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ORIGINAL PAPER

Kidney stone formation in exocrine pancreatic insufficient pigs fed an oxalate enriched diet

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ABSTRACT. Exocrine pancreatic insufficiency (EPI) causes fat malabsorption, which can increase intestinal oxalate uptake and promote calcium oxalate kidney stone formation. The aim of this study was to develop a porcine model of EPI-associated hyperoxaluria and nephrolithiasis, and to evaluate whether pancreatic enzyme replacement therapy (PERT; Creon®) reduces renal injury. A randomised controlled experiment included eight pigs: six underwent pancreatic duct ligation to induce EPI and were allocated to the EPI group (n = 3) or the EPI+Creon group (n = 3), while two healthy pigs served as comparators. All pigs were fed a high-fat diet; EPI groups additionally received potassium oxalate monohydrate supplementation (2%, followed by 3%). The EPI+Creon group was administered porcine pancreatic enzymes. Blood and urine samples were collected at predefined time points for measurement of oxalate and creatinine levels. At study termination, kidneys were assessed for calcium oxalate deposition using Pizzolato staining and for histopathological damage using semi-quantitative scoring. Oxalate supplementation significantly increased urinary and plasma oxalate concentrations in pigs with EPI ($P < 0.05$) and was associated with higher plasma creatinine at the 3% oxalate dose ($P = 0.005$). Calcium oxalate deposits were markedly more abundant in the renal cortex, medulla, and pelvis in EPI pigs than in EPI+Creon and healthy animals ($P < 0.001$). Severe glomerular sclerosis, tubular atrophy, and interstitial fibrosis were observed in EPI pigs and were only partially attenuated by PERT. In conclusion, oxalate-fed pigs with EPI develop hyperoxaluria, nephrolithiasis, and renal injury. PERT reduces but does not fully prevent these biochemical and structural kidney abnormalities.

Introduction

Hyperoxaluria is a pathological condition characterised by excessive urinary oxalate excretion, which can lead to kidney stones and renal dysfunction (Hoppe et al., 2009; Harambat et al., 2010; Cochat and Rumsby, 2013). It may result from increased dietary oxalate intake, impaired intestinal absorption, or metabolic disorders affecting oxalate metabolism (Holmes et al., 2001; Liebman and Al-Wahsh, 2011).

Three types of primary hyperoxaluria have been described. Primary hyperoxaluria types I and II are both autosomal recessive disorders of glyoxylic acid metabolism (Hoppe et al., 1998; 2005; 2009). Type I is a liver disease caused by a deficiency or loss of alanine:aminotransferase (AGT) activity, an enzyme involved in the final step of glyoxylic acid metabolism, leading to excessive production of oxalate and glycolic acid (Danpure, 2005; Cochat et al., 2006). Hyperoxaluria type II results from activity of glyoxylate reductase/hydroxypyruvate reductase (GRHPR), a cytosolic enzyme, causing excessive production and urinary excretion of both oxalate and L-glycerate (Cregeen and Rumsby, 1999). Primary hyperoxaluria type III is caused by loss of function of mitochondrial 4-hydroxy-2-oxoglutarate aldolase (HOGA1), resulting in oxalate overproduction that may also involve abnormal hydroxyproline metabolism (Belostotsky et al., 2010; Hoppe, 2012).

Secondary hyperoxaluria may arise either from excessive intestinal oxalate absorption (enteric hyperoxaluria) or from high dietary intake of oxalate-rich foods (dietary hyperoxaluria) (Glew et al., 2014). It is particularly relevant to clinical practice because it results from underlying conditions that disrupt normal oxalate homeostasis, including gastrointestinal disorders and malabsorption syndromes (Asplin, 2002; Kumar et al., 2004; Lieske et al., 2010). Secondary hyperoxaluria is often associated with diseases that impair fat digestion and absorption, such as exocrine pancreatic insufficiency (EPI), inflammatory bowel disease, or short bowel syndrome (Hatch and Freel, 2008; Bhasin et al., 2015; Mitchell et al., 2019).

In EPI, reduced pancreatic enzyme activity leads to fat malabsorption, which interferes with calcium binding to oxalate in the intestinal lumen (Asplin, 2002; Hatch and Freel, 2008; Liebman and Al-Wahsh, 2011). Unabsorbed fatty acids bind calcium, reducing its availability to form insoluble oxalate complexes. Consequently, free oxalate is more readily absorbed in the intestine, which increases its urinary excretion and predisposes individuals to nephrolithiasis and oxalate nephropathy (Mitchell et al., 2019). This mechanism underscores the clinical importance of secondary hyperoxaluria in patients with pancreatic disorders, as it may lead to progressive renal damage if not properly treated.

Pancreatic enzyme replacement therapy (PERT) is the standard treatment for EPI, aiming to restore digestive function by providing exogenous enzymes, including lipase, amylase and protease (Szkopek et al., 2024; Zaworski et al., 2025). While PERT effectively improves nutrient absorption and alleviates malabsorption symptoms, its impact on preventing or reducing hyperoxaluria remains poorly characterised. Elucidating the effects of pancreatic enzyme supplementation on oxalate metabolism is therefore important for the optimisation of treatment strategies in patients with EPI and associated renal complications.

