Heliyon 10 (2024) e40826

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Menaquinone-7 and its therapeutic potential in type 2 diabetes mellitus based on a Zucker diabetic fatty rat model

Ingo Mrosewski [a,*], Valeriya Mantel [a,b], Matthias Urbank [a], Gundula Schulze-Tanzil [c], Christian Werner [c], Clemens Gögele [c], Maria Kokozidou [c,1], Thomas Bertsch [d,1]

[a] MDI Limbach Berlin GmbH, Aroser Allee 84, 13407, Berlin, Germany
[b] Berlin University of Applied Sciences and Technology, Luxemburger Str. 10, 13353, Berlin, Germany
[c] Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg and Salzburg, Prof. Ernst Nathan Str. 1, 90419, Nuremberg, Germany
[d] Institute of Clinical Chemistry, Laboratory Medicine and Transfusion Medicine, Nuremberg General Hospital, Paracelsus Medical University, Prof. Ernst Nathan Str. 1, 90419, Nuremberg, Germany

ARTICLE INFO

Keywords:
Type 2 diabetes mellitus
Menaquinone-7
Nephropathy
Zucker diabetic fatty (ZDF) rats
Procollagen type III N-Terminal peptide (PIIINP)
Transforming growth factor beta 1 (TGF-β1)

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is marked by insulin resistance, low grade chronic inflammation, and endothelial dysfunction. Vitamin K2, especially menaquinone-7 (MK-7), might delay T2DM progression and alleviate its consequences. Hence, this study evaluated the effects of MK-7 on serum and urine markers of diabetes in an animal model of T2DM.

Methods: Hetero- (fa/+, control) and homozygous (fa/fa, diabetic) male Zucker diabetic fatty (ZDF) rats were supplemented or not with MK-7 for 12 weeks. After euthanasia, vitamin K1, menaquinone-4 and MK-7 serum concentrations were analyzed via reversed phase high pressure liquid chromatography. Glucose (serum), fructosamine (serum) and creatinine (serum and urine) levels were assessed photometrically, serum cystatin C and urinary total protein were turbidimetrically determined. Serum transforming growth factor beta 1 (TGF-β1) and procollagen type III N-terminal peptide (PIIINP) were quantified with enzyme-linked immunosorbent assay. Urinary marker proteins were analyzed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Nephropathy was assessed histologically.

Results: Supplementation led to significantly elevated MK-7 serum levels and a significant reduction of PIIINP serum levels in both hetero- and homozygous ZDF rats. Additionally, not statistically significant reductions of TGF-β1 serum levels, proteinuria as well as the nephropathy score were observed. *In vivo* body mass, serum fructosamine, glucose, cystatin C and creatinine levels were unaffected.

Conclusion: MK-7 reduced serum markers of fibrosis, histological features of nephropathy and urinary protein excretion, but failed to affect serum markers of T2DM. The therapeutic potential of MK-7 in T2DM and its mode of action should be further investigated in more detail.

* Corresponding author.
E-mail addresses: ingo.mrosewski@mvz-labor-berlin.de (I. Mrosewski), valeria_mantel@yahoo.de (V. Mantel), matthias.urbank@mvz-labor-berlin.de (M. Urbank), gundula.schulze@pmu.ac.at (G. Schulze-Tanzil), christian.werner@pmu.ac.at (C. Werner), clemens.goegele@pmu.ac.at (C. Gögele), maria.kokozidou@pmu.ac.at (M. Kokozidou), thomas.bertsch@klinikum-nuernberg.de (T. Bertsch).
[1] Shared last authorship.

https://doi.org/10.1016/j.heliyon.2024.e40826
Received 19 October 2024; Received in revised form 27 November 2024; Accepted 28 November 2024
Available online 3 December 2024

1. Introduction

Diabetes mellitus (DM) is one of the most important non-infectious diseases with approximately six million people worldwide dying from it yearly and a current prevalence of more than 530 million [1–4]. By 2045, its global prevalence will likely rise above 780 million, exacerbating healthcare challenges and economic burden [3–5].

Type 2 diabetes mellitus (T2DM) accounts for ca. 90 % of DM cases and is characterized by insulin resistance or relative insulin deficiency, hyperglycemia, chronic low-level inflammation, an accumulation of advanced glycation end products (AGE), and endothelial dysfunction/damage [2,5–9]. Additionally, T2DM is connected to obesity, progressive neuropathy, retinopathy, dysfunctional protein, and lipid metabolism as well as an increased susceptibility for stress fractures [1,10–12]. It is also the leading cause of chronic kidney disease (CKD) worldwide and a risk factor of intervertebral disc degeneration, osteoarthritis, and dementia [13–16]. A recent systematic review detailed that poor glycemic control occurred in 45–93 % of T2DM patients, emphasizing the necessity to evaluate adjuvant therapeutic options in addition to improvements in education, counseling, and continuous patient follow-up [17].

Natural vitamin K (VK) has two biologically active forms: vitamin K1 or phylloquinone (VK1) and Vitamin K2 (menaquinones – MK). MK consist of a methylated naphthoquinone-ring and an aliphatic isoprenyl sidechain of varying length (4–13 residues) [18]. They are partly of gut microbial origin but mostly found in animal products and in natto [19–22]. All VK subtypes serve as co-factors of γ-glutamylcarboxylase during the posttranslational modification of 15–18 currently known proteins, e.g., coagulation factors, osteocalcin (OC), periostin, matrix-Gla protein (MGP) or growth arrest-specific gene-6 (Gas6)-protein [19,23,24].

MK-7 contains seven isoprenyl residues, has a plasma half-life of approximately 70 h, is characterized by high intestinal absorption as well as bioavailability and exerts about 70 % of its effects in extra-hepatic tissues or organs [19,25–27].

Studies have shown that MK-7 can relieve symptoms of peripheral neuropathy, promote bone ossification through increase of carboxylated OC (cOC) to a greater extent than MK-4, and reduce fibrotic processes as well as vascular calcification via MGP, Gas6-protein and blockage of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) signaling pathways [25,28–33]. Additionally, it might increase the secretion of insulin from pancreatic β-cells and reduce the risk of T2DM development via increase of adiponectin levels, which in turn appear to be regulated by absolute un(der)carboxylated OC (uOC) and the uOC/cOC ratio [3,21,32]. This indicates a crosstalk between bone, adipose tissue, and glucose metabolism.

Zucker diabetic fatty (ZDF) rats, are the most popular model for studying the effects of T2DM according to a recent review and originate from the crossbreeding of Merck and Sherman rats in 1961 [2,10]. They are characterized by an autosomal-recessive leptin receptor gene mutation, hyperphagia and obesity from week of life four onwards [2,34,35]. Homozygous (fa/fa) male ZDF rats develop insulin resistance (week 3–8) and overt T2DM (week 8–10), if a specific high protein diet is fed, while heterozygous (fa/+) ZDF rats do not develop T2DM and can serve as control [10,36].

Procollagen type III N-terminal peptide (PIIINP) is an early biomarker for tissue remodeling and fibrosis in a variety of conditions such as heart failure, atherosclerosis, and renal fibrosis [37–39]. PIIINP has been used to predict and monitor moderate to advanced liver fibrosis in T2DM patients with nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH) [40–42]. Additionally, serum and urine levels of PIIINP are supposed to correlate with renal fibrosis, e.g., in diabetic nephropathy, making it an interesting biomarker in patients with T2DM [43].

The gene expression of PIIINP is influenced by pro-inflammatory cytokines (e.g., TNF-α and IL-1) via the mitogen-activated protein kinase (MAPK) pathway as well as by transforming growth factor beta 1 (TGF-β1) [38,39].

TGF-β1 is a cytokine involved in the regulation of cell growth, cell differentiation and extracellular matrix (ECM) turnover [44,45]. Additionally, it induces the expression of chemokines leading to tissue infiltration by pro-inflammatory macrophages [44]. TGF-β1 plays a vital role in fibrotic processes of liver, lungs, heart, and kidneys, especially in diabetic nephropathy. IL-24, hyperglycemia, hyperlipidemia, advanced glycation end products (AGE) and activated NF-κB induce the gene expression and protein synthesis of TGF-β1 in white adipocytes, endothelial cells, mesangial cells and proximal tubular epithelial cells and its effects are transduced via the small mothers against decapentaplegic homolog (SMAD) protein pathways, primarily via SMAD-2 and -3 [46–53]. IL-10 inhibits the synthesis of TGF-β1, at least in pulmonary and liver fibrosis [54,55].

In kidneys, TGF-β1 increases the synthesis of pro-fibrotic platelet-derived growth factor (PDGF) in tubular epithelial cells as well as the synthesis of type I, III and IV collagens, fibronectin, fibrillin, elastin and proteoglycans in myofibroblasts. Additionally, it induces the proliferation of mesangial cells, causing both focal segmental glomerulosclerosis and interstitial fibrosis [44,52,53,56,57]. Elevated TGF-β1 levels in serum and in urine appear to closely correlate with albuminuria, the degree of mesangial matrix expansion and tubulointerstitial fibrosis, even in early diabetic nephropathy [39,43,46,58,59].

The aim of this study was to evaluate the effects of MK-7 supplementation in hetero- and homozygous male ZDF rats with regard to typical markers of T2DM, diabetic nephropathy and associated fibrotic tissue remodeling. For this, serum levels of VK1, MK-4, MK-7, fructosamine, glucose, PIIINP, TGF-β1, creatinine and cystatin C were determined. Additionally, quantitative proteinuria and semi-quantitative distribution of urinary marker proteins were investigated.

2. Materials and methods

2.1. Ethics statement

Animal experimentation protocols were reviewed and approved by the internal review board of Paracelsus Medical University Nuremberg and the regional animal review board of Regierung von Unterfranken (Government of Lower Franconia) with the approval number: RUF 55.2.2-2532-2-729-17, dated January 02, 2020.

2.2. Diabetic animal model and sample taking

A total of forty-four 10- to 11-week-old, hetero- and homozygous male ZDF rats (Charles River Laboratories Inc., Châtillon-sur-Chalaronne, France) were procured for this study. They received a diabetogenic diet (Purina 5008, ssniff Spezialdiäten GmbH, Soest, Germany) *ad libitum*. Additionally, half the animals in each cohort were supplemented with MK-7 (100 mg per kg feed, Kappa Bioscience AS, Oslo, Norway) as soon as they reached an age of 14–15 weeks. This MK-7 concentration was chosen since it had worked well in other rodent experiments [60].

MK-7 was directly shipped to ssniff Spezialdiäten GmbH and incorporated into the diabetogenic diet according to the manufacturer's instructions before the feed was delivered to us. The MK-7-enriched chow was kept in the dark until feeding.

All ZDF rats were housed in groups of 2–3 animals under standardized living conditions (55 % humidity, 21 ± 2 °C, 12-h light and dark cycles). Body mass and phenotypical signs of T2DM (e.g., cataract) were assessed weekly *in vivo*. Progression of blood glucose levels (non-fasting and after 4 h of fasting) was observed via spot tests in each cohort using a veterinary glucometer for whole blood samples (AlphaTrak 2, Zoetis, Tullytown, PA, USA) from the age of 14 weeks onwards until euthanasia.

