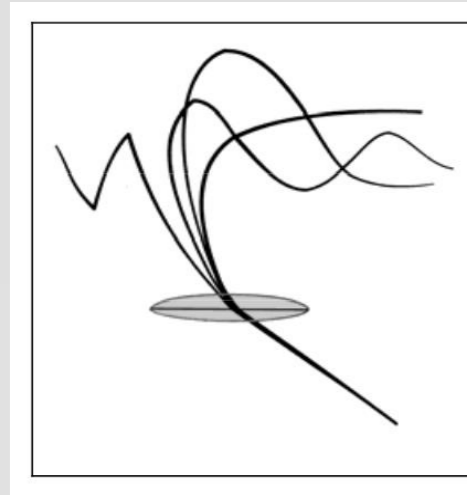


Histological analysis of renal morphology in wild-type and *Tff3* knock-out mice subjected to tunicamycin-induced endoplasmic reticulum stress



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INTRODUCTION

Tff3 protein is secreted by different mucosal epithelial cells and is important for protection and restoration of mucosal integrity and function. It is expressed in normal kidney tissue, but also in some renal diseases, such as oxidative stress-induced renal carcinoma and chronic kidney disease. Accumulation of unfolded proteins within the endoplasmic reticulum (ER) is one of the early events in pathological changes of different organs including kidney. *Tff3* deficiency has been linked to the function of ER.

OBJECTIVES

The aim of this study was to assess the effect of *Tff3* deficiency on microscopic morphology of renal cortex and medulla in case of induced acute ER stress.

MATERIALS & METHODS

Five 7 weeks-old male *Tff3*^{-/-}/C57BL6/NCrI mice (*Tff3* knock-out mice) and five male C57BL6/NCrI (wild-type) mice were used in the study. ER stress was induced with single dose of tunicamycin (Tm) (3 µg of Tm/g of body weight), a drug that blocks the initial step of N-glycosylation, leading to accumulation of misfolded proteins in the ER. Kidneys were collected after 24 h and prepared for histological analysis. The samples were fixed in 4% paraformaldehyde, embedded in paraffin, cut to 6 µm slides and stained using PAS method. Digital images were taken (5 images of the cortex and 3 images of the medulla for each animal) and measurements were performed on each image using FIJI software. In the cortex, glomeruli, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) were measured, and in the medulla, collecting ducts (CD) were measured (Fig. 1 A-D). Arithmetic means were obtained for each parameter and sample and used for further analysis.

RESULTS

The analysis of renal morphometric parameters did not show significant differences in the glomerular area, tubular diameter, height of tubular epithelium and diameter of tubular lumen between wild-type mice and *Tff3* knock-out mice after ER stress, although results for glomerular area and height of PCT epithelium showed borderline p value (Table 1 and 2).

CONCLUSION

Tff3 deficiency does not affect tubular and glomerular morphology significantly in applied acute ER stress model. This may point to the conclusion that *Tff3* protein deficiency does not affect the cellular mechanisms during 24 h after induction of ER stress. However, model of chronic ER stress occurring in course of normal aging could provide more valuable data of *Tff3* deficiency role in kidney function. Furthermore, borderline changes in glomerular area and PCT epithelium height might point to possible cortical hypertrophy in *Tff3* knock-out mice. This should be further investigated on a larger sample and following a longer period after ER stress induction.