In the present study, we examined the effects of EPI and PERT on hyperoxaluria in an experimental pig model. The main objective was to establish effective dietary induction of hyperoxaluria and nephrolithiasis in EPI pigs fed a high-fat diet. The secondary aim was to investigate the relationship between EPI, dietary oxalate supplementation and PERT.

We hypothesised that dietary oxalate loading in a large-animal model of EPI would induce hyperoxaluria and nephrolithiasis, and that PERT would attenuate, but not fully prevent, renal injury.

Material and methods

Animals

The pig model is widely used in biomedical research due to its physiological and anatomical similarities to humans, particularly regarding digestive and renal function (Lunney, 2007; Swindle et al., 2012). In this study, a well-established approach was employed involving pancreatic duct ligation (PDL) to induce EPI and evaluate the effects of PERT on oxalate metabolism, renal histopathology and biochemical parameters. The porcine model was selected *a priori* because pigs closely resemble human gastrointestinal physiology, including lipid digestion, bile and pancreatic enzyme secretion, renal architecture, and oxalate metabolism. In addition, this model allows repeated sampling and terminal histopathology under conditions consistent with ARRIVE 2.0 (Animal Research: Reporting of In Vivo Experiments) guidelines and EU Directive 2010/63/EU. The large-animal context provides translational relevance that cannot be achieved in rodents when studying EPI-associated enteric hyperoxaluria and potential renoprotective interventions.

The study was approved by the Second Local Ethics Committee for Animal Experiments in Warsaw (Approval No. WAW2/025/2022). All procedures involving animals were performed in accordance with the Polish Act on the Protection of Animals Used for Scientific or Educational Purposes, EU Directive 2010/63/EU, and relevant ethical guidelines for animal research.

Eight pigs (*Sus scrofa domesticus*; (Polish Large White × Yorkshire) × Hampshire) of both sexes, weighing 10.0 ± 1.0 kg at baseline, were enrolled and randomised to three groups: EPI (n = 3), EPI+Creon (n = 3), and healthy structural comparators (n = 2). All animals were housed individually in floor pens with constant visual, olfactory, and tactile contact, weighed weekly, and had *ad libitum* access to water. Environmental conditions were standardised (12 h light/12 h dark cycle, 21–25 °C, 70 ± 5% humidity, 10–12 air exchanges/h). Healthy comparators were maintained under the same husbandry conditions and high-fat diet schedule as the EPI groups but did not undergo PDL or oxalate supplementation. At the end of the study, all groups were subjected to the same anaesthesia, analgesia and euthanasia protocol to allow prespecified terminal histological assessment of structural endpoints.

Feeding and enzyme administration

Upon arrival at the experimental facility, the pigs were fed a cereal-based, pelleted, standard diet (Kcynia, Morawski Plant, Łódź, Poland). By day 7, the diet was gradually replaced with a high-fat diet (HFD) containing: %: crude protein 17.5, crude fibre 3.9, crude fat 20, ash 5.2; IU/kg: vitamin A 5000, vitamin D 500; mg/kg: vitamin E 85. The HFD (Kcynia, Morawski Plant, Łódź, Poland) was provided in an amount equivalent to 4% of body weight per day, with 1% administered in the morning meal (08:00–09:00) and 3% in the afternoon meal (16:00–17:00). Randomisation was performed on day 13 of the experiment (based on average body weight), after completion of the adaptation period (day 7) and before PDL surgery (day 14). In the EPI group (n = 3), pigs underwent PDL and received only the HFD. In the EPI+Creon group (n = 3), pigs underwent PDL and received the HFD supplemented with Creon® (2 × 10000 units per meal, per day; Abbot, IL, USA), administered with the morning and evening meals throughout the experiment. Both EPI groups additionally received potassium oxalate monohydrate (KOX; Sigma-Aldrich, Saint Louis, MO, USA) mixed into the HFD at 2% (days 45–59) and 3% (days 60–80). The healthy group (n = 2), consisting of age-matched pigs maintained on the same HFD, were used as comparators for renal structure assessment.

Blood and urine sampling

Blood and urine samples were collected 30 days after PDL (on day 45), corresponding to the period of established EPI, and again two weeks later (day 59). The final blood and urine samples were collected three weeks thereafter (day 80). Blood samples were collected via a jugular vein catheter before the morning meal, and subsequently at 2, 4, and 6 h postprandially. Samples were transferred into BD Vacutainer® glass tubes with aprotinin and K_3EDTA (BD Diagnostics, Franklin Lakes, NJ, USA). Blood samples were immediately placed on ice, and centrifuged at 3000 × *g* for 15 min at 4 °C. Plasma was separated and stored at −80 °C until further analysis.

Urine samples were collected over an 8-h period into sterile Falcon tubes (Eppendorf AG, Hamburg, Germany) and stored at −80 °C until further analysis. During urine collection, pigs were housed in metabolic cages.

