

Increased apoptosis and decreased expression of nephrin in aging Spontaneously Hypertensive Rats (SHR)

Aspasia Volakaki¹, Effie-Photini Tsilibary¹, Garyfallia Drossopoulou¹ and Demetrios Vlahakos²

¹ Institute of Biosciences and Applications, NCSR “Demokritos”, Athens, Greece

² Division of Nephrology, ATTIKON University Hospital, National and Kapodistrian University of Athens, Medical School, Athens, Greece



Objective

Hypertension leads to targeted organ damage of the heart, blood vessels, brain, eyes and kidneys with eventual development of renal diseases and is also a well-known risk factor for the rapid progression of kidney failure.

The present study aimed to investigate podocyte alterations and molecular mechanisms at different stages during progression of hypertensive glomerulosclerosis, by evaluating the role of foot process and slit diaphragm protein complexes, such as nephrin, its accompanying protein CD2AP, podocalyxin, and the discoidin domain receptor 1 (DDR1: a collagen receptor that displays tyrosine-kinase activity).

Methods

Spontaneously hypertensive rats (SHR) and normotensive control Wistar-Kyoto (WKY) rats were divided into groups referred to as SHR6W, SHR13W, SHR20W, WKY6W, WKY13W and WKY20W regarding he respective weeks of age. Systolic blood pressure (BP) was measured in conscious animals using a tail-cuff technique and the aforementioned proteins were evaluated in isolated glomeruli. Western blot and immunofluorescence analysis of nephrin, PODXL and CD2AP and DDR1 was performed in isolated rat glomeruli.

Results

❖ No significant differences were found in the weight of hypertensive rats compared to their controls (WKY rats). However, BP was 30-50% higher in SHR compared to WKY with the difference increasing with age. (Figure 1).

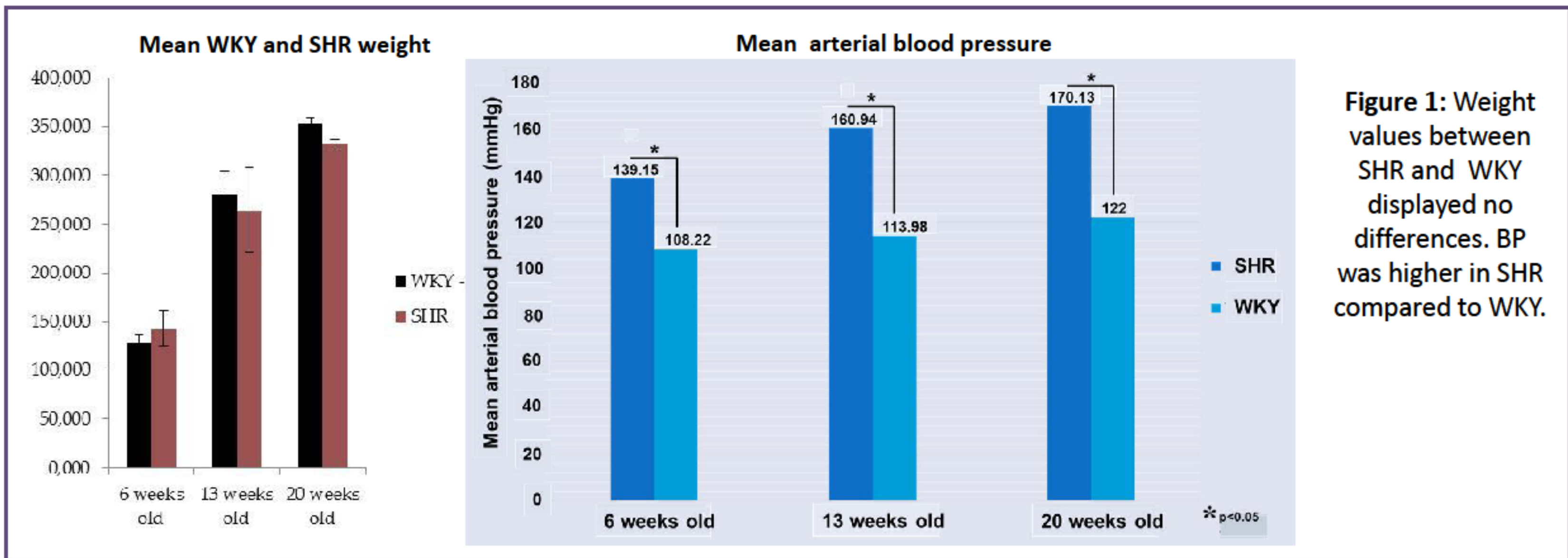


Figure 1: Weight values between SHR and WKY displayed no differences. BP was higher in SHR compared to WKY.

