., Cook, N. R., Alexander, E. K., Friedman, S., Walter, J., Bubes, V., Kotler, G., Lee, I. M., Manson, J. A. E., & Costenbader, K. H. (2022). Vitamin D and marine omega 3 fatty acid supplementation and incident autoimmune disease: VITAL randomized controlled trial. *BMJ (Clinical Research Ed.)*, *376*. https://doi.org/10.1136/BMJ-2021-066452

Harreiter, J., & Roden, M. (2019). Diabetes mellitus—Definition, classification, diagnosis, screening and prevention (Update 2019). *Wiener Klinische Wochenschrift*, *131*, 6–15. https://doi.org/10.1007/s00508-019-1450-4

Heizmann, C. W. (2019). S100 proteins: Diagnostic and prognostic biomarkers in laboratory medicine. *Biochimica et Biophysica Acta. Molecular Cell Research*, *1866*(7), 1197–1206. https://doi.org/10.1016/J.BBAMCR.2018.10.015

Heizmann, C. W., Fritz, G., & Schäfer, B. W. (2002). S100 proteins: structure, functions and pathology. *Frontiers in Bioscience : A Journal and Virtual Library*, *7*. https://doi.org/10.2741/A846

Herrmann, M., Farrell, C. J. L., Pusceddu, I., Fabregat-Cabello, N., & Cavalier, E. (2017). Assessment of vitamin D status - a changing landscape. *Clinical Chemistry and Laboratory Medicine*, *55*(1), 3–26. https://doi.org/10.1515/CCLM-2016-0264

Howells, L., Musaddaq, B., McKay, A. J., & Majeed, A. (2016). Clinical impact of lifestyle interventions for the prevention of diabetes: an overview of systematic reviews. *BMJ Open*, *6*(12), e013806. https://doi.org/10.1136/bmjopen-2016-013806

Hudson, B. I., Carter, A. M., Harja, E., Kalea, A. Z., Arriero, M., Yang, H., Grant, P. J., & Schmidt, A. M. (2008). Identification, classification, and expression of RAGE gene splice variants. *Wiley Online Library*, *22*(5), 1572–1580. https://doi.org/10.1096/fj.07-9909com

Hussien, N., El-Wakeel, H., … S. S.-G. P., & 2019, undefined. (2019). Alleviation of cardiac mitochondrial dysfunction and oxidative stress underlies the protective effect of vitamin D in chronic stress-induced cardiac dysfunction in. *Fmed.Stafpu.Bu.Edu.EgNI Hussien, HS El-Wakeel, SM Souror, IA AhmedGen Physiol Biophys, 2019•fmed.Stafpu.Bu.Edu.Eg*, *38*, 51–61. https://doi.org/10.4149/gpb_2018036

Iatcu, C. O., Steen, A., & Covasa, M. (2021). Gut Microbiota and Complications of Type-2 Diabetes. *Nutrients*, *14*(1). https://doi.org/10.3390/NU14010166

IDF news. (n.d.). Retrieved December 14, 2022, from https://www.idf.org/news/198:who-report-reveals-that-diabetes-is-among-the-top-10-causes-of-death-and-disability-worldwide.html

Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, *53*(6), 365–374. https://doi.org/10.1016/J.MEDICI.2018.02.001

International Diabetes Federation. (2021). *IDF_Atlas_10th_Edition_2021.pdf* (pp. 1–141). https://diabetesatlas.org/idfawp/resource-files/2021/07/IDF_Atlas_10th_Edition_2021.pdf

Jin, Y., Sharma, A., Carey, C., Hopkins, D., Wang, X., Robertson, D. G., Bode, B., Anderson, S. W., Reed, J. C., Steed, R. D., Steed, L., & She, J. X. (2013). The expression of inflammatory genes is upregulated in peripheral blood of patients with type 1 diabetes. *Diabetes Care*, *36*(9), 2794–2802. https://doi.org/10.2337/DC12-1986/-/DC1

Jones, G., Prosser, D. E., & Kaufmann, M. (2018). The Activating Enzymes of Vitamin D Metabolism (25- and 1α-Hydroxylases). *Vitamin D: Fourth Edition*, *1*, 57–79. https://doi.org/10.1016/B978-0-12-809965-0.00005-7

Jukic, A., Bakiri, L., Wagner, E. F., Tilg, H., & Adolph, T. E. (2021). Calprotectin: from biomarker to biological function. *Gut*, *70*, 1978–1988. https://doi.org/10.1136/gutjnl-2021-324855

Jung, J. Y., Sung, J. Y., Chung, W., Kim, A. J., Kim, H. S., Ro, H., Chang, J. H., & Lee, H. H. (2013). Calcitriol treatment increases serum levels of the soluble receptor of advanced glycation end products in hemodialysis patients with secondary hyperparathyroidism. *Jstage.Jst.Go.JpJY Sung, W Chung, AJ Kim, HS Kim, H Ro, JH Chang, HH Lee, JY JungThe Tohoku Journal of Experimental Medicine, 2013•jstage.Jst.Go.Jp*, *230*(1), 59–66. https://doi.org/10.1620/tjem.230.59