Autopsy

At the end of the study, all pigs were euthanised by a single dose of intravenous injection of sodium pentobarbiturate (140 mg/kg bwt; 'Morbital', Biowet, Puławy, Poland) following prior intravenous premedication with a mixture of medetomidine (7 mcg/kg bwt; 'Domitor', Orion Pharma, Warsaw, Poland) and butorphanol (0.2 mg/kg bwt; 'Torbugesic', Zoetis, Warsaw, Poland).

Histochemical and histopathological analysis

On autopsy, kidneys were collected and fixed in 10% neutral buffered formalin for 24 h. Healthy pigs (n = 2) were used as structural comparators. Tissue samples were longitudinally divided into eight parts. The fragments were dehydrated and embedded in paraffin according to standard histological procedures (Fischer et al., 2008). Paraffin-embedded tissues were cut into 4.5 µm thick sections using a rotary microtome. After drying overnight, the slides were deparaffinised in xylene and rehydrated through a descending ethanol series. Sections were processed for two staining protocols: (1) Pizzolato staining to detect calcium oxalate (CaC_2O_4) deposition (Pizzolato, 1964) (Figure 1), and (2) haematoxylin and eosin (H&E) staining to evaluate histopathological changes. Lesions were graded semi-quantitatively using a standardised scoring system, where 0 means no pathological changes,

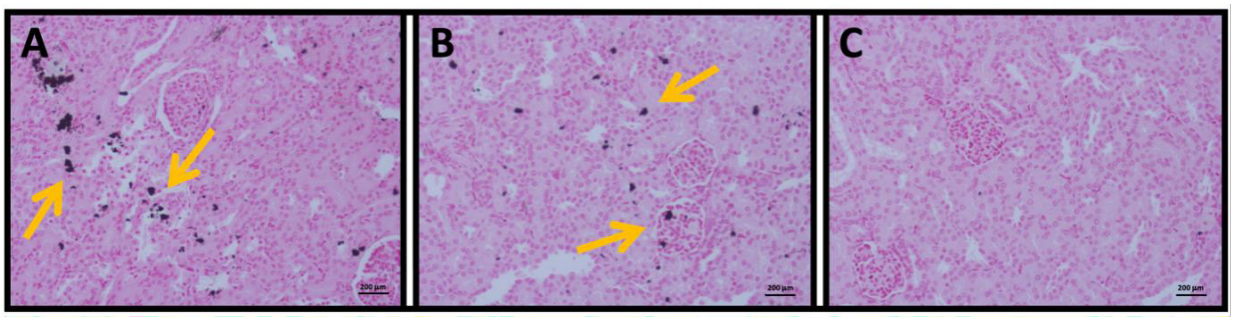

Figure 1. Examples of Pizzolato's method stained kidney tissue samples

Visible black dots present calcium oxalate deposits. Images were obtained using a light microscope (Axioskop 40, Zeiss, Germany). EPI – exocrine pancreatic insufficiency, A – group EPI, B – group EPI+Creon, C – group healthy.

1 – mild, 2 – moderate, and 3 – severe changes. Parameters assessed included glomerulosclerosis, microcystic tubules, tubular atrophy, interstitial fibrosis, inflammation and hydronephrosis (Figure 2). The analysis was performed using a Axioskop 40 light microscope (Zeiss, Jena, Germany) equipped with a Coolpix B700 digital camera (Nikon, Tokyo, Japan). Images were acquired using Axio Vision software version 4.2 (Zeiss, Germany). At least 20 measurements of each parameter were obtained for each section.

Statistical analysis

Statistical calculations were performed using GraphPad Prism 10.5.0 (San Diego, CA, USA). Data were tested for normal (Gaussian) distribution using the Shapiro-Wilk test. Differences in the parameters between experimental groups were assessed using one-way ANOVA (three-group comparisons) or an unpaired Welch's t-test (two-group comparisons). Differences were considered significant at $P < 0.05$, and at $P < 0.1$, they were regarded as trends.

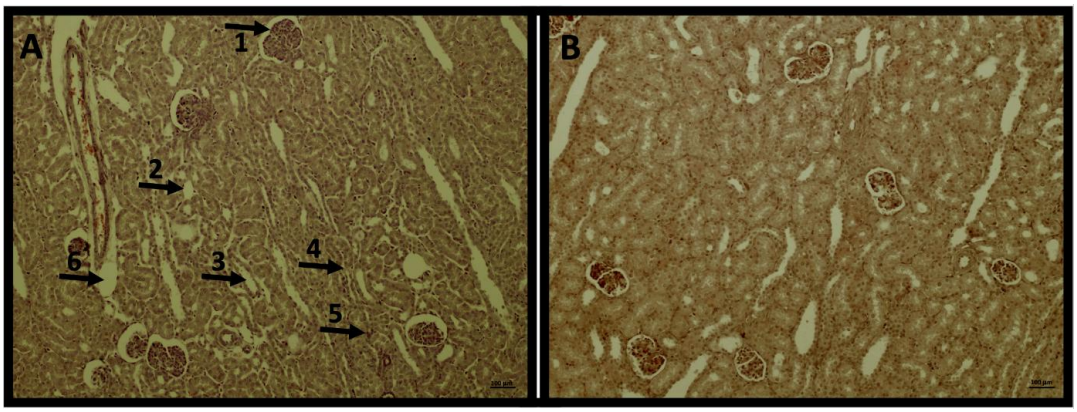

Figure 2. Representative examples of pathological changes in kidney tissue stained with haematoxylin and eosin (H&E)