Sacrifice occurred at 26–27 weeks via puncture of the left ventricle with exsanguination in deep narcosis (3 % isoflurane [Baxter, Deerfield, IL, USA], 1 h after applying 4 mg/kg carpofen s.c. [Zoetis, Sylvan Way Parsippany, NJ, USA]) in compliance with Federation of European Laboratory Animal Science Associations (FELASA) and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. At this time, blood samples were taken, and urine samples were obtained via bladder puncture. Due to stipulations of the regional animal review board, fasting of individual ZDF rats for 8–12 h prior to sacrifice was impossible.

During the animal experiment, three animals died while six others accidently received the wrong feed and had to be excluded, leaving thirty-five animals for inclusion in this study (Table 1).

2.3. Sample processing and storage

After sample taking, serum, lithium-heparin, and urine microtubes (Sarstedt, Nuembrecht, Germany) were stored at 4 °C until further processing for a maximum of 4–8 h dictated by the batch-wise finalization of rats. Microtubes were centrifuged at 2,000 *g* for 10 min (serum, lithium-heparin) or 400 *g* for 5 min (urine). Supernatants were transferred to 1.5- or 2.0-mL Eppendorf tubes (Eppendorf Vertrieb Deutschland GmbH, Wesseling-Berzdorf, Germany) and frozen at −80 °C until usage.

2.4. Quantification of vitamin K

VK1, menaquinone-4 (MK-4) and MK-7 were assessed via reversed phase high pressure liquid chromatography (HPLC) using a 4.6 mm × 50 cm C18-column with 2.7 μm particle size (Ascentis Express, Mainz, Germany) and an Agilent 1260 Infinity II system (Agilent, Santa Clara, USA). Standards and calibrators were supplied by Immuchrom (Immuchrom GmbH, Heppenheim, Germany). The mobile phase was composed of 0.5 g sodium acetate (Thermo Fisher Life Technologies GmbH, Darmstadt, Germany), 1.4 g of zinc chloride (Thermo Fisher Life Technologies GmbH), 920 mL methanol (VWR Chemicals, Darmstadt, Germany), 80 mL acetonitrile (Thermo Fisher Life Technologies GmbH) and 400 μL glacial acetic acid (Merck KGaA, Darmstadt, Germany), mixed and then added to another 1,000 mL acetonitrile. Methanol served as rinsing solution. The flow rate was 1.6 mL/min. Detection was done via fluorescence with an excitation wavelength of 248 nm and an emission wavelength of 418 nm. Chromatography time was ca. 13 min per sample. Lower limits of quantification were 0.1 μg/L for VK1 and MK-7 and 0.2 μg/L for MK-4. Evaluation was done with the software Chromeleon 7.3.2 (Thermo Fisher Life Technologies GmbH, Darmstadt, Germany).

2.5. Assessment of fructosamine and glucose

Serum fructosamine and glucose were assessed photometrically on a cobas 502 module [fructosamine] or a cobas 701 platform [glucose] using Roche reagent kits (Roche Diagnostics GmbH, Mannheim, Deutschland) according to manufacturer specifications.

Since the employed assays were not rat-specific, linearity was checked via sample pooling and a dilution series using distilled water (DW) [fructosamine] (Merck KGaA) or 0.9 % sodium chloride [glucose] (Roche Diagnostics GmbH). Results are given as concentrations of human equivalent due to the absence of a rat-specific calibration.

2.6. Measurements of procollagen type III N-terminal peptide and transforming growth factor β1

Rat-specific sandwich enzyme-linked immunosorbent assays (ELISA) were used for quantification of PIIINP (Novus Biologicals, LLC, Centennial, USA) and TGF-β1 (Thermo Fisher Life Technologies GmbH) on a MRX microplate absorbance reader (Dynex

Table 1
Experimental animal groups.

Non-diabetic rats			Diabetic rats	
group	fa/+ without MK-7	fa/+ with MK-7	fa/fa without MK-7	fa/fa with MK-7
n	9	11	7	8

fa/+: heterozygous ZDF rat, fa/fa: homozygous ZDF rat, MK-7: menaquinone-7, n: number.

Technologies Inc., Chantilly, VA, USA) at 450 nm with the software Revelation 4.25 (Dynex Technologies Inc.) according to manufacturer instructions. The measurement range was 31.25–2,000 pg/mL for both assays. Samples were diluted as necessary. All samples, standards and blanks were assessed as duplicates or triplicates.

2.7. Quantification of creatinine, cystatin C and total urinary protein

Serum and urinary creatinine (Jaffe method) were assessed photometrically on a cobas 701 platform (Roche Diagnostics GmbH), while serum cystatin C was determined via particle-enhanced immunologic turbidimetry on the Roche cobas 502 autoanalyzer using Roche reagent kits. The cystatin C assay is based on rabbit antibodies against human cystatin C and was successfully used in the past for cystatin C assessments in rats [61]. Urinary total protein was assessed turbidimetrically on the cobas 701 platform using the respective Roche reagent kit. For improved comparability, proteinuria was expressed as urine protein/creatinine ratio (UPCR).

Since these assays were not rat-specific, linearity was checked via sample pooling and a dilution series using 0.9 % sodium chloride [creatinine, urinary total protein] or DW [cystatin C]. Results were given as concentrations of human equivalent due to the absence of a rat-specific calibration.

2.8. Urinary protein marker analysis

The urinary marker proteins immunoglobulin G (IgG, 160 kDa), transferrin (80 kDa), albumin (67 kDa), α1-microglobulin (33 kDa) and β2-microglobulin (12 kDa) were assessed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). NuPage Gels 4–12 % (10 pockets), NuPage MES SDS Buffer Kit, Gel-Dry gel drying solution and SureLock Tandem Midi Gel Tank (all Thermo Fisher Life Technologies GmbH) were used.

Gels were loaded with 25 μL of prediluted samples and controls prepared as follows: 25 μL rat urine or control material were mixed with 50 μL DW and 25 μL NuPage SDS sample buffer. Afterwards, gels were run for 30 min at 200 V in 1x NuPage MES SDS running buffer, followed by Coomassie brilliant blue staining for 30 min (1 g Coomassie powder, 400 mL DW, 100 mL glacial acetic acid [Merck KGaA], 500 mL ethanol [VWR International GmbH]) and decoloration for 40 min (100 mL ethanol, 75 mL glacial acetic acid and 825 mL DW) on the Solaris 2000 orbital shaker (Thermo Fisher Life Technologies GmbH). Following decoloration, gels were rinsed in DW for 30 min. Subsequently, they were treated with Gel-Dry drying solution for 20 min, transferred between two DryEase Mini Cellophane sheets (Thermo Fisher Life Technologies GmbH) and inserted into a frame for assessment (Thermo Fisher Life Technologies GmbH).

Since hetero- and homozygous ZDF rats can potentially suffer from hydronephrosis [62,63], urine samples from four 15- to 16-week-old male Sprague Dawley (SD) rats obtained during another study (RUF 55.2.2-2532-2-1381-15) were included to serve as additional "normal" control in the assessment of proteinuria.

Semi-quantitative grading ("0" [no excretion], "1" [less], "2" [roughly equal], "3" [more]) of individual bands in comparison to the respective intra-run standard – a human ring trial sample with complete glomerular and tubular proteinuria (INSTAND e.V., Düsseldorf, Germany) and a known UPCR of 388 mg/g – was done via densitometry with ImageJ 1.54j (Wayne Rasband and contributors, National Institutes of Health, USA).

2.9. Histopathological assessment of nephropathy

After sacrifice, kidneys of all animals were resected and fixed in 4 % paraformaldehyde (PFA/PBS, Santa Cruz Biotechnology Inc., Dallas, TX, USA) for 48 h. Then, tissue samples were embedded into paraffin (Path Center: Shandon Pathcenter, Thermo Scientific, Waltham, MA, USA) before dissection of 7 μm thick sections. Sections were placed on the glass slides and kept in an oven (Memmert GmbH and Co.KG) at a temperature of 60 °C overnight. After being washed in xylol for 2 × 5 min (Carl Roth GmbH and Co.KG, Karlsruhe, Germany) for deparaffinization and rehydration in descending sequence of ethanol (ETOH) concentrations (99.8 %, 96 %, 80 %, 70 %) (Carl Roth GmbH and Co.KG), the specimens were stained with hematoxylin-eosin (HE) and Picrosirius red (SR). After the staining, the sections were dehydrated in an ascending ETOH series (70 %, 80 %, 96 %,99.8 %), immersed in xylol (10 min) and embedded in Entellan (Merck KGaA). For examination, the 200x to 400x magnification of a light microscope (DM1000 LED with integrated camera, Leica Microsystems GmbH, Wetzlar, Germany) was used.

2.10. Hematoxylin-eosin stain

After incubation for 6 min in Harry's hematoxylin (Carl Roth GmbH and Co.KG), the prepared kidney sections were rinsed in running tap water and counterstained with eosin (Carl Roth GmbH and Co.KG) for 4 min.

2.11. Picrosirius red stain

SR staining was performed to depict collagen distribution. Deparaffinized sections were washed in tap water for 4 min. Cell nuclei were stained with Weigert's hematoxylin (MORPHISTO GmbH, Frankfurt am Main, Germany) for 8 min. Afterwards, they were washed with DW for 5 s, followed by rinsing in tap water for 10 min and in DW for 1 min. Then, the sections were stained using SR (MORPHISTO GmbH) for 60 min, before being incubated twice in 30 % acetic acid (Carl Roth GmbH and Co.KG) for 1 min each and then twice in 96 % EtOH for 4 min each. Finally, they were transferred to isopropanol for 4 min.

2.12. Histopathological nephropathy score

For the evaluation of renal tissue slices, a nephropathy score was developed, and continuously used (Supplementary Table 1). Histological evaluation was done by a single investigator.

2.13. Statistical testing

MedCalc 23.0.6 (MedCalc Software Ltd, Ostend, Belgium) was used for linearity assessments with Passing-Bablok regression and Spearman rank correlation coefficient including the respective diagrams for non-rat-specific assays.

Testing for statistical significance and graphical depiction was done with GraphPad Prism 10.4.0 (GraphPad Software, San Diego, CA, USA). Normality of distribution was assessed via Shapiro-Wilk test and visual inspection of quantile-quantile plots. Outliers were detected with "robust regression and outlier removal" (ROUT; Q = 1 %). *In vivo* body mass was evaluated via mixed effects model analysis with Geisser-Greenhouse correction and Tukey's multiple comparisons test. Welch's ANOVA with Dunnett T3 multiple comparison test was used for creatinine, cystatin C, fructosamine, glucose, MK-4, MK-7, PIIINP, TGF-β1 and UPCR. For VK1, urinary marker proteins (IgG, transferrin, albumin, α1-microglobulin and β2-microglobulin) and the nephropathy score, Kruskal-Wallis test with Dunn's multiple comparison test was applied, because they did not pass the normality test. *P*-values <0.05 were regarded as statistically significant.

3. Results

3.1. Characteristics of ZDF rats in vivo

Homozygous ZDF rats showed typical features of T2DM like polydipsia, polyuria, obesity, and cataracts. Heterozygous animals developed no cataracts and showed higher levels of physical activity. Glucometer spot tests were consistent with T2DM in homo- but not in heterozygous animals with no detectable effect of MK-7 supplementation (data not shown).

