

INFLUENCE OF INSULIN IN THE GENE EXPRESSION OF RAS COMPONENTS OF THE PODOCYTE IN A DIABETIC SITUATION

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Introduction

- **Diabetic nephropathy (DN)** is the leading cause of end-stage renal disease in our environment.
- **Renin-Angiotensin System (RAS)** blockade has been shown to delay the progression of chronic kidney disease and specifically in DN.
- The **podocytes** are key cells involved in the **development of albuminuria** with a **functionally active local RAS** and **insulin receptors**.
- In the diabetic milieu, podocytes are influenced by circulating RAS and remarkable by its own RAS activation. *Figure 1*

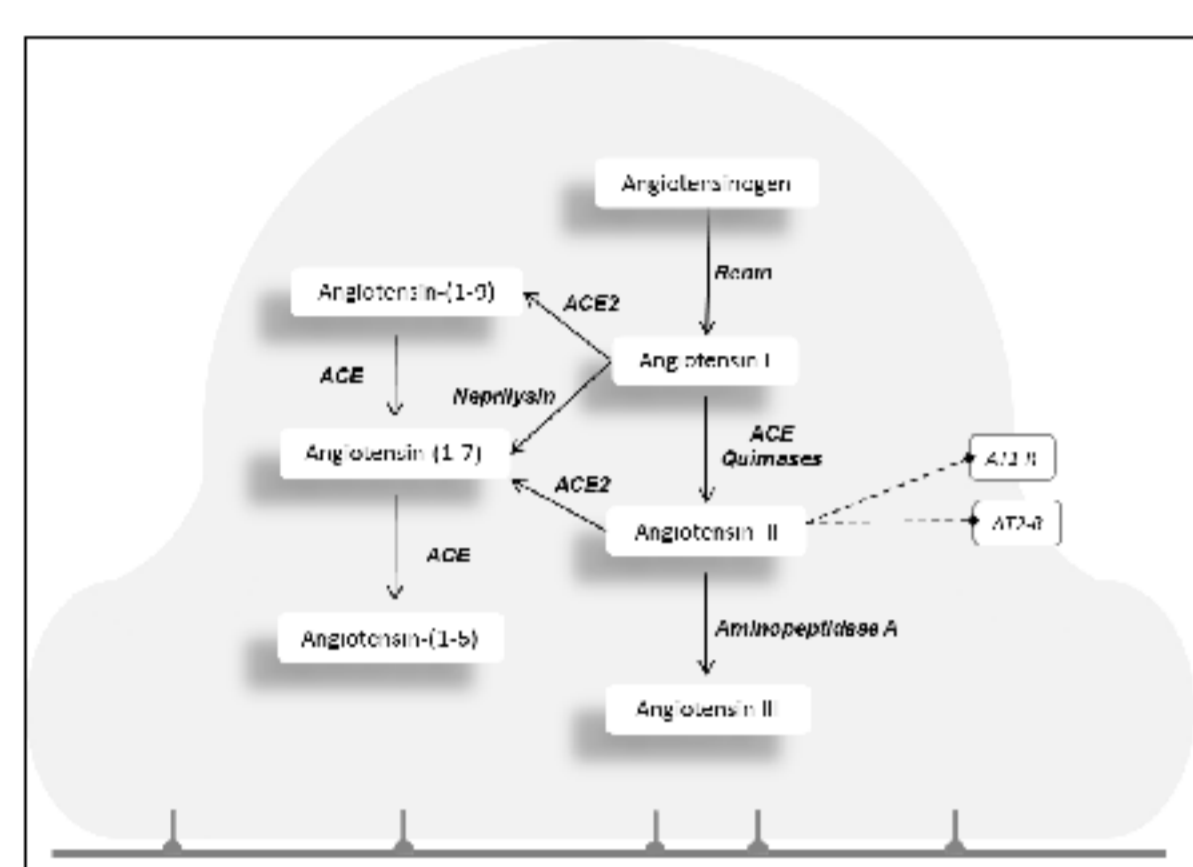


Figure 1. RAS system within the podocyte

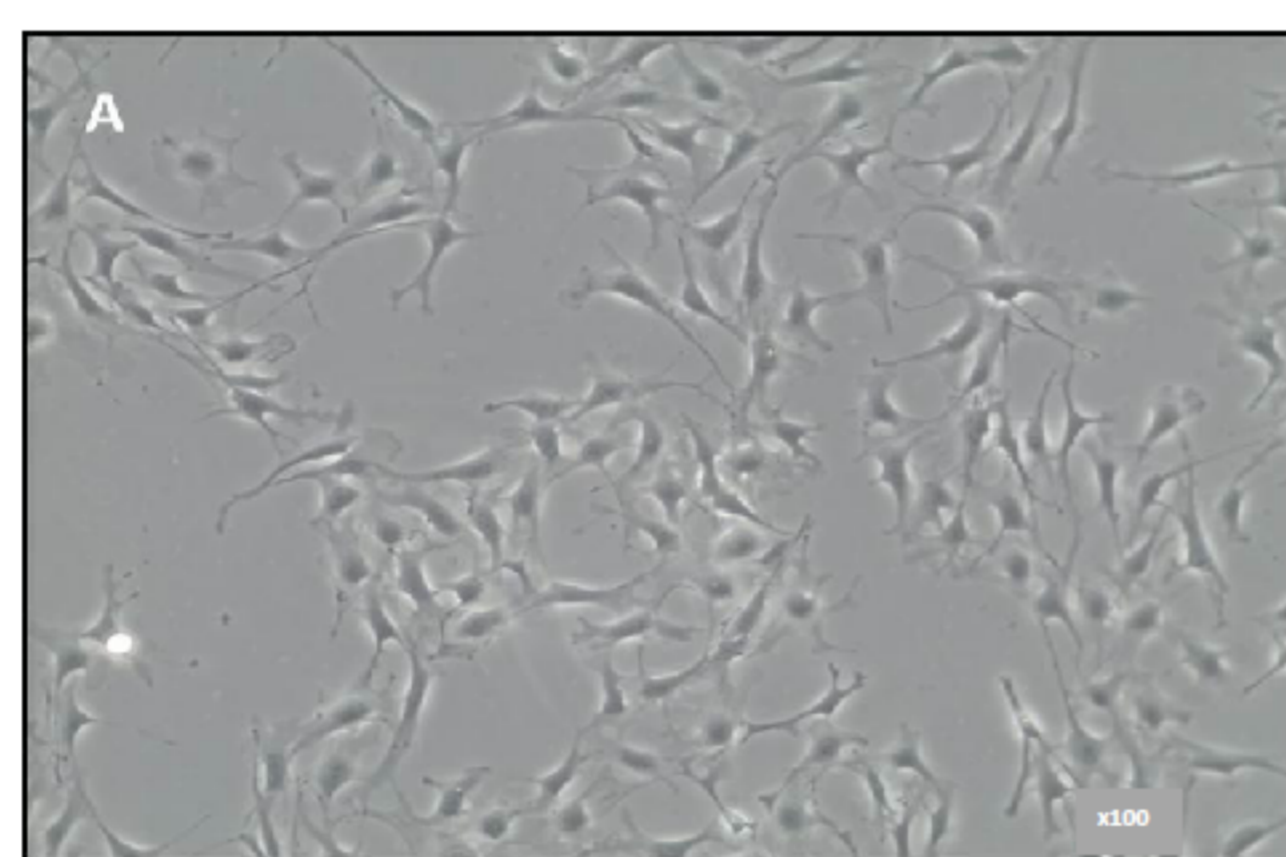


Figure 2 A. Cells growing. Permissive conditions