Kelley, N., Jeltema, D., Duan, Y., molecular, Y. H.-I. journal of, & 2019, undefined. (2019). The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Mdpi.Com*, *20*(13). https://doi.org/10.3390/ijms20133328

Khalid, M., Petroianu, G., & Adem, A. (2022). Advanced Glycation End Products and Diabetes Mellitus: Mechanisms and Perspectives. *Biomolecules 2022, Vol. 12, Page 542*, *12*(4), 542. https://doi.org/10.3390/BIOM12040542

Kögel, D., Peters, M., König, H. G., Hashemi, S. M. A., Bui, N. T., Arolt, V., Rothermundt, M., & Prehn, J. H. M. (2004). S100B potently activates p65/c-Rel transcriptional complexes in hippocampal neurons: Clinical implications for the role of S100B in excitotoxic brain injury. *Neuroscience*, *127*(4), 913–920. https://doi.org/10.1016/J.NEUROSCIENCE.2004.06.013

Kolb Mandrup-Poulsen, H. T. (2005). An immune origin of type 2 diabetes? *Diabetologia*, *48*, 1038–1050. https://doi.org/10.1007/s00125-005-1764-9

Kosaraju, J., Madhunapantula, S. R. V., Chinni, S., Khatwal, R. B., Dubala, A., Muthureddy Nataraj, S. K., & Basavan, D. (2014). Dipeptidyl peptidase-4 inhibition by Pterocarpus marsupium and Eugenia jambolana ameliorates streptozotocin induced Alzheimer's disease. *Behavioural Brain Research*, *267*, 55–65. https://doi.org/10.1016/J.BBR.2014.03.026

Kottaisamy, C. P. D., Raj, D. S., Kumar, V. P., & Sankaran, U. (2021). Experimental animal models for diabetes and its related complications—a review. *Laboratory Animal Research*, *37*(1). https://doi.org/10.1186/S42826-021-00101-4

Kubis-Kubiak, A., Dyba, A., & Piwowar, A. (2020). The Interplay between Diabetes and Alzheimer's Disease-In the Hunt for Biomarkers. *International Journal of Molecular Sciences*, *21*(8). https://doi.org/10.3390/IJMS21082744

Kumar, P. T., Antony, S., Nandhu, M. S., Sadanandan, J., Naijil, G., & Paulose, C. S. (2011). Vitamin D3 restores altered cholinergic and insulin receptor expression in the cerebral cortex and muscarinic M3 receptor expression in pancreatic islets of streptozotocin induced diabetic rats. *The Journal of Nutritional Biochemistry*, *22*(5), 418–425. https://doi.org/10.1016/J.JNUTBIO.2010.03.010

Lasič, E., Galland, F., Vardjan, N., Šribar, J., Križaj, I., Leite, M. C., Zorec, R., & Stenovec, M. (2016). Time-dependent uptake and trafficking of vesicles capturing extracellular S100B in cultured rat astrocytes. *Journal of Neurochemistry*, *139*(2), 309–323. https://doi.org/10.1111/JNC.13754

Latimer, C. S., Brewer, L. D., Searcy, J. L., Chen, K. C., Popović, J., Kraner, S. D., Thibault, O., Blalock, E. M., Landfield, P. W., & Porter, N. M. (2014). Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(41), E4359–E4366. https://doi.org/10.1073/PNAS.1404477111

Lee, E. J., & Park, J. H. (2013). Receptor for Advanced Glycation Endproducts (RAGE), Its Ligands, and Soluble RAGE: Potential Biomarkers for Diagnosis and Therapeutic Targets for Human Renal Diseases. *Genomics & Informatics*, *11*(4), 224–229. https://doi.org/10.5808/GI.2013.11.4.224

Lee, S., Clark, S. A., Gill, R. K., & Christakos, S. (1994). 1,25-Dihydroxyvitamin D3 and pancreatic beta-cell function: vitamin D receptors, gene expression, and insulin secretion. *Endocrinology*, *134*(4), 1602–1610. https://doi.org/10.1210/ENDO.134.4.8137721

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216–226. https://doi.org/10.1007/S00125-007-0886-7

Li, K., Li, J., Zheng, J., & Qin, S. (2019). Reactive Astrocytes in Neurodegenerative Diseases. *Aging and Disease*, *10*(3), 664–675. https://doi.org/10.14336/AD.2018.0720

Lim, J. H., Lee, H. J., Ho Jung, M., & Song, J. (2009). Coupling mitochondrial dysfunction to endoplasmic reticulum stress response: A molecular mechanism leading to hepatic insulin resistance. *Cellular Signalling*, *21*(1), 169–177. https://doi.org/10.1016/J.CELLSIG.2008.10.004

Lim, M., Park, L., Shin, G., Hong, H., Kang, I., & Park, Y. (2008). Induction of apoptosis of Beta cells of the pancreas by advanced glycation end-products, important mediators of chronic complications of diabetes mellitus. *Annals of the New York Academy of Sciences*, *1150*, 311–315. https://doi.org/10.1196/ANNALS.1447.011

Lin, N., Zhang, H., & Su, Q. (2012). Advanced glycation end-products induce injury to pancreatic beta cells through oxidative stress. *Diabetes & Metabolism*, *38*(3), 250–257. https://doi.org/10.1016/J.DIABET.2012.01.003