Weekly body mass showed statistically significant differences between hetero- and homozygous ZDF rats regardless of MK-7 feeding until week of life 16 and between heterozygous (both cohorts) and homozygous supplemented animals until week of life 17 (Fig. 1, Supplementary Table 2).

3.2. Vitamin K serum concentrations

Homozygous non-supplemented ZDF rats showed statistically significant increases of VK1 and MK-4 in comparison to their supplemented counterparts, heterozygous MK-7-fed ZDF rats (VK1) and heterozygous non-supplemented animals (MK-4) (Fig. 2a–b, Supplementary Table 2). MK-7 feeding led to a statistically significant increase of MK-7 serum levels in hetero- and homozygous ZDF rats when compared to their non-supplemented counterparts. Additionally, MK-7 serum levels in homozygous supplemented ZDF rats were significantly higher than in heterozygous supplemented animals. MK-7 levels in heterozygous supplemented ZDF rats in turn were significantly higher than in homozygous non-supplemented animals. Additionally, homozygous non-supplemented ZDF rats showed a statistically significant MK-7 elevation in comparison to heterozygous non-supplemented animals (Fig. 2c–Supplementary Table 3).

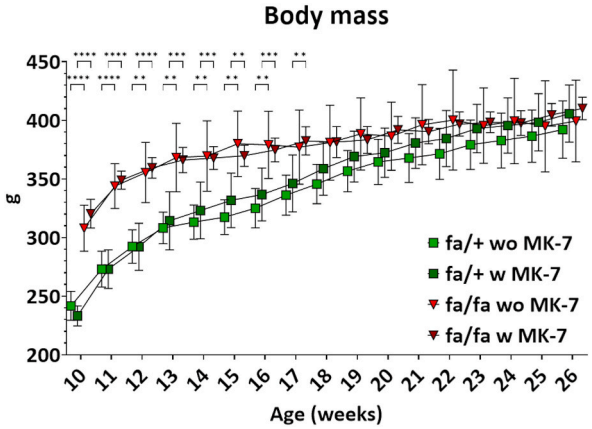

Fig. 1. Weekly body mass of hetero- and homozygous male ZDF rats without or with MK-7 supplementation depicted as means with standard deviations. Please note that the origins of x- and y-axes are not zero. *P*-values: ** <0.01, *** <0.001, **** <0.0001. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, w: with, wo: without. n = 5–11.

3.3. Fructosamine and non-fasting glucose serum levels

Homozygous ZDF rats showed significantly higher serum levels of fructosamine and non-fasting glucose in comparison to heterozygous animals. MK-7 feeding did not have any statistically significant influence. Glucose measurements via cobas c701 and AlphaTrak2 revealed similar trends, but glucometer measurements showed lower absolute glucose concentrations (Fig. 3a–c, Supplementary Table 3). Linearity tests revealed almost perfect alignment with the line of identity (Supplementary Fig. 1).

3.4. Procollagen type III N-terminal peptide and transforming growth factor β1 serum concentrations

Hetero- and homozygous ZDF rats with MK-7 feeding showed a statistically significant reduction in serum concentrations of PIIINP when compared to their non-supplemented counterparts. Serum PIIINP concentrations in both homozygous subgroups were statistically significantly higher than in supplemented heterozygous animals (Fig. 4a, Supplementary Table 3).

TGF-β1 showed similar trends which were not statistically significant. Only the comparison between supplemented hetero- and homozygous ZDF rats showed statistical significance (Fig. 4b–Supplementary Table 3).

3.5. Serum creatinine and cystatin C

There was no statistically significant difference between hetero- and homozygous ZDF rats with regard to serum creatinine concentrations, regardless of MK-7 feeding (Fig. 5a, Supplementary Table 2). Supplemented and non-supplemented homozygous ZDF rats showed statistically significant increases of cystatin C in comparison to heterozygous supplemented animals and an identical but not statistically significant trend in comparison to heterozygous non-supplemented animals. MK-7 feeding did not significantly influence cystatin c serum levels within hetero- and homozygous subgroups (Fig. 5b–Supplementary Table 3). Linearity tests revealed almost perfect alignment with the line of identity (Supplementary Fig. 1).

3.6. Total urinary protein excretion and relative excretion of urinary marker proteins

Homozygous non-supplemented ZDF rats showed a significant UPCR elevation in comparison to both heterozygous cohorts. When the UPCR of homozygous non-supplemented ZDF rats was compared to that of SD rats, statistical significance was scantly missed ($P = 0.053$). In homozygous MK-7-supplemented ZDF rats, UPCR was lower than in their non-supplemented counterparts ($P = 0.238$) but remained significantly elevated in comparison to heterozygous ZDF rats and SD rats. The UPCR of heterozygous non-supplemented ZDF rats was statistically significantly lower than in SD rats (Fig. 6a, Supplementary Table 3). Linearity tests revealed almost perfect alignment with the line of identity for urinary creatinine and total protein (Supplementary Fig. 1).

For IgG, there was no significant difference in relative excretion between SD rats and ZDF rats. Relative transferrin excretion was significantly increased in homozygous non-supplemented ZDF rats in comparison to SD rats and heterozygous ZDF rats. Relative albuminuria was higher in homozygous animals but failed to reach statistical significance. Relative α1-microglobulin and β2-microglobulin excretion was lower in homozygous ZDF rats than in heterozygous ZDF rats and SD rats. This reduction was statistically significant for homozygous non-supplemented ZDF rats in comparison to heterozygous MK-7-supplemented ZDF rats.

MK-7 feeding led to a trend of reduced relative albuminuria in homozygous ZDF rats, while the relative excretions of transferrin, α1-microglobulin and β2-microglobulin were no longer significantly different from heterozygous ZDF rats and SD rats (Fig. 6b–f, Supplementary Table 4).

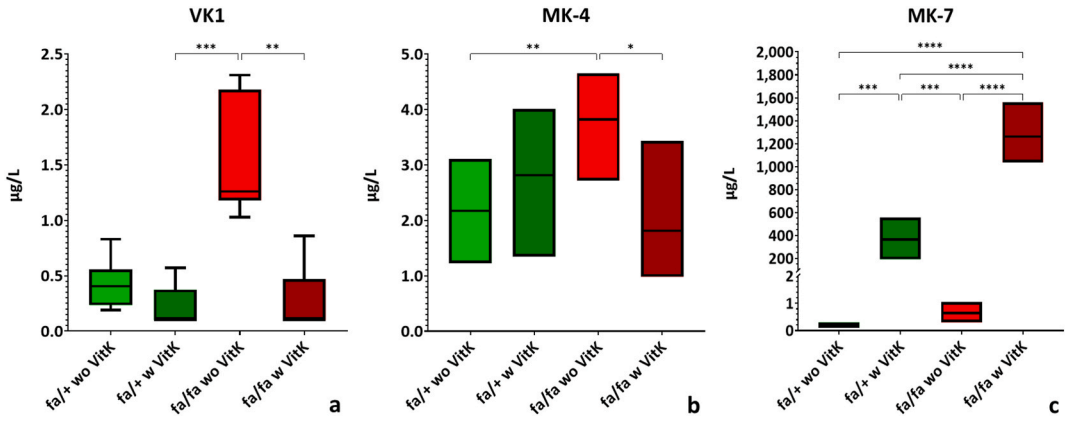

Fig. 2. Quantification of vitamin K1 (VK1) [a], menaquinone-4 (MK-4) [b] and menaquinone-7 (MK-7) [c] in serum of hetero- and homozygous ZDF rats without or with MK-7 supplementation depicted as median, 25th/75th percentile, minimum, and maximum (VK1) or means, minimum and maximum values (MK-4, MK-7). *P*-values: * <0.05, ** <0.01, *** <0.001, **** <0.0001. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, w: with, wo: without. n = 7–8.

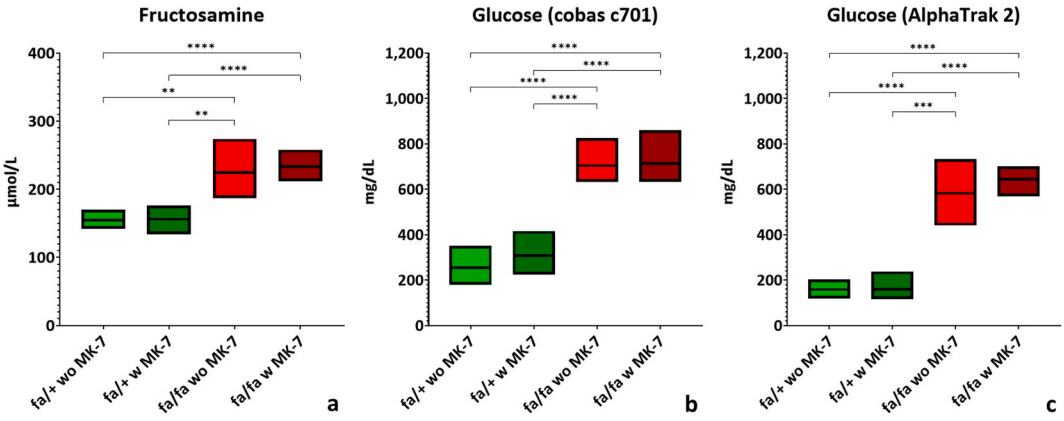

Fig. 3. Quantification of serum fructosamine [a] and non-fasting glucose [b, c] in hetero- and homozygous ZDF rats without or with MK-7 supplementation depicted as means, minimum, and maximum values. *P*-values: ** <0.01, *** <0.001, **** <0.0001. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, w: with, wo: without. n = 7–8.

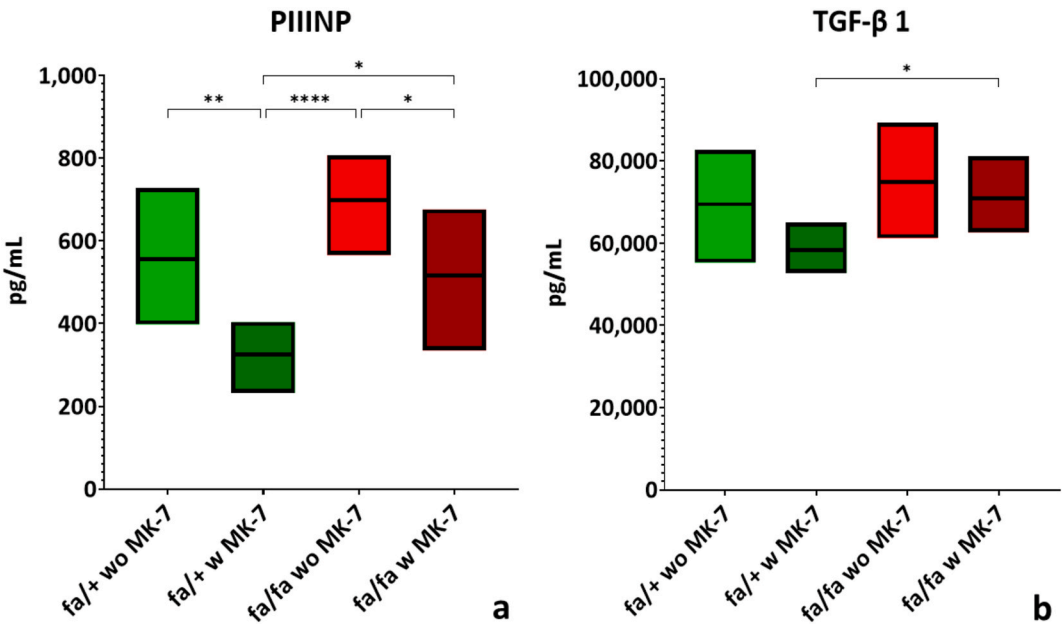

Fig. 4. Quantification of serum procollagen type III N-terminal peptide (PIIINP) [a] and transforming growth factor beta1 (TGF-β1) [b] in hetero- and homozygous ZDF rats without or with MK-7 supplementation depicted as means, minimum, and maximum values. *P*-values: * <0.05, ** <0.01, **** <0.0001. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, w: with, wo: without. n = 7–8.