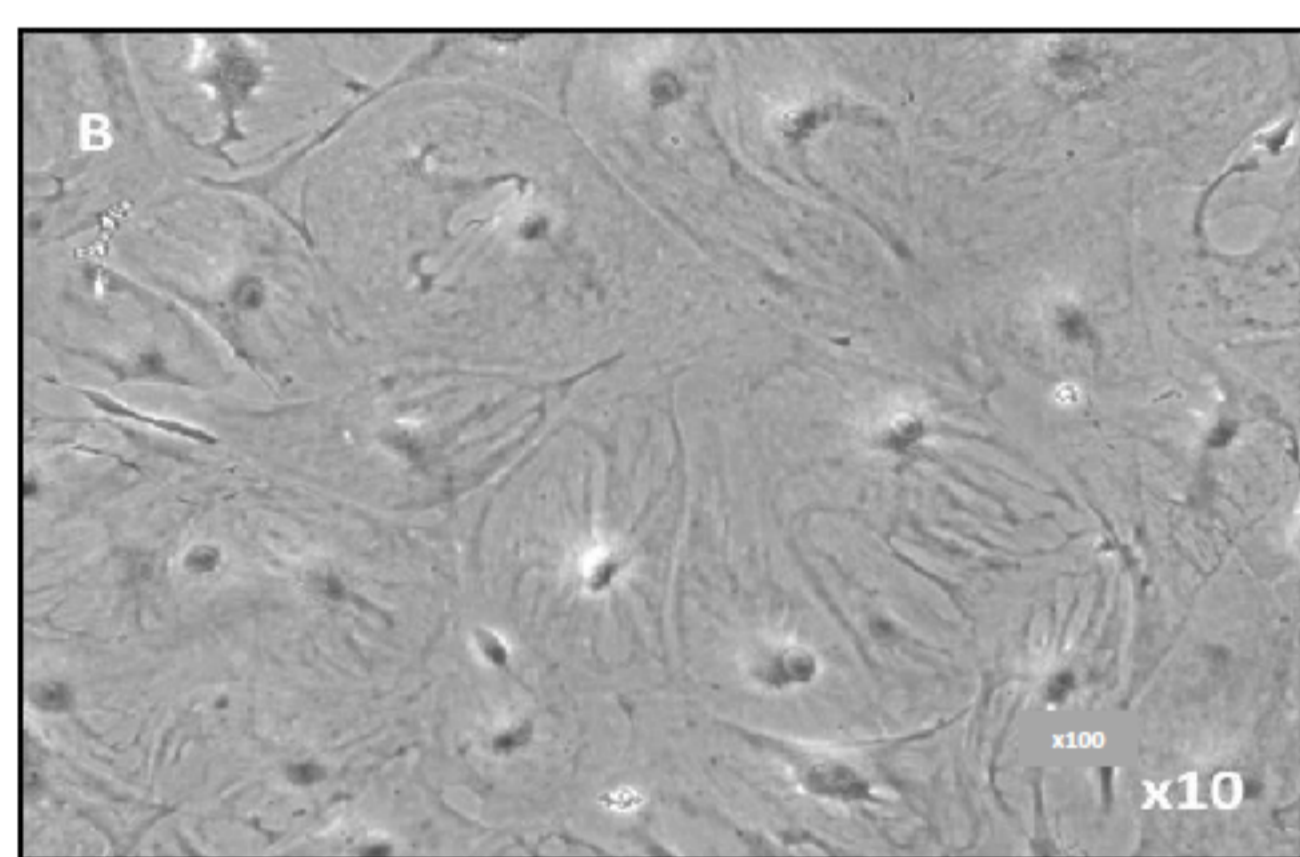


Figure 2 B. Cells on 14th day of differentiation

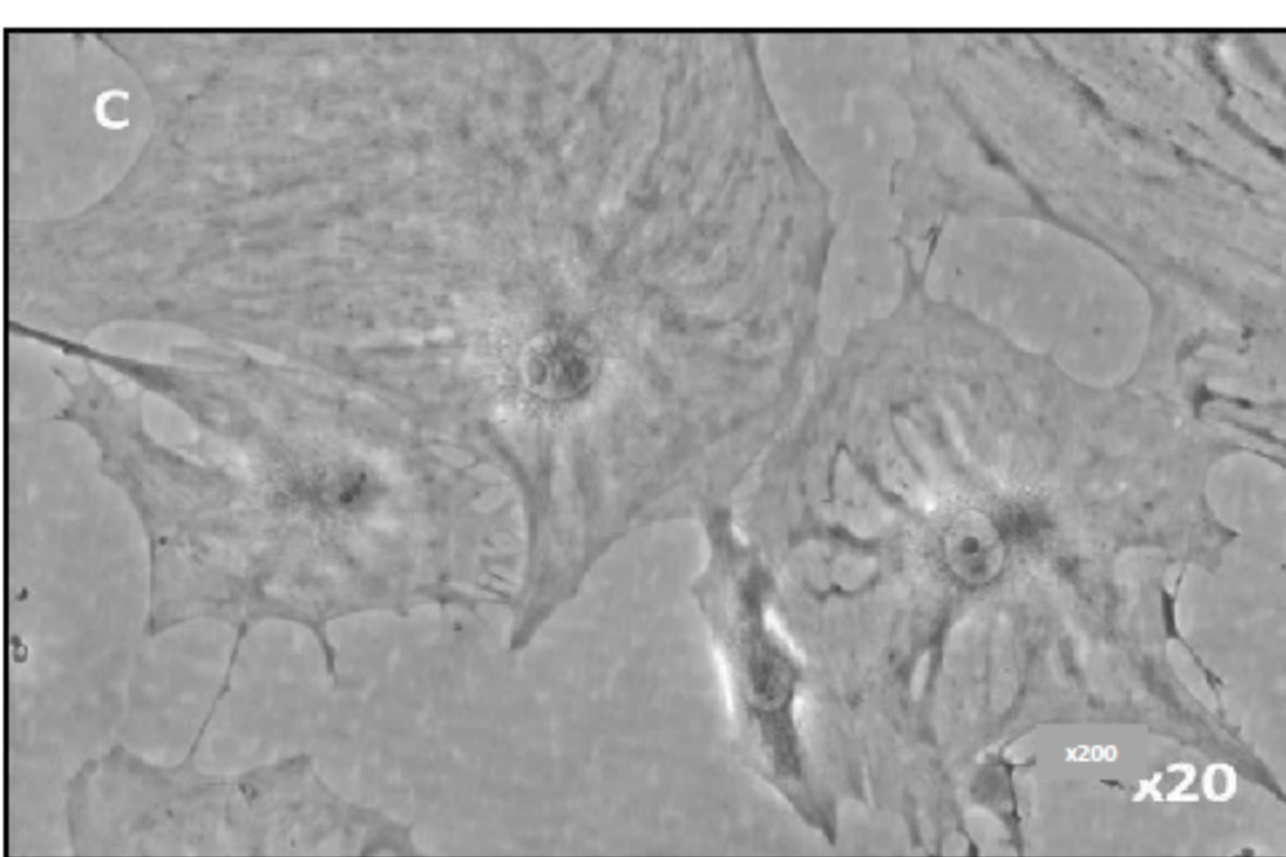


Figure 2 C. Cells on 14th day of differentiation

Aim

Study the effect of **long term insulin** incubation in several **RAS** components gene expression in podocytes in **high glucose media after insulin and/or albumin incubation**, mimicking diabetes situation.

