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Psichologinio streso poveikis inkstų morfologiniams pokyčiams sergant I tipo cukriniu diabetu

Morphological Changes of Kidney in Type 1 Diabetes Mellitus Influenced by Psychological Stress

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1. ABBREVIATIONS

AKI	Acute kidney injury
CKD	Chronic kidney disease
CVD	Cardiovascular disease
CD4+	Cluster of differentiation 4
CD8+	Cluster of differentiation 8
DCT	Distal convoluted tubule
DKD	Diabetic kidney disease
ESKD	End stage kidney disease
FF	Filtration fraction
GAD65	Glutamic acid decarboxylase 65
GBM	Glomerular basement membrane
GM	Glomerulus
GFR	Glomerular filtration rate
HAL DQ2	Human leucocyte antigen DQ2
HAL DQ8	Human leucocyte antigen DQ8
HbA1c	Haemoglobin A1C
IA-2	Islet antigen 2
IDF	International Diabetes Federation
IQR	Interquartile range
OGTT	Oral glucose tolerance test
PCT	Proximal convoluted tubules
RAAS	Renin-Angiotensin-Aldosterone System
SD	Standard deviation
T1DM	Type 1 Diabetes mellitus
T2DM	Type 2 Diabetes mellitus
TAL	Thick ascending limb
TDL	Thin descending limb
WHO	World Health Organisation
ß-cells	Beta Cells

2. SUMMARY

Author: Lorenz Kauth

Title: Morphological Changes of Kidney in Type 1 Diabetes Mellitus Influenced by Psychological Stress.

Aim: The aim of this study was to investigate how psychological stress influences the morphological changes in the kidneys of individuals with Type 1 Diabetes mellitus.

Objectives: 1) To conduct a comprehensive literature analysis to identify existing research on the relationship between psychological stress, Type 1 Diabetes mellitus, and kidney morphology. 2) To examine the specific morphological changes in the kidneys of individuals with Type 1 Diabetes mellitus and compare with control. 3) To assess the impact of psychological stress on renal morphological changes among experimental groups.

Research Methodology: The research included 35 mature healthy female Wistar rats, randomly divided into 4 groups: Control (n=9), Stress (n=10), DM (n=8), and the DM+Stress (n=8). Each group underwent different experimental conditions for 28 days. Diabetes was induced using streptozotocin, while chronic stress was simulated by daily restraint stress. Tissue samples were analysed for the size of renal corpuscles, Bowman's capsule size, and the total number of renal corpuscles using QuPath 04.4 software. The data was statistically analysed using R Studio, with significance levels set at $p < 0.05$.

Results: The results indicated that the size of renal corpuscles varied across the four groups. The stress group exhibited the largest renal corpuscles, with $p < 0.05$, followed by the Control group, while the DM ($p > 0.05$) group and the DM+Stress group ($p < 0.05$) had the smallest renal corpuscles. These findings suggest that stress alone may cause hypertrophy, whereas the combination of diabetes and stress might result in reduced size of renal corpuscles, indicating a possible compensatory response by the kidneys to the combined stressors. The analysis of Bowman's capsule size showed that DM+Stress group resulted in the largest capsule, $p < 0.05$. The stress group showed the smallest Bowman's capsule size, $p < 0.05$. These results suggest that chronic stress by itself may cause the capsule to shrink, but when stress is combined with diabetes, it seems to make the capsule enlarge even more, possibly because stress speeds up the process. In terms of the number of renal corpuscles, no statistical significance was observed between Control, the DM and DM+Stress groups. The stress group had the lowest number of renal corpuscles, $p < 0.05$, suggesting that stress alone can lead to a reduction in the number of renal corpuscles.

Conclusions: The research findings suggest that both diabetes and psychological stress independently impact kidney morphology, even when the combination of the two does not always

lead to more pronounced changes. While chronic stress significantly increases the size of renal corpuscles, it reduces the size of the Bowman's capsule and the total number of renal corpuscles. DM and stress only appeared to increase the Bowman's capsule size and decrease the size of the renal corpuscles. This supports the idea that both stress and DM impair the morphological effects of diabetes on the kidneys.

Autorius: Lorenz Kauth

Pavadinimas: Psichologinio streso poveikis inkstų morfologiniams pokyčiams sergant I tipo cukriniu diabetu.

Tikslas: Šio tyrimo tikslas - ištirti, kaip psichologinis stresas veikia inkstų morfologinius pokyčius, sergantiems 1 tipo cukriniu diabetu.

Uždaviniai: 1. Atlikti išsamią literatūros analizę, siekiant nustatyti esamus tyrimus apie psichologinio streso, 1 tipo cukrinio diabeto ir inkstų morfologijos tarpusavio ryšį. 2. Ištirti morfologinius inkstų pokyčius, sergantiems 1 tipo cukriniu diabetu ir palyginti su kontrole 3. Įvertinti psichologinio streso poveikį inkstų morfologiniams pokyčiams skirtingose eksperimentinėse grupėse.

Tyrimo metodika: Tyrimas buvo atliktas su 35 Wistar veislės žiurkėmis, kurios atsitiktiniu būdu buvo padalintos į 4 grupes: kontrolė (n=9), stresas (n=10), diabetas (n=8) ir diabetas+stresas (n=8). Kiekvienai grupei 28 dienas buvo taikytos skirtingos eksperimentinės sąlygos. Diabetas buvo sukeltas naudojant streptozotociną, o lėtinis stresas imituotas kasdieniu imobilizacijos streso modeliu. Inkstų mėginiai buvo analizuojami vertinant inksto kūnelių dydį, Bowmano kapsulės dydį ir inksto kūnelių skaičių, naudojant „QuPath 0.4.4" programinę įrangą. Duomenys buvo statistiškai analizuoti naudojant „R Studio" programą. Skirtumai buvo laikomi statistiškai reikšmingais kai $p < 0,05$.

Rezultatai: Tyrimo rezultatai parodė, kad inkstų kūnelių dydis skyrėsi tarp keturių grupių. Streso grupėje buvo nustatyti didžiausi inkstų kūneliai, $p < 0,05$, po to sekė kontrolinė grupė, o diabeto ($p > 0,05$) ir diabeto+streso grupėje ($p < 0,05$) inkstų kūneliai buvo mažiausi. Šie rezultatai rodo, kad vien stresas gali sukelti hipertrofiją, tuo tarpu diabeto ir streso derinys gali lemti sumažėjusį inkstų kūnelių dydį, kas gali reikšti inkstų kompensacinį atsaką į abu stresorius. Baumano kapsulės dydžio analizė parodė, kad diabeto+streso grupėje buvo didžiausia Baumano kapsulė, $p < 0,05$. Streso grupėje nustatytas mažiausias kapsulės dydis, $p < 0,05$. Šie rezultatai leidžia manyti, kad lėtinis stresas gali lemti kapsulės sumažėjimą, tačiau streso ir diabeto derinys gali skatinti kapsulės padidėjimą, galimai dėl to, kad stresas skatina šį morfologinį pakitimą. Kalbant apie inksto kūnelių skaičių, statistiškai

reikšmingų skirtumų tarp kontrolės, diabeto ir diabeto+streso grupių nebuvo. Streso grupė turėjo mažiausią inkstų kūnelių skaičių, p <0,05, kas rodo, kad tik stresas gali lemti jų skaičiaus sumažėjimą.

Išvados: Tiek diabetas, tiek psichologinis stresas nepriklausomai veikia inkstų morfologiją, net kai jų derinys ne visada sukelia ryškesnius pokyčius. Nors lėtinis stresas reikšmingai padidina inksto kūnelius, jis sumažina Baumano kapsulės dydį ir bendrą kūnelių skaičių. Diabetas ir stresas tik padidina Baumano kapsulės dydį ir sumažina kūnelių dydį. Tai patvirtina idėją, kad tiek stresas, tiek diabetas neigiamai veikia inkstų morfologinius pokyčius.

3. KEY WORDS

Type 1 Diabetes mellitus, psychological stress, kidney morphology.

4. INTRODUCTION

Diabetes is one of the most prevalent diseases in the world and occurs in all regions all over the world [1]. It evolved to one of the most common and most serious diseases of our time and is characterised by hyperglycaemia [2]. In 2021, 536,6 million people in the age group from 20 to 79 years old suffered from diabetes worldwide, and the estimated number for diabetic patients in 2045 rises to 629 million people, up to 783,2 million [1,2]. That's 12,2% of the whole population in this age group [1]. Additionally, the International Diabetes Federation (IDF) estimates the number of children and adolescents with type 1 diabetes in the age group of 14-19 years to be up to 1,1 million [1, 2]. According to the World Health Organisation (WHO), the annual death rate due to high blood glucose is up to 4 million annually. That's a percentage increase of 95% since 2000 [2, 3]. Diabetes mellitus can lead to life-threatening complications, incur significant costs, cause disabling conditions, and reduce life expectancy. [1]. Diabetes mellitus patients are susceptible to complications in the long term, like nephropathy, retinopathy or neuropathy [4]. In patients with diabetes, kidney tissue begins to deteriorate [4, 5].

Nowadays, chronic stress is becoming a part of everyday life, and chronic pathological stress has reached an endemic level in Western society [6]. Chronic stress is associated with many different diseases, such as cardiovascular diseases, endocrine diseases and Diabetes mellitus [7, 8]. Different studies showed the impact of stress of Diabetes mellitus on the kidney's function and its morphology. The aim of the study is to investigate how chronic psychological stress influences the morphology of the kidneys of individuals with Type 1 Diabetes mellitus.

5. AIM AND OBJECTIVES

Aim

The aim of this study is to investigate how psychological stress influences the morphological changes in the kidneys of individuals with Type 1 Diabetes mellitus.

Objectives

1) To conduct a comprehensive literature analysis to identify existing research on the relationship between psychological stress, Type 1 Diabetes mellitus, and kidney morphology.

2) To examine the specific morphological changes in the kidneys of individuals with Type 1 Diabetes mellitus and compare with control.
3) To assess the impact of psychological stress on renal morphological changes among experimental groups.