Panel A shows advanced renal lesions observed: 1. Glomerulosclerosis, 2. Microcystic tubules with numerous markedly dilated tubular lumina, 3. Tubular atrophy characterized by flattened epithelium and loss of normal tubular architecture, 4. Interstitial fibrosis with pronounced expansion of the interstitium, 5. Interstitial inflammation with scattered inflammatory cell infiltrates, and 6. Hydronephrosis, reflected by extensive dilation of tubular structures and parenchymal disorganization. Panel B shows normal renal morphology without pathological changes. EPI - exocrine pancreatic insufficiency. A – group EPI, B – group healthy. Images were acquired using a light microscope (Axioskop 40, Zeiss, Germany).

Blood analyses

Creatinine levels in blood and urine were measured using the Liquick Cor-CREATININE 60 diagnostic kit (Catalogue No: 2-233; PZ Cormay, Warsaw, Poland) according to the manufacturer's recommendations. Oxalate concentrations in blood and urine were measured using the Oxalate Assay Kit colorimetric test (Catalogue No: MAK315; Sigma-Aldrich, Saint Louis, MO, USA) according to the manufacturer's recommendations.

Results

Oxalate concentration in blood and urine

In the EPI groups, the addition of 2% KOX (potassium oxalate monohydrate) to the diet resulted in a significant increase ($P = 0.0377$) in 8-h urinary oxalate excretion, compared to the group without KOX. Administration of Creon did not significantly affect urinary oxalate levels; values in the EPI+Creon group were similar to

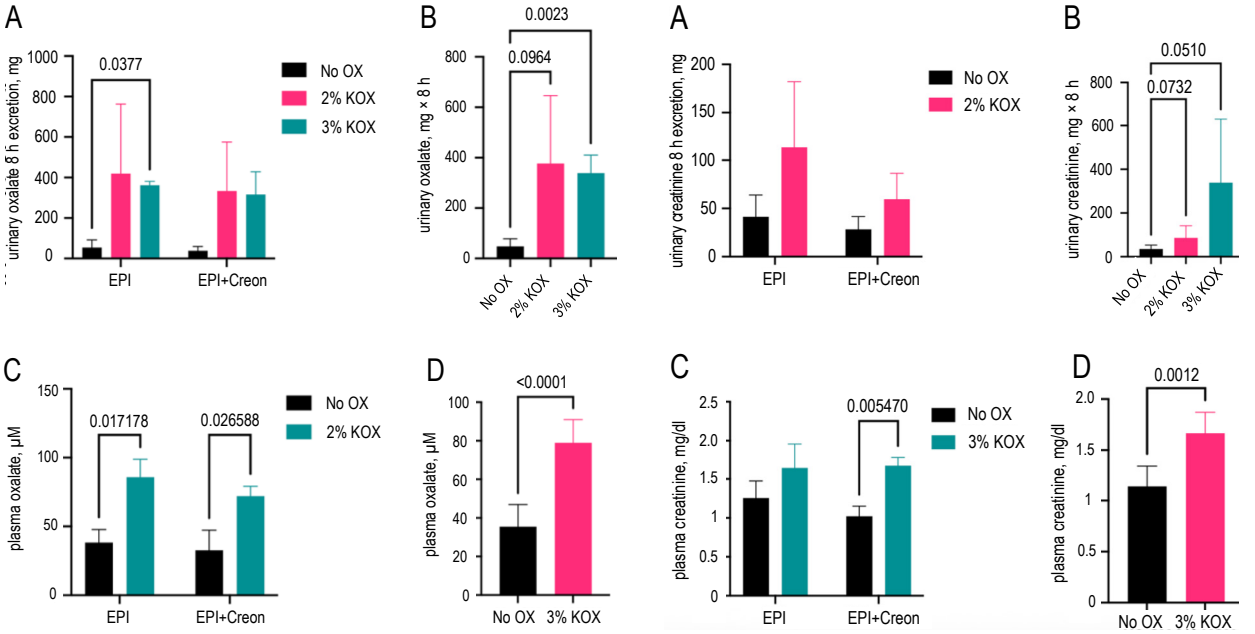

Figure 3. Blood and urine oxalate concentrations in exocrine pancreatic insufficiency (EPI) pigs, with or without Creon® treatment. (A) 8-h urinary oxalate excretion (mg) in the EPI and EPI+Creon groups receiving no oxalate (No OX), 2% potassium oxalate monohydrate (KOX), or 3% KOX added to the high-fat diet (HFD). (B) Pooled 8-h urinary oxalate excretion (mg × 8 h) compared between the No OX, 2% KOX, and 3% KOX dietary regimens (data combined across the EPI and EPI+Creon groups). (C) Plasma oxalate concentration (µM) in the EPI and EPI+Creon groups receiving No OX or 3% KOX in the HFD. (D) Pooled plasma oxalate concentration (µM) compared between the No OX and 3% KOX dietary regimens (data combined across the EPI and EPI+Creon groups). EPI group (n = 3): pigs after pancreatic duct ligation (PDL) fed exclusively a high-fat diet (HFD). EPI+Creon group (n = 3): pigs after PDL fed HFD + Creon® (2 × 10 000 units per day), administered with the morning and evening meals throughout the experiment. Data are expressed as mean ± standard deviation (± SD). Differences were considered significant at $P < 0.05$; *P*-values are given above the result bars.