Fig. 7a–e details exemplary SDS-PAGE with heterozygous ZDF rats and SD rats showing a predominantly tubular pattern of excreted proteins (<67 kDa) while homozygous ZDF rats showed less tubular protein excretion but more prominent glomerular proteinuria (≥67 kDa). Inter-individual variability within the groups was high.

3.7. Histopathological assessment of nephropathy

The kidneys of homozygous ZDF rats showed swelling of the glomeruli and widening of Bowman's capsulular space in the renal cortex more often than in heterozygous controls. Furthermore, atrophy of tubular epithelial cells and interstitial hemorrhage became evident in homozygous animals when compared to the heterozygous counterparts. An increased number of inflammatory cells could not be detected, regardless of genotype or MK-7 supplementation. In summary, the histopathological observations revealed a statistically significant elevation of the nephropathy score when homozygous ZDF rats were compared to heterozygous animals. Within heterozygous ZDF rats, the span was greater in supplemented rats while median values were identical. Within homozygous subgroups, the median and span were lower in supplemented ZDF rats, but this difference missed statistical significance (Fig. 8, Supplementary

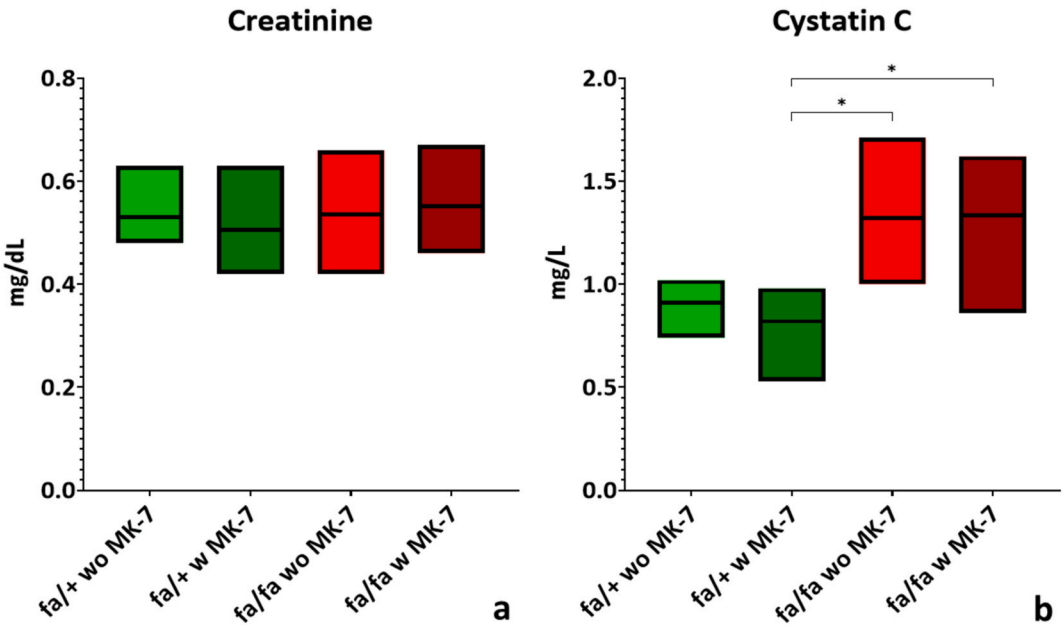

Fig. 5. Quantification of serum creatinine [a] and cystatin C [b] in hetero- and homozygous ZDF rats without or with MK-7 supplementation depicted as means, minimum, and maximum values. *P*-values: * <0.05. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, w: with, wo: without. n = 7–8.

Table 3). Fig. 9 shows representative staining of renal slices in 400x magnification. SR staining revealed an enhanced collagen deposition in the renal cortex (red).

4. Discussion

4.1. Characteristics of ZDF rats in vivo

Gradual body mass alignment between hetero- and homozygous ZDF rats agreed with the results of other authors [64–66]. The one week extended statistical significance in the body mass comparison of homozygous supplemented animals versus heterozygous subgroups was caused by lower standard deviations in homozygous supplemented ZDF rats, which were already present before supplementation started. MK-7 feeding had no statistically significant influence.

The age-dependent increase of T2DM symptoms in homozygous ZDF rats with an increasing inability to utilize consumed calories is the probable explanation for their lack of additional weight gain [36,66]. While glucometer spot test results are not reported here in detail, non-fasting results in homozygous ZDF rats at 26 weeks of age (607 ± 43 mg/dL) were similar to the results of other authors at 24–26 weeks [65,66]. At the same time, glucometer results in heterozygous animals (162 ± 49 mg/dL) were distinctly lower than reported [65,66].

The basic experimental setup was regarded as successful which could not be assumed a priori since a marked clinical heterogenicity in ZDF rats with some homozygous animals not developing T2DM has been described [36,65].

4.2. Vitamin K serum concentrations

Supplemented hetero- and homozygous ZDF rats showed massively increased MK-7 levels. Homozygous animals exceeded their heterozygous counterparts by a factor of ca. 3.5. This is explainable by the lack of satiety and resulting hyperphagia in homozygous ZDF rats and agrees with published linearity between MK-7 dosage and MK-7 serum levels [25]. Currently, no therapeutic target range for MK-7 has been established, but the MK-7 reference interval in humans is 0.07–0.76 μg/L [67]. Previous studies indicated that VK1 and MK-4 plasma concentrations in rodents are similar to those in humans, which likely extends to MK-7 [68,69]. Therefore, observed MK-7 serum concentrations in supplemented ZDF rats must be regarded as very high. This might raise concern of toxicity, but MK-4 supplementation with up to 45 mg daily showed no adverse effects in humans while trials in rodents revealed no toxicity after one-time MK-7 doses of up to 5,000 mg/kg bodyweight or MK-7 application of up to 4,500 mg/kg bodyweight for 90 days [70,71]. Additionally, we observed no obvious *in vivo* toxicity or adverse effects on investigated analytes.

For VK1 and MK-4, homozygous non-supplemented animals showed the highest serum levels, probably caused by hyperphagia leading to increased VK1 serum levels from their feed. VK1 in turn is converted to MK-4 via UbiA prenyltransferase domain-containing protein-1 in extrahepatic tissues like pancreas, arterial walls, and testes [18,68]. MK-7 is also converted to MK-4 in rodents and

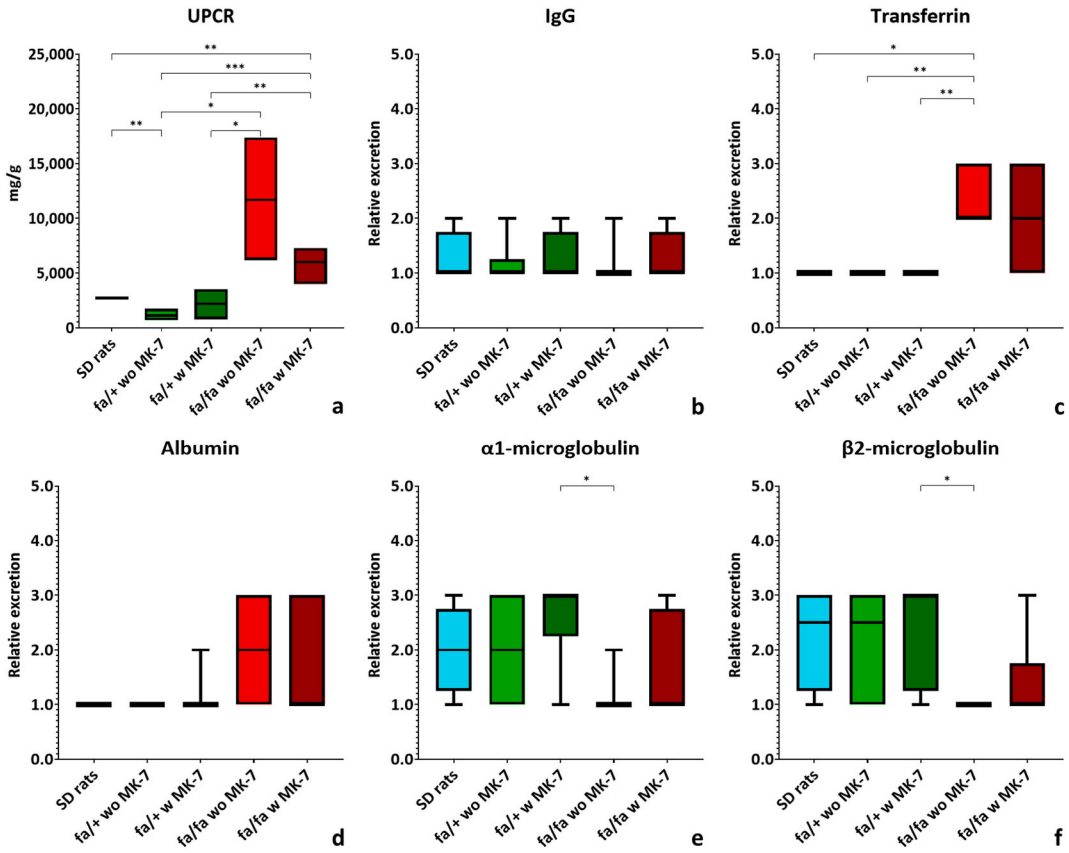

Fig. 6. Quantification of urinary protein excretion as urine protein/creatinine ratio (UPCR) [a] and semiquantitative urinary excretion of immunoglobulin G (IgG) [b], transferrin [c], albumin [d], α1-microglobulin [e] and β2-microglobulin [f] of hetero- and homozygous ZDF rats without or with MK-7 supplementation depicted as means, minimum and maximum values (UPCR) or medians, 25th/75th percentile, minimum and maximum values (all others). *P*-values: * <0.05, ** <0.01, *** <0.001. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, SD: Sprague Dawley, w: with, wo: without. 7a: n = 4 for SD rats, n = 5–6 for ZDF rats, 7b–f: n = 4 for SD rats, n = 6–8 for ZDF rats.

humans, which suggests that this conversion is regulated (e.g., via a feedback loop) since supplemented hetero- and homozygous ZDF rats showed lower serum levels of MK-4 than homozygous non-supplemented ZDF rats [72].

4.3. Fructosamine and non-fasting glucose serum levels

Fructosamine concentrations in diabetic ZDF rats were comparable to results in animals aged 14 weeks in other studies [73,74]. In heterozygous controls, fructosamine results were markedly above ranges reported by the same authors [73,74]. This discrepancy can be explained by the additional weight gain in heterozygous ZDF rats between week 14 and 26, leading to progression of hyperglycemia, obesity, and exhaustion of pancreatic β-cells.