❖ Western blot analysis of CD2AP and nephrin was performed in isolated rat glomeruli of WKY and SHR (Figure 2). Actin and CD2AP levels remained unaffected. Unaltered CD2AP levels may indicate unperturbed interaction with actin in the formation of the podocytic cytoskeleton. Biochemical data showed that in isolated glomeruli, nephrin was downregulated as early as 6 weeks of age in the SHR6W compared to the relevant WKY control, while in SHR20W nephrin levels were reduced by 70% compared to WKY20W (Figure 2).

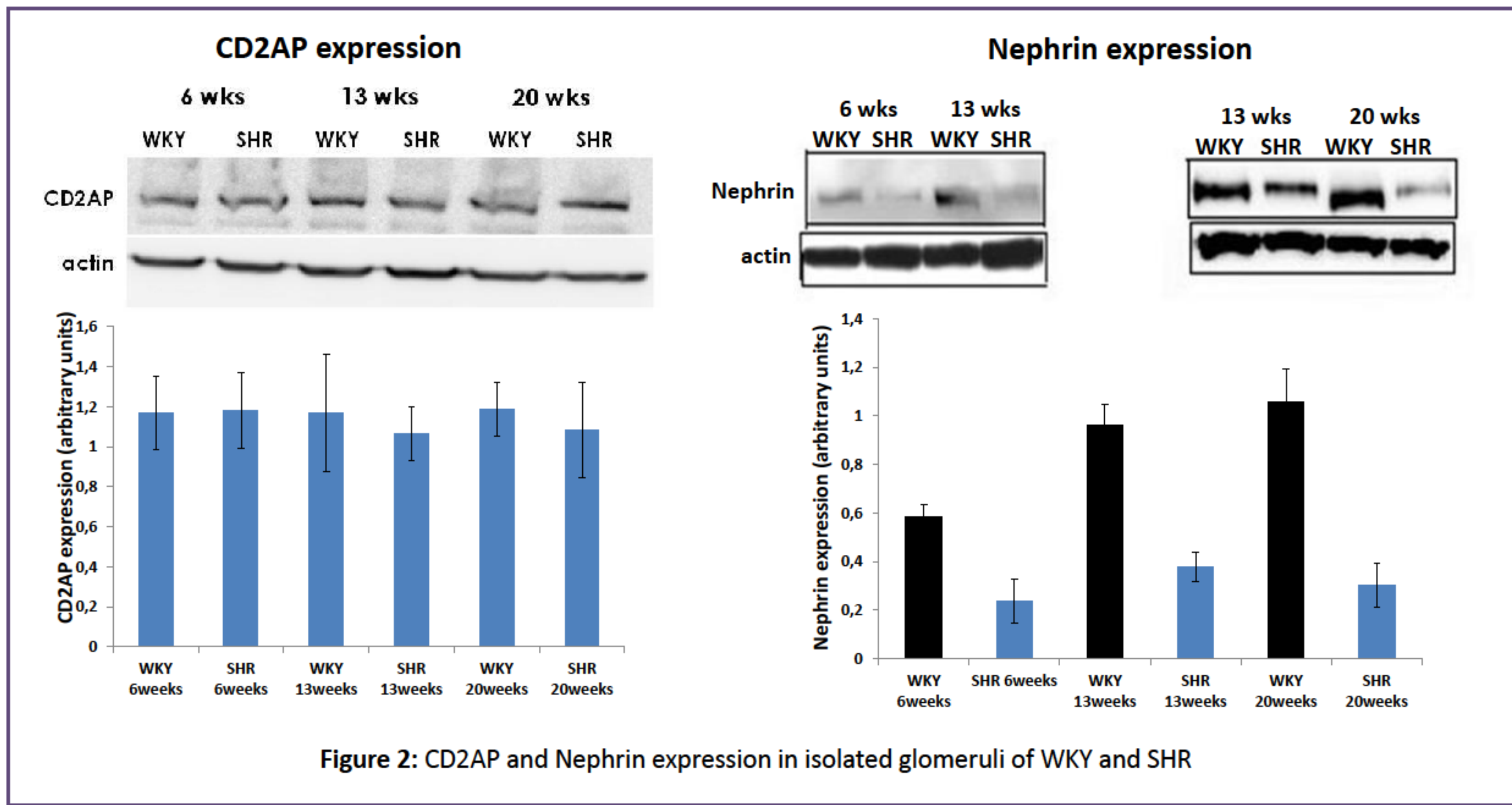


Figure 2: CD2AP and Nephrin expression in isolated glomeruli of WKY and SHR

Conclusions

We conclude that age-dependent development of hypertension and hypertensive nephrosclerosis in SHR rats is associated with increased cell apoptosis, increased matrix accumulation, and age-dependent down-regulation of the vital podocytic protein nephrin, rendering podocytes susceptible to early apoptosis.

Acknowledgements

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❖ Immunofluorescence analysis was performed in isolated rat glomeruli in order to determine alterations in podocalyxin expression (Figure 3). Podocalyxin levels showed progressive decrease from 20% (at 6weeks) to 50% (at 13 weeks).

