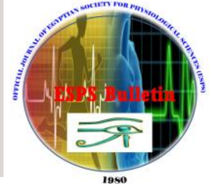
Bull. Egypt. Soc. Physiol. Sci. *Vol. (45), Issue (2)*,258-268

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)



Metrnl impact in streptozotocin induced diabetic nephropathy in rats, targeting Nrf2/HO-1 pathway

Suzan A. Khodir[1*], Amany Wahb[2], Mohamed Ali Ibrahim[3], Noha M. Abd El-aziz[4], Amany Tawfik Elfakhrany[5], Haitham M. Sewilam[6], Noha O. Shawky[7], Shaimaa K. Dawa[8], Radwa Hassan Ali[9,10]

[1] Medical Physiology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[2] Medical Biochemistry and Molecular Biology Department, Faculty of medicine, HelwanUniversity, Egypt.
[3] Internal Medicine Department, Faculty of Medicine, Menoufia University Shebin El-Kom, Menoufia, Egypt
[4]Anatomy and Embryology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[5] Clinical Pharmacology Department, Faculty of medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.
[6]Histology Department, Faculty of Medicine, Helwan University, Egypt.
[7] Medical Physiology Department, Faculty of Medicine, Helwan University, Egypt.
[8]PathologyDepartment, Faculty of Medicine, Banha University, Egypt
[9]Medical Physiology Department, Faculty of Medicine, Ain Shams University,Cairo Egypt
[10]Medical Physiology Department, Faculty of Medicine, Armed Forces College of Medicine (AFCM), Cairo, Egypt

Submit Date : 02 Jan. 2025
Revised Date : 21 Jan. 2025
Accept Date: 27 Jan. 2025

Keywords

- Bax
- Diabetic Nephropathy
- HO-1
- Metrnl
- NF-kB
- Nrf2

Abstract

Introduction: The most prevalent complications in diabetes mellitus is diabetic nephropathy (DN). Metrnl's role in endocrine metabolism has been well investigated. It has been demonstrated to contribute to the healing of inflammatory injury. **Objective**: to assess the potential underlying processes and the protective impact of Metrnl in DN caused by STZ **Materials and Methods:** Thirty adult male albino rats were split into: control, DN, and DN+Metrnl (10/group).Urine albumin, serum glucose, serum glycosylated Hb A1c, serum urea, serum creatinine, serum cystatin C, creatinine clearance, renal MDA, renal GSH, renal TNF-α, renal IL-10, and renal gene expression of HO-1 and Nrf2 were assessed. Additionally, immunohistochemical and renal histopathology studies were carried out. **Results:** The DN group had significantly higher serum levels of glucose, glycosylated HbA1c, urea, creatinine, cystatin C, urinary albumin, renal MDA, and renal TNF-α than the control group, but they also had significantly lower renal GSH, renal IL-10, creatinine clearance values, and Nrf2 and HO-1 gene expression. Furthermore, NF-kB and Bax immunoreaction were upregulated in the DN compared to control. The STZ-induced DN alterations significantly improved in the DN+Metrnl group. ***Conclusion***: Metrnl offers substantial protection against STZ-induced DN through anti-oxidant and anti-inflammatory pathways, apoptosis inhibition, and the up-regulation of Nrf2 and HO-1 renal gene expressions.

Corresponding author: Suzan A. Khodir, Email: suzan.abdalhameed.12@med.menofia.edu.eg, ORCID iD: 0000-0002-2535-9445.Medical PhysiologyDepartment, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt

Introduction

One of the most prevalent complications in diabetes mellitus (DM) is diabetic nephropathy (DN) [1]. End-stage kidney disease (ESKD) is largely caused by DN, which has become the primary cause of ESKD [2]. Hyperglycemia is the cause of DN, a chronic kidney disease that can be fatal and causes glomerular inflammation, hypertension, and several other serious symptoms [1].

The characteristic pathological alterations of DN involve many aspects, including severe albuminuria, glomerular basement membrane thickening, glomerular and tubular hypertrophy, glomerulosclerosis, and tubulointerstitial fibrosis [3]. Other variables, including as chronic inflammation, dyslipidemia, and the buildup of advanced glycation end products (AGEs), can also significantly share in the initiation and progression of DN in addition to chronic continuous hyperglycemia [4].AGEs raises reactive oxygen species (ROS) levels [5]. AGEs cross-talk with other pathways in addition to interacting with their receptor and changing intracellular signaling to encourage the production of proinflammatory cytokines. Diabetes-related AGEs trigger the apoptosis pathway, and AGE-induced apoptosis results in basement membrane thickening and eventual glomerular function loss [6]. Despite tremendous progress in understanding the fundamental processes of DN, successfully addressing these harmful pathways remains a huge issue.