After euthanasia, non-fasting serum glucose concentrations in hetero- and homozygous ZDF rats determined by Roche analyzer agreed with results at week of life 24 to 26 in other studies, albeit being at the upper ends of these ranges [65,66]. This is explainable by the fact that we used blood from the left ventricle while the other authors might have used veinous blood – an information not provided in the cited articles. Glucose levels in arterial blood are approximately 8 % higher than in venous blood [75].

For glucometer assessments, we made an identical observation as during the *in vivo* spot tests: Measurements in homozygous animals fell within reported ranges, while results in heterozygous animals were markedly below expectations [65,66]. Glucose measurements in whole blood samples are ca. 15 % lower than those in serum or plasma [75]. A study comparing feline and canine venous glucose concentrations in whole blood (glucometer, glucose dehydrogenase reaction) to serum (biochemical analyzer, hexokinase reaction) found a mean relative difference of 25.5 % (28.9 % for lower values, 22.1 % for higher values) with glucometer measurements showing lower results [76]. We made similar observations with mean differences between glucometer and biochemical analyzer results being 41.8 % in non-diabetic and 13.4 % in diabetic animals. This supports the conclusions drawn by Tauk et al. that glucometer measurements of whole blood samples can lead to misclassifications of the glycemic state [76]. Additionally, glucometer measurements might not conform to the same strict internal quality controls and external quality assessments as biochemical analyzers.

In ZDF rats, glucometer measurements appear unsuitable for comparison with biochemical analyzer results. However, they should

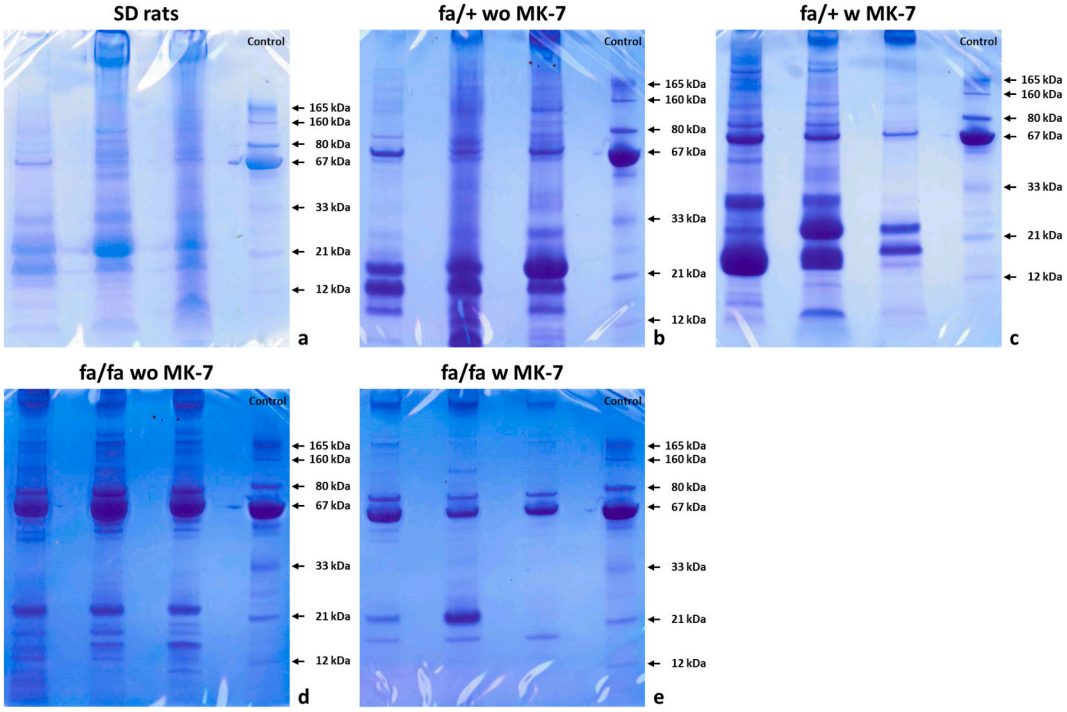

Fig. 7. Exemplary urine sodium dodecyl sulfate-polyacrylamide gel electrophoreses (SDS-PAGE) of SD rats [a], heterozygous ZDF rats without menaquinone-7 (MK-7) supplementation [b], heterozygous ZDF rats with MK-7 supplementation [c], homozygous ZDF rats without MK-7 supplementation [d] and homozygous ZDF rats with MK-7 supplementation [e]. In each example, urine of 3 different rats of the respective group as well as a standard lane (far right) are visible. The marker standard bands correspond to proteins as following: 165 kDa = IgA, 160 kDa = IgG, 80 kDa = transferrin, 67 kDa = albumin, 33 kDa = α1-microglobulin, 21 kDa = retinol-binding protein, 12 kDa = β2-microglobulin. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, SD: Sprague Dawley, w: with, wo: without. Full, non-cropped and unedited images of the SDS-PAGEs can be found in Supplementary Fig. 2.

be suitable for regular *in vivo* monitoring due to fast analyzation times and the need for minor amounts of blood which is advantageous in small animals [76].

MK-7 feeding had no statistically significant effect on markers of short- or mid-term glucose metabolism [77]. This contrasts with several animal and human studies indicating beneficial effects of MK-4 and MK-7 on glucose metabolism in T2DM [3,12,21,78]. We hypothesize that due to the very high MK-7 levels and its effects on the gene expression, protein synthesis and γ-carboxylation of OC, absolute uOC and cOC levels were too high, paradoxically causing a reduction in pancreatic insulin gene expression and release as well as a reduced adiponectin release from white adipose tissue [3,21,78–81]. Ferron et al. could demonstrate this effect in β cell islets of mice: Up until 0.3 ng/mL uOC, the gene expression of insulin 1 and 2 genes was steadily increased. Application of higher uOC concentrations led to a rapid decline with insulin gene expression falling below control levels when 30 ng/mL uOC were used [80]. The authors also demonstrated that an increase in adiponectin gene expression in adipocytes required higher doses of uOC than insulin but after administration of 30 ng/mL uOC, the gene expression of adiponectin started to be less increased than after application of 10 ng/mL uOC [80]. Taken together with the more advanced age of our ZDF rats at the time of sacrifice, a combination of senescence and aforementioned effects likely led to the lack of therapeutic impact with regard to fructosamine and glucose [81].

4.4. Procollagen type III N-terminal peptide and transforming growth factor β1 serum concentrations

MK-7 feeding led to a statistically significant reduction of PIIINP serum levels and a similar but not statistically significant reduction of TGF-β1 serum levels in hetero- and homozygous ZDF rats, which might indicate nephroprotective and anti-fibrotic MK-7 effects, especially since blockage of TGF-β1 signaling has led to significant improvements of structural changes in animal models of diabetic nephropathy as well as cell cultures, even when hyperglycemia was not treated [37–39,44,46,58,82]. Mesangial cells and tubular epithelial cells of patients with diabetic nephropathy have already been shown to synthesize increased amounts of type III collagen and TGF-β1 [39,46–53].

However, Barchetta et al. concluded in their study that PIIINP levels in T2DM patients without NASH, NAFLD or liver fibrosis were primarily associated with adipose tissue inflammation [37]. Similarly, TGF-β1 serum levels were shown to positively correlate with body mass index and fat mass in humans [48]. Since both hetero- and homozygous ZDF rats were quite adipose at the time of euthanasia, the reduction of PIIINP and TGF-β1 serum levels could also be caused by anti-inflammatory effects in adipose tissue.

Histologic assessments of possible fibrotic changes in liver and pancreas as well as of organ-specific PIIINP and TGF-β1 gene

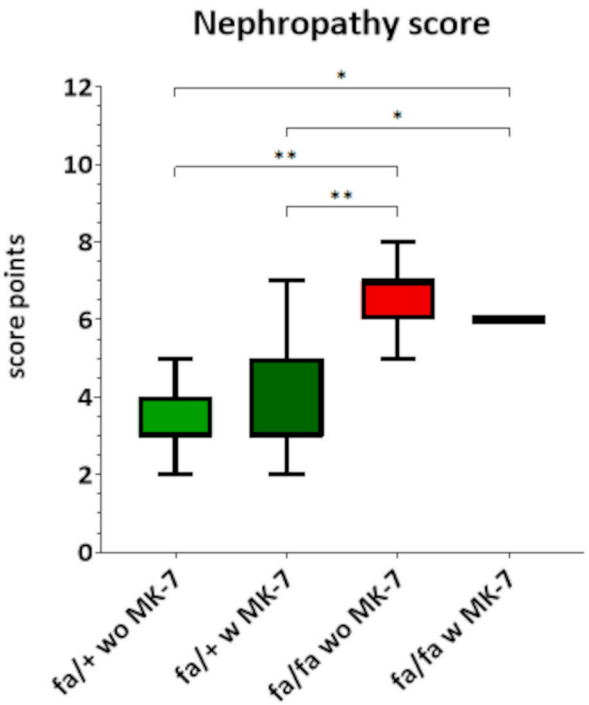

Fig. 8. Histopathological nephropathy scores of hetero- and homozygous ZDF rats without or with MK-7 supplementation depicted as medians, 25th/75th percentile, minimum and maximum values. *P*-values: * <0.05, ** <0.01. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, w: with, wo: without. n = 7–11.

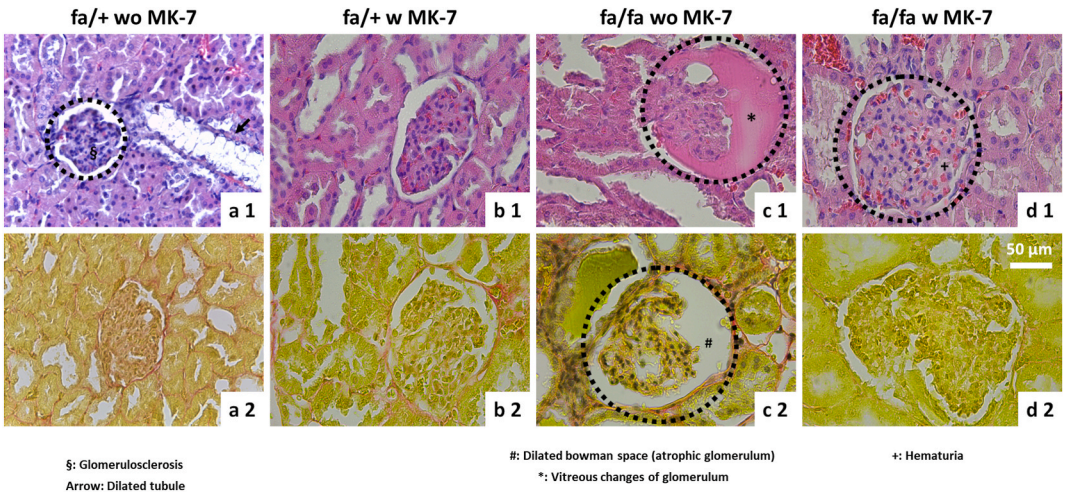

Fig. 9. Representative histological staining of renal cortex of heterozygous ZDF rats without menaquinone-7 (MK-7) supplementation [a], heterozygous ZDF rats with MK-7 supplementation [b], homozygous ZDF rats without MK-7 supplementation [c] and homozygous ZDF rats with MK-7 supplementation [d]. The sections were stained with Hematoxylin-eosin stain (**a1**–**d1**) to acquire information on the overall histological structure or Picrosirius red stain (**a2**–**d2**) for collagen structure depiction visualized with light microscopy. Scale bar: 50 µm. Arrow: dilated tubule, *: vitreous changes of glomerulus, #: dilated Bowman's capsule (atrophic glomerulus), +: glomerulus with hematuria, $ glomerulosclerosis. fa/+: heterozygous ZDF rats; fa/fa: homozygous ZDF rats, w: with, wo: without.

expression during the next phase of our study will shed additional light on this.