Lips, P., Eekhoff, M., van Schoor, N., Oosterwerff, M., de Jongh, R., Krul-Poel, Y., & Simsek, S. (2017a). Vitamin D and type 2 diabetes. *The Journal of Steroid Biochemistry and Molecular Biology*, *173*, 280–285. https://doi.org/10.1016/J.JSBMB.2016.11.021

Lips, P., Eekhoff, M., van Schoor, N., Oosterwerff, M., de Jongh, R., Krul-Poel, Y., & Simsek, S. (2017b). Vitamin D and type 2 diabetes. In *Journal of Steroid Biochemistry and Molecular Biology* (Vol. 173, pp. 280–285). Elsevier Ltd. https://doi.org/10.1016/j.jsbmb.2016.11.021

Lv, Y. Q., Yuan, L., Sun, Y., Dou, H. W., Su, J. H., Hou, Z. P., Li, J. Y., & Li, W. (2022). Long-term hyperglycemia aggravates α-synuclein aggregation and dopaminergic neuronal loss in a Parkinson's disease mouse model. *Translational Neurodegeneration*, *11*(1). https://doi.org/10.1186/S40035-022-00288-Z

Mattson, M. P., Culmsee, C., Yu, Z., & Camandola, S. (2000). Roles of nuclear factor κB in neuronal survival and plasticity. *Journal of Neurochemistry*, *74*(2), 443–456. https://doi.org/10.1046/J.1471-4159.2000.740443.X

Mehran, A. E., Templeman, N. M., Brigidi, G. S., Lim, G. E., Chu, K. Y., Hu, X., Botezelli, J. D., Asadi, A., Hoffman, B. G., Kieffer, T. J., Bamji, S. X., Clee, S. M., & Johnson, J. D. (2012). Hyperinsulinemia drives diet-induced obesity independently of brain insulin production. *Cell Metabolism*, *16*(6), 723–737. https://doi.org/10.1016/J.CMET.2012.10.019

Mitrašinović-Brulić, M., Dervišević, A., Začiragić, A., Fočak, M., Valjevac, A., Hadžović-Džuvo, A., & Suljević, D. (2021). Vitamin D3 attenuates oxidative stress and regulates glucose level and leukocyte count in a semi-chronic streptozotocin-induced diabetes model. *Journal of Diabetes and Metabolic Disorders*, *20*(1), 771–779. https://doi.org/10.1007/S40200-021-00814-2

Mohammadzadeh, F., Tsoporis, J. N., Izhar, S., Desjardins, J. F., & Parker, T. G. (2018). Deficiency of S100B confers resistance to experimental diabetes in mice. *Experimental Cell Research*, *365*(1), 129–137. https://doi.org/10.1016/J.YEXCR.2018.02.030

Monville, C., Torres, E. M., & Dunnett, S. B. (2006). Comparison of incremental and accelerating protocols of the rotarod test for the assessment of motor deficits in the 6-OHDA model. *Journal of Neuroscience Methods*, *158*(2), 219–223. https://doi.org/10.1016/J.JNEUMETH.2006.06.001

Moretti, R., Morelli, M. E., & Caruso, P. (2018). Vitamin D in Neurological Diseases: A Rationale for a Pathogenic Impact. *International Journal of Molecular Sciences*, *19*(8). https://doi.org/10.3390/IJMS19082245

Mortensen, O. H., Nielsen, A. R., Erikstrup, C., Plomgaard, P., Fischer, C. P., Krogh-Madsen, R., Lindegaard, B., Petersen, A. M., Taudorf, S., & Philip, C. (2009). Calprotectin--a novel marker of obesity. *PloS One*, *4*(10). https://doi.org/10.1371/JOURNAL.PONE.0007419

Most, P., Seifert, H., Gao, E., Funakoshi, H., Völkers, M., Heierhorst, J., Remppis, A., Pleger, S. T., DeGeorge, B. R., Eckhart, A. D., Feldman, A. M., & Koch, W. J. (2006). Cardiac S100A1 Protein Levels Determine Contractile Performance and Propensity Toward Heart Failure After Myocardial Infarction. *Circulation*, *114*(12), 1258–1268. https://doi.org/10.1161/CIRCULATIONAHA.106.622415

Mousa, A., Naderpoor, N., Teede, H., Scragg, R., & de Courten, B. (2018). Vitamin D supplementation for improvement of chronic low-grade inflammation in patients with type 2 diabetes: A systematic review and meta-analysis of randomized controlled trials. *Nutrition Reviews*, *76*(5), 380–394. https://doi.org/10.1093/NUTRIT/NUX077

Muneeb, M., Mansou, S. M., Saleh, S., & Mohammed, R. A. (2022a). Vitamin D and rosuvastatin alleviate type-II diabetes-induced cognitive dysfunction by modulating neuroinflammation and canonical/noncanonical Wnt/β-catenin signaling. *PLOS ONE*, *17*(11). https://doi.org/10.1371/JOURNAL.PONE.0277457

Muneeb, M., Mansou, S. M., Saleh, S., & Mohammed, R. A. (2022b). Vitamin D and rosuvastatin alleviate type-II diabetes-induced cognitive dysfunction by modulating neuroinflammation and canonical/noncanonical Wnt/β-catenin signaling. *PLoS ONE*, *17*(11 November). https://doi.org/10.1371/journal.pone.0277457