Figure 4. Creatinine concentrations in blood and urine of exocrine pancreatic insufficiency (EPI) pigs, with or without Creon® treatment. (A) 8-h urinary creatinine excretion (mg) in the EPI and EPI+Creon groups receiving no oxalate (No OX) or 2% potassium oxalate monohydrate (KOX) added to the high-fat diet (HFD). (B) Pooled 8-h urinary creatinine excretion (mg × 8 h) compared between the No OX, 2% KOX, and 3% KOX dietary regimens (data combined across the EPI and EPI+Creon groups). (C) Plasma creatinine concentration (mg/dl) in the EPI and EPI+Creon groups receiving No OX or 3% KOX in the HFD. (D) Pooled plasma creatinine concentration (mg/dl) compared between the No OX and 3% KOX dietary regimens (data combined across the EPI and EPI+Creon groups). EPI group (n = 3): pigs after PDL fed exclusively a HFD. EPI+Creon group (n = 3): pigs after PDL fed HFD + Creon® (2 × 10 000 units per day), administered with the morning and evening meals throughout the experiment. Data are expressed as mean ± standard deviation (± SD). Differences were considered significant at $P < 0.05$; *P*-values are given above the result bars.

those in the EPI group without enzyme supplementation (Figure 3A). Both KOX-treated groups (2 and 3%) showed significantly higher urinary oxalate levels than the control group (No OX, $P = 0.0023$). The difference between 2% and 3% KOX was not statistically significant ($P = 0.0964$) (Figure 3B). Dietary supplementation with 3% KOX to significantly increased plasma oxalate concentrations in both the EPI ($P = 0.017178$) and EPI+Creon ($P = 0.026588$) groups compared to the respective controls. The application of Creon did not significantly reduce plasma oxalate concentrations (Figure 3C). The diet containing 3% KOX also caused a significant increase in plasma oxalate levels compared to the No OX group ($P < 0.0001$) (Figure 3D).

Creatinine concentration in blood and urine

In pigs with EPI, supplementation with 2% KOX did not significantly increase urinary creatinine excretion when compared to the group without KOX. Administration of Creon in the EPI+Creon group had no effect on urinary creatinine levels (Figure 4A). In animals receiving 3% KOX, urinary creatinine excretion increased ($P = 0.0510$) compared to the control group (No OX). Urinary creatinine levels in the 2% KOX group were similar to those in the control group ($P = 0.0732$) (Figure 4B), while plasma creatinine concentrations in the EPI and EPI+Creon groups were approximately 1.5 and 1.8 mg/dl, respectively. After administration of 3% KOX in the diet, plasma creatinine concentration increased to approximately 2.0 mg/dl ($P = 0.005470$) (Figure 4C) in the EPI+Creon group. The diet containing 3% KOX resulted in a significant increase ($P = 0.0012$) in plasma creatinine concentration (average 1.9 mg/dl) compared to the control group (No OX, 1.2 mg/dl) (Figure 4D).

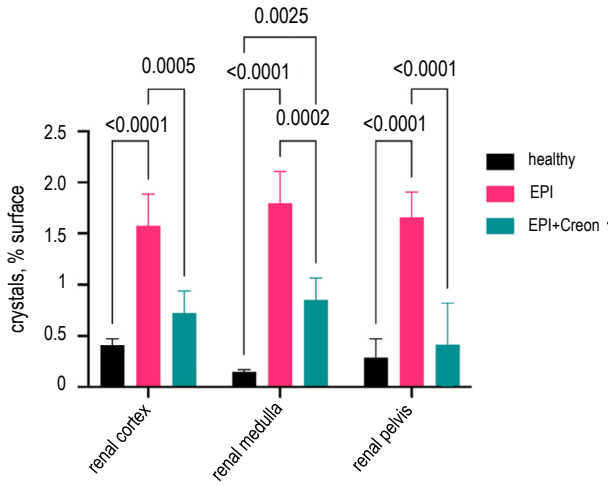

Figure 5. CaC_2O_4 deposits in the kidney tissue of exocrine pancreatic insufficiency (EPI) pigs, with or without Creon® treatment, following histochemical Pizzolato staining

Group EPI (n = 3): pigs after pancreatic duct ligation (PDL) fed exclusively a high-fat diet (HFD). Group EPI+Creon (n = 3): pigs after PDL fed a HFD + Creon (2 × 10 000 units per day), respectively, at the morning and evening meals throughout the experiment. Group Healthy Control (n = 2): pigs were used as a comparator only for the structural assessment. Data are expressed as mean ± standard deviation (± SD). Differences were considered significant at $P < 0.05$; *P*-values are given with the result bars.