6. LITERATURE REVIEW

6.1. Diabetes mellitus: Definition, types and diagnosis

Diabetes mellitus (DM) is a group of metabolic disorders with inadequate blood glucose control characterised by hyperglycaemia resulting from defects in insulin action, insulin secretion, or both, and disturbances of carbohydrate, fat, and protein metabolism [2, 9, 10]. Chronic hyperglycaemia is associated with dysfunction, long-term damage, and failure of different organs, especially the nerves, heart, pancreas, liver, blood vessels, eyes, and the kidneys [9]. Characteristics such as thirst, polyuria, weight loss, and blurred vision may be present. The most fatal clinical manifestation is the non-ketonic hyperosmolar state or diabetic ketoacidosis. Those states can lead to severe dehydration or coma. Without adequate medical treatment, both states can lead to death [2, 9].
Diabetes is classified into 5 different types, with the two main types being Type 1 Diabetes mellitus (T1DM) and Type 2 Diabetes mellitus (T2DM). Gestational diabetes, hybrid forms and other specific subtypes are other types in the classification. Drug or chemical-induced DM, diseases of the exocrine part of the pancreas, endocrinopathies and infections are just a few examples from the specific subtypes [2, 9].
T1DM is caused by ß-cell destruction, which usually leads to absolute insulin deficiency. It's divided into immune-mediated diabetes and idiopathic diabetes. Immune-mediated diabetes, which has a prevalence of around 5-10% of all diabetes patients, results from a cellular-mediated autoimmune destruction of the ß-cell of the pancreas. Idiopathic diabetes is a form of diabetes not associated with

autoantibodies, yet it requires insulin therapy for survival. Although insulin dependence is essential, the need for insulin and episodes of ketoacidosis may occur intermittently. Those patients need lifelong insulin therapy [9, 11]. T2DM ranges from an insulin secretory defect with insulin resistance to insulin resistance with relative insulin deficiency. It occurs in around 90-95 % of all patients with T2DM. Most patients with T2DM are obese or overweight. It often remains undiagnosed for a longer time due to non-noticeable symptoms [2, 9, 11]. To diagnose DM, currently, four diagnostic tests are recommended. The fasting plasma glucose test, the 2h oral glucose tolerance test (OGTT), the haemoglobin A1c (HbA1c) test and a random blood glucose test in presence of signs and symptoms of diabetes [2, 11]. The criteria for diagnosis of diabetes are a fasting plasma glucose test ≥7,0 mmol/L (126 mg/dl) or 2h-postload glucose test during an oral glucose tolerance test ≥ 11,1 mmol/l (200 mg dl) or HbA1c ≥ 6,5% (48 mmol/mol) or a patient with hyperglycaemia or hyperglycaemic crisis with classic symptoms with a random plasma glucose ≥ 11,1 mmol/l (200 mg/dl) [11, 12]. The diagnosis of DM plays an important role in the life of the individuals [2, 13]. It's not only important for their health and the prevention of further complications, but it also may affect their daily life, social opportunities, employment and their life and health insurance [2]. Biochemical testing is a fast and easy tool to diagnose diabetes [2, 12].

6.2. Type 1 Diabetes mellitus: Epidemiology and pathophysiology

Type 1 Diabetes Mellitus (T1DM) is among the most prevalent chronic autoimmune diseases in children, affecting both genders equally, though there is a slight male predominance in younger age groups [7]. The prevalence and incidence of T1DM vary according to factors such as age, season, geographic region, and ethnicity [2, 7]. Two notable peaks in occurrence are seen before the age of 20: one between 5 and 7 years old, and another during puberty and the mid-teen years. Nevertheless, the T1DM manifestation is as common in childhood as in adulthood. The appearance in adulthood is characterised by a milder course. A seasonal variation is also observed, most of the manifestations are diagnosed in autumn and winter. The incidence of T1DM also varies globally, with the lowest rates in Venezuela and China (0,1 per 100.000) and the highest in Sardinia and Finland (50-60 per 100.000). The annual T1DM prevalence and incidence have been growing at a rate of about 3%. It's suggested that this rise is explained by environmental risk factors and cannot be explained by genetic factors [7]. In high-income countries, T1DM decreases life expectancy by around 13 years, and the prognosis is far worse in countries with limited access to insulin. Distinguishing between T1DM and T2DM in adults can be difficult and may affect the accuracy of prevalence and incidence estimations [2]. According to the WHO, in 2016, 9.7% of the Lithuanian population aged 30 and older was affected by DM, and the condition accounted for 1% of the proportional mortality across all age

groups [14]. The International Diabetes Federation (IDF) Diabetes Atlas stated, as of 2021, Lithuania had approximately 186.900 adults aged 20–79 living with diabetes, representing about 5.8% of the adult population. Additionally, around 62.600 individuals (33.5% of those with diabetes) were estimated to be undiagnosed. An estimated 7.657 individuals in all age groups are diagnosed with T1DM [15].

The pathophysiology of T1DM results from complex interactions between pancreatic ß-cells and the innate and adaptive immune system and is not fully understood yet. The exact trigger for the immune response against ß-cells remains unknown, there are debates on whether it is caused by a specific event or is a random occurrence. Genetic susceptibility to T1DM might play a big role [7, 16]. Viral infections, especially enteroviruses, are often associated with T1DM. The adaptive immune system, especially T-lymphocytes, plays an important role in T1DM. It begins with the presentation of ß-cell autoantibodies to cluster of differentiation 4 (CD4+) T-lymphocytes by antigen-presenting cells, which triggers an inflammatory response [7]. This leads to the recruitment of cluster of differentiation 8 (CD8+) T-lymphocytes. CD8+ T-lymphocytes target the islet of Langerhans antigens, and decreased regulated immune functions are observed. The ß-cells are getting damaged through various mechanisms, leading to dysfunction and ultimately to T1DM [7, 16]. Around 70-90% of the population with T1DM shows evidence of immune-mediated inflammation, including ß-cell antigens against Glutamic acid decarboxylase 65 (GAD65), islet antigen 2 (IA-2), or insulin, together with genetic links to immune response genes. The primary genetic associations in in European population are with human leucocyte antigen DQ8 (HAL DQ8) and human leucocyte antigen DQ2 (HAL DQ2). In individuals without these immune features, the pathogenesis remains unclear [16].

6.3. Type 1 Diabetes mellitus and psychological stress

Stress is a biological response evoked by any intrinsic or extrinsic stimulus. Based on stimulus, timing, and severity, stress can create various actions on the body, ranging from disruptions in homeostasis to potentially fatal effects and death [17]. The ability to adapt to stress has evolved as an important factor for survival. The fight-or-flight-response activates catabolic, anti-reproductive, anti-growth, and immunosuppressive mechanisms, and is typically temporary and ensures immediate survival. However, if stress becomes chronic, those mechanisms can become harmful and contribute to the onset or exacerbation of various diseases. Chronic stress can also lead to behavioural changes, such as increased eating and unhealthy dietary habits, which can result in weight gain and disruptions in the glucose and lipid metabolism [18]. Psychological stress has also been suggested as one of the potential trigger factors for T1DM. Stress increases catecholamines and serum glucocorticoid concentrations, which lead to an increase in insulin resistance and need [18, 19]. Several studies

showed the link between stressful life situations and an increased risk of developing T1DM. They indicated that psychological, social, and physical triggers lead to increased release of catecholamines and cortisol. This may increase the insulin need, leading to overloading the ß-cells and triggering the onset of T1DM. On the other hand, other studies report a positive association between T1DM and stressors and do not support this hypothesis [18].

Stress might not only be a potential trigger for T1DM manifestation, but it also might affect the patients' outcomes and glycaemic Control. Many children and caregivers suffer from stress due to T1DM, and stress is common in those families. Additionally, to the general life stress, many patients with T1DM suffer from additional diabetes-specific stress due to the disease and daily life management [18]. Both higher levels of disease-related stress and general stress are associated with higher levels of HbA1c, poorer self-management, and lower diabetes specific quality of life. High diabetic specific stress levels also showed an influence on the variance in HbA1c. General stress did not [18, 20].

T1DM in young children poses exceptional challenges, with ongoing disease management, increased parental stress, and the fear of hypoglycaemia. Technology related to diabetes management has improved significantly and contributes to improvement in glycaemic Control, which also improves the family's psychological functioning. Despite that, research about the connection between psychological stress and T1DM remains limited in quantity [21].

6.4. Kidney's morphology and function

From the beginning of its embryonic development, the kidney is a structurally complex organ vital for human survival [22]. Humans possess a pair of kidneys, which are bilateral, bean-shaped organs located retroperitoneally on either side of the vertebral column. Each kidney has a concave area called the hilum where the ureter, renal artery, and renal vein enter (Fig.1).

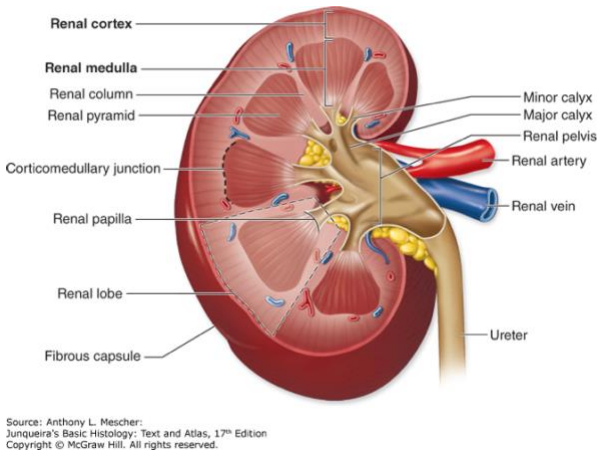

Fig. 1. Structure of the kidney.
Source: The Urinary System | Junqueira's Basic Histology: Text and Atlas, 17th Edition | AccessMedicine | McGraw Hill Medical

The parenchyma of each kidney has two main parts: the renal cortex, the outer part, and the medulla, the inner part, each containing different parts of the nephron - the functional unit of the kidney [22, 23]. The nephron (Fig. 2) includes the renal corpuscle (Fig.3), consisting of the glomerulus (GM), Bowman's capsule, and a complex tubular system [22].

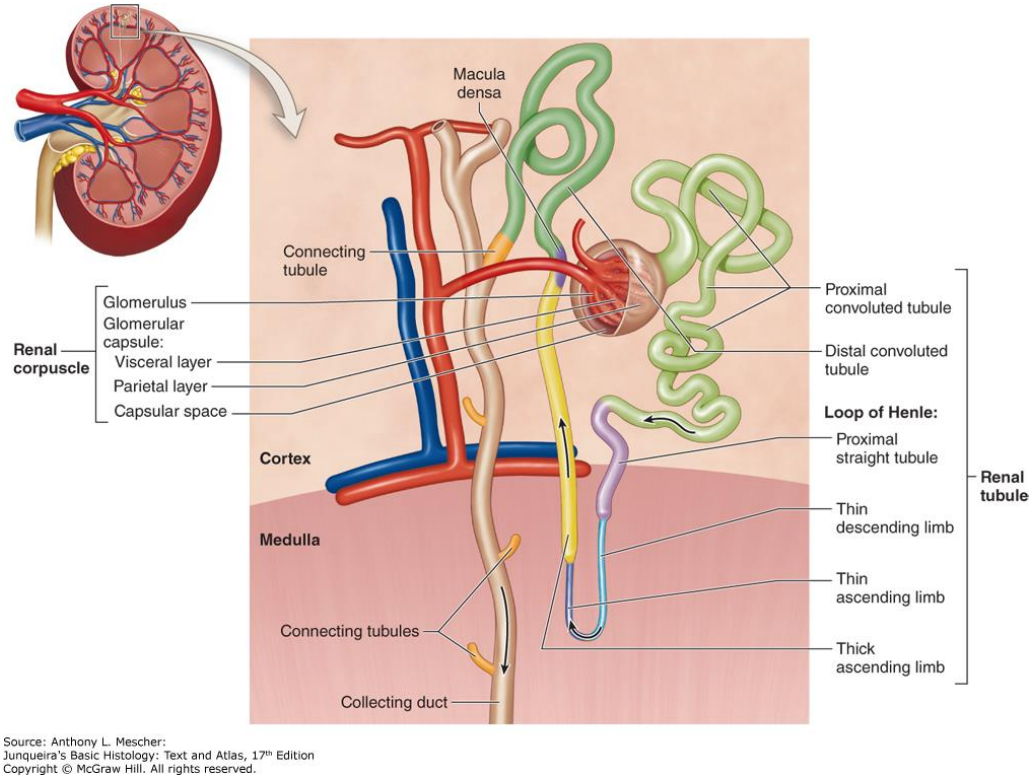

Fig. 2. A nephron and its parts.
Source: The Urinary System | Junqueira's Basic Histology: Text and Atlas, 17th Edition | AccessMedicine | McGraw Hill Medical

The GM and the proximal convoluted tubule (PCT) are located within the renal cortex. From the PCT, the nephron continues to the loop of Henle, which extends into the medulla before returning to the cortex to connect with the distal convoluted tubule (DCT). The nephron concludes at the collecting duct via connecting tubules. Nephrons are categorized into two types: superficial nephrons, where the GM is located near the cortex with short loops of Henle, and juxtamedullary nephrons, where the GM is situated near the cortico-medullary junction with long loops of Henle that penetrate deep into the medulla [22].