Muneer, M., & Akbar, I. (2021). Acute Metabolic Emergencies in Diabetes: DKA, HHS and EDKA. *Advances in Experimental Medicine and Biology*, *1307*, 85–114. https://doi.org/10.1007/5584_2020_545

Muñoz, A., & Grant, W. B. (2022). Vitamin D and Cancer: An Historical Overview of the Epidemiology and Mechanisms. *Nutrients*, *14*(7). https://doi.org/10.3390/NU14071448

Muramatsu, K. (2020). Diabetes mellitus-related dysfunction of the motor system. In *International Journal of Molecular Sciences* (Vol. 21, Issue 20, pp. 1–26). MDPI AG. https://doi.org/10.3390/ijms21207485

Nandipati, K. C., Subramanian, S., & Agrawal, D. K. (2017). Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance. *Molecular and Cellular Biochemistry*, *426*(1–2), 27–45. https://doi.org/10.1007/S11010-016-2878-8

Nemere, I., Safford, S. E., Rohe, B., DeSouza, M. M., & Farach-Carson, M. C. (2004). Identification and characterization of 1,25D3-membrane- associated rapid response, steroid (1,25D3-MARRS) binding protein. *Journal of Steroid Biochemistry and Molecular Biology*, *89–90*(1–5), 281–285. https://doi.org/10.1016/j.jsbmb.2004.03.031

Nimitphong, H., & Holick, M. F. (2011). Vitamin D, neurocognitive functioning and immunocompetence. *Current Opinion in Clinical Nutrition and Metabolic Care*, *14*(1), 7–14. https://doi.org/10.1097/MCO.0B013E3283414C38

Nishiyama, H., Knöpfel, T., Endo, S., & Itohara, S. (2002). Glial protein S100B modulates long-term neuronal synaptic plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(6), 4037–4042. https://doi.org/10.1073/PNAS.052020999

Norton, L., Shannon, C., Gastaldelli, A., & DeFronzo, R. A. (2022). Insulin: The master regulator of glucose metabolism. *Metabolism: Clinical and Experimental*, *129*. https://doi.org/10.1016/J.METABOL.2022.155142

Nowotny, K., Jung, T., Höhn, A., Weber, D., & Grune, T. (2015a). Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. *Biomolecules*, *5*(1), 194–222. https://doi.org/10.3390/BIOM5010194

Nowotny, K., Jung, T., Höhn, A., Weber, D., & Grune, T. (2015b). Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. *Biomolecules*, *5*(1), 194. https://doi.org/10.3390/BIOM5010194

Ogbonnaya Eleazu, C., Chinedum Eleazu, K., Chukwuma, S., & Essien, U. N. (2013). *Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans*. http://www.jdmdonline.com/content/12/1/60

Oosterwerff, M. M., Eekhoff, E. M. W., Heymans, M. W., Lips, P., & Van Schoor, N. M. (2011). Serum 25-hydroxyvitamin D levels and the metabolic syndrome in older persons: a population-based study. *Clinical Endocrinology*, *75*(5), 608–613. https://doi.org/10.1111/J.1365-2265.2011.04110.X

Organization, W. H., & Federation, I. D. (n.d.). *Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia : report of a WHO/IDF consultation*. World Health Organization. https://apps.who.int/iris/handle/10665/43588

Ouma, S., Suenaga, M., Bölükbaşı Hatip, F. F., Hatip-Al-Khatib, I., Tsuboi, Y., & Matsunaga, Y. (2018). Serum vitamin D in patients with mild cognitive impairment and Alzheimer's disease. *Brain and Behavior*, *8*(3). https://doi.org/10.1002/brb3.936

Ozaki, K. I., Awazu, M., Tamiya, M., Iwasaki, Y., Harada, A., Kugisaki, S., Tanimura, S., & Kohno, M. (2016). Targeting the ERK signaling pathway as a potential treatment for insulin resistance and type 2 diabetes. *American Journal of Physiology - Endocrinology and Metabolism*, *310*(8), E643–E651. https://doi.org/10.1152/AJPENDO.00445.2015

Pandey, S., & Dvorakova, M. C. (2019). Future Perspective of Diabetic Animal Models. *Endocrine, Metabolic & Immune Disorders - Drug Targets*, *20*(1), 25–38. https://doi.org/10.2174/1871530319666190626143832

Patel, P., & Shah, J. (2017). Role of Vitamin D in Amyloid clearance via LRP-1 upregulation in Alzheimer's disease: A potential therapeutic target? *Journal of Chemical Neuroanatomy*, *85*, 36–42. https://doi.org/10.1016/J.JCHEMNEU.2017.06.007

Peeyush, K. T., Savitha, B., Sherin, A., Anju, T. R., Jes, P., & Paulose, C. S. (2010). Cholinergic, dopaminergic and insulin receptors gene expression in the cerebellum of streptozotocin-induced diabetic rats: Functional regulation with Vitamin D3 supplementation. *Pharmacology Biochemistry and Behavior*, *95*(2), 216–222. https://doi.org/10.1016/J.PBB.2010.01.008