4.5. Serum creatinine and cystatin C

Serum creatinine values observed in our study were comparable to the results of Coimbra et al. in 18 and 40 weeks-old hetero- and

homozygous ZDF rats [51]. The lack of any difference in creatinine results could be misinterpreted as absence of renal impairment but tubular secretion of creatinine can be increased in renal injuries which explains the "creatinine-blind range" until glomerular filtration is impaired by $\geq$ 50 % [83,84].

Serum cystatin C levels in homozygous ZDF rats were elevated when compared to heterozygous animals, indicating reduced kidney function in diabetic animals. This agrees with published properties of creatine and cystatin C as markers of kidney function [83–85].

The lack of improvement in creatinine and cystatin C results after MK-7 supplementation contrasts with our observations regarding PIIINP, TGF-β1 and proteinuria, which indicated nephroprotective effects of MK-7. Nonetheless, beneficial effects of MK-7 on vascular stiffness and renal function have been published [26,32]. Effects on vascular calcification have been divergent with some studies showing no beneficial effects while others found a long-term protective effect [32,86–88].

4.6. Total urinary protein excretion and relative excretion of urinary marker proteins

Similar to other studies, we found significant proteinuria with glomerular focus in diabetic ZDF rats, that far outweighed the physiologic mixed proteinuria in heterozygous controls and SD rats [51,64,89–94]. Additionally, the excretion of tubular proteins was reduced in homozygous ZDF rats. Tubular proteinuria (especially small proteins of 17–21 kDa) is believed to be a physiologic indicator of sexual maturity and fertility in male rats, which explains their decrement in senescence or after castration as well as their reduction in homozygous ZDF rats, which are also used in infertility studies [90,91,95].

MK-7 feeding of homozygous ZDF rats led to an UPCR reduction, which missed statistical significance in comparison to homozygous non-supplemented animals, but the resulting UPCR was far more comparable to SD rats and heterozygous ZDF rats. Relative transferrin excretion and albuminuria showed similar but not as distinctive results while tubular proteins in supplemented homozygous ZDF rats were returned to levels more comparable to heterozygous ZDF rats and SD rats. These observations most likely indicate nephroprotective effects of MK-7 and corroborate the results of PIIINP and TGF-β1 discussed above.

Regardless of the correlation with these fibrosis markers, improvements in proteinuria were probably caused by a different effect than TGF-β1 reduction. Other authors already demonstrated that TGF-β1 blockade did not improve albuminuria, in all probability because proteinuria is mediated via other messengers like vascular endothelial growth factor [96,97]. It stands to reason that MK-7 also influenced other cytokines, interleukins, or growth factors, possibly through its influence on NF-κB signaling pathways [98]. PCR-based evaluation of cytokines in the kidneys will reveal additional information. Quantitative detection of urinary marker proteins would have been preferable but was not possible due to insufficient sample volumes.

4.7. Histopathological assessment of nephropathy

In correspondence to the increased proteinuria with glomerular focus in diabetic ZDF rats described above and similar to the reports of other authors, we found glomerular sclerosis with thickening of Bowman's capsule as well as tubular atrophy and dilation or tubulointerstitial damage in homozygous ZDF rats while the structure of heterozygous controls remained largely normal [1,51,64,90]. Siwy et al. also observed an interstitial accumulation of type III collagen [64].

With the combination of the statistically significant reduction of PIIINP, the trend of reduced TGF-β1 and the UPCR reduction in supplemented homozygous ZDF rats, high concentrations of MK-7 appear to reduce the detrimental effects of T2DM on kidney morphology and function, likely via influencing cytokine levels through NF-κB [98].

4.8. Study limitations

The results in our study often showed broad 95 % confidence intervals of respective means or medians due to inter-individual variability (Supplementary Tables 3 and 4). It is likely that this precluded statistical significance in several cases when hetero- and homozygous subgroups were compared to assess the differences between supplemented and non-supplemented animals, for example in the analysis of TGF-β1, UPCR, and nephropathy score. Other authors observed a similar phenomenon in their studies with ZDF rats [36,99]. Greater sample sizes likely would have led to statistical significance.

Additionally, our current research was not definitive in answering how high MK-7 concentrations conveyed its protective impact, since we did not investigate the effects on signaling pathways like SMAD-2 and -3 as well as uOC and cOC.

5. Conclusions

Our current study failed to show positive MK-7 effects with regard to *in vivo* body mass, serum fructosamine, glucose, creatinine and cystatin C levels. However, reductions of serum PIIINP and TGF-β1 concentrations as well as improvements in proteinuria and nephropathy score could be demonstrated, possibly indicating protective effects against fibrotic tissue remodeling and/or adipose tissue inflammation.

Since serum and urine marker results do not allow definitive conclusions, additional (immuno-)histologic assessments will be required to complement the presented observations. Further investigations into MK-7 effects on cytokine signaling will also be needed to supplement our understanding of MK-7 effects in T2DM.

CRediT authorship contribution statement

Ingo Mrosewski: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valeriya Mantel:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Matthias Urbank:** Writing – review & editing, Investigation, Formal analysis. **Gundula Schulze-Tanzil:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Christian Werner:** Investigation. **Clemens Gögele:** Writing – review & editing, Investigation, Data curation. **Maria Kokozidou:** Writing – review & editing, Visualization, Supervision, Resources, Investigation, Data curation. **Thomas Bertsch:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation.

Data availability statement

All raw data are available from the "Mendeley Data" repository: Mrosewski, Ingo (2024), "Menaquinone-7 and its therapeutic potential in type 2 diabetes mellitus based on a Zucker diabetic fatty rat model", Mendeley Data, V1, https://doi.org/10.17632/2j2jkn4knv.1.

Funding

The authors would like to acknowledge the funding of animal costs by Kerscher'sche Stiftung (grant numbers SZ_FP_008.18 and SZ_FP_164.20).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors express their gratitude to Kappa Bioscience AS for the complimentary provision of MK-7.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40826.

References

[1] Y.L. Pang, J.W. Hu, G.L. Liu, et al., Comparative medical characteristics of ZDF-T2DM rats during the course of development to late stage disease, Animal Model Exp Med 1 (3) (2018) 203–211, https://doi.org/10.1002/ame2.12030.
[2] S. Pandey, M.C. Dvorakova, Future perspective of diabetic animal models, Endocr., Metab. Immune Disord.: Drug Targets 20 (1) (2019) 25–38, https://doi.org/10.2174/1871530319666190626143832.
[3] F. Rahimi Sakak, N. Moslehi, M. Niroomand, et al., Glycemic control improvement in individuals with type 2 diabetes with vitamin K2 supplementation: a randomized controlled trial, Eur. J. Nutr. 60 (5) (2021) 2495–2506, https://doi.org/10.1007/s00394-020-02419-6.
[4] International Diabetes Federation, in: Diabetes Atlas, tenth ed., 2021. Available from: https://diabetesatlas.org/atlas/tenth-edition/. (Accessed 15 August 2024).
[5] K. Ogurtsova, J.D. da Rocha Fernandes, Y. Huang, et al., IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040, Diabetes Res. Clin. Pract. 128 (2017) 40–50, https://doi.org/10.1016/j.diabres.2017.03.024.
[6] M. Prentki, C.J. Nolan, Islet β cell failure in type 2 diabetes, J. Clin. Invest. 116 (7) (2006) 1802–1812, https://doi.org/10.1172/JCI29103.
[7] D.M. Muoio, C.B. Newgard, Mechanisms of disease:Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes, Nat. Rev. Mol. Cell Biol. 9 (2008) 193–205, https://doi.org/10.1038/nrm2327.
[8] F. Paneni, J.A. Beckman, M.A. Creager, et al., Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I, Eur. Heart J. 34 (31) (2013) 2436–2443, https://doi.org/10.1093/eurheartj/eht149.
[9] J.D. Glaeser, D. Ju, W. Tawackoli, et al., Advanced glycation end product inhibitor pyridoxamine attenuates IVD degeneration in type 2 diabetic rats, Int. J. Mol. Sci. 21 (24) (2020) 9709, https://doi.org/10.3390/ijms21249709.
[10] A. Al-Awar, K. Kupai, M. Veszelka, et al., Experimental diabetes mellitus in different animal models, J. Diabetes Res. 2016 (2016) 9051426, https://doi.org/10.1155/2016/9051426.
[11] N.D.P.K. Manne, G.K. Ginjupalli, K.M. Rice, et al., Long-term treatment with empagliflozin attenuates renal damage in obese zucker rat, Exp. Clin. Endocrinol. Diabetes 128 (8) (2020) 512–519, https://doi.org/10.1055/a-0815-4908.
[12] J.I. Aguayo-Ruiz, T.A. García-Cobián, S. Pascoe-González, et al., Effect of supplementation with vitamins D3 and K2 on undercarboxylated osteocalcin and insulin serum levels in patients with type 2 diabetes mellitus: a randomized, double-blind, clinical trial, Diabetol Metab Syndr 12 (2020) 73, https://doi.org/10.1186/s13098-020-00580-w.
[13] L. Rinaldo, B.A. McCutcheon, H. Gilder, et al., Diabetes and back pain: markers of diabetes disease progression are associated with chronic back pain, Clin. Diabetes 35 (3) (2017) 126–131, https://doi.org/10.2337/cd16-0011.
[14] T. Sozen, N. Calik Basaran, M. Tinazli, et al., Musculoskeletal problems in diabetes mellitus, Eur J Rheumatol 5 (4) (2018) 258–265, https://doi.org/10.5152/eurjrheum.2018.18044.