The glomerulus is surrounded by Bowman’s capsule and multiple tufts of interconnected capillaries originating from branches of the afferent glomerular arteriole. The Bowman’s capsule is composed of two distinct layers. The visceral layer, which closely adheres to the glomerular capillaries, consists of specialized epithelial cells known as podocytes, supported by a basal lamina. The parietal layer, on the other hand, lines the outer surface of the capsule, facing the connective tissue stroma, and is made up of simple squamous epithelium. This layer transitions into the simple cuboidal epithelium of the

proximal convoluted tubule. Between these layers lies the urinary (capsular) space, which is responsible for collecting the plasma ultrafiltrate, or primary urine. This space opens into the proximal convoluted tubule at the urinary pole, while the opposite vascular pole is where the afferent and efferent arterioles enter and leave the glomerulus. There are three main cell types: podocytes, which form the visceral layer of Bowman's capsule; fenestrated endothelial cells, which line the glomerular capillaries; and mesangial cells, which are located within the mesangial matrix. These mesangial cells together form the mesangium, a structure that provides support to the glomerulus and plays a role in regulating filtration [23].

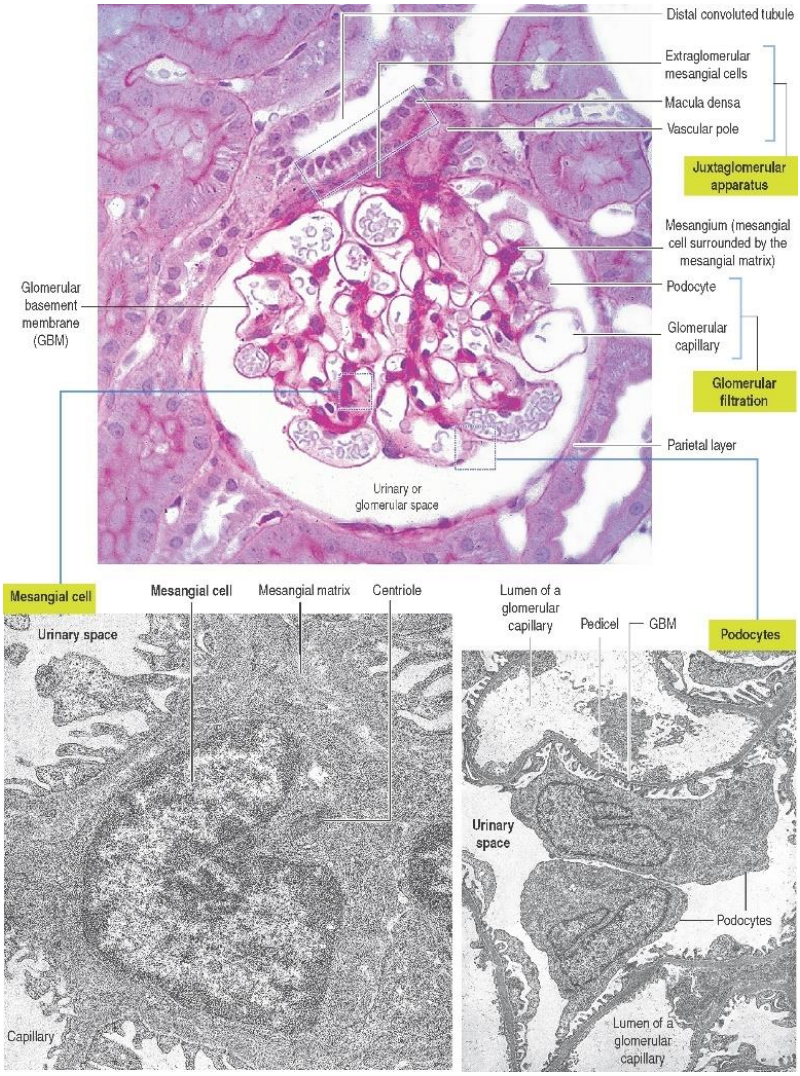

Fig. 3. Structure of renal corpuscle.
Source: Leslie P. Gartner. Textbook of Histology, 5th Edition; 2020

Each cell within the renal parenchyma is specialised to maintain homeostasis of electrolytes, fluid volume, and waste products [22, 24]. The kidneys' primary function is blood filtration [24], but they also play a key role in regulating water and electrolyte balance and maintaining blood pressure. The kidneys are also crucial for clearing bacterial components and cytokines from the bloodstream [22].

The GM filters large amounts of blood, and the tubular system processes this filtrate into urine by reabsorbing and secreting water and solutes [22, 24]. It's a cluster of capillaries surrounded by the Bowman's capsule, with afferent and efferent arterioles regulating intraglomerular pressure. The glomerular capillaries have specialised features which enable them to filter large volumes of blood [22], and the filtration barrier, which is made up of endothelial cells of fenestrated capillaries, the glomerular basement membrane (GBM), and podocytes [24], supports the formation of primary filtration [22].

Bowman's capsule leads to the PCT in the renal cortex, where epithelial cells with microvilli enhance the reabsorption of water, electrolytes, and nutrients from the blood [22, 24]. It is located next to the GM in the renal cortex, filtering between 160 to 180 L of blood daily and producing about 1.5 to 2 L of urine per day [20]. The proximal convoluted tubule (PCT) then transitions into the thin descending limb (TDL) of the loop of Henle, which gradually narrows and is characterized by smaller cells with fewer mitochondria and less prominent microvilli. As the tubule ascends toward the cortex, it becomes the thick ascending limb (TAL), where the cells become more distinct, with increased microvilli and mitochondria. This structural change supports active sodium transport and aids in urine dilution. Approximately 30 to 40% of sodium reabsorption occurs in this segment, playing a critical role in altering urine osmolarity. [22]. The juxtaglomerular apparatus is located at the entry of the glomerulus, and it is a specialised sensory structure that has feedback mechanisms to regulate glomerular blood flow and filtration rate [24]. The DCT plays an essential role in maintaining body sodium balance and blood pressure [25] and has the most significant number of mitochondria among other cells in the nephron. The final part of the nephron is the collecting tubules. They consist of intercalated and connecting tubule cells and regulate hydrogen and bicarbonate secretion [22].

6.5. Impact of Type 1 Diabetes mellitus on morphological changes of the kidney

The kidney is considered one of the most important targets of microvascular damage in DM. Around half of all patients with T2DM and one-third with T1DM could eventually develop kidney disease [8]. From a histopathological point of view, diabetic kidney disease (DKD) can underlie a broad spectrum of different histopathological features. These changes may include nodular or diffuse glomerulosclerosis, tubulointerstitial fibrosis, tubular atrophy, and renal arteriolar hyalinosis, either individually or in combination [4, 8]. Although the presence and severity of each of these features are independently linked to the risk of progressing renal disease, they do not necessarily occur together [4]. To get a better understanding of the underlying process, various studies focused on the impact of DM and the histopathological changes of the kidney.

In diabetic rats, the kidney shows various structural abnormalities that are visible under light microscopy. The main morphological changes include dilatation of Bowman's capsule space [5, 26, 27], glomerular lobulation, and tubular degeneration. Dilatation of Bowman's capsule space is a common feature in diabetic kidney damage [5, 26]. Bowman's capsule space is typically characterised by normal structure and size. In diabetic patients, it can become enlarged, which is called dilatation of Bowman's capsule space [5, 27]. Furthermore, glomerular lobulation is another abnormality, in which the normal smooth outside of the glomerulus is disrupted, leading to the formation of lobed GM, an indicator of pathological changes [26]. Rats with untreated diabetes showed a degenerated glomerulus, infiltration of inflammatory cells, and a thickened basement membrane. Proximal convoluted tubules showed oedema and deposits of mucopolysaccharides and hyaline substances [5]. Furthermore, histological analysis revealed the expansion of mesangial cells and tubular atrophy in the kidneys of rats with diabetes. Also, damage to the glomeruli has been observed, which leads to the impairment of normal glomerular function [27], which is important for maintaining fluid and electrolyte balance [28]. As a result of this dysfunction, glycogen deposits in the renal cells, leading to further cellular damage. Furthermore, cast formation has been observed within the tubular area in the diabetic kidney, which obstructs its normal function. This feature wasn't observed in the Control groups [27].

Another study highlighted the morphological changes over time. Different Control groups from diabetic rats and healthy rats have been observed and examined at the 9th week and the 16th week. The examination of diabetic rats at the 9th week showed that no significant morphological changes of the kidney had appeared compared to the Control group. By the 16th week, notable changes in the diabetic rats have been observed. The GM volume was significantly increased in the rats with DM at the 16th week, and the mean area of GM was larger than in the Control group. Nevertheless, there was no significant difference in the GM morphology between the 9th week mark for the Control group and diabetic rats [29]. This indicates that the progression of diabetic kidney damage is slow, with more significant morphological changes developing over time.

Other studies indicate that the GM contains mesangial cells within normal limits, with a slight thickening of the glomerular basement membrane (GBM). There are no mesangial cells or accumulation of mesangial matrix forming nodules, and no signs of global glomerulosclerosis. The tubulointerstitial region shows thickening of the tubular basement membrane along with some atrophic tubules. Moreover, the blood vessels exhibit a build-up of hyaline material in the tunica intima of the arterioles. These findings were categorized as class I diabetic nephropathy [30].

A significant alteration in kidney structure is the dysfunction of podocytes, which are highly specialized cells that cover the urinary side of the glomerular GBM. Along with glomerular endothelial cells, podocytes are essential for maintaining the GBM, its charge barrier, and the integrity

of the glomerular capillary loop. These functions are impaired in diabetic glomeruli. The diabetic environment triggers "patho-adaptive" changes in podocytes, such as cytoskeletal reorganization, de-differentiation, apoptosis, and autophagy. These changes are reflected in morphological changes such as widening, retraction, and flattening, reduced motility, increased intercellular tight junction formation, a decrease in slit diaphragm length, glomerular hypertrophy, detachment, and dropout. The thickening of GBM is present in almost all diabetic patients within a few years of diagnosis and is one of the earliest and most characteristic changes [8].