Prasad, K. (2014). Low levels of serum soluble receptors for advanced glycation end products, biomarkers for disease state: myth or reality. *The International Journal of Angiology : Official Publication of the International College of Angiology, Inc*, *23*(1), 11–16. https://doi.org/10.1055/S-0033-1363423

Primavera, M., Giannini, C., & Chiarelli, F. (2020). Prediction and Prevention of Type 1 Diabetes. *Frontiers in Endocrinology*, *11*. https://doi.org/10.3389/FENDO.2020.00248

Pruenster, M., Vogl, T., Roth, J., & Sperandio, M. (2016). S100A8/A9: From basic science to clinical application. *Pharmacology & Therapeutics*, *167*, 120–131. https://doi.org/10.1016/J.PHARMTHERA.2016.07.015

Pugazhenthi, S., Qin, L., & Reddy, P. H. (2017). Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochimica et Biophysica Acta. Molecular Basis of Disease*, *1863*(5), 1037–1045. https://doi.org/10.1016/J.BBADIS.2016.04.017

Quialheiro, A., D Orsi, E., Moreira, J. D., Xavier, A. J., & Peres, M. A. (2023). The association between vitamin D and BDNF on cognition in older adults in Southern Brazil. *Revista de Saude Publica*, *56*, 109. https://doi.org/10.11606/S1518-8787.2022056004134

Rad, M. G., Sharifi, M., Meamar, R., & Soltani, N. (2022). The role of pancreas to improve hyperglycemia in STZ-induced diabetic rats by thiamine disulfide. *Nutrition and Diabetes*, *12*(1). https://doi.org/10.1038/s41387-022-00211-5

Ramasamy, R., … S. Y.-A. of the N. Y., & 2011, undefined. (2011). Receptor for AGE (RAGE): signaling mechanisms in the pathogenesis of diabetes and its complications. *Wiley Online Library*, *1243*(1), 88–102. https://doi.org/10.1111/j.1749-6632.2011.06320.x

Reddy, V. P., & Beyaz, A. (2006). Inhibitors of the Maillard reaction and AGE breakers as therapeutics for multiple diseases. *Drug Discovery Today*, *11*(13–14), 646–654. https://doi.org/10.1016/J.DRUDIS.2006.05.016

research, C. V. N.-M. nutrition & food, & 2006, undefined. (2006). Toxicity of the AGEs generated from the Maillard reaction: on the relationship of food-AGEs and biological-AGEs. *Wiley Online Library*, *50*(12), 1140–1149. https://doi.org/10.1002/mnfr.200600144

Rheinheimer, J., de Souza, B. M., Cardoso, N. S., Bauer, A. C., & Crispim, D. (2017). Current role of the NLRP3 inflammasome on obesity and insulin resistance: A systematic review. *Metabolism: Clinical and Experimental*, *74*, 1–9. https://doi.org/10.1016/J.METABOL.2017.06.002

Rothermundt, M., Peters, M., Prehn, J. H. M., & Arolt, V. (2003). S100B in brain damage and neurodegeneration. *Microscopy Research and Technique*, *60*(6), 614–632. https://doi.org/10.1002/JEMT.10303

Sanajou, D., Ghorbani Haghjo, A., Argani, H., & Aslani, S. (2018). AGE-RAGE axis blockade in diabetic nephropathy: Current status and future directions. *European Journal of Pharmacology*, *833*, 158–164. https://doi.org/10.1016/J.EJPHAR.2018.06.001

Sastry, M., Ketchem, R. R., Crescenzi, O., Weber, C., Lubienski, M. J., Hidaka, H., & Chazin, W. J. (1998). The three-dimensional structure of Ca(2+)-bound calcyclin: implications for Ca(2+)-signal transduction by S100 proteins. *Structure (London, England : 1993)*, *6*(2), 223–231. https://doi.org/10.1016/S0969-2126(98)00023-9

Sędzikowska, A., & Szablewski, L. (2021). Insulin and insulin resistance in alzheimer's disease. *International Journal of Molecular Sciences*, *22*(18). https://doi.org/10.3390/IJMS22189987

Shabani, S., & Mirshekar, M. A. (2018). *Diosmin is neuroprotective in a rat model of scopolamine-induced cognitive impairment*. https://doi.org/10.1016/j.biopha.2018.09.127

Sharma, M., Chan, H. K., Lavilla, C. A., Uy, M. M., Froemming, G. R. A., & Okechukwu, P. N. (2023). Induction of a single dose of streptozotocin (50 mg) in rat model causes insulin resistance with type 2 diabetes mellitus. *Fundamental and Clinical Pharmacology*, *37*(4), 769–778. https://doi.org/10.1111/fcp.12892

Sharma, P., Garg, A., Garg, S., Res, V. S.-A. J. B., & 2016, undefined. (2016). Animal model used for experimental study of Diabetes Mellitus: an overview. *Researchgate.Net*. https://www.researchgate.net/profile/Sweta-Garg-2/publication/311081095_Animal_Model_Used_For_Experimental_Study_of_Diabetes_Mellitus_An_Overview/links/59817b47a6fdccb31004a6d0/Animal-Model-Used-For-Experimental-Study-of-Diabetes-Mellitus-An-Overview.pdf