Erythroid-derived 2-related factor 2 (Nrf2) is a crucial cellular defense mechanism against oxidative damage.[7]. Heme oxygenase-1 (HO-1), a crucial Nrf2 target gene, is regarded as a kidney protective gene. In addition to producing anti-inflammatory, antioxidant, and antiapoptotic chemicals, it contributes to the breakdown of oxidized heme [8]. Numerous studies have shown that Nrf2/HO-1 pathway activation can substantially ameliorate oxidative stress and inflammatory response while preventing renal impairment in DN. Numerous antioxidant factors can have their expression controlled by the Nrf2/HO-1 signaling pathway. For illnesses where oxidative stress is elevated, stimulating these antioxidants is a crucial treatment approach [9].

It is essential to find new drugs for the therapy of DN and characterize them in preclinical DN models. Metrnl is a secreted protein that is expressed in the kidney and other human organs. Metrnl's role in endocrine metabolism has been well investigated [10].It has been demonstrated to help improve insulin sensitivity and heal inflammatory damage [11].According to clinical research, there is a negative correlation between markers of declining renal function and lower levels of Metrnl expression in DKD patients. In addition, Metrnl has been shown in several trials to have a preventive effect against coronary artery disease and diabetes mellitus [12]. Metrnl controls inflammation and energy expenditure [13].According to a recent study, Metrnl inhibited apoptosis and endoplasmic reticulum stress [14].

The precise function of Metrnl in DN is still not well understood in spite of these discoveries. Determining the importance of Metrnl as a possible therapeutic agent or biomarker for certain disorders requires a thorough study. Here, our goal was to look into the possible function and molecular underpinnings of Metrnl in STZ-induced DN.

Material & Methods

Animals

This study was carried out in accordance with the rules established by the Animal Experimentation Ethics Committee of Menoufia University's Faculty of Medicine with IRB NO: 1/2025BIO 19-1.The weight of the thirty mature male Wister rats ranged from 150 to 180 g. The rats were obtained from a local animal providing facility housed in housing that had a 12-hour light and 12-hour dark cycle and ranged in temperature from 20 to 24 degrees Celsius. Regular rat food and tap water were also freely available to them.

DM induction and grouping: Streptozotocin (STZ), (65 mg/kg) administered intraperitoneally in 0.2 ml of citrate buffer (Sigma-Aldrich Chemical Co., USA) resulted in DM [15].

DM was proven to have occurred 48 hours after the STZ injection. Participating in the trial were animals with a fasting blood glucose level of more than 200 mg/dl that were diagnosed with diabetes.

Thirty rats were divided into three groups (n = 10); Control group, rats were given a single i.p. injection of 10 mmol/L citrate buffer and for eight weeks 1 ml phosphate buffer saline (PBS) (Sigma-Aldrich Company, St. Louis, USA) intravenously daily.

Diabetic Nephropathy (DN) group: As noted before, DM was induced. For eight weeks, rats were given 1 ml of PBS intravenously every day.

Metrnl-treated DN group (DN+Metrnl): Every day after diabetes was established, Metrnl (Adipogen, San Diego, CA, USA) was dissolved in 1 mL of PBS and administered intravenously (2 µg/rat/day) [16,17,18] for eight weeks.

Urine collection and biochemical analysis

Urine samples were collected for a whole day from rats housed in various metabolic cages at the end of the experiment. Urine samples were centrifuged at 3000 rpm for 12 minutes after their volume was established. After that, the supernatant was taken out and kept at -20°C to measure creatinine and microalbuminuria.

To determine the quantity of albumin in the urine, the Microalbuminuria ELISA kit (Exocell Inc., Philadelphia, USA) was utilized.The creatinine clearance (ml/min), with values expressed in mL/min, was calculated by multiplying the urine creatinine concentration (mg/dL) by the urine volume (mL/min) to the plasma creatinine concentration (mg/dL) [19].

Blood sampling and biochemical analysis

Blood samples were drawn from the retroorbital venous plexus under mild anesthesia after a 12-hour overnight fast, and they were split evenly between two tubes. The first tube was centrifuged at 2,000 rpm for 10 minutes. The obtained serum was stored at -20 degrees until further examination was required. When the blood was transferred to the second tube, an EDTA tube, this proportion was utilized to determine the HbA1c.After the rats were killed by cervical dislocation, the right kidney was taken for histological examination and the left kidney was used for a biochemical test.