Overall, the damage and the changes in glomerular morphology, the tubular degeneration, and the presence of glycogen deposits and casts indicate that the kidney is affected in patients with DM [5, 8, 24-31]. The findings also show that DM and the morphological changes appear with time, and DM is a progressive disease [29].

6.6. Impact and complications of Type 1 Diabetes mellitus on the function of the kidney

DM can cause a variety of kidney-related complications, including diabetic kidney disease (DKD) [32], glomerular hyperfiltration and loss of podocytes [8], proteinuria [33], hypertension [34], diabetic nephropathy [35], chronic kidney disease (CKD) and eventually end stage kidney disease (ESKD) and kidney failure [33, 35, 36]. DKD is a common long-term complication of DM and globally the leading cause of CKD and ESKD, it's responsible for 50% of all cases [36]. It leads to structural changes such as thickening of the GMB, fusion of foot processes, podocyte loss, causing loosening of the GBM, and expansion of the mesangial matrix. In DKD, the chronic hyperglycaemia increases osmolarity within glomerular capillaries, leading to higher glomerular pressure, afferent arteriole dilation, and glomerular hyperfiltration, which raises the glomerular filtration rate (GFR) [32]. As kidney damage progresses in diabetes, particularly glomerular injury, the glomerular filtration barrier loses its size and charge-selective properties, leading to protein loss in the urine. Albumin, the most abundant blood protein, is typically found in urine as albuminuria, which is an early sign of kidney damage [32]. Even small amounts of albumin in the urine are a hallmark of DKD. The level of albuminuria is directly linked to the progression of DKD [32, 33]. Hypertension in diabetes is related to maladaptive changes and interactions between the autonomic nervous system and increased activation of the renin-angiotensin-aldosterone system (RAAS), and negative environmental factors. Angiotensin II and aldosterone are known to interfere with insulin signalling in insulin-sensitive tissues, potentially leading to impaired endothelial-mediated vascular relaxation and the onset of hypertension. These hormones may also contribute to insulin resistance, which reduces insulin signalling and hinders nitric oxide-mediated vascular relaxation. Growing evidence indicates that elevated aldosterone levels, coupled with hyperinsulinemia commonly associated with

obesity and insulin resistance, can lead to increased vascular stiffness, raising blood pressure and the risk of cardiovascular disease (CVD). Other factors like renal dysfunction, the autonomic nervous system dysregulation, elevated intravascular volume, premature vascular ageing, and lifestyle in diabetic patients contribute to the development of hypertension [34].

Diabetic nephropathy is the leading cause of end-stage renal disease in developed countries and affects individuals with both T1DM and T2DM diabetes as a microvascular complication. It involves a combination of metabolic and hemodynamic disturbances, which lead to kidney damage. Hyperglycaemia triggers the accumulation of advanced glycation end-products (AGEs) and activation of various pathways, such as the polyol pathway and the RAAS. These factors contribute to glomerular hypertrophy, mesangial expansion, thickening of the GMB, and podocyte dysfunction. Increased glomerular filtration pressure and hyperfiltration result in further kidney damage. Additionally, inflammatory and fibrotic processes are activated, leading to fibrosis and progressive scarring of kidney tissue. Over time, this combination of factors leads to a decline in kidney function, ultimately leading to end-stage renal disease [35].

6.7. Impact of stress on morphological changes of the kidney

Stress is a biological response evoked by any intrinsic or extrinsic stimulus [16]. Based on stimulus, timing, and severity, stress induces an organic response, which is not only responsible for physiological changes, but also for morphological changes in different organs, including the kidney [16, 37]. The impact of stress on the kidney's morphology is not well known yet [37]. Benchimol de Souza, in his study about the influence of chronic psychological stress on the kidneys of prepubertal and adult rats, applied stress conditions by keeping rats in a rigid plastic tube for two hours to restrain their movements. The Control group was kept under normal conditions without any stress. Both groups had access to water and food for two hours per day. The studies showed that the total number of glomeruli was significantly smaller in chronically stressed pubertal rats killed after 10 weeks of stimulus than in the age-matched Control groups. Group, which underwent stress conditions, showed 20,9 % fewer glomeruli compared to the Control group. Another group of pubertal rats was exposed to chronic stress for 10^{th} weeks, followed by 6 more weeks, and used for later evaluation under standard circumstances at the 16^{th} week of life. Also, this group demonstrated 19,8 % fewer glomeruli compared to the Control group. The study was carried out under the same conditions in adult rats. The chronically stressed group of adult rats was killed after 10 weeks and showed a glomerular reduction of 33,1 % compared to the Control group. Meanwhile, the group of rats which got exposed for 10 weeks to chronic stress and were killed 6 weeks after the last event of stress only showed a glomerular loss of 16,6 % compared to the Control group. The study showed a significant difference

in the total glomerular count with a p value <0.0001. Those morphological alterations may have a serious implication for predisposing individuals to renal disease and hypertension in adult life [38].
In another study, mice were kept for 6 hours a day in a restraining container for 5 or 21 days based on the procedure. The control group and the group that underwent stress conditions had access to food and water the whole time. Histological examination of the kidney in the control group showed normal tissue structures, well-preserved Bowman's capsules and GM, and the convoluted tubules were intact. In the group of mice with stress exposure for 5 days, mild degeneration with hypercellularity of the glomerulus and convoluted tubules was observed. More severe degeneration of the GM and tubules was found in the mice which underwent 21 days of stress [39].
Also, in a study aiming at histopathological changes in liver and kidney tissues from chronic unpredictable mild stress, the histology of the kidney tissue in the control group revealed intact GM, Bowman's capsule and distal convoluted tubules. The chronic unpredictable mild stress induced stress-depression group demonstrated the presence of dilated intratubular capillaries, inflammation and absence of cell lining [40].
Several studies highlighted that psychological stress has an impact on morphological changes of the kidney. Stress resulted in a notable reduction in glomeruli, which was partially reversible several weeks after the last stressor [37]. Further studies revealed mild to severe degeneration of GM and tubules, with damage increasing with longer stress exposure [39]. Also, dilatation of intratubular capillaries, inflammation, and loss of cell lining could be observed [40], like reduction of Bowman's capsule [41]. These findings highlight the effects of chronic stress on kidney morphology, with more severe changes occurring with prolonged stress exposure.

6.8. Impact of stress on the function of the kidney

Stress-related disorders (SRDs), which are psychiatric conditions triggered by significant life stressors, may increase susceptibility to various health issues. However, it's suggested that SRDs are linked to an increased risk of acute kidney injury (AKI) and CKD, but it remains unclear [42]. Over the past two decades, the prevalence of chronic kidney disease (CKD) has increased substantially. While the rise in CKD rates has primarily been associated with comorbidities such as diabetes, hypertension, and obesity, recent research has begun to explore the role of social, economic, and psychological factors in the prevalence and progression of CKD [43]. Various studies have made a link between chronic stress and the increased risk for KD and AKI [44, 45] or associated it with a rapid decline in kidney function [42, 44].
Furthermore, at study stated that mental stress may contribute to the development of hypertension. Stress leads to a significant increase in both blood pressure and heart rate (P <0.05). While the GFR

and renal plasma flow remained unchanged, there was a notable increase in the filtration fraction (FF) ($P < 0.05$), and a trend toward reduced sodium excretion was observed. The reduction in sodium excretion was linked to a notable increase in proximal sodium reabsorption ($P < 0.05$), likely due to changes in renal blood flow. The substantial increase in FF suggests heightened resistance in the post-glomerular arterioles, which could explain the increased sodium reabsorption in the proximal tubules, potentially due to changes in the peritubular Starling forces [45].

7. METHODS

7.1. Subjects of the study

This experimental study involved a total of 35 healthy female Wistar rats, aged 7 months (n=19) or 5 months (n=16), with an average weight of 253 g ± 13.1 g. The animals were randomly assigned to cages containing three animals and housed under controlled, standard laboratory conditions. These conditions included a room temperature of 24 °C ± 1 °C, a 12-hour light/dark cycle (with lights on at 8:00 and off at 20:00), and a relative humidity of 55% ± 5%. The animals were given unrestricted access to food and water throughout the experiment. Additionally, cages and bedding were replaced on a weekly basis. All the animals used in this study were sourced from The Centre for Innovative Medicine, Vilnius, Lithuania, with permission from the State Food and Veterinary Service No. G2-214 [46].

7.2. Study design

In the first two weeks, an adaptive period for the animals took place (n=35). The animals were provided unrestricted access to standard laboratory rodent food and water *ad libitum*, under stress-free conditions. After the adaptive period, the animals were divided into four groups: Control, Stress, DM, and DM+Stress. They were housed in groups of three animals per cage and were treated for the next 28 days in different experimental conditions. The first group of rats (n=9), the Control group, received water and standard feed *ad libitum* without any restrictions throughout the whole period. No actions have been applied to the rats during the 28-day experimental period. In the second Stress group, rats (n=10) were subjected to chronic psychological stress. Restraint stress was induced daily for two hours by placing the animal in a small Plexiglas restraint cage. The cage was adequately ventilated with holes on both ends. During those two hours, the animals did not have access to food and water. The third group (n=8) was the group with DM. DM was induced using streptozotocin [46]. A single dose of 65 mg/kg of streptozotocin (MilliporeSigma, USA) was administered to induce

diabetes, following the procedure outlined in Furman's published protocol [47], with minor modifications. Finally, the rats in the fourth group – DM+Stress (n=8) were administered a single dose of 65 mg/kg streptozotocin to induce T1DM by Furman's protocol. Also, they were exposed to stress under the same conditions as in the stress group [46]. On the 29th day, rats were euthanised using a CO_2 gas chamber in accordance with established euthanasia protocols.

7.3. Tissue processing for light microscopy

The kidneys were removed and weighed. The transverse and longitudinal sections were made (Fig. 4) and were prepared for tissue processing for light microscopy.

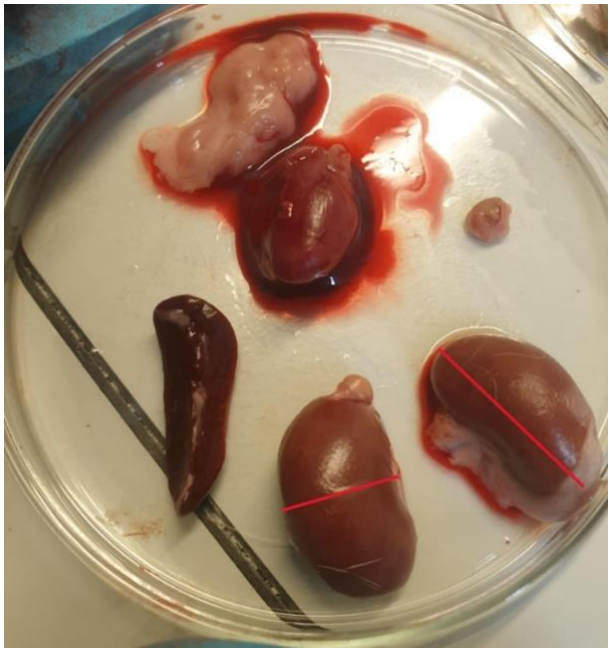

Fig. 4. Excised kidneys and other organs.