Shen, C. Y., Lu, C. H., Wu, C. H., Li, K. J., Kuo, Y. M., Hsieh, S. C., & Yu, C. L. (2020). The Development of Maillard Reaction, and Advanced Glycation End Product (AGE)-Receptor for AGE (RAGE) Signaling Inhibitors as Novel Therapeutic Strategies for Patients with AGE-Related Diseases. *Molecules 2020, Vol. 25, Page 5591*, *25*(23), 5591. https://doi.org/10.3390/MOLECULES25235591

Sidarala, V., & Kowluru, A. (2016). The Regulatory Roles of Mitogen-Activated Protein Kinase (MAPK) Pathways in Health and Diabetes: Lessons Learned from the Pancreatic β-Cell. *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*, *10*(2), 76–84. https://doi.org/10.2174/1872214810666161020154905

Smith, S. P., & Shaw, G. S. (1998). A novel calcium-sensitive switch revealed by the structure of human S100B in the calcium-bound form. *Structure*, *6*(2), 211–222. https://doi.org/10.1016/S0969-2126(98)00022-7

Snelson, M., Nutrients, M. C.-, & 2019, undefined. (2019). Dietary advanced glycation end products: digestion, metabolism and modulation of gut microbial ecology. *Mdpi.Com*, *11*(2). https://doi.org/10.3390/nu11020215

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathologica*, *119*(1), 7–35. https://doi.org/10.1007/S00401-009-0619-8

Stanojevic, J., Dragic, M., Stevanovic, I., Ilic, T., Stojanovic, I., Zeljkovic, M., & Ninkovic, M. (2022). Intermittent theta burst stimulation ameliorates cognitive impairment and hippocampal gliosis in the Streptozotocin-induced model of Alzheimer's disease. *Behavioural Brain Research*, *433*. https://doi.org/10.1016/J.BBR.2022.113984

Sterenczak, K. A., Nolte, I., & Escobar, H. M. (2013). Rage splicing variants in mammals. *Methods in Molecular Biology*, *963*, 265–276. https://doi.org/10.1007/978-1-62703-230-8_16

Sultan, S., Taimuri, U., Basnan, S. A., Ai-Orabi, W. K., Awadallah, A., Almowald, F., & Hazazi, A. (2020). Low Vitamin D and Its Association with Cognitive Impairment and Dementia. *Journal of Aging Research*, *2020*. https://doi.org/10.1155/2020/6097820

Sun, B., & Kekenes-Huskey, P. M. (2020). Molecular Basis of S100A1 Activation and Target Regulation Within Physiological Cytosolic Ca2+ Levels. *Frontiers in Molecular Biosciences*, *7*, 529082. https://doi.org/10.3389/FMOLB.2020.00077/BIBTEX

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J., & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Research and Clinical Practice*, *183*, 109119. https://doi.org/10.1016/j.diabres.2021.109119

Suryono, Kido, J.-I., Hayashi, N., Kataoka, M., & Nagata, T. (2005). Calprotectin Expression in Human Monocytes: Induction by Porphyromonas gingivalis Lipopolysaccharide, Tumor Necrosis Factor-α, and Interleukin-1β. *Journal of Periodontology*, *76*(3), 437–442. https://doi.org/10.1902/jop.2005.76.3.437

Sutherland, J. P., McKinley, B., & Eckel, R. H. (2004). The metabolic syndrome and inflammation. *Metabolic Syndrome and Related Disorders*, *2*(2), 82–104. https://doi.org/10.1089/MET.2004.2.82

Tabák, A. G., Herder, C., Rathmann, W., Brunner, E. J., & Kivimäki, M. (2012). Prediabetes: a high-risk state for diabetes development. *Lancet (London, England)*, *379*(9833), 2279–2290. https://doi.org/10.1016/S0140-6736(12)60283-9

Talmor, Y., Golan, E., Benchetrit, S., Bernheim, J., Klein, O., Green, J., & Rashid, G. (2008). Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. *American Journal of Physiology - Renal Physiology*, *294*(5). https://doi.org/10.1152/AJPRENAL.00051.2008

Tan, K. C., Shiu, S. W., Chow, W. S., Leng, L., Bucala, R., & Betteridge, D. J. (2006). Association between serum levels of soluble receptor for advanced glycation end products and circulating advanced glycation end products in type 2 diabetes. *Diabetologia*, *49*(11), 2756–2762. https://doi.org/10.1007/S00125-006-0394-1

Tan, R., Bölükbaşi Hatip, F., Açikalin, Ö., Yamauchi, A., Kataoka, Y., & Hatip-Al-Khatib, I. (2018). Effect of varenicline on behavioral deficits in a rat model of Parkinson's disease induced by unilateral 6-hydroxydopamine lesion of substantia nigra. *Behavioural Pharmacology*, *29*(4), 327–335. https://doi.org/10.1097/FBP.0000000000000355

Taylor, S. I., Yazdi, Z. S., & Beitelshees, A. L. (2021). Pharmacological treatment of hyperglycemia in type 2 diabetes. *The Journal of Clinical Investigation*, *131*(2). https://doi.org/10.1172/JCI142243

Teigelkamp, S., Bhardwaj, R. S., Roth, J., Meinardus-Hager, G., Karas, M., & Sorg, C. (1991). Calcium-dependent complex assembly of the myeloic differentiation proteins MRP-8 and MRP-14. *The Journal of Biological Chemistry*, *266*(20), 13462–13467.