Materials and Methods

Cell Culture

- **Conditionally immortalized mouse podocyte cell line.**
- Proliferated in permissive conditions (mouse γ -interferon, 32°C) for 3-5 days (*Figure 2a*). Induced to differentiate for 14 days in non-permissive conditions (37°C and removing γ -interferon). *Figure 2b/2c*.
- Incubation for 48 hours in high glucose media (25mM) with insulin (200nM; PODi), albumin (10mg/ml; PODa) or both (PODai). There was a control group without intervention (PODc). Each group with an n=8.

Gene expression studies

- RAS elements studied: **Angiotensinogen (Agt)**, **Angiotensin converting enzyme (ACE)**, **Angiotensin converting enzyme 2 (ACE2)** and **angiotensin receptors AT1 and AT2 (AT1-R, AT2-R)**.
- Total RNA extraction \rightarrow Reverse transcription \rightarrow qPCR.
- β -actin was used as housekeeping gene. Results were expressed in Ratio gene/ β -actin

Results

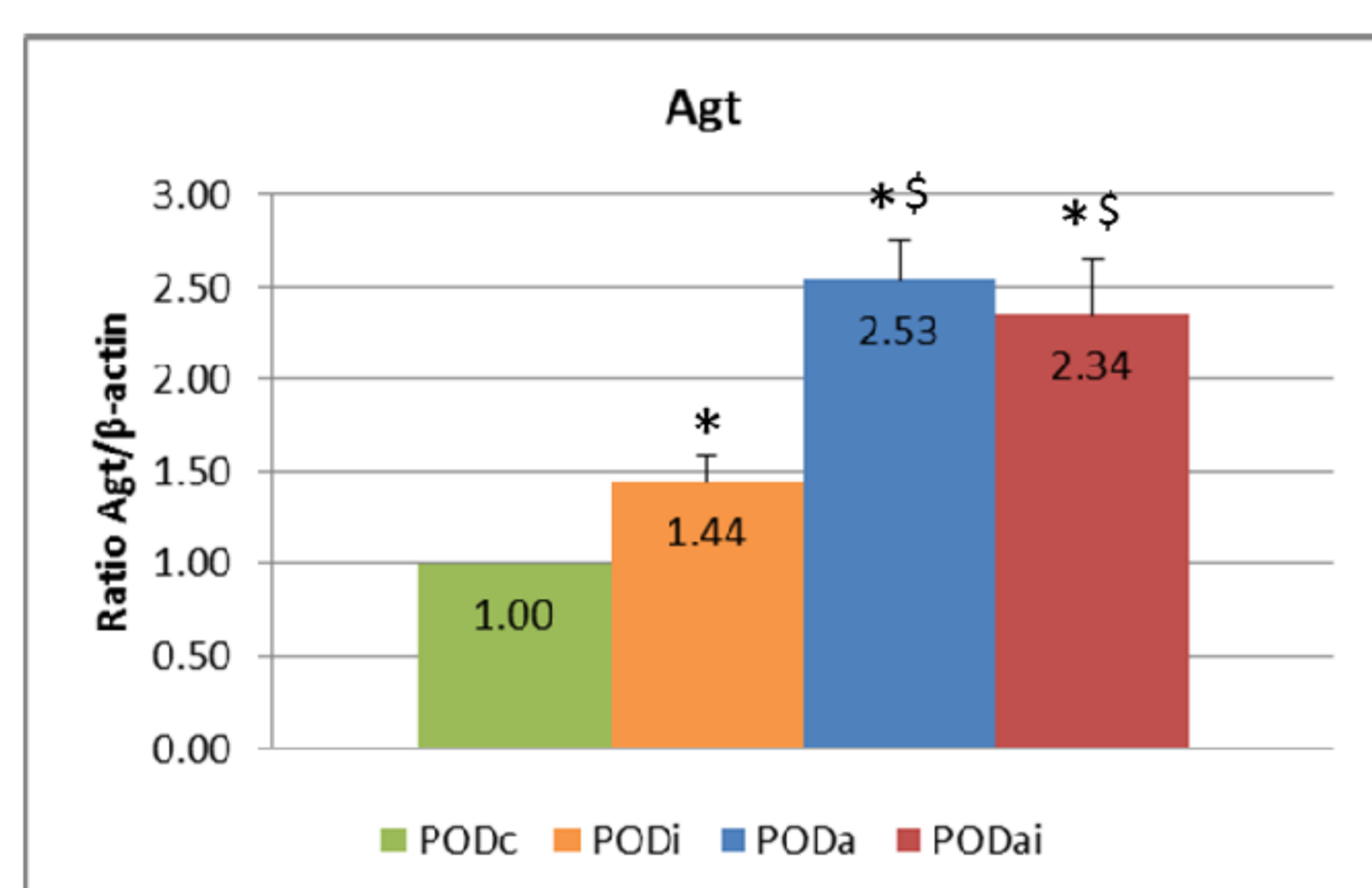


Figure 3. Agt gene expression

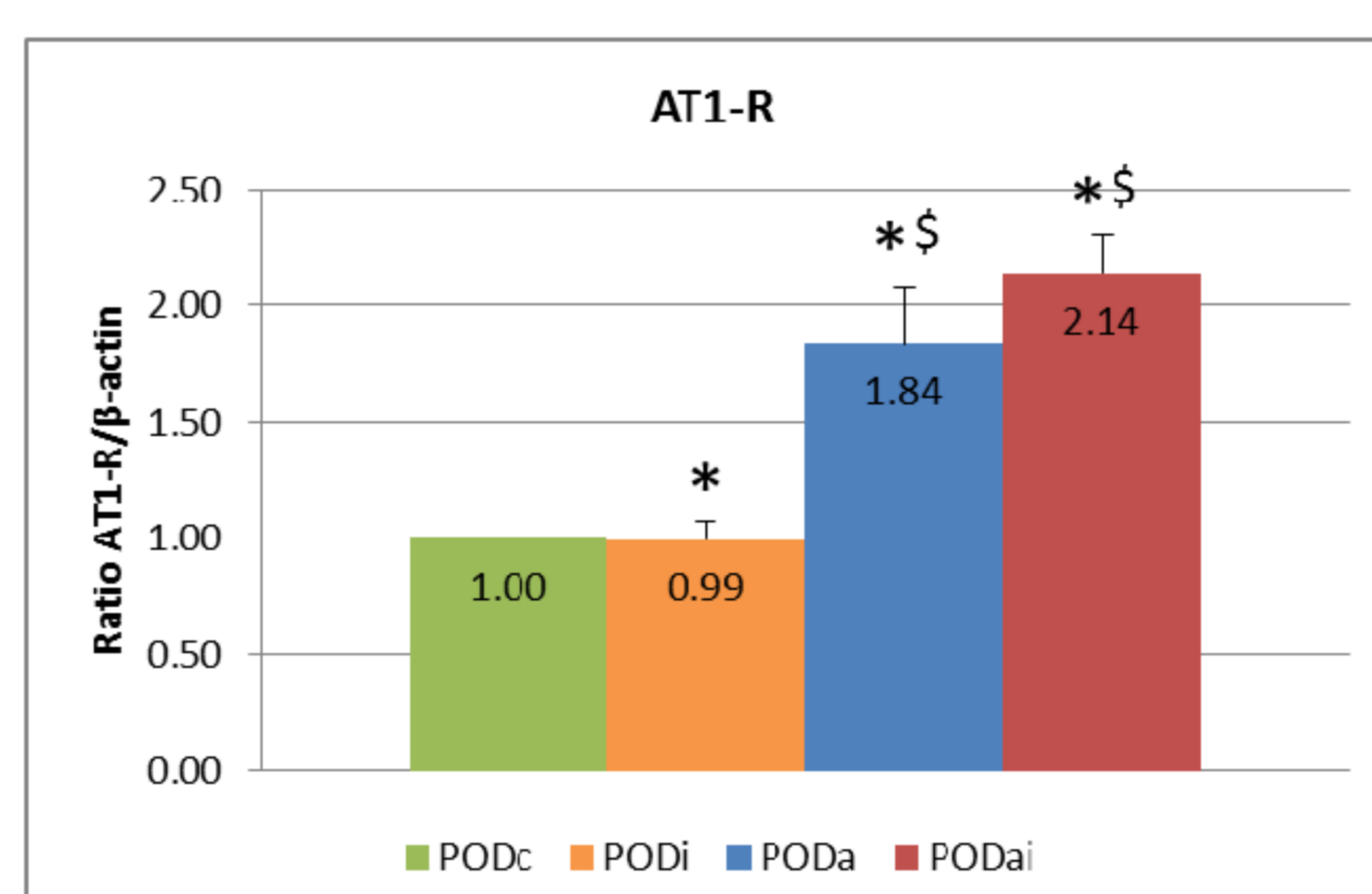


Figure 4. AT1-R gene expression

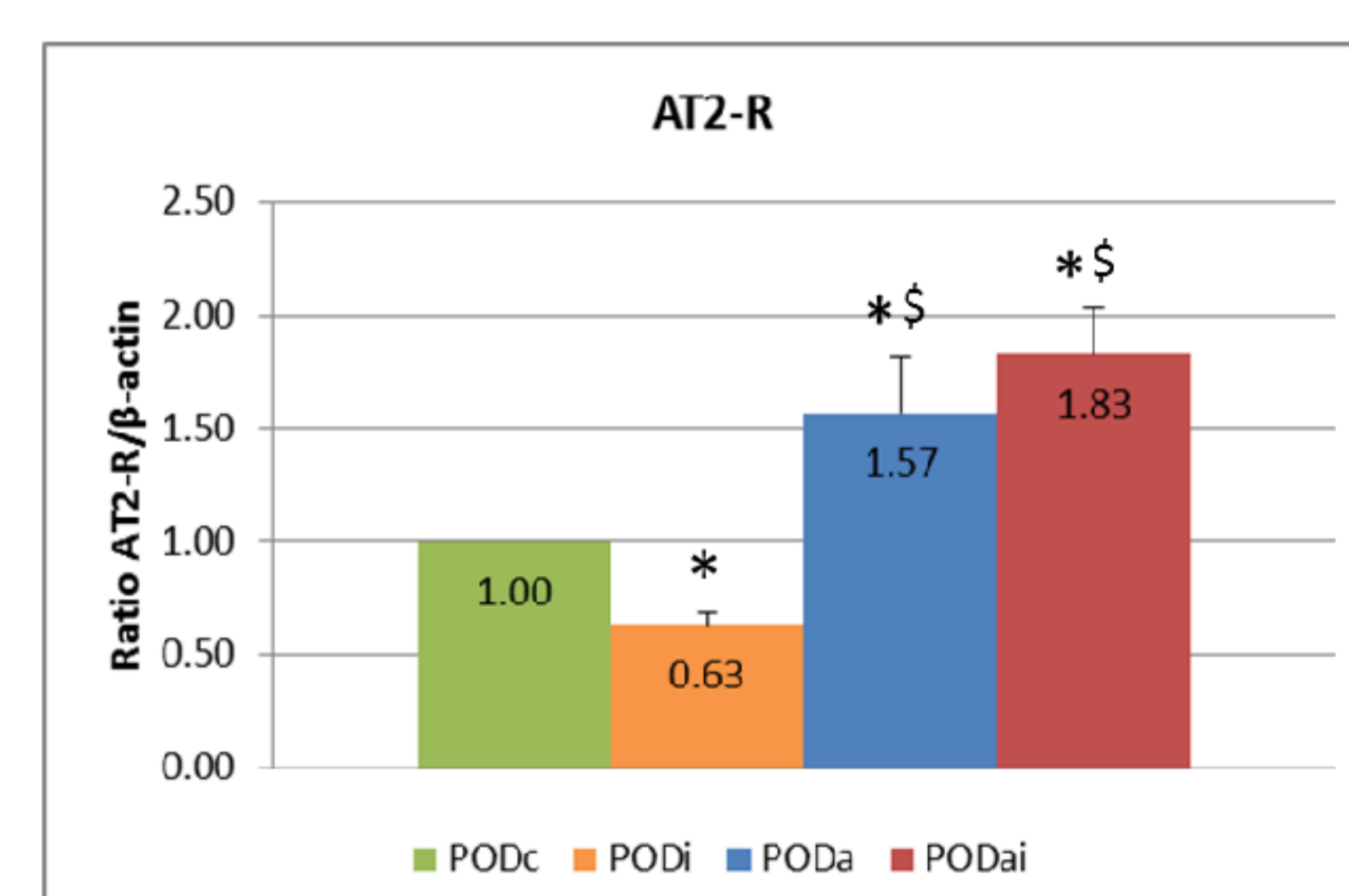


Figure 5. AT2-R gene expression

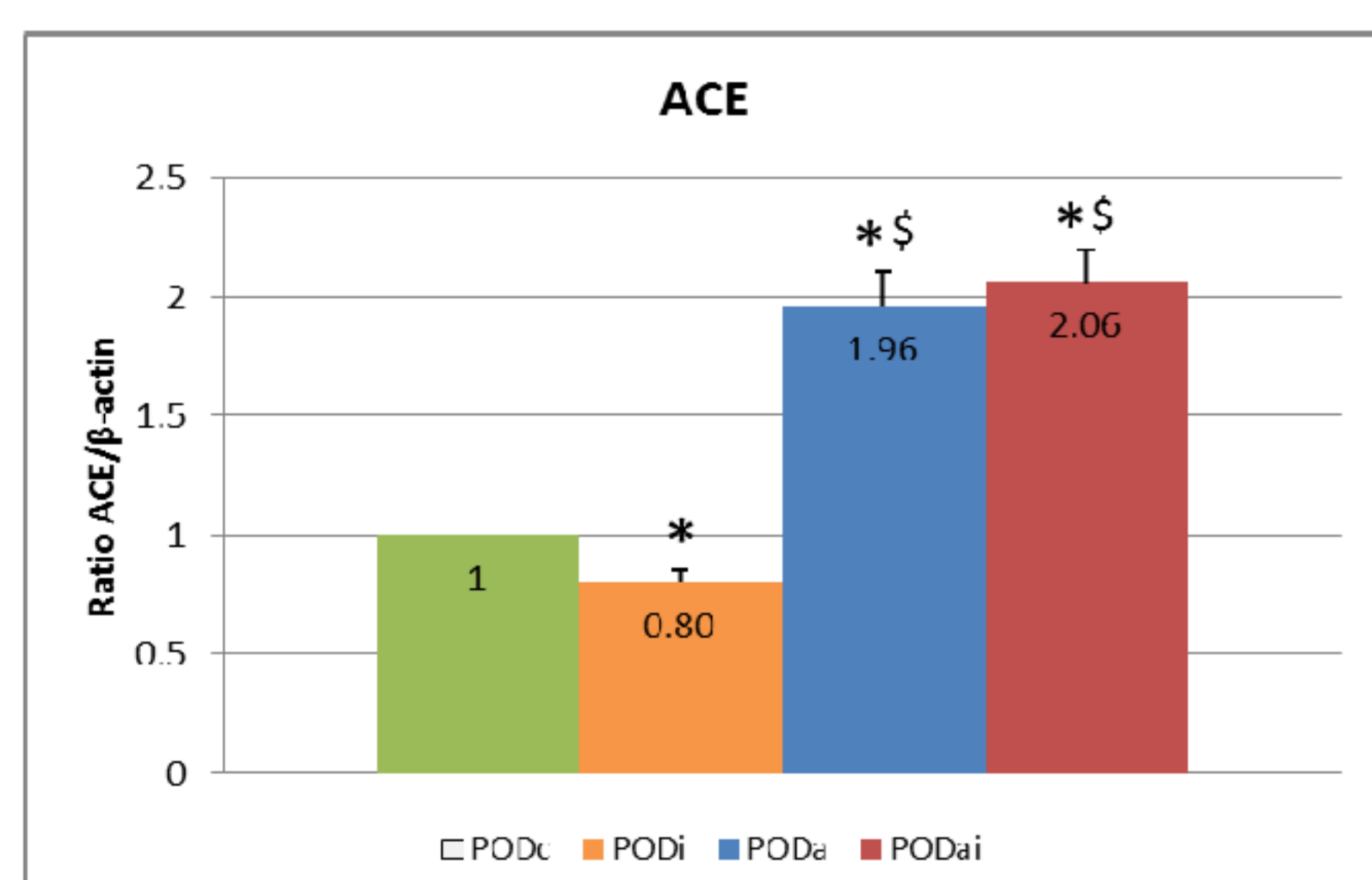


Figure 6. ACE gene expression