[15] D. Cozachenco, M.C. Selles, F.C. Ribeiro, Interferon- γ as a potential link between diabetes mellitus and dementia, J. Neurosci. 39 (24) (2019) 4632–4635, https://doi.org/10.1523/jneurosci.3046-18.2019.
[16] S. Thipsawat, Early detection of diabetic nephropathy in patient with type 2 diabetes mellitus: a review of the literature, Diab Vasc Dis Res 18 (6) (2021) 14791641211058856, https://doi.org/10.1177/14791641211058856.
[17] S.A. Bin Rakhis, N.M. AlDuwayhis, N. Aleid, et al., Glycemic control for type 2 diabetes mellitus patients: a systematic review, Cureus 14 (6) (2022) e26180, https://doi.org/10.7759/cureus.26180.
[18] P. Mladěnka, K. Macáková, L. Kujovská Krčmová, et al., Vitamin K - sources, physiological role, kinetics, deficiency, detection, therapeutic use, and toxicity, Nutr. Rev. 80 (4) (2022) 677–698, https://doi.org/10.1093/nutrit/nuab061.
[19] J.W.J. Beulens, S.L. Booth, E.G.H.M. Van Den Heuvel, et al., The role of menaquinones (vitamin K2) in human health, Br. J. Nutr. 110 (8) (2013) 1357–1368, https://doi.org/10.1017/s0007114513001013.
[20] J.W.J. Beulens, A.D.L. vander, D.E. Grobbee, et al., Dietary phylloquinone and menaquinones intakes and risk of type 2 diabetes, Diabetes Care 33 (8) (2010) 1699–1705, https://doi.org/10.2337/dc09-2302.
[21] Y. Li, J. Peng Chen, L. Duan, et al., Effect of vitamin K2 on type 2 diabetes mellitus: a review, Diabetes Res. Clin. Pract. 136 (2018) 39–51, https://doi.org/10.1016/j.diabres.2017.11.020.
[22] M.K. Shea, S.L. Booth, Vitamin K, Adv. Nutr. 13 (1) (2022) 350–351, https://doi.org/10.1093/advances/nmab133.
[23] Y. Mi, X. Xiao, D. Liu, et al., Establishing a rat model for the study of vitamin K deficiency, Int. J. Exp. Pathol. 97 (2) (2016) 187–193, https://doi.org/10.1111/iep.12178.
[24] J. Lacombe, K. Guo, J. Bonneau, et al., Vitamin K-dependent carboxylation regulates Ca2+ flux and adaptation to metabolic stress in β cells, Cell Rep. 42 (5) (2023) 112500, https://doi.org/10.1016/j.celrep.2023.112500.
[25] L.J. Schurgers, K.J. F Teunissen, K. Hamulyák, et al., Vitamin K-containing dietary supplements: comparison of synthetic vitamin K 1 and natto-derived menaquinone-7, Blood 109 (8) (2007) 3279–3283, https://doi.org/10.1182/blood-2006-08-040709.
[26] A.C. Akbulut, A. Pavlic, P. Petsophonsakul, et al., Vitamin K2 needs an RDI separate from vitamin K1, Nutrients 12 (6) (2020) 1852, https://doi.org/10.3390/nu12061852.
[27] T. Sato, N. Inaba, T. Yamashita, MK-7 and its effects on bone quality and strength, Nutrient 12 (4) (2020) 965, https://doi.org/10.3390/nu12040965.
[28] K. Kawana, M. Takahashi, H. Hoshino, et al., Circulating levels of vitamin K1, menaquinone-4, and menaquinone-7 in healthy elderly Japanese women and patients with vertebral fractures and patients with hip fractures, Endocr. Res. 27 (3) (2001) 337–343, https://doi.org/10.1081/erc-100106011.
[29] G.C.M. Gast, N.M. de Roos, I. Sluijs, et al., A high menaquinone intake reduces the incidence of coronary heart disease, Nutr Metab Cardiovasc Dis 19 (7) (2009) 504–510, https://doi.org/10.1016/j.numecd.2008.10.004.
[30] N. Jadhav, S. Ajgaonkar, P. Saha, et al., Molecular pathways and roles for vitamin K2-7 as a health-beneficial nutraceutical: challenges and opportunities, Front. Pharmacol. 13 (2022) 896920, https://doi.org/10.3389/fphar.2022.896920.
[31] M. Yamaguchi, H. Taguchi, Y.H. Gao, et al., - Effect of vitamin K2 (menaquinone-7) in fermented soybean (natto) on bone loss in ovariectomized rats, J Bone Miner Metab 17 (1) (1999) 23–29, https://doi.org/10.1007/s007740050059.
[32] M. Halder, P. Petsophonsakul, A.C. Akbulut, et al., Vitamin K: double bonds beyond coagulation insights into differences between vitamin K1 and K2 in health and disease, Int. J. Mol. Sci. 20 (4) (2019) 896, https://doi.org/10.3390/ijms20040896.
[33] R. Ruze, T. Liu, X. Zou, et al., Obesity and type 2 diabetes mellitus: connections in epidemiology, pathogenesis, and treatments, Front. Endocrinol. 14 (2023) 1161521, https://doi.org/10.3389/fendo.2023.1161521.
[34] M.S. Phillips, Q. Liu, H.A. Hammond, et al., Leptin receptor missense mutation in the fatty Zucker rat, Nat. Genet. 13 (1) (1996) 18–19, https://doi.org/10.1038/ng0596-18.
[35] S.C. Griffen, J. Wang, M.S. German, A genetic defect in beta-cell gene expression segregates independently from the fa locus in the ZDF rat, Diabetes 50 (1) (2001) 63–68, https://doi.org/10.2337/diabetes.50.1.63.
[36] X. Wang, D.C. Dubois, S. Sukumaran, et al., Variability in Zucker diabetic fatty rats: differences in disease progression in hyperglycemic and normoglycemic animals, Diabetes Metab Syndr Obes 7 (2014) 531–541, https://doi.org/10.2147/dmso.s69891.
[37] I. Barchetta, F.A. Cimini, R. De Gioannis, et al., Procollagen-III peptide identifies adipose tissue-associated inflammation in type 2 diabetes with or without nonalcoholic liver disease, Diabetes Metab Res Rev 34 (5) (2018) e2998, https://doi.org/10.1002/dmrr.2998.
[38] D. Singh, V. Rai, D. K Agrawal, Regulation of collagen I and collagen III in tissue injury and regeneration, Cardiol Cardiovasc Med 7 (1) (2023) 5–16, https://doi.org/10.26502/fccm.92920302.
[39] H. Kuivaniemi, G. Tromp, Type III collagen (COL3A1): gene and protein structure, tissue distribution, and associated diseases, Gene 707 (2019) 151–171, https://doi.org/10.1016/j.gene.2019.05.003.
[40] H. Hyogo, S.I. Yamagishi, S. Maeda, et al., Serum asymmetric dimethylarginine levels are independently associated with procollagen III N-terminal peptide in nonalcoholic fatty liver disease patients, Clin. Exp. Med. 14 (1) (2014) 45–51, https://doi.org/10.1007/s10238-012-0223-4.
[41] F. Bril, D.J. Leeming, M.A. Karsdal, et al., Use of plasma fragments of propeptides of type III, V, and VI procollagen for the detection of liver fibrosis in type 2 diabetes, Diabetes Care 42 (7) (2019) 1348–1351, https://doi.org/10.2337/dc18-2578.
[42] G. Marchesini, M. Brizi, G. Bianchi, et al., Nonalcoholic fatty liver disease: a feature of the metabolic syndrome, Diabetes 50 (8) (2001) 1844–1850, https://doi.org/10.2337/diabetes.50.8.1844.
[43] S.G. Mansour, J. Puthumana, S.G. Coca, et al., Biomarkers for the detection of renal fibrosis and prediction of renal outcomes: a systematic review, BMC Nephrol. 18 (1) (2017) 72, https://doi.org/10.1186/s12882-017-0490-0.
[44] R.E. Gilbert, A. Akdeniz, T.J. Allen, et al., Urinary transforming growth factor-beta in patients with diabetic nephropathy: implications for the pathogenesis of tubulointerstitial pathology 16 (12) (2001) 2442–2443, https://doi.org/10.1093/ndt/16.12.2442.
[45] X. Xu, L. Zheng, Q. Yuan, et al., Transforming growth factor-β in stem cells and tissue homeostasis, Bone Res 6 (2018) 2, https://doi.org/10.1038/s41413-017-0005-4.
[46] S. Chen, B. Jim, F.N. Ziyadeh, Diabetic nephropathy and transforming growth factor-beta: transforming our view of glomerulosclerosis and fibrosis build-up, Semin. Nephrol. 23 (6) (2003) 532–543, https://doi.org/10.1053/s0270-9295(03)00132-3.
[47] H.M. Lin, J.H. Lee, H. Yadav, et al., Transforming growth factor-beta/Smad3 signaling regulates insulin gene transcription and pancreatic islet beta-cell function, J. Biol. Chem. 284 (18) (2009) 12246–12257, https://doi.org/10.1074/jbc.m805379200.
[48] H. Yadav, C. Quijano, A.K. Kamaraju, et al., Protection from obesity and diabetes by blockade of TGF-β/Smad3 signaling, Cell Metab. 14 (1) (2011) 67–79, https://doi.org/10.1016/j.cmet.2011.04.013.
[49] R. Raguraman, A. Srivastava, A. Munshi, et al., Therapeutic approaches targeting molecular signaling pathways common to diabetes, lung diseases and cancer, Adv. Drug Deliv. Rev. 178 (2021) 113918, https://doi.org/10.1016/j.addr.2021.113918.
[50] R. Bataller, D.A. Brenner, Liver fibrosis, J. Clin. Invest. 115 (2) (2005) 209–218, https://doi.org/10.1172/jci24282.
[51] T.M. Coimbra, U. Janssen, H.J. Gröne, et al., Early events leading to renal injury in obese Zucker (fatty) rats with type II diabetes, Kidney Int. 57 (1) (2000) 167–182, https://doi.org/10.1046/j.1523-1755.2000.00836.x.
[52] Y.S. Kim, D.H. Jung, E. Sohn, et al., Glycoxidised LDL induced the upregulation of Axl receptor tyrosine kinase and its ligand in mouse mesangial cells, PLoS One 7 (11) (2012) e50297, https://doi.org/10.1371/journal.pone.0050297.
[53] S. Lizakowski, L. Tylicki, M. Renke, et al., Aliskiren and perindopril reduce the levels of transforming growth factor-β in patients with non-diabetic kidney disease, Am. J. Hypertens. 25 (6) (2012) 636–639, https://doi.org/10.1038/ajh.2012.14.
[54] K.S. Hung, T.H. Lee, W.Y. Chou, et al., Interleukin-10 gene therapy reverses thioacetamide-induced liver fibrosis in mice, Biochem. Biophys. Res. Commun. 336 (1) (2005) 324–331, https://doi.org/10.1016/j.bbrc.2005.08.085.
[55] K. Nakagome, M. Dohi, K. Okunishi, et al., In vivo IL-10 gene delivery attenuates bleomycin induced pulmonary fibrosis by inhibiting the production and activation of TGF-beta in the lung, Thorax 61 (10) (2006) 886–894, https://doi.org/10.1136/thx.2005.056317.

[56] K. Sharma, Y. Jin, J. Guo, F.N. Ziyadeh, Neutralization of TGF-p by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice, Diabetes 45 (4) (1996) 522–530, https://doi.org/10.2337/diab.45.4.522.

[57] D. Pap, E. Sziksz, Z. Kiss, et al., Microarray analysis reveals increased expression of matrix metalloproteases and cytokines of interleukin-20 subfamily in the kidneys of neonate rats underwent unilateral ureteral obstruction: a potential role of IL-24 in the regulation of inflammation and tissue remodeling, Kidney Blood Press. Res. 42 (1) (2017) 16–32, https://doi.org/10.1159/000464317.

[58] Korpinen Eija, Teppo Anna-Maija, Hukkanen Leena, et al., Urinary transforming growth factor-beta1 and alpha1-microglobulin in children and adolescents with type 1 diabetes, Diabetes Care 23 (5) (2000) 664–668, https://doi.org/10.2337/diacare.23.5.664.

[59] S.W. Ha, H.J. Kim, J.S. Bae, et al., Elevation of urinary betaig-h3, transforming growth factor-beta-induced protein in patients with type 2 diabetes and nephropathy, Diabetes Res. Clin. Pract. 65 (2) (2004) 167–173, https://doi.org/10.1016/j.diabres.2004.01.007.