The red line shows how the kidneys were cut.

Tissue processing for light microscopy involves several key steps to prepare tissue samples for examination under the microscope. The first step is the fixation, which prevents the object from autolysis and putrefaction, damages pathogenic microorganisms and makes the object susceptible to further procedures. Formalin, a common fixative, is used to stabilise the tissue and keep its structure intact for further analysis [48]. This step typically takes 6 to 24 hours [49]. In the next step, the tissue needs to be dehydrated, in which the water in the tissue is replaced by using Ethanol, from 70 to 100%. This step helps to stiffen the tissue and to prepare it for the next stage [48, 49]. After dehydration, the tissue undergoes clearing, where the alcohol is replaced with a clearing agent. Paraffin wax is used for the next step of embedding and is insoluble in alcohol and water. Therefore,

the alcohol is replaced by a clearing agent. Hereby, the tissues get more transparent, making them ready for embedding [48]. The next step is impregnation and embedding. The tissue is flooded with dissolved paraffin. After the sample was cooled and a paraffin block was formed, the samples were cut with a microtome. In the sectioning, microscopy, suitable translucent micro-sections were made, 4 µm thick [49]. All these procedures were performed by the researchers from the Centre for Innovative Medicine, Vilnius, Lithuania. Those sections were either transverse sections or longitudinal sections, like in Fig. 5.

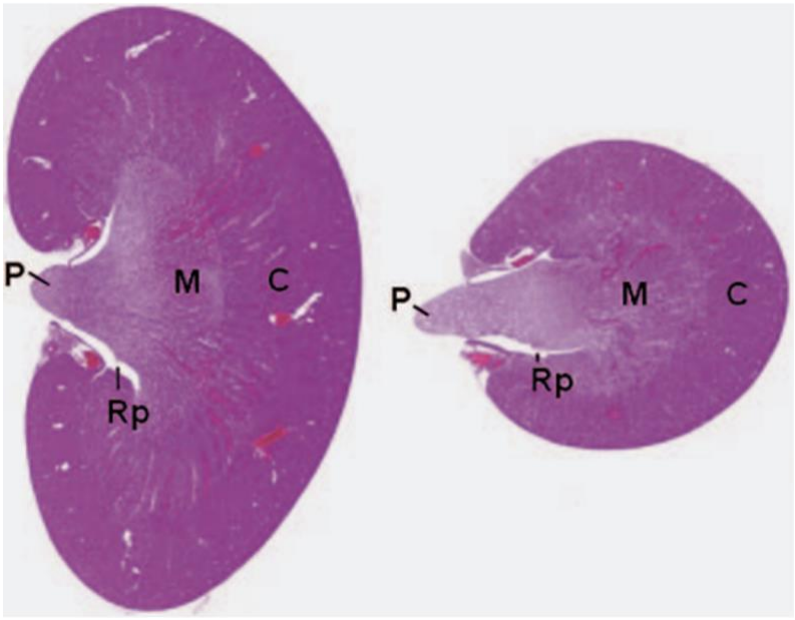

Fig. 5. Longitudinal and transverse cuts of the kidney.
P - papilla, M - medulla, C - cortex, Rp - renal pelvis.
Source: Revised guides for organ sampling and trimming in rats and mice Part 3. A joint publication of the RITA and NACAD groups. Gerd Morawietz, Christine Ruehl-Fehlert, Birgit Kittel, Axel Bube, Kevin Keane, Sabine Halm, Anke Heuser, and Jürgen Hellmann. Exp Toxic Pathol 2004; 55: 433–449.

Once the sections were prepared, they were stained to highlight the structural parts of tissues and cells. Haematoxylin and eosin (H+E staining) was used for staining in our experiment. Haematoxylin serves as the basic dye, showing basophilic structures like nucleus, ribosomes, extracellular matrix and rough endoplasmic reticulum and stains the sample blue or purple [48, 49]. Eosin is the acidic dye, giving the sample a pink or red colour and highlighting the acidic structures like cytoplasm and fibres of the extracellular matrix [49]. In the end, a coverslip was placed on top of the stained tissue section to protect it from the external environment and mechanical damage. This step is important because it ensures the tissue is preserved and protected for viewing under the microscope [48]. In this study, all the stained and prepared slides were scanned. All these procedures were performed by the researchers from the Centre for Innovative Medicine, Vilnius, Lithuania.

7.4. Data analysis

For data analysis, all scanned samples were analysed by using the QuPath 04.4 program. It's an open-source software platform designed for digital pathology and whole slide image analysis [50]. Each slide contained a longitudinal and transverse cut of the kidney. For analysis, a transverse cut was chosen and analysed. The focus was on the size of a renal corpuscle and the size of the Bowman's capsule (Fig. 6).

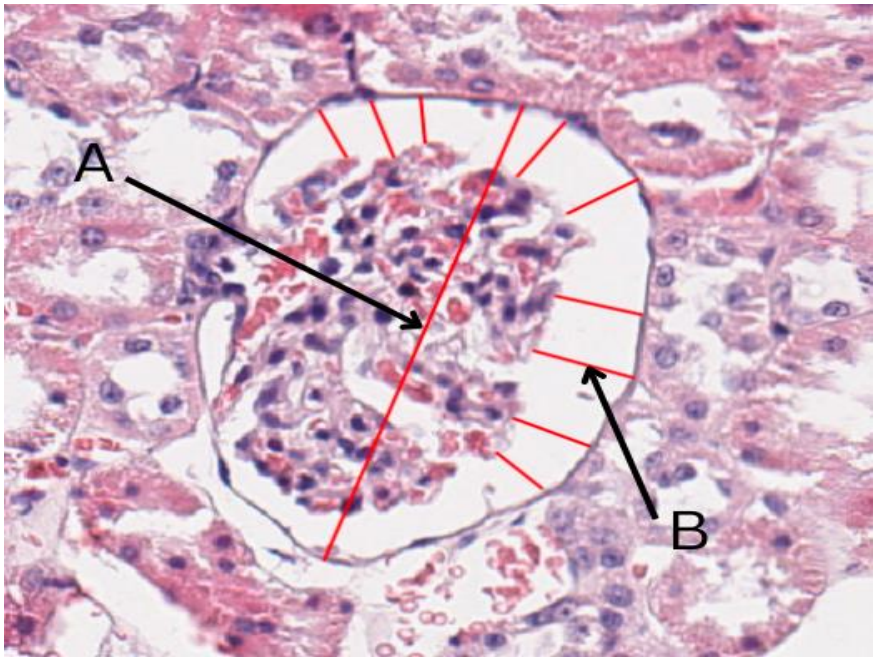

Fig. 6. Example of analysed renal corpuscle and its evaluation.

A – size of renal corpuscle, B – Bowman's capsule size (longest distance from visceral to parietal layers)

To evaluate all the collected data, R Studio was used to examine the statistical differences between Control group, Stress group, DM group and the DM+Stress group. The objective was to assess whether each group showed a statistically significant difference from the Control group, with a specified significance level at p-value of < 0.05. This methodology ensured comparison and accurate interpretation of the results, showing the reliability of the findings [51]. H_0 states that there is no difference in renal corpuscle size and Bowman's capsule size between Control group, Stress group, DM group and the DM+Stress group.

Furthermore, the total number of renal corpuscles per slide was evaluated. Due to variability in slide preparation, where some slides had incomplete transverse sections, a random evaluation method was performed. For each slide, a 1500 x 1500 µm-sized area was randomly selected, and the total number of renal corpuscles in that area was counted (Fig. 7). Statistical significance was calculated using R Studio with a p-value of < 0.05.

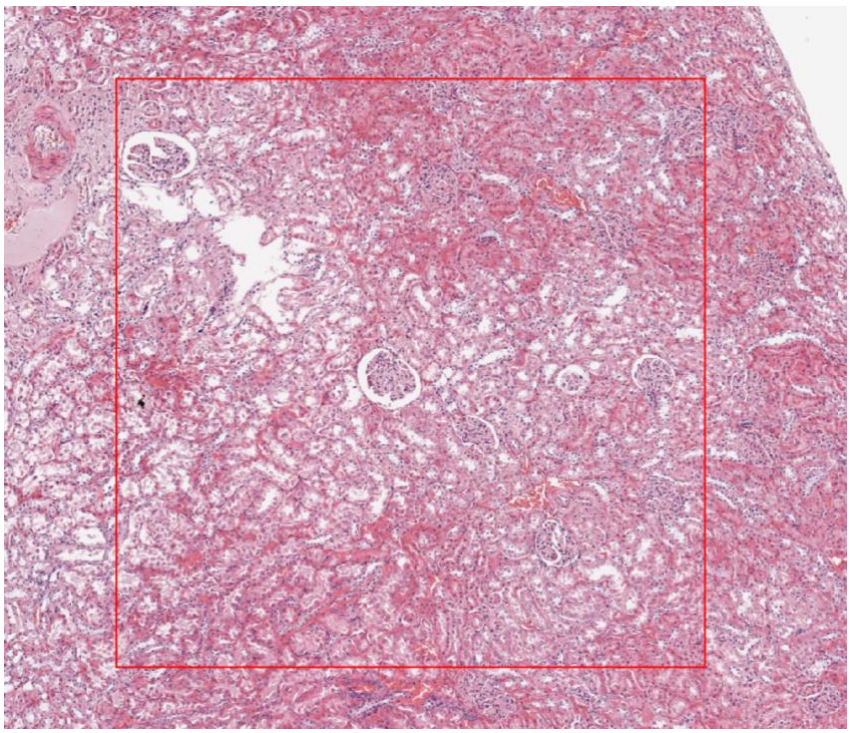

Fig. 7. Example of a 1500 x 1500 µm area for evaluation of the number of renal corpuscles.

8. RESEARCH RESULTS AND DISCUSSION

8.1. Size of the renal corpuscles

The four groups, Control (n=1280), Stress (n=1671), DM (n=1289) and DM + stress (n=1128), where n is the number of the measurements made in each group, showed different trends in size of renal corpuscle, which was evaluated using measures such as mean, median, standard deviation (SD), interquartile range (IQR) (Table 1).

Table 1. Descriptive statistics of the size of renal corpuscles in µm across the different experimental groups.

	Control	Stress	DM	DM+Stress
Mean	112.16	118.53	111.41	109.75
Median	110.72	117.49	110.72	108.80
SD	23.54	23.26	21.28	21.48
IQR	28.49	26.99	27.25	27.22
25%	97.29	103.96	97.57	96.25
75%	125.77	130.95	124.84	123.47

The mean of the size of renal corpuscles for the Control group is 112.16 µm, while the Stress group has a higher mean of 118.53 µm. The DM group has a mean of the size of renal corpuscles of 111.41 µm, and the DM+Stress group has a mean of 109.75 µm (Fig. 8).