CaC_2O_4 deposits in kidney tissue estimated with Pizzolato staining

Significant differences between groups in CaC_2O_4 deposition were observed in all kidney regions (Figure 5). In the renal cortex, the mean percentage of the area occupied by crystals was significantly higher ($P < 0.001$) in the EPI control group (~1.8%) than in the EPI+Creon group (~0.8%) and healthy controls (~0.4%). A similar trend was observed in the renal medulla, where the mean percentage of the area occupied by crystals was significantly higher ($P < 0.001$) in the EPI control group (~1.9%) compared to the EPI+Creon (~0.9%) and healthy controls (~0.2%). Significantly higher levels ($P < 0.0001$) of deposits were also observed in the renal pelvis of the EPI Control group (~1.7%), compared to that observed in the EPI+Creon (~0.6%) and healthy control (~0.3%) groups.

Histopathological scoring of renal lesions

Pigs in the EPI group had significantly higher pathological scores ($P < 0.0001$) for glomerular sclerosis, tubular atrophy and interstitial fibrosis (Figure 6). The EPI+Creon group showed moderate pathological changes. Although their overall scores were lower than in the EPI group, certain features, e.g., microcystic tubules, remained similar ($P = 0.0789$), indicating that Creon only partially mitigated renal damage. In the EPI+Creon group, no clear reduction was observed for glomerular sclerosis ($P = 0.0587$) and interstitial fibrosis ($P = 0.0121$). Differences were as also less pronounced for inflammation ($P = 0.0966$) and hydronephrosis ($P = 0.1135$). Healthy pigs showed minimal or no histopathological lesions (Figure 6).

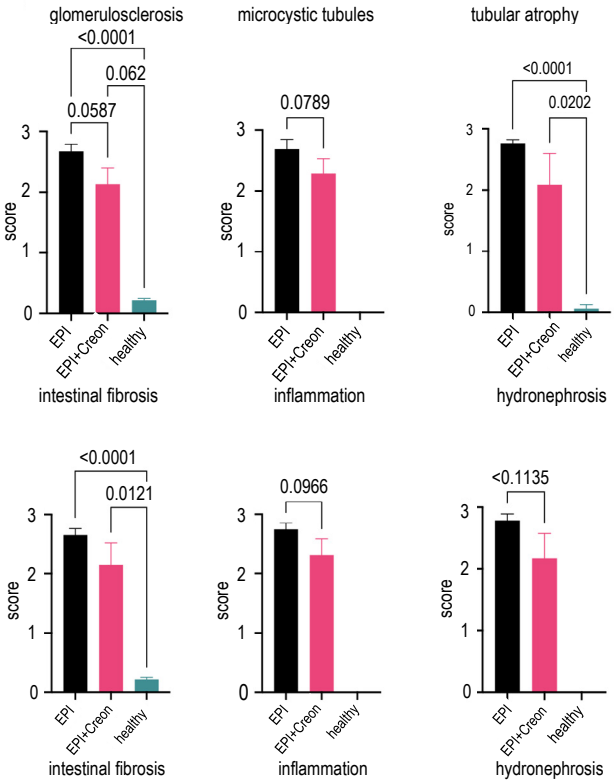

Figure 6. The scoring of the pathological changes in the kidneys of exocrine pancreatic insufficiency (EPI) pigs, with or without Creon® treatment

Standard histopathological grading was used, where 0 means no pathological changes found, 1 – mild changes found, 2 – moderate changes found and 3 – severe changes observed. These observations included glomeruloscrelosis, microcystic tubules, tubular athrophy, interstitial fibrosis, inflammation and hydronephrosis. Group EPI (n = 3): pigs after pancreatic duct ligation (PDL) fed exclusively a high-fat diet (HFD). Group+EPI+Creon (n = 3): pigs after PDL fed a HFD + Creon® (2 × 10 000 units per day), respectively, at the morning and evening meals throughout the experiment. Group Healthy Control (n = 2): pigs were used as a comparator only for the structural assessment. Data are expressed as mean ± standard deviation (± SD). Differences were considered significant at $P < 0.05$; *P*-values are given with the result bars.