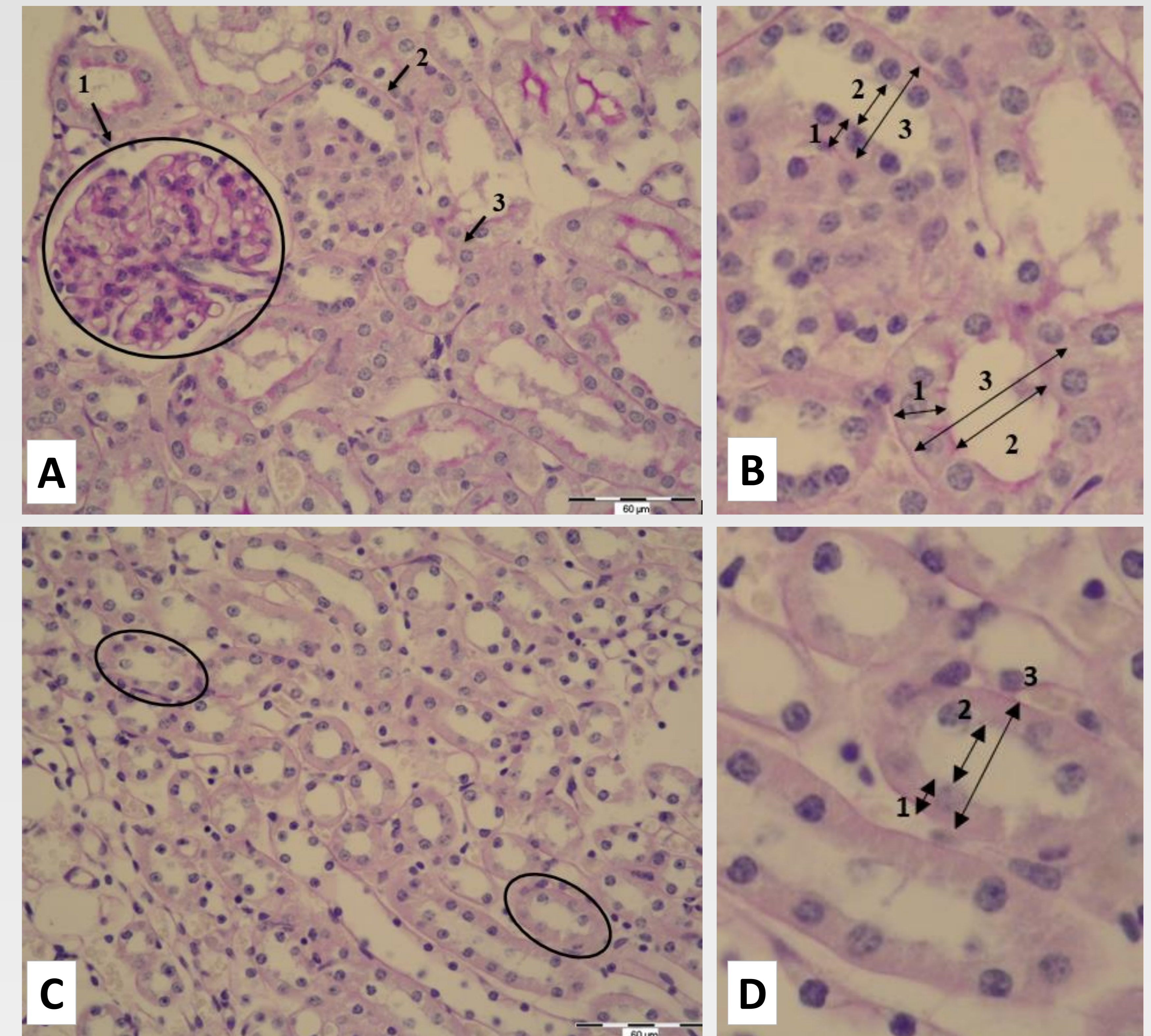


Figure 1. Cortex and medulla measurements. A, B – cortex; C, D – medulla. A: 1 – glomerulus, 2 – DCT, 3 – PCT. B: 1 – height of epithelium, 2 – lumen diameter, 3 – tubule diameter. C: ellipse – CD. D: 1 – height of epithelium, 2 – lumen diameter, 3 – duct diameter. Scale bar: 60 µm.

Table 1. Results of cortical morphology measurements.

Table 2. Results of medullar morphology measurements.

CORTEX	Median (interquartile range)		p*
Measured value	Wild-type mice	<i>Tff3</i> knock-out mice	
Glomerular area	3919,4426 (3468,6759 – 4312,3850)	4647,2160 (4509,0954 – 5011,6911)	0,06
PCT diameter	37,6168 (36,3515 – 0,1848)	40,6602 (38,5780 – 40,9264)	0,30
Height of PCT epithelium	11,1616 (10,4270 – 12,2428)	13,3326 (12,3541 – 13,9013)	0,06
PCT lumen diameter	11,4849 (11,0670 – 11,9922)	11,3622 (8,2027 – 13,2374)	> 0,95
DCT diameter	24,6166 (22,3063 – 25,2296)	23,2768 (21,9764 – 26,4916)	> 0,95
Height of DCT epithelium	6,6066 (6,2116 – 6,9319)	6,9802 (6,4838 – 8,5596)	0,42
DCT lumen diameter	9,0290 (8,5137 – 9,7888)	9,5492 (7,4467 – 10,7306)	> 0,95

*Mann-Whitney U test

MEDULLA	Median (interquartile range)		p*
Measured value	Wild-type mice	<i>Tff3</i> knock-out mice	
CD diameter	23,7308 (22,2575 – 25,5453)	23,2740 (22,8229 – 23,4525)	> 0,95
Height of CD epithelium	5,6582 (5,4822 – 5,9963)	5,6953 (5,5697 – 5,9095)	0,90
CD lumen diameter	13,0150 (11,8638 – 14,1245)	11,3543 (11,2968 – 12,0018)	0,06

*Mann-Whitney U test