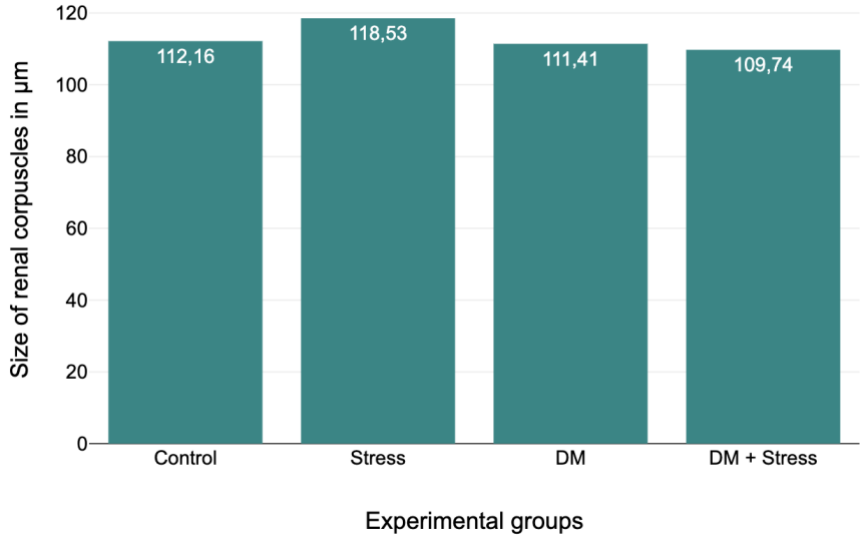

Fig. 8. Mean size of renal corpuscles across different experimental groups.

The median values are similar to the mean values within the groups, showing a relatively similar distribution of the size of renal corpuscles, but the spread of the data is also important to consider. In the Control group, the standard deviation (SD) of 23.54 µm suggests a significant variability in the size of renal corpuscles, with the values ranging from 49.22 µm to 210.02 µm. The interquartile range (IQR) is 28.49 µm, showing the spread of data as well. The Stress group shows a similarly wide range of the size of renal corpuscles, with an SD of 23.26 µm and an IQR of 26.99 µm. The DM group shows slightly less variance in its size of renal corpuscles, with an SD of 21.28 µm and an IQR of 27.25 µm. The DM+Stress group shows the lowest mean of the size of renal corpuscles and a SD of 21.48 µm, indicating a lower variability than the other groups.

The results of non-parametric statistical tests, such as the Kruskal-Wallis test and the Mann-Whitney U test, can be seen in Table 2.

Table 2. Descriptive statistics of the size of renal corpuscles in µm across the different experimental groups and p-values across the different experimental groups.

	Median	Minimum	Maximum	p-value
Control (n=1280)	110.72	49.22	210.02	Control vs. Stress < 0.001[#]
Stress (n=1671)	117.49	52.11	222.64	Control vs. DM = 0.561[#]
DM (n=1289)	110.72	55.21	232.76	Control vs. DM+Stress = 0.021[#]

DM+Stress (n=1128)	108.80	52.75	180.41	Stress vs. DM < 0.001[#] Stress vs. DM+Stress < 0.001[#] DM vs. DM+Stress = 0.072[#] Control vs. Stress vs. DM vs. DM+Stress < 0.001[$]

n - number of measurements made in each group; # - Mann-Whitney U Test; $ - Kruskal-Wallis rank sum test.

To assess whether the observed differences in the size of renal corpuscles across the four groups were statistically significant, a Kruskal-Wallis rank sum test was performed. The results of the Kruskal-Wallis test showed a highly significant difference between the four groups, with a chi-squared value of 122.94 (df = 3) and a p-value of < 0.05. This suggests that at least one of the groups differs significantly from the others in the size of renal corpuscles. To further investigate which specific groups differ compared to each other, pairwise comparisons were performed using the Mann-Whitney U Test. When comparing the Control group to the Stress group, a significant difference was observed, with a p-value of < 0.05. This result indicates that chronic psychological stress influences kidney morphology, leading to a noticeable increase in the size of renal corpuscles. The significant increase in the size of renal corpuscles in the Stress group suggests that stress alone can cause morphological changes in the kidneys. This finding is important because it highlights the impact of stress on the kidneys' structure, independent of the factor of diabetes. On the other hand, when comparing the Control group with the DM group, the p-value of > 0.05 indicates no significant difference between these two groups. This suggests that diabetes, at least in the short time frame of this study, does not lead to significant differences in the size of renal corpuscles. The lack of significant difference between the Control and DM groups indicates that diabetes alone does not have a major impact on kidney morphology in this experimental setup, at least not in terms of the size of renal corpuscles. This could mean that the effects of diabetes on kidney structure may take longer to develop or could involve other factors than just the size of renal corpuscle, or that there is no impact on that in diabetes. Comparing the Control group and the DM+Stress group, the p-value of < 0.05 indicates that there is a significant difference between these two groups. This suggests that the combination of diabetes and chronic stress results in a significant reduction in the size of renal corpuscles compared to the Control group. This might show that the effects of diabetes and stress together might affect the size, but not be as severe as might be expected compared to the Stress group. Those results may also suggest that the combination of stress and diabetes leads to an effect on kidney morphology, but might not show more expressed changes in the duration of the experimental study. Comparing the Stress group with the DM group, a significant difference was found, with a p-value of < 0.05. This result further supports the statement that chronic psychological stress has an independent effect on kidney morphology, increasing the size of renal corpuscles significantly compared to diabetes alone. It shows the

significant role of stress in changing the kidneys' morphology, suggesting that stress response could have a more immediate and severe effect on the kidneys than diabetes has. Similarly, when comparing the Stress group with the DM+Stress group, the p-value of < 0.05 showed a significant difference. This suggests that the presence of diabetes decreases the effect of stress on kidney morphology. Lastly, when comparing the DM group to the DM+Stress group, the p-value was < 0.05, showing a significant difference between these two groups. This result shows that the addition of chronic stress to diabetes leads to a greater effect on the size of renal corpuscles than diabetes by itself. The absence of a significant difference between these two groups may indicate that the morphological changes observed in the renal corpuscles due to diabetes are not worsened by the presence of stress in the short term. This finding could show the possibility that the effects of diabetes on kidney morphology may be responsible for the observed changes, and that stress does not worsen the impact of diabetes when both stress and diabetes are present.

The results from these pairwise comparisons suggest that chronic psychological stress alone can lead to significant reductions in the size of renal corpuscles. Diabetes does not appear to have a major effect on the size of renal corpuscles within the time frame of this study. Furthermore, the combination of DM and stress does not seem to show a bigger morphological impact than DM alone, indicating that the influence of stress may be the more relevant factor in changing kidney structure in terms of the size of renal corpuscles.

8.2. Size of Bowman's capsule

The size of the Bowman's capsule is another important morphological feature of the kidneys. Changes in the capsule size can provide further insight into morphological kidney changes under the different experimental conditions (Table 3).

Table 3. Descriptive statistics of the Bowman's capsule size in μm across the different experimental groups.

	Control	Stress	DM	DM+Stress
Mean	12.39	9.75	11.24	12.77
Median	10.75	7.78	10.72	12.04
SD	6.96	6.27	5.84	6.03
IQR	9.54	8.15	8.00	7.94
25%	6.81	4.82	6.58	8.27
75%	16.35	12.97	14.58	16.22

Table 3 provides an overview of the collected data on the size of the Bowman's capsule for each group. It shows the central tendency and spread of the capsular size (Fig. 9). The DM+Stress group has the highest mean of capsule size (12.77 µm), followed by the Control group with a mean of 12.39 µm. The DM group has the third highest mean (11.24 µm), and the Stress group has the lowest mean (9.75 µm). This shows that the combination of diabetes and stress results in the biggest average capsule size, while stress alone leads to the smallest.

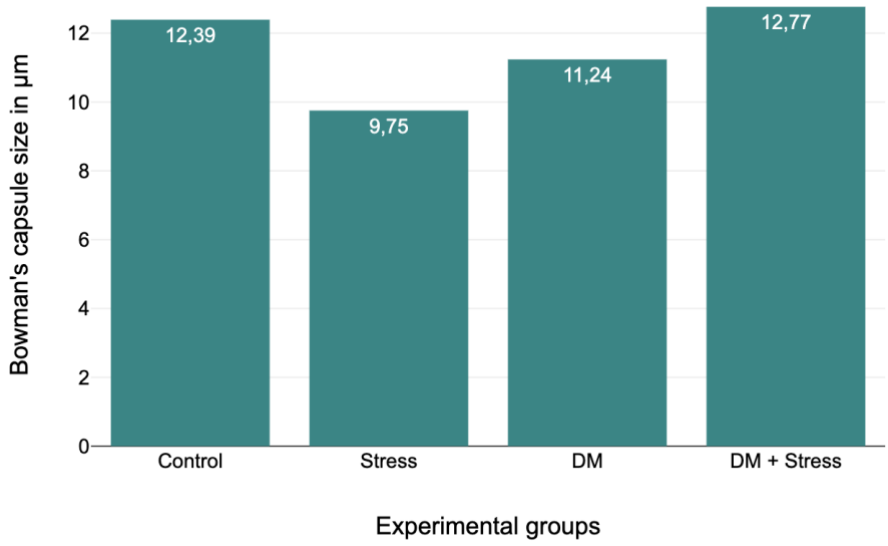

Fig. 9. The mean of Bowman's capsule size across different experimental groups.

In terms of median values, the DM+Stress group leads with a median of 12.04 µm, followed by the Control group with a median of 10.75 µm. The DM group has a median of 10.72 µm, and the Stress group has the lowest median of 7.78 µm. This finding supports that stress alone results in the smallest capsules, while the combination of DM and stress leads to the largest capsules.

The SD values give insights into the variability of capsule size within each group. The Control group shows the highest SD (6.96 µm), indicating a greater variation in capsule size compared to other groups. The Stress group has an SD of 6.27 µm, which is slightly lower than the Control group, while the DM group has the smallest SD (5.84 µm), showing that capsule sizes in this group are more consistent compared to the other groups. The DM+Stress group shows an SD of 6.03 µm, which is a bit lower than the Control group and in between the DM group and the Stress group.

The IQR reveals the spread of capsule sizes within each group. The Control group has the largest IQR (9.54 µm), showing a wide range between the 25^{th} and 75^{th} percentiles. The Stress group has an IQR of 8.15 µm, while the DM group has an IQR of 8.00 µm, giving a more concentrated range of values. The DM+Stress group has an IQR of 7.94 µm, showing a smaller spread compared to the Control group, but still showing a degree of variation in capsule sizes.

The Control group has the largest maximum value (48.24 µm), with a minimum of 2.23 µm, showing the presence of extreme values. The Stress group has the smallest range, with capsule sizes between 2.02 µm and 42.46 µm. The DM+Stress group shows a range from 2.83 µm to 45.86 µm, and the DM group has a range from 2.56 µm to 47.29 µm. This suggests that even if the Control group shows the widest variation, the other groups also have a big variability in capsule size.

The results of non-parametric statistical tests, such as the Kruskal-Wallis test and the Mann-Whitney U test, can be seen in Table 4.

Table 4. Descriptive statistics of the Bowman's capsule size in µm across the different experimental groups and p-values across the different experimental groups.