Teissier, T., & Boulanger, É. (2019). The receptor for advanced glycation end-products (RAGE) is an important pattern recognition receptor (PRR) for inflammaging. *Biogerontology*, *20*(3), 279–301. https://doi.org/10.1007/S10522-019-09808-3

Tobon-Velasco, J., Cuevas, E., & Torres-Ramos, M. (2014). Receptor for AGEs (RAGE) as Mediator of NF-kB Pathway Activation in Neuroinflammation and Oxidative Stress. *CNS & Neurological Disorders - Drug Targets*, *13*(9), 1615–1626. https://doi.org/10.2174/1871527313666140806144831

Torreggiani, M., Liu, H., Wu, J., Zheng, F., Cai, W., Striker, G., & Vlassara, H. (2009). Advanced glycation end product receptor-1 transgenic mice are resistant to inflammation, oxidative stress, and post-injury intimal hyperplasia. *The American Journal of Pathology*, *175*(4), 1722–1732. https://doi.org/10.2353/AJPATH.2009.090138

Tuomilehto, J., Ogle, G. D., Lund-Blix, N. A., & Stene, L. C. (2020). Update on Worldwide Trends in Occurrence of Childhood Type 1 Diabetes in 2020. *Pediatric Endocrinology Reviews : PER*, *17*(Suppl 1), 198–209. https://doi.org/10.17458/per.vol17.2020.tol.epidemiologychildtype1diabetes

Vlassara, H., Endocrinology, G. S.-N. R., & 2011, undefined. (2011). AGE restriction in diabetes mellitus: a paradigm shift. *Nature.Com*, *526*(9), 526–539. https://doi.org/10.1038/nrendo.2011.74

Vogl, T., Gharibyan, A. L., & Morozova-Roche, L. A. (2012). Pro-Inflammatory S100A8 and S100A9 Proteins: Self-Assembly into Multifunctional Native and Amyloid Complexes. *International Journal of Molecular Sciences*, *13*(3), 2893. https://doi.org/10.3390/IJMS13032893

Vogl, T., Tenbrock, K., Ludwig, S., Leukert, N., Ehrhardt, C., Van Zoelen, M. A. D., Nacken, W., Foell, D., Van Der Poll, T., Sorg, C., & Roth, J. (2007). Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nature Medicine*, *13*(9), 1042–1049. https://doi.org/10.1038/NM1638

Wang, J. Q., Yin, J., Song, Y. F., Zhang, L., Ren, Y. X., Wang, D. G., Gao, L. P., & Jing, Y. H. (2014). Brain aging and AD-like pathology in streptozotocin-induced diabetic rats. *Journal of Diabetes Research*, *2014*. https://doi.org/10.1155/2014/796840

Wang, Y., He, D., Ni, C., Zhou, H., Wu, S., Xue, Z., & Zhou, Z. (2016). Vitamin D induces autophagy of pancreatic β-cells and enhances insulin secretion. *Molecular Medicine Reports*, *14*(3), 2644–2650. https://doi.org/10.3892/MMR.2016.5531

Weiss, M. A. B. T.-V. & H. (2009). Chapter 2 The Structure and Function of Insulin: Decoding the TR Transition. In *Insulin and IGFs* (Vol. 80, pp. 33–49). Academic Press. https://doi.org/https://doi.org/10.1016/S0083-6729(08)00602-X

Winocur, G., Roder, J., & Lobaugh, N. (2001). Learning and memory in S100-beta transgenic mice: an analysis of impaired and preserved function. *Neurobiology of Learning and Memory*, *75*(2), 230–243. https://doi.org/10.1006/NLME.2000.3961

Wu, J., & Yan, L. J. (2015). Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. *Diabetes, Metabolic Syndrome and Obesity : Targets and Therapy*, *8*, 181–188. https://doi.org/10.2147/DMSO.S82272

Wu, M., Xu, L., Wang, Y., Zhou, N., Zhen, F., Zhang, Y., Qu, X., Fan, H., Liu, S., Chen, Y., & Yao, R. (2018). S100A8/A9 induces microglia activation and promotes the apoptosis of oligodendrocyte precursor cells by activating the NF-κB signaling pathway. *Brain Research Bulletin*, *143*, 234–245. https://doi.org/10.1016/J.BRAINRESBULL.2018.09.014

Wu, Y., Ding, Y., Tanaka, Y., & Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International Journal of Medical Sciences*, *11*(11), 1185–1200. https://doi.org/10.7150/ijms.10001

Xu, H., Baracskay, P., O'Neill, J., & Csicsvari, J. (2019). Assembly Responses of Hippocampal CA1 Place Cells Predict Learned Behavior in Goal-Directed Spatial Tasks on the Radial Eight-Arm Maze. *Neuron*, *101*(1), 119-132.e4. https://doi.org/10.1016/J.NEURON.2018.11.015