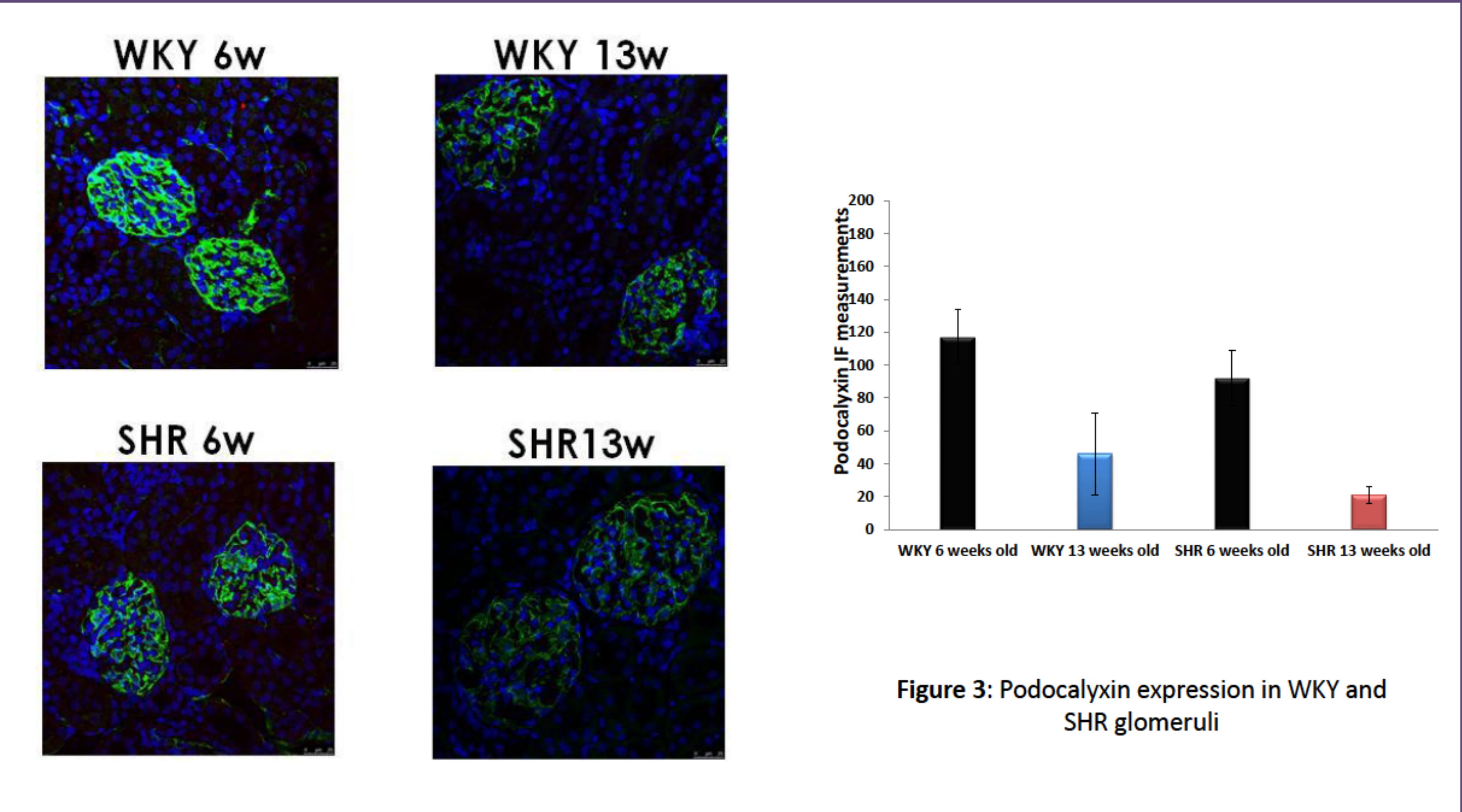


Figure 3: Podocalyxin expression in WKY and SHR glomeruli

❖ The observed nephrin downregulation in SHR6W was followed by increased podocyte apoptosis. More specifically, cleaved PARP appeared in SHR13W and SHR20W compared to the relevant controls. No difference in cleaved PARP levels was found between WKY and SHR rats at 6 weeks (Figure 4).