	Median	Minimum	Maximum	P-value
Control (n=1280)	10.75	2.23	48.24	Control vs. Stress < 0.001[#] Control vs. DM = 0.002[#] Control vs. DM+Stress = 0.001[#] Stress vs. DM < 0.001[#] Stress vs. DM+Stress < 0.001[#] DM vs. DM+Stress < 0.001[#] Control vs. Stress vs. DM vs. DM+Stress < 0.001[$]
Stress (n=1671)	7.78	2.02	42.46	
DM (n=1289)	10.72	2.56	47.29	
DM+Stress (n=1128)	12.04	2.83	45.86	

n: number of measurements made in each group; #: Mann-Whitney U Test, $: Kruskal-Wallis rank sum test

The Kruskal-Wallis rank sum test resulted in a chi-squared value of 274.65 (df = 3) and a p-value of < 0.05, which is highly significant. This indicates that there is significant difference in Bowman's capsule size across at least one of the groups, suggesting that the factors being tested have a significant impact on capsule size. To further investigate which specific groups differed from each other, pairwise comparisons using the Mann-Whitney U test was performed. The results revealed significant differences between multiple experimental groups.

The comparison of the Control group and the Stress group showed a p-value of $p < 0.05$, which means there is a significant difference between these two groups. The stress group had much smaller capsule sizes than the Control group. This result suggests that chronic psychological stress has a significant impact on kidney structure, leading to a reduction in capsule size. The kidneys of rats exposed to stress were significantly smaller in terms of capsule size than those of the Control group. This finding shows how stress alone can change the kidneys' morphology, possibly through hormonal changes or inflammation that affect kidney morphology. The Control group and the DM group showed a p-value of < 0.05, which is also statistically significant. This states that diabetes alone also leads to a reduction in capsule size compared to the Control group, but the effect isn't as pronounced as the effect of stress alone. The DM group had smaller capsule sizes than the Control group, but not as small as in the

Stress group. This suggests that while diabetes affects kidney morphology, the changes might be smaller compared to the high effects of stress. It's possible that diabetes leads to structural changes in the kidneys that take longer to develop, or that they involve different mechanisms compared to stress. Looking at the comparison between the Control group and the DM+Stress group, the p-value was < 0.05, showing that the combination of diabetes and chronic stress leads to a significant difference in capsule size when compared to the Control group. Furthermore, the DM+Stress group had higher mean and median capsule sizes than the Control group. This result suggests that the combination of diabetes and stress might lead to changes in kidney structure, which are different from what we see with each factor alone. The increase in capsule size in this group could indicate some form of compensatory mechanism, where the kidneys adjust in response to both the metabolic stress of diabetes and the hormonal stress from the psychological stress factor. It's also possible that this combined effect doesn't just add the effects of stress and diabetes but changes the overall way the kidneys adapt to both conditions, leading to slightly larger capsules compared to the Control group. The comparison between the Stress group and the DM group gave a p-value of < 0.05. The difference between the Stress group and the DM group is significant. The DM group had a significantly larger capsule size compared to the Stress group. This result shows that stress alone caused a bigger reduction in capsule size compared to diabetes alone. The kidneys in the Stress group were smaller, which suggests that stress may have a stronger negative effect on kidney morphology than diabetes in this short-term study. It could also mean that the mechanisms through which stress affects kidney size are more immediate or more intense compared to those seen in diabetes alone in that timeframe. Comparing the Stress group to the DM+Stress group, the p-value was < 0.05. This result shows that adding diabetes to chronic stress leads to a significant increase in capsule size compared to the stress group. The DM+Stress group had a larger mean and median capsule size than the Stress group, indicating that the presence of diabetes increases the effect of stress on kidney morphology. It seems that while stress alone reduces kidney size, adding diabetes to this condition results in a larger capsule size. This could be due to the interaction between the two factors, where diabetes may alter the response of the kidneys to stress, potentially leading to compensatory growth or structural changes that are more pronounced than stress alone. Finally, when we compare the DM group to the DM+Stress group, a significant difference was found with a p-value of < 0.05. This shows that adding stress to diabetes causes a significant increase in capsule size. The DM+Stress group showed larger capsule sizes than the DM only group. This suggests that stress might increase the effect of diabetes on kidney morphology, leading to more pronounced changes in capsule size. It's possible that the combination of stress and diabetes could have a synergic effect, where the kidneys react to both factors in a way that leads to more significant morphological change than diabetes alone.

The results of this study suggest that both stress and diabetes significantly affect kidney morphology, with the combination of these two factors leading to more pronounced changes in capsule size compared to each factor alone. The presence of chronic stress results in the smallest capsule sizes, while diabetes and the combination of stress and diabetes show larger capsule sizes, possibly indicating compensatory changes in kidney structure. The data highlights the importance of considering both physiological and psychological factors in the study of kidney health and underscores the potential combined effects that stress and diabetes may have on kidney morphology.

8.3. Total number of renal corpuscles

The total number of renal corpuscles is a morphological feature of the kidneys that can provide further insight into morphological kidney changes (Table 5).

Table 5. Descriptive statistics of the total number of renal corpuscles across different experimental groups.

	Control (n=120)	Stress (n=127)	DM (n=113)	DM+Stress (n=103)
Mean	17.14	14.11	16.29	17.17
Median	17.00	14.00	16.00	17.00
SD	1.46	1.36	1.89	2.56
IQR	1.50	2.00	2.50	1.50
25%	16.00	13.00	15.00	15.50
75%	17.50	15.00	17.50	17.00

n: number of renal corpuscles counted in each group; SD: standard deviation; IQR: interquartile range

The DM+Stress group, with a mean of 17.17, has the highest number of renal corpuscles, followed closely by the Control group, which has the highest mean number of renal corpuscles (17.14). The DM group has the third highest mean (16.29), while the Stress group has the lowest mean (14.11). The Stress group has a decrease of 21,48% of total number of renal corpuscles compared to the Control group. This suggests that the combination of diabetes and stress results in a slightly higher number of renal corpuscles on average, while stress alone leads to a lower number.

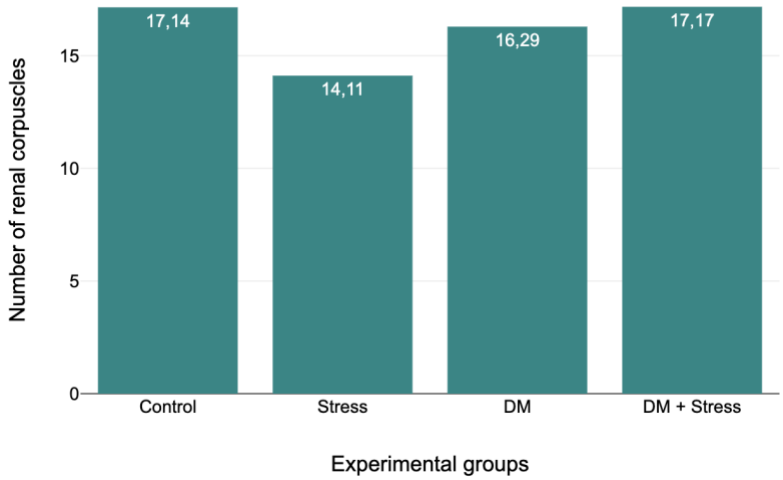

Fig. 10. Mean of number of renal corpuscles per slide across different experimental groups.

In terms of median values, DM+Stress group and the Control group have the highest median with a median of 17.00. The DM group has a median of 16.00, and the Stress group has the lowest median of 14.00. This supports the idea that the combination of diabetes and stress leads to the highest number of renal corpuscles, while stress alone results in the smallest number of renal corpuscles. The DM+Stress group shows the highest SD (2.56), showing the greatest variety in the number of renal corpuscles, followed by the DM group with an SD of 1.89. The Control group has an SD of 1.46, and the Stress group has the lowest SD (1.36), showing that number of renal corpuscles in this group are more consistent compared to the others. The Control group shows a range from 16.00 to 20.00, while the Stress group has a range from 12.00 to 16.00. The DM group spans from 14.00 to 19.00, and the DM+Stress group has the widest range, from 15.00 to 22.00. This suggests that while the Control and DM+Stress groups show the broadest spread of values, the stress group has a more limited range and the lowest values.

The statistical analysis was performed using the Kruskal-Wallis rank sum test and the Mann-Whitney U Test (Table 6).

Table 6. Descriptive statistics of the number of renal corpuscles across the different experimental groups and p-values across the different experimental groups.

	Median	Minimum	Maximum	P-value
Control (n=120)	17.00	16.00	20.00	Control vs. Stress = 0.003[#]
Stress (n=127)	14.00	12.00	16.00	Control vs. DM = 0.469[#]
DM (n=114)	16.00	14.00	19.00	Control vs. DM+Stress = 0.824[#]

DM+Stress (n=103)	17.00	15.00	22.00	Stress vs. DM = 0.030[#] Stress vs. DM+Stress = 0.010[#] DM vs. DM+Stress = 0.663[#] Control vs. Stress vs. DM vs. DM+Stress = 0.007[$]

n: number of renal corpuscles per group; #: Mann-Whitney U Test, $: Kruskal-Wallis rank sum test

The results revealed several significant findings. To compare statistically the number of renal corpuscles, the Kruskal-Wallis rank sum test was used. The test resulted in a chi-squared value of 12.21 (df = 3) and a p-value of < 0.05. This indicates that there are significant differences in a total number of renal corpuscles in at least one of the groups, suggesting that the factors being tested have a significant impact on the number of renal corpuscles. To further investigate which specific groups differed from each other, pairwise comparisons using the Mann-Whitney U test was performed. The results revealed significant differences between multiple experimental groups. First, comparing the Control group with the Stress group, a significant difference was observed with a p-value of < 0.05. This indicates that chronic psychological stress significantly affected the number of renal corpuscles in the kidney. However, when comparing the Control group to the DM group, no significant difference was found, a p-value of > 0.05, suggesting that diabetes alone did not result in a statistically significant change in the number of renal corpuscles compared to the Control group. Similar to the comparison between the Control group and the DM+Stress group also showed no significant difference, p-value of > 0.05, indicating that the combined effects of diabetes and stress did not significantly alter the number of renal corpuscles in relation to the Control group. The comparison between the Stress group and the DM group showed a significant difference, a p-value of < 0.05, suggesting that the presence of stress significantly reduced the number of renal corpuscles with psychological stress compared to those with diabetes alone. Comparing the Stress group and the DM+Stress group, a statistical significance was observed, with a p-value of < 0.05, showing that the presence of stress significantly reduced the number of renal corpuscles in the Stress group compared to the DM+Stress group. Finally, the comparison between the DM group and the DM+Stress group showed no significant difference, with a p-value of > 0.05, indicating that the combined effects of diabetes and stress did not produce a statistically significant change in the number of renal corpuscles when compared to diabetes alone.

The statistical analysis indicates that chronic psychological stress has a significant impact on the number of renal corpuscles. Diabetes alone or the combination of diabetes and stress did not show statistically significant effects in comparison to the Control group. However, it is important to acknowledge that the findings of this study may be limited by the relatively small sample size, which

could impact the statistical power and reliability of the results. Larger studies are needed to validate these findings.

8.4. Discussion

The aim of this study was to investigate how chronic psychological stress influences kidney morphology in patients with T1DM. This research focused on evaluating the size of renal corpuscles, the size of Bowman's capsule and the number of renal corpuscles across four experimental groups: Control group (n=9), Stress group (n=10), DM group (n=8), and the DM+Stress group (n=8). The results showed several important findings that help to understand the interaction between psychological stress, T1DM, and kidney morphology. These findings align with some aspects of the existing literature while also provide new insights.