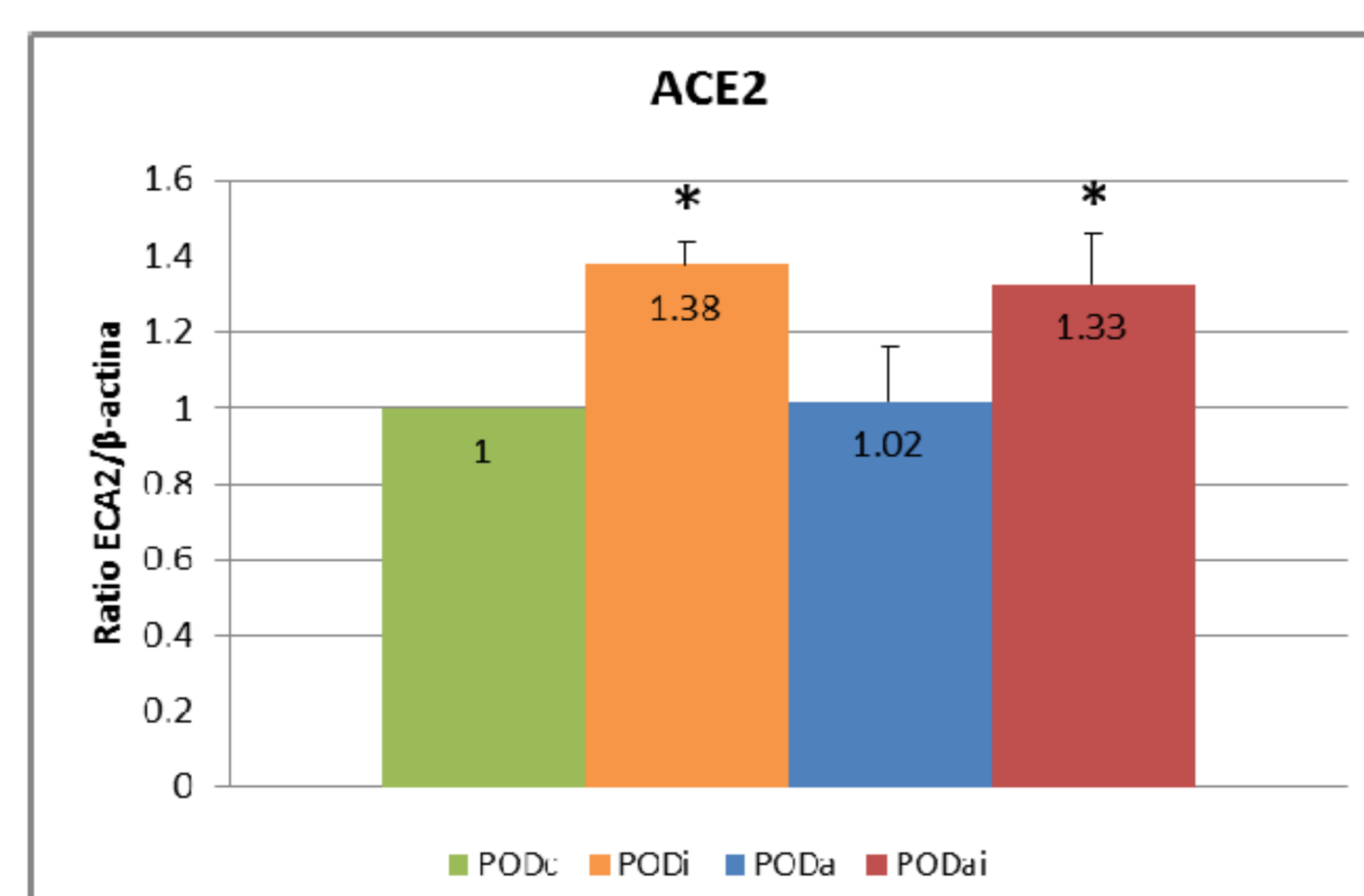


Figure 7. ACE2 gene expression

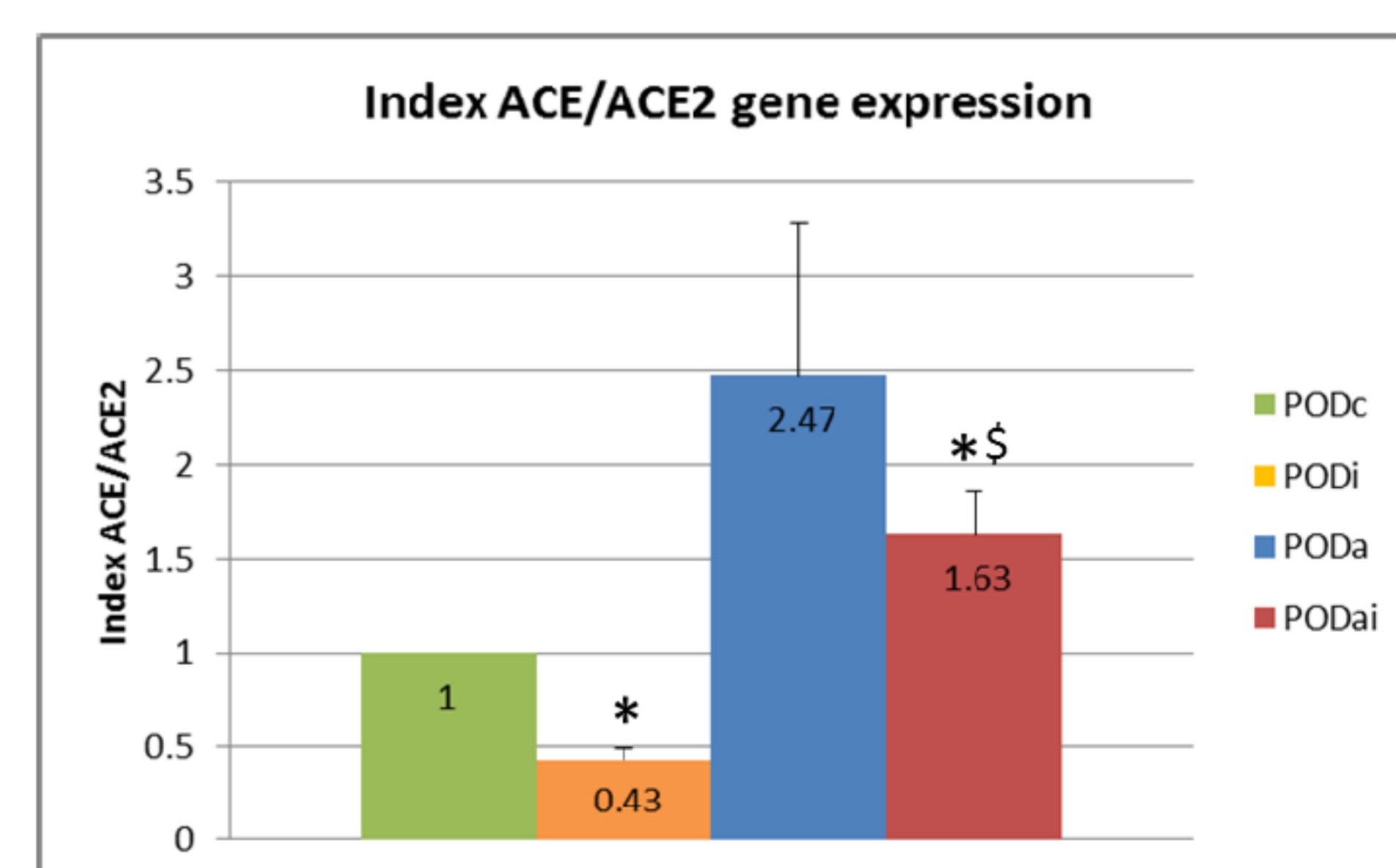


Figure 8. The balance in RAS enzymes: Index ACE/ACE2 gene expression

*p<0.05 vs PODc; \$p<0.05 vs PODi.

Conclusions

- Podocytes react in a **diabetic milieu** modifying their RAS gene expression profile.
- Insulin favors an “anti-Angiotensin II” gene expression profile, but is not capable of reversing the “pro-Angiotensin II” effect caused by the presence of albumin.

References

- Pavenstadt H et al. *Physiol Rev.* 2003 Jan;83(1):253-307.
- Shankland SJ et al. *KI 72*, 26-36 July (1) 2007.
- Liebau MC et al. *Am J Physiol Renal Physiol.* 2006 Mar;290(3):F710-9.
- Velez JC et al. *Am J Physiol Renal Physiol.* 2007 Jul;293(1):F398-407.
- Coward RJ et al. *Diabetes.* 2005 Nov;54(11):3095-102.