Fasting serum glucose (Diamond Diagnostic, Egypt), serum HbA1c (Stanbio Glycohemoglobin, Egypt), were measured by colorimetric kits. Colorimetric kits (Spectrum Diagnostics, Egypt) were used to measure the concentrations of creatinine in plasma, urine, and urea. ELISA kits for cystatin C (ab201281, Abcam, Cambridge, UK) were used to measure its serum concentration.

Tissue Homogenate preparation

Each weighted kidney specimen was homogenized independently using a tissue homogenizer (MPW120, MPW Medical Instruments, China).The crude tissue homogenate was spun for 15 minutes at 10,000 rpm in an ice-cold centrifuge before being stored at -80°C for testing. Using readily accessible commercial kits (Biodiagnostic CO., Dokki, Giza, Egypt), renal MDA and reduced glutathione (GSH) were determined by spectrophotometry in accordance with the manufacturer's instructions. As directed by the manufacturer, ELISA kits for renal TNF-α (TNF-α: ERT2010 1, Assaypro LLC, Saint Charles, Missouri, USA) and renal IL-10 (ERI3010-1, Assaypro LLC, Saint Charles, Missouri, USA) were utilized.

Gene expression quantification using RT-PCR

RT-PCR was used to analyze the relative mRNA levels of the Nrf2 and HO-1 genes in the renal material. The TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from tissues in accordance with the manufacturer's instructions. The extracted RNA was stored at -80 C until it was needed. The quantity and purity of RNA were determined by measuring the absorbance at 260 nm (A260) with a nanophotometerN60.In the first PCR step, complementary DNA (cDNA) synthesis (reverse transcription phase) was carried out using ThermoScript RT reagent kits (Invitrogen).cDNA was then amplified using SYBR Green Mix kits (Stratagene-USA).Each amplification curve's cycle threshold, or Ct, values were determined.Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the reference gene. Data analysis was conducted using the 7500 ABI PRISM 2.0.1 version (Applied Biosystems-USA).The comparative ΔΔCt method was used to measure and express the relative amounts of Nrf2, and HO-1.

The following primers were used for the Nrf2 gene:

(1)Forward primer: 5-GGTTGCCCACATTCCCAAATC-3

(2) Reverse primer: 5-CAAGTGACTGAAACGTAGCCG-3

The following primers were used for the HO-1 gene:

(1) Forward primer: 5-AGGTGCACATCCGTGCAGAG-3

(2) Reverse primer: 5-CTTCCAGGGCCGTATAGATATGGTA-3

Histopathological method

Kidney tissue slices were fixed at 10% neutral buffered formalin for histological investigations. They were then dried in ethyl alcohol, washed in xylol, and lastly placed with paraffin. Haematoxylin and Eosin was used to stain the 4 μm-thick sections.

For immunohistochemical studies, the kidney paraffin sections (4 μm) were stained by Bax (rabbit polyclonal antibody, Dako, Carpinteria California, USA), and NF-kB (monoclonal, dilution 1:200, Abcam).

Statistical analysis

The data was tabulated and analyzed using SPSS Statistical Package for Social Science software, version 16 (SPSS, Inc., USA).Use the Shapiro test to determine if the data has a normal distribution. The statistical data was displayed as X±SD, or mean ± standard deviation. To determine the significance of the group differences, a one-way analysis of variance (ANOVA) was followed by a post hoc Tukey test. A probability value below 0.05 was considered statistically significant ($p<0.05$).

Results

While the DN group's serum levels of glucose, glycosylated HbA1c, urea, creatinine, cystatin C, urinary albumin, renal MDA and renal TNF-α were dramatically higher than those of the control, the group's renal GSH, renal IL-10,creatinine clearance values, and the expression of the Nrf2 and HO-1 genes were dramatically lower. While renal GSH, renal IL-10,creatinine clearance values and renal gene expression of Nrf2 and HO-1 of DN+Metrnl were dramatically higher than those of DN but still dramatically lower than those of control, serum levels of glucose, glycosylated Hb A1c, urea, creatinine, cystatin C, urinary albumin, renal MDA and renal TNF-αof the DN+Metrnl group were dramatically lower than those of DN but still dramatically higher than those of the control group.(Table 1).

Table (1): The measured serum glucose, glycosylated HbA1$_c$, urea, creatinine, cystatin C, creatinine clearance, urinary albumin, renal TNF-α, renal IL-10, renal MDA, renal GSH, renal Nrf2 gene expression and renal HO-1 gene expression in all studied groups.