Discussion

The results of the current study clearly demonstrate that pigs with EPI fed a high-fat diet with KOX accumulate significant amounts of calcium oxalate (CaC_2O_4) crystals in their kidneys, including the cortex, medulla, and renal pelvis. These observations are consistent with previous reports describing the mechanism of secondary hyperoxaluria in EPI patients, in which impaired fat

digestion leads to increased bioavailability of free oxalate in the intestinal lumen and its excessive absorption (Glew et al., 2014). Population-based studies and systematic reviews have also shown an increased incidence of kidney stones in patients with cystic fibrosis (CF), particularly calcium oxalate deposits (CaC_2O_4). Their pathogenesis appears consistent with mechanisms described in experimental models of EPI (Higgins, 2023). In CF, impaired digestion and absorption of lipids and minerals, including calcium, result mainly from EPI. This phenomenon leads to enteric hyperoxaluria and an increased risk of urinary oxalate crystallisation. In addition, chronic dehydration and hypovolemia, secondary to salt loss with sweat in CF contribute to the formation of concentrated urine with increased lithogenic potential (Ozçelik et al., 2004). It should also be noted that the use of PERT in CF patients can modify calcium and oxalate metabolism. There is evidence that some enzyme preparations may increase calcium absorption, leading to hypercalciuria, a phenomenon also observed in patients with hyperparathyroidism or idiopathic calcium stones (Aris et al., 1999; Putman et al., 2019). Although PERT in CF patients improves nutrient digestion and absorption, its effects on calcium oxalate homeostasis is complex and may vary depending on the specific enzyme formulation as well as the composition of the intestinal microbiome (Nayir Buyuksahin et al., 2023).

The present study showed that pancreatic enzyme supplementation (Creon) in pigs with experimentally induced EPI caused a significant reduction in calcium oxalate depositions in the renal cortex, medulla, and pelvis. The observed reduction in crystal accumulation and lower severity of histopathological lesions, including glomerular sclerosis and interstitial fibrosis, confirm the partial efficacy of enzyme therapy in limiting the consequences of secondary hyperoxaluria. The proposed mechanism involves improved lipid digestion, which reduces the concentration of free fatty acids in the intestinal lumen. A study by Zaworski et al. (2025) showed that the supply of exogenous pancreatic enzymes, particularly α-amylase, improved the structure of the small intestinal epithelium and restored physiological enterocyte turnover, which may indirectly reduce the absorption of metabolites such as oxalates. Despite the observed improvement, analysis of biochemical and histopathological parameters indicated that enzyme treatment did not restore full metabolic homeostasis. In the EPI+Creon group, moderate CaC_2O_4 accumulation and features of microcalcification and fibrosis were still observed, suggesting incomplete compensation for the enzymatic deficiency. In addition, plasma and urinary oxalate levels in this group did not return to baseline levels, which confirms the limited efficacy of enzyme monotherapy. A review by Szkopek et al. (2024) emphasised that the effectiveness of enzyme therapy in the treatment of EPI in humans and animals may depend not only on the dose but also on enzyme bioavailability, pH stability, and their duration of activity in the intestinal lumen. Glew et al. (2014) also reported that improving lipid digestion alone may not be sufficient to prevent hyperoxaluria unless combined with dietary modification and correction of the intestinal microflora.

The current study demonstrated a significant increase in urinary and plasma oxalate concentrations in pigs with EPI fed a diet enriched with KOX. The correlation between dietary oxalate content and the severity of hyperoxaluria suggests a direct causal relationship, which is consistent with previous reports on secondary hyperoxaluria. The resultant hyperoxaluria increases urine saturation with calcium oxalate, leading to crystallisation and deposit formation. These deposits can damage the renal tubular epithelium and trigger a local inflammatory response followed by interstitial fibrosis (Cochat and Rumsby, 2013). Glew et al. (2014) reported that mechanical damage to tubular epithelium induced by CaC_2O_4 deposits caused necrosis and activation of a cascade of pro-inflammatory cytokines such as TNF-α and IL-1β, which contributed to the progression of kidney damage.

The increase in plasma and urine creatinine levels in EPI animals in this study represents an additional indicator of declining renal function associated with nephrotoxic effects of oxalate deposition and progressive structural injury. Creatinine, a product of muscle metabolism, is cleared by glomerular filtration, and elevated plasma concentrations reflect impairment of this process. Harambat et al. (2010) showed that creatinine levels correlated with disease severity and renal outcomes under hyperoxaluric conditions. Here, supplementation with pancreatic enzymes (Creon) had no significant effect on creatinine concentrations, suggesting that PERT provides insufficient protection against advanced functional deterioration. The lack of therapeutic effect on filtration function likely reflects the irreversible nature of established nephron damage, and reducing intestinal oxalate absorption cannot reverse pre-existing glomerular sclerosis or tubular atrophy. In addition, it is possible that the duration of therapy was inadequate to permit meaningful regeneration of glomerular-tubular structures.