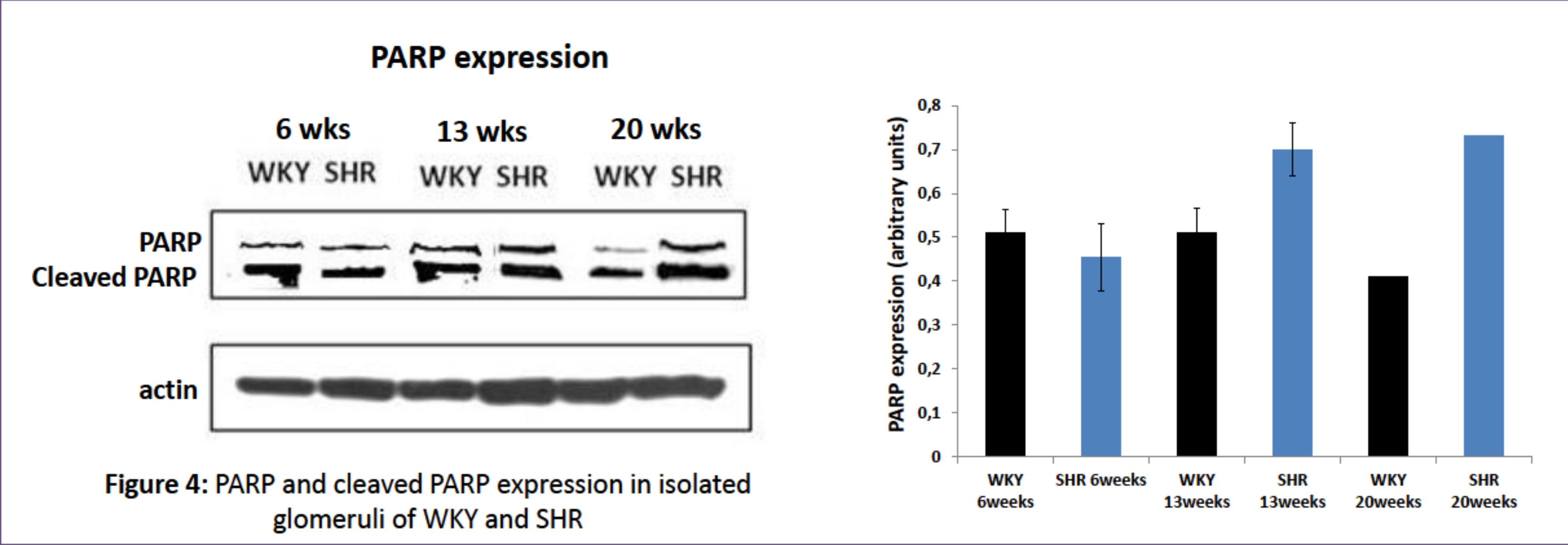


Figure 4: PARP and cleaved PARP expression in isolated glomeruli of WKY and SHR

❖ Finally, DDR1 levels appeared significantly upregulated in SHR6W, indicating that collagen