Starting with the size of renal corpuscles, the data showed significant differences between the experimental groups. The Control group had a mean size of renal corpuscles of 112.16 μm, while the Stress group exhibited a significantly higher mean of 118.53 μm. In this study, the significant increase in the size of renal corpuscles in the Stress group supports the hypothesis that stress alone can induce morphological changes in the kidney, potentially through mechanisms involving renal blood flow alterations and hyperfiltration. Interestingly, when comparing the Control group with the DM group, no significant difference in the size of renal corpuscles was observed. The mean size of renal corpuscles in the DM group (111.41 μm) was similar to that of the Control group (112.16 μm), suggesting that the short-term effects of diabetes on kidney morphology may not be as much as expected. This finding contrasts with the literature, which generally links diabetes to glomerular hypertrophy as part of the early stages of diabetic nephropathy [35]. However, this result may be due to the relatively short duration of the study, as the effects of hyperglycaemia on kidney morphology are typically slow to develop. While diabetes could eventually lead to significant renal corpuscle changes, it may take longer for these alterations to become prominent in terms of the size of the renal corpuscle. The most surprising finding came from the comparison between the Control group and the DM+Stress group, where a significant difference in the size of renal corpuscles was found. The mean length in the DM+Stress group (109.75 μm) was slightly lower than in the Control group, and the DM+Stress group showed a statistically significant reduction in the size of renal corpuscles. This suggests that the combination of stress and diabetes may influence the size of renal corpuscle in the short term, but in a decrease and not an increase, as initially could be expected. Previous studies have suggested that stress can affect the kidneys' structure, potentially leading to greater renal damage [38]. However, this study's findings suggest that the kidneys may have compensatory mechanisms that soften the combined impact of both stress and diabetes and lower the size of renal corpuscles, at least over the short duration of the study. Comparing the stress group with the DM group, a significant

difference in the size of renal corpuscles was observed, with the stress group showing a larger mean size. This finding further supports the suggestion that stress can lead to glomerular hypertrophy, potentially due to the activation of pathways that affect renal vasculature and glomerular filtration. However, diabetes alone did not show the same impact on the size of renal corpuscles within the study period, indicating that the effects of stress on kidney morphology may be faster than the effects of diabetes. Furthermore, comparing the DM group and the DM+Stress group, no statistically significant difference was found. This shows that stress as an additional factor did not lead to a significant change in the size of renal corpuscles.

Moving on to Bowman's capsule size, the data revealed significant differences between the groups, providing further insights into the effects of stress and diabetes on kidney structure. The DM+Stress group had the largest mean Bowman's capsule size (12.77 μm), followed closely by the Control group at 12.39 μm. In contrast, the DM group had the smallest mean Bowman's capsule size (9.75 μm), while the DM group had a mean of 11.24 μm. These results suggest that the combination of diabetes and stress results in an increase in Bowman's capsule size, possibly as a compensatory response to the combined metabolic and hormonal stresses exposed to the kidneys. The larger Bowman's capsules in the DM+Stress group could reflect adaptive changes aimed at maintaining kidney function. This finding aligns with studies that suggest diabetes can induce thickening of the glomerular basement membrane [35] and Bowman's capsule [26], while stress might lead to a reduction in Bowman's capsule size due to renal dysfunction [41]. The smaller Bowman's capsule observed in the Stress group is notable. Previous research has shown that chronic stress can lead to glomerular damage and reduced kidney function, potentially by inducing glomerular atrophy and increasing inflammation. The results of this study support this idea, as the Stress group exhibited significantly smaller capsules compared to the other groups. The reduction in Bowman's capsule size may reflect the damaging effects of stress on kidney health, which could involve mechanisms such as increased renal vascular resistance and altered glomerular filtration. The DM group showed a mean capsule size of 11.24 μm, which was slightly smaller than the Control group but larger than the Stress group. This suggests that while diabetes alone has effects on Bowman's capsule size, the changes may not be as pronounced as those caused by stress. Diabetes is known to cause a range of morphological changes in the kidneys, including mesangial expansion and glomerular hypertrophy, which can contribute to capsule thickening over time [8]. However, in this study, the effects of diabetes on Bowman's capsule size were not as prominent as those of stress, possibly because the short duration of the study did not allow enough time for significant structural changes to manifest in the Bowman's capsule.

In contrast, the combination of DM+Stress led to a significant increase in Bowman's capsule size compared to the Stress group, also having a bigger total number of renal corpuscles. This suggests that while stress might reduce the number of renal corpuscles and lead to Bowman's capsule atrophy,

the addition of diabetes could induce compensatory changes in the kidney, such as capsule thickening. This finding is interesting because it suggests that the kidneys might react to both diabetes and stress together in a way that's different from how they would react to each of these factors on their own.

Finally, examining the number of renal corpuscles, the data revealed a significant reduction in the number of renal corpuscles in the Stress group, with a mean of 14.11 compared to 17.14 in the Control group. This finding goes with previous research suggesting that chronic stress can lead to a significant reduction in the number of glomeruli [38], likely because of stress hormones on renal vasculature and glomerular filtration. The decreased number of renal corpuscles in the Stress group supports the hypothesis that stress negatively impacts kidney morphology by reducing the number of functioning renal corpuscles. This result also shows the importance of stress management in maintaining kidney function, as the reduction in the number of renal corpuscles could lead to impaired kidney function over time, which means chronic stress could be a potential trigger for kidney changes and kidney dysfunction. The DM group showed a slight reduction in the number of renal corpuscles compared to the Control group, with a mean of 16.29. However, this difference was not statistically significant, suggesting that the effects of diabetes on the number of renal corpuscles may not be as strong as the effects of stress. Diabetes has long been associated with glomerulosclerosis and other forms of kidney damage, but these changes may take longer to develop and may not be reflected in the short-term number of renal corpuscles changes observed in this study. This finding aligns with the literature, which reports that the most significant changes in kidney morphology due to diabetes occur over longer periods of hyperglycaemia [29]. The combination of DM and chronic stress did not lead to a more significant reduction in the number of renal corpuscles compared to either factor alone. This suggests that the kidneys might use compensatory mechanisms to balance out the effects of both stress and diabetes. The absence of a significant change in the number of renal corpuscles in the DM+Stress group might indicate that the kidneys are able to adapt to the combined effects of these two stressors, possibly through changes in glomerular filtration and renal blood flow. These mechanisms may help to prevent glomerular dysfunction, at least in the short term.

Interestingly, the Stress group shows the largest renal corpuscles, has the smallest size of Bowman’s capsule, and the lower number of the renal corpuscles overall. This suggests a fast response of the kidneys to chronic stress, potentially indicating a form of glomerular enlargement or hypertrophy, while also showing signs of glomerular loss.

In conclusion, this study provides significant insights into the effects of chronic psychological stress on kidney morphology in the context of Type 1 Diabetes mellitus. The findings from this study suggest that chronic psychological stress has a significant impact on kidney morphology, particularly by reducing the number of renal corpuscles, reducing Bowman’s capsule size, and increasing the size of renal corpuscles. Stress alone appears to have a more immediate effect on the size of renal

corpuscles and number, while the effects of diabetes on kidney morphology may take longer to develop. Interestingly, the combination of stress and diabetes results in a statistically significantly larger Bowman's capsule size than diabetes alone, suggesting that the kidneys may adapt to these combined stressors in a way that softens their effects. These findings underscore the need for further research to explore the long-term consequences of these interactions on kidney function.

8.5. Strengths and weaknesses of the study

One of the key strengths of this study is its approach to exploring the combined effects of chronic psychological stress and T1DM on kidney morphology. By using a good, structured experimental design with different groups (Control, Stress, DM, and DM+Stress), the study provides a decent analysis of how each factor contributes to kidney changes, both individually and in combination. The use of histological techniques, such as the evaluation of the number of renal corpuscles, size of the renal corpuscles, and size of the Bowman's capsule, allowed a good examination of kidney morphology. Additionally, the study provides data to an under-researched area, specifically, how psychological stress influences kidney health in individuals with T1DM. This adds new findings to the understanding of the potential mechanisms. Furthermore, by evaluating statistical analyses using R Studio, the study ensures that the data interpretations are both precise and reliable, providing good insights into the effects of stress and T1DM on kidney morphology.

Despite the valuable findings from this study, several limitations must be considered when interpreting the results. The small sample size, with 8 to 10 animals per group, limited the statistical power of the analysis and reduced the reliability of the findings. Larger sample sizes would provide more precise data and improve the reliability of the results. Additionally, the short experimental duration may not have been long enough to fully see the long-term effects of chronic stress on kidney morphology in the context of T1DM. The study was conducted over a relatively short period of 28 days, and long-term studies are needed to assess the impact of stress and T1DM on kidney function. Additionally, variability in tissue preparation and slide evaluation could have introduced some degree of bias, despite efforts to standardize the methods. It's also important to acknowledge that the results may vary slightly depending on the individual performing the measurements, as different interpretations or techniques in slide evaluation can lead to small discrepancies. Furthermore, not every histological component of the kidney was included in the study. These limitations show the need for further research with more comprehensive experimental designs, including larger sample sizes, extended durations, and additional functional assessments to better understand the complex relationship between chronic stress, T1DM, and kidney morphology and function.

9. CONCLUSIONS

1. The comprehensive literature analysis revealed a significant amount of research linking Type 1 Diabetes mellitus with kidney damage, although the effects of chronic psychological stress on kidney morphology in Type 1 Diabetes mellitus patients remain unexplored. Most studies focus on the individual impacts of diabetes and stress, but there is limited understanding of their combined effects on renal health.
2. Type 1 Diabetes mellitus leads to a statistically significant reduction of Bowman's capsule size ($p < 0.05$), with no significant changes observed in the size of renal corpuscles or total number of renal corpuscles ($p > 0.05$). Furthermore, additional exposure to psychological stress not only decreased the size of renal corpuscles ($p < 0.05$) but also significantly increased Bowman's capsule size ($p < 0.05$). Similar to diabetes alone, no changes in the total number of renal corpuscles were observed through the addition of stress ($p > 0.05$).
3. Psychological stress was shown to exacerbate kidney damage in individuals with Type 1 Diabetes mellitus, specifically reducing the number of renal corpuscles ($p < 0.05$) and Bowman's capsule size ($p < 0.05$), but also increasing the size of the renal corpuscles ($p < 0.05$). However, the combined effect of diabetes and stress did not lead to more severe damage than stress alone, suggesting that stress may independently affect kidney morphology more significantly than diabetes in the short term.

10. PRACTICAL RECOMMENDATIONS

The results show the importance of managing psychological stress in individuals with Type 1 Diabetes mellitus, but also in individuals without Type 1 Diabetes mellitus, as chronic stress appears to worsen kidney morphology. Managing stress in diabetic patients could be an important step in preventing kidney damage and improving overall kidney health. Further research is needed to explore the underlying mechanisms of stress-induced kidney damage and to assess the long-term effects of chronic stress in Type 1 Diabetes mellitus.

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