	Control group	DN group	DN+Metrnl group
Serum Glucose (mg/dl)	80.99±7.36	318±9.1*	239±6.3$^{*\#}$
Serum glycosylated Hb A1$_c$(% of normal Hb)	3.75±0.15	10.9±0.91*	7.99±0.29$^{*\#}$
Serum Urea (mg/dl)	30.92±1.87	99.2±3.14*	59.6±2.16$^{*\#}$
Serum Creatinine (mg/dl)	0.51±.08	1.61±0.1*	0.99±0.11$^{*\#}$
Serum cystatin C (mg/L)	0.65±0.05	1.99±0.04*	1.08±0.13$^{*\#}$
Creatinine clearance (mL/min)	1.09±0.05	0.41±0.03*	0.77±0.08$^{*\#}$
urinary albumin (mg/day)	49.2±2.3	159.1±3.4*	98.36±2.48$^{*\#}$
Renal MDA (nmol/ gm. Tissue)	8.2±2.9	28.3± 2.14*	19.23± 1.25$^{*\#}$
Renal GSH (µmol/gm. Tissue)	27.9±2.01	8.25±1.02*	18.35±0.47$^{*\#}$
Renal TNF-α (ng/ml)	19.2±1.2	47.5±0.9*	29.1±2.041$^{*\#}$
Renal IL-10 (ng/mL)	20.25±1.25	6.32±0.9*	15.89±1.02$^{*\#}$
Renal Nrf2 gene expression	1	0.29±0.03*	0.61±0.06$^{*\#}$
Renal HO-1 gene expression	1	0.42±0.04*	0.72±0.01$^{*\#}$

* Significant compared with control, # Significant compared with DN.

Hematoxylin and Eosin staining:

Sections from control group showed normal renal glomerulus and tubules (Fig. 1A). The DN group showed mesangial expansion with tubular atrophy and interstitial cellular proliferation (Fig. 1B). The DN+Metrnl showed improvement of renal glomerulus and tubules (Fig. 1C).

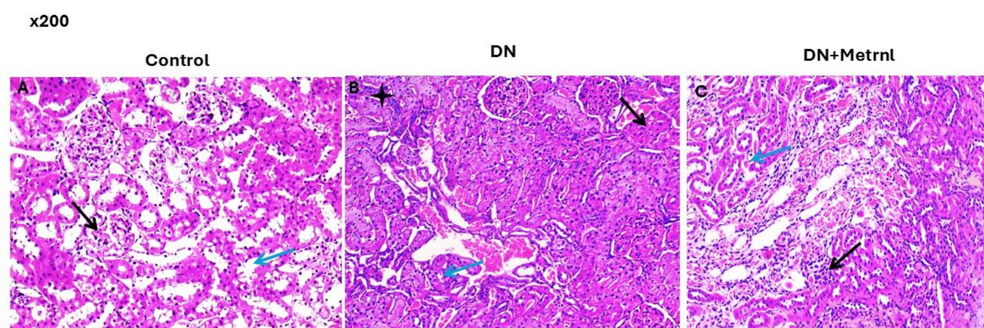


Fig (1): H& E-stained kidney sections in the studied groups (H&E ×200): (A) are photomicrographs of the control group showed normal renal glomerulus (black arrow) and tubules (blue arrow). (B) are photomicrographs of the diabetic group showed mesangial expansion (black arrow) with tubular atrophy (blue arrow) and interstitial cellular proliferation (star). (C) is a photomicrograph of the DN+Metrnl showing marked improvements in the renal glomerulus (black arrow) and tubules (blue arrow).

Immunohistochemical results

In Bax stain, The DN group showed substantial elevation in the percentage area of Bax when compared to control (68.5±0.02 vs 13.2±0.15, respectively, *P*<0.05). However, the DN+Metrnl group showed a substantial decrease in this percentage compared to DN (32.8±0.33 vs 68.5±0.02, respectively, *P*<0.05), but still increased from the control group. (Fig. 2: A-D).

In NF-kB stain, The DN group showed a substantial elevation in the percentage area of NF-kB compared to control (70±0.05 vs15.1±0.03., respectively, *P*<0.05). However, the DN+Metrnl group showed a substantial decrease in this percentage when compared to DN group (33.4±0.12 vs 70±0.1, *P*<0.05),but still increased from the control. (Fig. 2: E-H).