accumulation leading to glomerulosclerosis starts early during the development of hypertensive nephropathy (Figure 5).

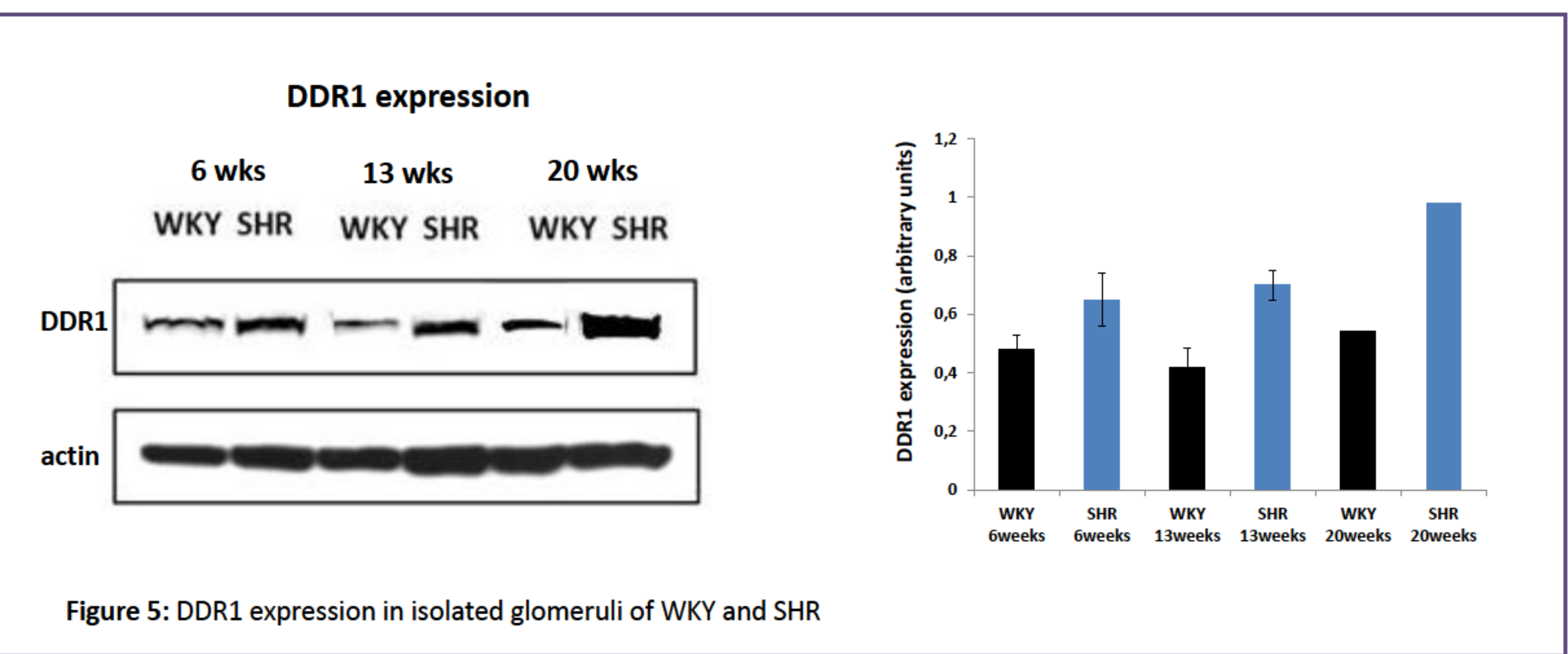


Figure 5: DDR1 expression in isolated glomeruli of WKY and SHR