[60] A. Neradova, G. Wasilewski, S. Prisco, et al., Combining phosphate binder therapy with vitamin K2 inhibits vascular calcification in an experimental animal model of kidney failure, Nephrol. Dial. Transplant. 37 (2022) 652–662, https://doi.org/10.1093/ndt/gfab314.

[61] A. Bökenkamp, G. Ciarimboli, C. Dieterich, Cystatin C in a rat model of end-stage renal failure, Ren. Fail. 23 (3–4) (2001) 431–438, https://doi.org/10.1081/jdi-100104726.

[62] J.P. Vora, S.M. Zimsen, D.C. Houghton, et al., Evolution of metabolic and renal changes in the ZDF/Drt-fa rat model of type II diabetes, J. Am. Soc. Nephrol. 7 (1) (1996) 113–117, https://doi.org/10.1681/asn.v71113.

[63] S.A. Marsh, P.C. Powell, A. Agarwal, et al., Cardiovascular dysfunction in Zucker obese and Zucker diabetic fatty rats: role of hydronephrosis, Am. J. Physiol. Heart Circ. Physiol. 293 (1) (2007) H292–H298, https://doi.org/10.1152/ajpheart.01362.2006.

[64] J. Siwy, C. Zoja, J. Klein, et al., Evaluation of the Zucker diabetic fatty (ZDF) rat as a model for human disease based on urinary peptidomic profiles, PLoS One 7 (12) (2012) e51334, https://doi.org/10.1371/journal.pone.0051334.

[65] G.E. Monahan, J. Schiavi-Tritz, M. Britton, et al., Longitudinal alterations in bone morphometry, mechanical integrity and composition in Type-2 diabetes in a Zucker diabetic fatty (ZDF) rat, Bone 170 (2023) 116672, https://doi.org/10.1016/j.bone.2023.116672.

[66] P. Wang, J.C. Chatham, Onset of diabetes in Zucker diabetic fatty (ZDF) rats leads to improved recovery of function after ischemia in the isolated perfused heart, Am. J. Physiol. Endocrinol. Metab. 286 (5) (2004) E725–E736, https://doi.org/10.1152/ajpendo.00295.2003.

[67] K. Dunovska, E. Klapkova, B. Sopko, et al., LC-MS/MS quantitative analysis of phylloquinone, menaquinone-4 and menaquinone-7 in the human serum of a healthy population, PeerJ 7 (2019) e7695, https://doi.org/10.7717/peerj.7695.

[68] T. Okano, Y. Shimomura, M. Yamane, et al., Conversion of phylloquinone (Vitamin K1) into menaquinone-4 (Vitamin K2) in mice: two possible routes for menaquinone-4 accumulation in cerebra of mice, J. Biol. Chem. 283 (17) (2008) 11270–11279, https://doi.org/10.1074/jbc.m702971200.

[69] H.H.W. Thijssen, M.J. Drittij-Reijnders, Vitamin K distribution in rat tissues: dietary phylloquinone is a source of tissue menaquinone-4, Br. J. Nutr. 72 (3) (1994) 415–425, https://doi.org/10.1079/bjn19940043.

[70] K. Pucaj, H. Rasmussen, M. Møller, et al., Safety and toxicological evaluation of a synthetic vitamin K2, menaquinone-7, Toxicol. Mech. Methods 21 (7) (2011) 520–532, https://doi.org/10.3109/15376516.2011.568983.

[71] J. Iwamoto, Y. Sato, T. Takeda, et al., Bone quality and vitamin K2 in type 2 diabetes: review of preclinical and clinical studies, Nutr. Rev. 69 (3) (2011) 162–167, https://doi.org/10.1111/j.1753-4887.2011.00380.x.

[72] T. Sato, L.J. Schurgers, K. Uenishi, Comparison of menaquinone-4 and menaquinone-7 bioavailability in healthy women, Nutr. J. 11 (2012) 93, https://doi.org/10.1186/1475-2891-11-93.

[73] H.B. Jones, D. Nugent, R. Jenkins, Variation in characteristics of islets of Langerhans in insulin-resistant, diabetic and non-diabetic-rat strains, Int. J. Exp. Pathol. 91 (3) (2010) 288–301, https://doi.org/10.1111/j.1365-2613.2010.00713.x.

[74] N. Gowda, A. Dandu, J. Singh, et al., Treatment with CNX-011-67, a novel GPR40 agonist, delays onset and progression of diabetes and improves beta cell preservation and function in male ZDF rats, BMC Pharmacol Toxicol 14 (2013) 28, https://doi.org/10.1186/2050-6511-14-28.

[75] H. Keller, Klinisch-chemische Labordiagnostik für die Praxis – Analyse. Befund, Interpretation, second ed., Georg Thieme Verlag, Stuttgart, New York, 1991, pp. 249–259.

[76] B.S. Tauk, K.J. Drobatz, K.A. Wallace, et al., Corrleation between glucose concentrations in serum, plasma, and whole blood measured by a point-of-care glucometer and serum glucose concentration measured by an automated biochemical analyzer for canine and feline blood samples, J. Am. Vet. Med. Assoc. 246 (129) (2015) 1327–1333, https://doi.org/10.2460/javma.246.12.1327.

[77] D.A. Armbruster, Fructosamine: structure, analysis, and clinical usefulness, Clin. Chem. 33 (12) (1987) 2153–2163, https://doi.org/10.1093/clinchem/33.12.2153.

[78] A.G. Hussein, R.H. Mohamed, S.M. Shalaby, et al., Vitamin K2 alleviates type 2 diabetes in rats by induction of osteocalcin gene expression, Nutrition 47 (2018) 33–38, https://doi.org/10.1016/j.nut.2017.09.016.

[79] T. Kocak, N. Tek, Osteocalcin: a new phenomenon for type 2 diabetes and obesity, Eur J Environ Public Health 7 (3) (2023) 1–10, https://doi.org/10.29333/ejeph/12799.

[80] M. Ferron, E. Hinoi, G. Karsenty, et al., Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice, Proc Natl Acad Sci U S A. 105 (13) (2008) 5266–5270, https://doi.org/10.1073/pnas.0711119105.

[81] N. Suksomboon, N. Poolsup, Ko Ko H. Darli, Effect of vitamin K supplementation on insulin sensitivity: a meta-analysis, Diabetes Metab Syndr Obes 10 (2017) 169–177, https://doi.org/10.2147/dmso.s137571.

[82] H.S. Jeong, K.K. Park, S.P. Kim, et al., Effect of antisense TGF-β1 oligodeoxynucleotides in streptozotocin-induced diabetic rat kidney, J Korean Med Sci. 19 (3) (2004) 374–383, 10.3346%2Fjkms.2004.19.3.374.

[83] E.J. Lamb, C.R.V. Tomson, P.J. Roderick, Estimating kidney function in adults using formulae, Ann. Clin. Biochem. 42 (Pt 5) (2005) 321–345, https://doi.org/10.1258/0004563054889936.

[84] M.S.N. Murty, U.K. Sharma, V.B. Pandey, et al., Serum cystatin C as a marker of renal function in detection of early acute kidney injury, Indian J. Nephrol. 23 (3) (2013) 180–183, https://doi.org/10.4103/0971-4065.111840.

[85] Y. Xu, Y. Ding, X. Li, et al., Cystatin C is a disease-associated protein subject to multiple regulation, Immunol. Cell Biol. 93 (5) (2015) 442–451, https://doi.org/10.1038/icb.2014.121.

[86] M.H.J. Knapen, L.A.J.L.M. Braam, N.E. Drummen, et al., Menaquinone-7 supplementation improves arterial stiffness in healthy postmenopausal women. A double-blind randomised clinical trial, Thromb Haemost 113 (5) (2015) 1135–1144, https://doi.org/10.1160/th14-08-0675.

[87] A.G. Mansour, E. Hariri, Y. Daaboul, et al., Vitamin K2 supplementation and arterial stiffness among renal transplant recipients-a single-arm, single-center clinical trial, J Am Soc Hypertens 11 (9) (2017) 589–597, https://doi.org/10.1016/j.jash.2017.07.001.

[88] S.R. Zwakenberg, P.A. de Jong, J.W. Bartstra, et al., The effect of menaquinone-7 supplementation on vascular calcification in patients with diabetes: a randomized, double-blind, placebo-controlled trial, Am. J. Clin. Nutr. 110 (4) (2019) 883–890, https://doi.org/10.1093/ajcn/nqz147.

[89] M. Kitada, Y. Ogura, D. Koya, Rodent models of diabetic nephropathy: their utility and limitations, Int J Nephrol Renovasc Dis 9 (2016) 279–290, https://doi.org/10.2147/ijnrd.s103784.

[90] J.M. Alt, H. Hackbarth, F. Deerberg, et al., Proteinuria in rats in relation to age-dependent renal changes, Lab Anim 14 (2) (1980) 95–101. https://journals.sagepub.com/doi/10.1258/002367780780942809.

[91] O.S. Olukiran, R.O. Akomolafe, Oset al Ilesanmi, Age-related changes in urinary protein excretion in relation to indices of renal function in Wistar rats, Animal Model Exp Med 1 (4) (2018) 295–304, https://doi.org/10.1002/ame2.12035.

[92] W.K. Bolton, F.R. Benton, J.G. Maclay, et al., Spontaneous glomerular sclerosis in aging Sprague-Dawley rats I. Lesions associated with mesangial IgM deposits, Am. J. Pathol. 85 (2) (1976) 277–302. https://pubmed.ncbi.nlm.nih.gov/793408.

[93] W.G. Couser, M.M. Stilmant, Mesangial lesions and focal glomerular sclerosis in the aging rat, Lab. Invest. 33 (5) (1975) 491–501. https://pubmed.ncbi.nlm.nih.gov/127075/.

[94] O.W. Neuhaus, W. Flory, Age-dependent changes in the excretion of urinary proteins by the rat, Nephron 22 (4–6) (1978) 570–576, https://doi.org/10.1159/000181537.
[95] F. Benko, M. Chomová, O. Uličná, et al., ZDF rats: a suitable model to study male reproductive dysfunction in diabetes mellitus type 2 patients, Available from. Animal Models in Medicine and Biology, IntechOpen, 2020, https://doi.org/10.5772/intechopen.89939. (Accessed 16 August 2024). Last accessed.
[96] F.N. Ziyadeh, B.B. Hoffman, D.C. Han, et al., Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice, Proc Natl Acad Sci U S A. 97 (14) (2000) 8015–8020, https://doi.org/10.1073/pnas.120055097.
[97] G. Remuzzi, T. Bertani, Pathophysiology of progressive nephropathies, N. Engl. J. Med. 339 (20) (1998) 1448–1456, https://doi.org/10.1056/nejm199811123392007.
[98] Y. Xie, S. Li, D. Wu, et al., Vitamin K: infection, inflammation, and auto-immunity, J. Inflamm. Res. 17 (2024) 1147–1160, https://doi.org/10.2147/jir.s445806.
[99] M. Hamidi Shishavan, End-organ damage in diabetes: exploring innovative targets and therapies. [Thesis Fully Internal (DIV), University of Groningen], University of Groningen, 2015. https://research.rug.nl/en/publications/end-organ-damage-in-diabetes-exploring-innovative-targets-and-the. (Accessed 12 November 2024). Last accessed.