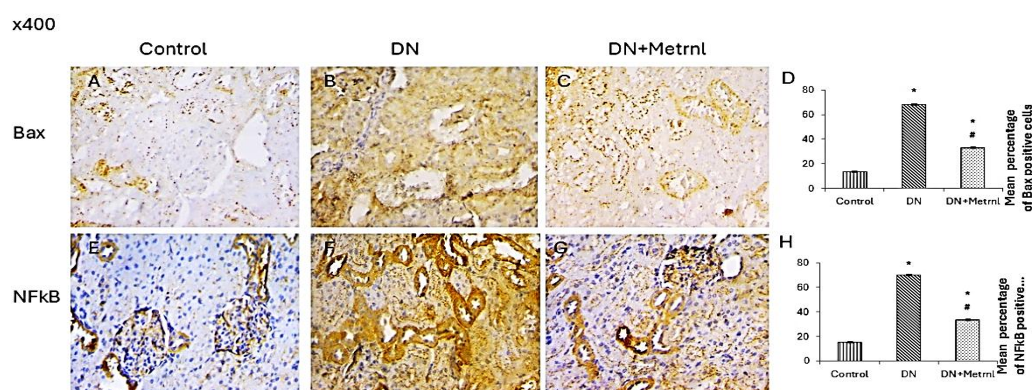


Fig (2): Representative micrographs of the different experimental groups showing significant increase of the Bax (A-D) and NF-kB (E-H) immunoreaction in DN and a significant decline in the DN+Metrnl.

Discussion

Numerous research on the preventive impact of Metrnl in DM, and other conditions have been conducted in recent years. We identified Metrnl as a new renoprotective impact in our investigation. Numerous physiological processes, such as insulin sensitivity, immunology, and inflammation, are regulated by adipokines [20]

Animals that received STZ injections showed substantially worse glycemic states than those in the control group. Pancreatic β-cells are the preferred location for the accumulation of STZ, a hazardous glucose analogue that causes cell toxicity, apoptosis, and increased generation of reactive oxygen species.[21]

When compared to the DN group, DN+Metrnl significantly reduced the glycemic impairment brought on by STZ. Adipokines and other secretory proteins may directly control glucose homeostasis in humans by modifying glucose absorption and release. Previous research found a negative correlation between Metrnl and a glycemic index, which includes fasting C-peptide, FBG and HbA1c [22,23]. Previous studies revealed that the concentration of Metrnl was lower in diabetic patients than in healthy people [24],Who reported also an inverse correlation between Metrnl level and TNF-a and hs-CRP.

Asleh et al. found that podocytes were more susceptible to the adverse effects of hyperglycemia and were primarily linked to DN with proteinuria due to changes in podocyte morphology. These outcomes supported the DN group's findings that

their renal function was lower than that of the control [25].

Metrnl significantly improved the DN group's renal impairment brought on by STZ. Metrnl has been shown in previous studies to protect kidneys with DN by lowering the synthesis of fibrotic molecules and blocking the TGF-β1/Smads signaling pathway[12]. In contrast to the normoalbuminuria group, the albuminuria group's serum Metrnl concentrations were considerably lower, according to the primary finding of the prior study [20]. Previous study revealed that A/G and serum Metrnl level were protective factors against DN. The study also discovered a correlation between the severity of DN and serum Metrnl levels, indicating that Metrnl may be a viable new treatment target for individuals with DN [20].

Oxidative stress has an important role in the pathogenesis of DN. Our findings are consistent with other research showing that STZ-induced diabetes in rats resulted in oxidative damage. This is seen in the DN group by a substantial increase in renal MDA levels and a decrease in GSH [26],because hyperglycemia increases free radicals and decreases cellular antioxidants, it can exacerbate oxidative stress and lipid peroxidation. Oxidative stress causes structural damage to proteins, lipids, DNA, and RNA, which leads to DN symptoms.[27].

Metrnl significantly reduced the oxidative stress brought on by STZ. Metrnl's antioxidant action is consistent with a prior work [28] that found that Metrnl autocrinely inhibits DOX-induced oxidative stress, apoptosis, and cardiac dysfunction by activating SIRT1.Another study attributed the antioxidant activity of metrnl to its ability to scavenge free radicals and increased anti-oxidant production via up-regulation of Nrf2/HO-1 pathway [18].

Complex pathway interaction enhanced renal tubular apoptosis and elevated inflammation and oxidative stress in the pathogenesis of renal damage [29]. According to earlier research, the DN group revealed a significant rise in renal Bax immunoreaction levels [30]. Renal Bax immunoreaction was downregulated in the DN+Metrnl group compared to the DN group. Previous studies have already established the anti-apoptotic effects of Metrnl [28,31].