Yagihashi, S., … H. M.-J. of diabetes, & 2011, undefined. (1124). Mechanism of diabetic neuropathy: where are we now and where to go? *Wiley Online LibraryS Yagihashi, H Mizukami, K SugimotoJournal of Diabetes Investigation, 2011•Wiley Online Library*. https://doi.org/10.1111/j.2040

Yamagishi, S. I., & Matsui, T. (2010). Soluble form of a receptor for advanced glycation end products (sRAGE) as a biomarker. *Frontiers in Bioscience (Elite Edition)*, *2*(4), 1184–1195. https://doi.org/10.2741/E178

Yang, Q., O'Hanlon, D., Heizmann, C. W., & Marks, A. (1999). Demonstration of heterodimer formation between S100B and S100A6 in the yeast two-hybrid system and human melanoma. *Experimental Cell Research*, *246*(2), 501–509. https://doi.org/10.1006/EXCR.1998.4314

Yu, H., Li, H., Liu, X., Du, X., & Deng, B. (2020a). Levels of serum S100B are associated with cognitive dysfunction in patients with type 2 diabetes. *Aging (Albany NY)*, *12*(5), 4193. https://doi.org/10.18632/AGING.102873

Yu, H., Li, H., Liu, X., Du, X., & Deng, B. (2020b). Levels of serum S100B are associated with cognitive dysfunction in patients with type 2 diabetes. *Aging*, *12*(5), 4193–4203. https://doi.org/10.18632/AGING.102873

Zaulkffali, A. S., Razip, N. N. M., Alwi, S. S. S., Jalil, A. A., Mutalib, M. S. A., Gopalsamy, B., Chang, S. K., Zainal, Z., Ibrahim, N. N., Zakaria, Z. A., & Khaza'ai, H. (2019). Vitamins D and E Stimulate the PI3K-AKT Signalling Pathway in Insulin-Resistant SK-N-SH Neuronal Cells. *Nutrients*, *11*(10). https://doi.org/10.3390/NU11102525

Zervides, K. A., Jern, A., Nystedt, J., Gullstrand, B., Nilsson, P. C., Sundgren, P. C., Bengtsson, A. A., & Jönsen, A. (2022). Serum S100A8/A9 concentrations are associated with neuropsychiatric involvement in systemic lupus erythematosus: a cross-sectional study. *BMC Rheumatology*, *6*(1). https://doi.org/10.1186/S41927-022-00268-W

Zhang, B., Lai, G., Wu, J., Sun, R., Xu, R., Yang, X., Qi, Y., & Zhao, Y. (2016). 20-HETE attenuates the response of glucose-stimulated insulin secretion through the AKT/GSK-3β/Glut2 pathway. *Endocrine*, *54*(2), 371–382. https://doi.org/10.1007/S12020-016-1031-5

Zhao, Z., Zhao, C., Xu, H. Z., Zheng, F., Cai, W., Vlassara, H., & Ma, Z. A. (2009). Advanced glycation end products inhibit glucose-stimulated insulin secretion through nitric oxide-dependent inhibition of cytochrome c oxidase and adenosine triphosphate synthesis. *Endocrinology*, *150*(6), 2569–2576. https://doi.org/10.1210/EN.2008-1342

Zhu, Y., Shu, T., Lin, Y., Wang, H., Yang, J., Shi, Y., & Han, X. (2011). Inhibition of the receptor for advanced glycation endproducts (RAGE) protects pancreatic β-cells. *Biochemical and Biophysical Research Communications*, *404*(1), 159–165. https://doi.org/10.1016/J.BBRC.2010.11.085

Zilliox, L. A., Chadrasekaran, K., Kwan, J. Y., & Russell, J. W. (2016). Diabetes and Cognitive Impairment. In *Current Diabetes Reports* (Vol. 16, Issue 9). Current Medicine Group LLC 1. https://doi.org/10.1007/s11892-016-0775-x

Zwadlo, G., Brüggen, J., Gerhards, G., Schlegel, R., & Sorg, C. (1988). Two calcium-binding proteins associated with specific stages of myeloid cell differentiation are expressed by subsets of macrophages in inflammatory tissues. *Clinical and Experimental Immunology*, *72*(3), 510–515.

APPENDICES

Appendix-1. Ethics committee approval

Evrak Tarih ve Sayısı: 02.09.2022-E.250346

T.C.
PAMUKKALE ÜNİVERSİTESİ
Hayvan Deneyleri Etik Kurulu

Sayı : E-60758568-020-250346
Konu : Başvurunuz Hk.

Sayın Prof. Dr. Funda Fatma BÖLÜKBAŞI HATİP

İlgi : 28/08/2022 tarihli dilekçeniz. 10.150.1.99 106831 2.09.2022

"Vitamin D3 Streptozotosin (STZ) ile İndüklenen Diyabetik Sıçan Modelinde S100 Protein Alt Tiplerine Etkisi" (PAUHADYEK-2022/29) konulu çalışmanız **01.09.2022 tarih ve 2022/07 sayılı** toplantımızda görüşülmüş olup

Yapılan görüşmelerden sonra, söz konusu çalışmanın **Hayvan Deneyleri Etiği açısından uygun olduğuna** ve **33 adet sıçan kullanılarak yapılmasına** oy birliği ile karar verildi.

Gereğini bilgilerinize rica ederim.

Prof. Dr. Gülçin METE
Başkan

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