The results clearly indicate a significant relationship between dietary oxalate content and the severity of hyperoxaluria and accompanying kidney damage. An increase in both plasma and urinary oxalate levels was observed even at 2% KOX supplementation of a high-fat diet. Increasing the KOX content to 3% resulted in a further rise in plasma oxalate levels, which correlated with the severity of histopathological changes in the kidneys, especially progressive interstitial fibrosis, tubular atrophy and glomerular sclerosis. Surprisingly, the difference in oxalate levels between diets containing 2 and 3% KOX was markedly more pronounced in plasma than in urine, suggesting that as renal injury advances, the kidney's capacity to excrete oxalate becomes increasingly compromised. Such a mechanism is consistent with the 'impaired clearance' hypothesis described in the literature, according to which the kidneys initially adapt to an increased oxalate load, but prolonged exposure leads to functional saturation and structural damage (Cochat and Rumsby, 2013). An important consideration from a clinical perspective is the toxicity associated with even moderate but prolonged oxalate exposure. Mitchell et al. (2019) reported that in patients with chronic gastrointestinal disorders (e.g., IBD, EPI), increased oxalate intake together with impaired binding mechanisms (e.g., reduced calcium availability) contribute to progressive renal deposition and stone risk. Consistent with this, Siener et al. (2024) reported elevated intestinal oxalate absorption and increased urinary stone-forming risk in patients with Crohn's disease, underscoring that enteric hyperoxaluria is a shared complication across malabsorptive conditions of diverse aetiology This condition, referred to as 'silent hyperoxaluria,' may remain clinically unrecognised until advanced nephron damage occurs. The findings of the present study are consistent with observations of Glew et al. (2014), who documented the development of both acute and chronic oxalate nephropathy in patients hospitalised for diarrhoea, malabsorption and intestinal resection, even in the absence of increased dietary oxalate intake. These observations support the hypothesis that in patients with impaired intestinal barrier function, even a small increase in oxalate supply may exceed the buffering capacity and lead to systemic oxalate retention.

In the present study, the semi-quantitative assessment of renal lesions, including glomerular sclerosis, tubular atrophy, interstitial fibrosis, microaneurysms and oxalate deposits allowed precise differentiation of the degree of renal damage between the experimental groups. The results clearly show that histopathological changes in EPI animals without enzyme supplementation (EPI group) were advanced, multifocal and involved almost all analysed parameters. Importantly, these histopathological findings are consistent with the lesions typically observed in human oxalate nephropathy secondary to chronic pancreatitis (Cochat and Rumsby, 2013). In the EPI+Creon group, although significantly less severe changes were observed, some features, including microcystic tubules and moderate fibrosis, were still present, indicating that enzyme therapy confers only partial protection and incomplete structural regeneration. Pathophysiologically, persistent tubular atrophy and glomerular sclerosis reduce the pool of functional nephrons, leading to compensatory hyperfiltration in the remaining nephrons and further damage exacerbation (Danpure, 2005). Bhasin et al. (2015) showed that histopathological lesions associated with secondary hyperoxaluria, may be partially reversible when detected at an early stage and treated with combined therapy, including PERT and dietary oxalate restriction. Similar conclusions were presented by Mitchell et al. (2019), who emphasised the importance of early intervention to limit progression to chronic kidney disease.

The use of a pig (*Sus scrofa domesticus*) model in the study of EPI and secondary hyperoxaluria represents a robust translational approach due to the close anatomical, physiological and biochemical similarities between pigs and humans. In contrast to classical rodent models, pigs show high similarity to humans in terms of body weight, gastrointestinal tract length, gut microflora composition, kidney structure and glomerular filtration parameters (Swindle et al., 2012). In particular, the PDL model employed in this study allows for stable and reproducible induction of EPI in pigs. As demonstrated by some studies, the physiological response of pigs to PDL, including loss of pancreatic function, weight loss, fat malabsorption and changes in gut microflora, resembles that observed in humans with chronic pancreatitis or EPI after pancreatectomy (Lunney, 2007; Pierzynowski et al., 2012). Unlike mouse or rat models, which often differ in metabolic pathways, nephron structure and intestinal microbiota complexity, pigs more closely reflect human oxalate metabolism and excretion. Moreover, the presence of a comparable profile of intestinal oxalate transporters further supports the value of this model in studying oxalate uptake and its effects on the kidney (Hatch and Freel, 2008). In research on oxalate nephropathy, the porcine model enables assessment of both acute and chronic renal alterations, including calcium oxalate microcalcifications, tubular damage, interstitial fibrosis and glomerulopathy, making it an ideal tool for evaluating potential therapeutic interventions (Asplin, 2002; Lieske et al., 2010).

The study was designed as an exploratory, proof-of-concept investigation; group sizes were intentionally small to estimate variability and assess feasibility rather than to support definitive efficacy claims. Healthy control animals were included solely for structural (histopathological) comparison, and metabolic endpoints were not measured in this group to comply with the Reduction principle. A sham-operated group was not included for ethical reasons and will be considered in future studies if supported by power estimates derived from the present work. Consequently, the findings should be interpreted as initial evidence that EPI combined with oxalate loading induces renal crystal accumulation and injury in pigs, and that PERT partially attenuates these effects. Definitive validation will require adequately powered, confirmatory experiments.

Conclusions

In conclusion, the study confirms the key role of pancreatic insufficiency and an oxalate-enriched diet in the development of oxalate nephropathy and nephrolithiasis. Pancreatic enzyme supplementation (PERT) partially reduced hyperoxaluria and renal damage but was insufficient to fully preserve renal function, especially in advanced cases. The results emphasize the importance of an integrated therapeutic approach that includes dietary management, PERT and potential modification of the gut microbiota. In addition, kidney histopathology proved useful not only for diagnosis but also for prognosis, enabling early detection of lesions and more timely and effective intervention.

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Conflict of interest

The authors declare that there is no conflict of interest.

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