Inflammation has a significant part in the pathophysiology of DN, according to mounting data. The pathophysiology of certain inflammatory diseases seems to be significantly influenced by the NF-κB pathway [32]. A NF-κB-dependent inflammatory response may worsen renal failure in both acute and chronic kidney disorders [33].According to our findings, renal NF-kB immunoreaction was upregulated in DN compared to control with increase in TNF-α and decrease in the anti-inflammatory IL-10, this agreed with prior study [32]

In contrast to the DN group, Metrnl significantly reduced proinflammatory cytokines and downregulated NF-kB renal immunoreaction with an increase in IL-10. A prior research found a substantial negative connection between Metrnl levels and TNF-α [34].

Through the elevation of IL-14 gene expression, Metrnl can provide an anti-inflammatory impact. Another method that Metrnl reduces inflammation is by upregulating the peroxisome proliferator-activated receptor-delta (PPAR-d) pathways. Metrnl promotes the expression of PPAR-d [35], a member of the PPARs family that inhibits the

expression of IL-1β and IL-6 in monocytes and reduces inflammation by downregulating the gene expression of matrix metalloproteinase-9, inducible nitric oxide synthase, and scavenger type A receptors in macrophages [36]. In addition, Metrnl knockout results caused inflammatory activation by increasing the NF-kB pathway and impairs vasodilation function by decreasing the eNOS phosphorylation level[37].

The transcriptional regulation of the genes encoding endogenous antioxidant enzymes is mostly governed by Nrf2 signaling [38].HO-1 is one of the phase II antioxidant enzymes. According to reports, Nrf2-mediated antioxidant enzymes have promise as therapeutic targets for reducing oxidative stress and enhancing renoprotection [39].

Our results revealed that DN dramatically downregulated Nrf2/HO-1 pathway and this goes in line with previous study [6].

In contrast to the DN group, Metrnl activated the Nrf2/HO-1 pathway and this in line with previous study [18]. According to a prior study, Metrnl enhanced nuclear accumulation and cardiac Nrf2 protein abundance, which in turn activated downstream antioxidant enzymes and reduced DOX-induced cardiac damage [28].More studies are needed on Metrnl impact on DN to further elucidate its underlying mechanisms involved.

Conclusion

Metrnl offers substantial protection against STZ-induced DN through anti-oxidant and anti-inflammatory pathways, apoptosis inhibition, and the up-regulation of Nrf2 and HO-1 renal gene expressions.

Acknowledgment: The authors would like to thank the Faculty of Medicine, Menoufia University, for providing the necessary facilities.

Funding: This paper was not funded.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose

References

1- **SulaimanMK** Diabetic nephropathy: recent advances in pathophysiology and challenges in dietary management. DiabetolMetabSyndr(2019), 11, 7.

2- **Zhao L, Han Q, Zhou L et al.** Addition of glomerular lesion severity improves the value of anemia status for the prediction of renal outcomes in Chinese patients with type 2 diabetes. Renal Failure 2022; 44:346–357.

3- **Tervaert, T.W.; Mooyaart, A.L.; Amann, K.; Cohen, A.H.; Cook, H.T.; Drachenberg, C.B.; Ferrario, F.;** Fogo, A.B.; Haas, M.; de Heer, E.; et al. Pathologic classification of diabetic nephropathy. J. Am. Soc. Nephrol. 2010, 21, 556–563.

4- **Yamagishi, S.; Matsui, T.** Advanced glycation end products, oxidative stress and diabetic nephropathy. Oxid. Med. Cell. Longev. 2010, 3, 101–108.

5- **Dounousi, E.; Duni, A.; Leivaditis, K.; Vaios, V.; Eleftheriadis, T.; Liakopoulos, V.** Improvements in the Management of Diabetic Nephropathy. Rev. Diabet. Stud. 2015, 12, 119–133.

6- **Zhang, B., Zhang, X., Zhang, C., Shen, Q., Sun, G., & Sun, X..**Notoginsenoside R1 protects db/db mice against diabetic

nephropathy via upregulation of Nrf2 mediated HO-1 expression. *Molecules*(2019),*24*(2), 247.

7- **Jaiswal AK Nrf2** signaling in coordinated activation of antioxidant gene expression. Free RadicBiol Med (2004), 36, 1199–1207.

8- **Zhang C, Li Q, Lai S, Yang L, Shi G, Wang Q, Luo Z, Zhao R and Yu Y** Attenuation of diabetic nephropathy by Sanziguben granule inhibiting EMT through Nrf2-mediated anti-oxidative effects in streptozotocin (STZ)-induced diabetic rats. J Ethnopharmacol (2017), 205, 207–216.

9- **Feng X, Zhao J, Ding J, Shen X, Zhou J and Xu Z** LncRNA Blnc1 expression and its effect on renal fibrosis in diabetic nephropathy. Am J Transl Res(2019), 11, 5664–5672.

10- **Ding X, Chang X, Wang J et al.** Serum Metrnl levels are decreased in subjects with overweight or obesity and are independently associated with adverse lipid profile. Frontiers in Endocrinology 2022; 13. PMID: 36133314.

11- **Javaid HMA, Sahar NE, ZhuGe D-L, Huh JY.** Exercise Inhibits NLRP3 Inflammasome Activation in Obese Mice via the Anti-Inflammatory Effect of Meteorin-like. Cells 2021; 10. PMID: 34943988.

12- **Lin, L., Huang, S., Lin, X., Liu, X., Xu, X., Li, C., & Chen, P.** Upregulation of Metrnl improves diabetic kidney disease by inhibiting the TGF-β1/Smads signaling pathway: A potential therapeutic target. *Plos one*,(2024),*19*(8), e0309338

13- **G.S. Baht, A. Bareja, D.E. Lee, R.R. Rao, R. Huang, J.L.** Huebner, et al., Meteorin-like facilitates skeletal muscle repair through a Stat 3/IGF-1 mechanism, Nat Metab 2 (2020) 278–289.

14- **L. Xu, Y. Cai, Y. Wang, C. Xu,** Meteorin-like (METRNL) attenuates myocardial ischemia/reperfusion injury-induced cardiomyocytes apoptosis by alleviating endoplasmic reticulum stress via activation of AMPK-PAK2 signaling in H9C2 cells, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 26 (2020), e924564.

15- **Al-Awar A, Kupai K, Veszelka M et al.** Experimental diabetes mellitus in different animal models. Journal of Diabetes Research 2016; 1–12. DOI 10.1155/2016/9051426.

16- **Jung TW, Lee SH, Kim H-C, et al.** METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARδ-dependent pathways in skeletal muscle of mice. Experimental & molecular medicine. 2018;50(9):1-11.

17- **Li ZY, Song J, Zheng SL, et al.** Adipocyte Metrnl Antagonizes Insulin Resistance ThroughPPARγ Signaling. Diabetes. 2015 Dec;64(12):4011-22.

18- **Khodir, S. A., El-Haroun, H., Gaafar, A. M., A Zayed, M., Rizk, S. K., S Mohamed, A., & Ahmed, K. E. S.** Nrf2/HO-1 Pathway Mediates the Protective Impact of Metrnl on Oxaliplatin-Induced Peripheral Neuropathy in Rats. *Bulletin of Egyptian*

Society for Physiological Sciences,(2024) 1-17

19- **T. Bazzano, T. I. Restel, L. C. Porfirio, A. S. D. Souza, and I. S. Silva,** "Renal biomarkers of male and female Wistar rats (*Rattusnorvegicus*) undergoing renal ischemia and reperfusion," *ActaCirurgicaBrasileira*, vol. 30, no. 4, pp. 277–288, 2015

20- **Chen, J., Li, Z. Y., Xu, F., Wang, C. Q., Li, W. W., Lu, J., & Miao, C. Y..** Low Levels of Metrnl are Linked to the Deterioration of Diabetic Kidney Disease. *Diabetes, Metabolic Syndrome and Obesity*,(2024), 959-967.

21- **Pinheiro L. S., Melo A. D. d., Andreazzi A. E., Junior L. C. d. C., Costa M. B., et al.:** Protocol of Insulin Therapy For Streptozotocin-Diabetic Rats Based on a Study of Food Ingestion and Glycemic Variation. Scand. J. Lab. Anim. Sci.(2011),38 (2):117-127.

22- **Onalan E, Cavlı C, Dogan Y, Onalan E, Gozel N, Buran I, et al.** Low serum levels of meteorin-like/subfatin: An indicator of diabetes mellitus and insulin resistance? Endokrynol Pol (2020) 71(5):397–403. doi: 10.5603/EP.a2020.0038

23- **Tuncer Kara K , Kizil M , Çakmak E , Yildirim TT , Kilinc F , Kuloğlu T , et al.** Comparison of plasma and salivary meteorin-like protein levels in patients with newly diagnosed type-2 diabetes and treated with metformin. Eur Rev Med PharmacolSci (2022) 26(19):7145–50. doi: 10.26355/eurrev_202210_29900.

24- **El-Ashmawy, H.M., Selim, F.O., Hosny, T.A. and Almassry, H.N.,** "Association of low serum meteorin like (metrnl) concentrations withworsening of glucose tolerance, impaired endothelial function and atherosclerosis", Diabetes Research and Clinical Practice,(2019),Vol. 150, pp. 57-63.

25- **Asleh R, Nakhoul FM, Miller-Lotan R et al.** Poor lysosomal membrane integrity in proximal tubule cells of haptoglobin 2-2 genotype mice with diabetes mellitus," Free Radical Biology and Medicine, 2012; 53(4): 779–786, 2012.

26- **Mahmoud AM, Ashour MB, Abdel-Moneim A et al.** Hesperidin and naringin attenuate hyperglycemia- mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats, Journal of Diabetes and its Complications, 2012; 26(6):483–490.

27- **Manda G, Checherita AI, Comanescu MV et al.** Redox signaling in diabetic nephropathy: hypertrophy versus death choices in mesangial cells and podocytes, Mediators of Inflammation, 2015; vol. 2015, Article ID 604208, 13 pages, 2015.

28- **Hu, C., Zhang, X., Song, P., Yuan, Y. P., Kong, C. Y., Wu, H. M., ...& Tang, Q. Z..** Meteorin-like protein attenuates doxorubicin-induced cardiotoxicity via activating cAMP/PKA/SIRT1 pathway. *Redox Biology*,(2020)*37*, 101747.

29- **Martınez-Castelao A, Hueso M, Sanz V et al.** Treatment of hypertension after renal transplantation: long-term efficacy of

verapamil, enalapril, and doxazosin. Kidney Int. 1998; 68: S130–S134

30- **Ahmed OM, Ali TM, Abdel Gaid MA et al.** Effects of enalapril and paricalcitol treatment on diabetic nephropathy and renal expressions of - -TNF-α, p53, caspase-3 and Bcl-2 in STZ-induced diabetic rats. PloS one, 2019;14(9), e0214349.

31- **Li, J., Hong, Y., Zhong, Y., Yang, S., Pei, L., Huang, Z., ...& Wang, T..** Meteorin-like (METRNL) attenuates hypertensive induced cardiac hypertrophy by inhibiting autophagy via activating BRCA2. *BiochimicaetBiophysicaActa (BBA)-Molecular Basis of Disease*,(2024)*1870*(4), 167113.

32- **Zhu, L., Han, J., Yuan, R., Xue, L., & Pang, W.** Berberineameliorates diabetic nephropathy by inhibiting TLR4/NF-κB pathway. *Biological research*, (2018),*51*, 1-12

33- **Kruger B, Krick S, Dhillon N, Lerner SM, Ames S, Bromberg JS, et al.** Donor Toll-like receptor 4 contributes to ischemia and reperfusion injury following human kidney transplantation. ProcNatlAcadSci USA. 2009;106(9):3390–5.

34- **Khajebishak, Y., Madani, S., Faghfouri, A. H., Soleimani, A., Ilaei, S., Peyrovi, S., &Payahoo, L.** Relationship between meteorin-like peptide (Metrnl) serum levels and inflammatory cytokines, oxidative stress biomarkers and body composition parameters in type 2 diabetes patients. *Nutrition & Food Science*, (2023),*53*(5), 861-872

35- **Jung, T.W., Lee, S.H., Kim, H.-C., Bang, J.S., Abd El-Aty, A., Hacımüftüo_glu, A., Shin, Y.K. and Jeong, J.H**., “METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARd-dependent pathways in skeletal muscle of mice”, Experimental and Molecular Medicine,(2018), Vol. 50 No. 9, pp. 1-11.

36- **Hu, J., He, A., Yue, X., Zhou, M. and Zhou, Y.,** “METRNL reduced inflammation in sepsis-induced renal injury via PPARd-dependent pathways”, Food Science and Technology**, (2021),**Vol. 42, pp. 1-8.

37- **Zheng S, Li Z, Song J, et al.** Endothelial METRNL determines circulating METRNL level and maintains endothelial function against atherosclerosis. *ActapharmaceuticaSinica B*. 2023;13:1568–1587.

38- **Ahmed, S.M.; Luo, L.; Namani, A.;Wang, X.J.; Tang, X.** Nrf2 signaling pathway: Pivotal roles in inflammation. Biochim. Biophys. Acta Mol. Basis Dis. 2017, 1863, 585–597.

39- **Cui, W.; Min, X.; Xu, X.; Du, B.; Luo, P.** Role of nuclear factor erythroid 2-related

40- **factor 2 in diabetic nephropathy**. J. Diabetes Res. 2017, 2017.