ANGIOTENSIN CONVERTING ENZYME 2 DELETION INCREASES ACE EXPRESSION AND OXIDATIVE STRESS IN PANCREAS FROM NOD MICE

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INTRODUCTION AND AIMS

- Angiotensin converting enzyme 2 (ACE2) acts as a negative regulator of the renin-angiotensin system (RAS)[1]. ACE2 is altered in diabetic nephropathy (DN)[2,3]. Downregulation of ACE2 either by gene deletion or by pharmacological inhibition worsens renal injury and arterial hypertension[4,5,6].
- The non-obese diabetic (NOD) mice is a strain which spontaneously develops autoimmune diabetes, mimicking type 1 diabetes (DM1) in human[7].
- The effect of ACE2 deletion on pancreas from NOD mice in an early pre-diabetic stage on glucose homeostasis, insulin secretion, RAS and oxidative stress has not been previously studied.

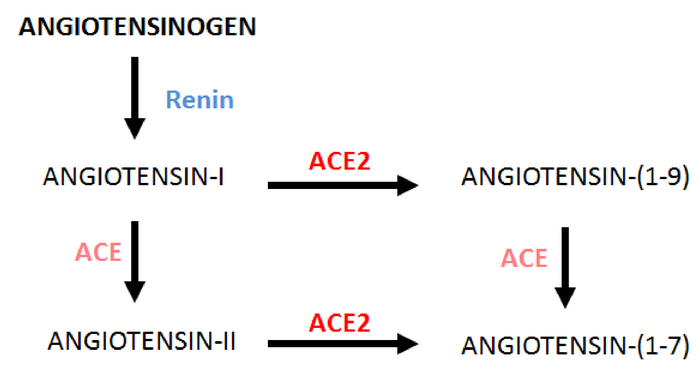


Figure 1: Schematic diagram of main processing pathways for angiotensin peptide generation and degradation through ACE and ACE2.

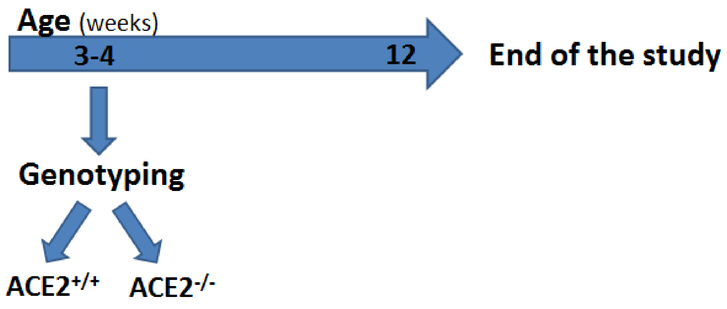
METHODS

NOD/ShiLtJ strain was crossed with ACE2KO mice to obtain the new mouse strain (NOD-ACE2$^{+/+}$ and NOD-ACE2$^{-/-}$). Mice genotype was determined at 3-4 weeks. NOD animals were euthanized at 12 weeks old (before diabetes development).

Parameters assessed:

- Glucose tolerance test by intraperitoneal administration of D-glucose (2g/Kg). Circulating glucose levels were determined at 0, 15, 30, 60 and 120 minutes after glucose bolus.
- Insulin secretion by ELISA at 0, 2 and 5 minutes after glucose bolus.
- Immunohistochemistry for insulin, ACE, angiotensin II receptor 1 (AT1R) and nitrotyrosine in pancreas.


Female NOD mice



RESULTS

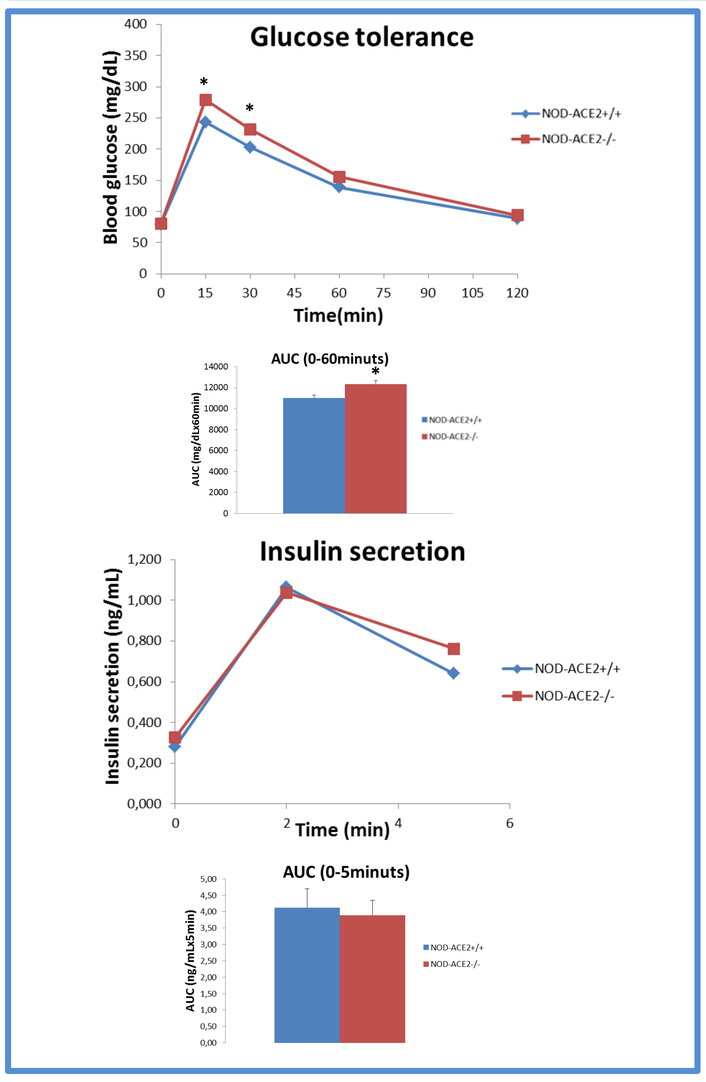


Figure 1: Glucose tolerance and insulin secretion in NOD-ACE2$^{+/+}$ and NOD-ACE2$^{-/-}$ mice. NOD-ACE2$^{-/-}$ mice show less tolerance to glucose bolus administration and less insulin secretion in response to glucose bolus. *p≤0.05 NOD-ACE2$^{-/-}$ vs NOD-ACE2$^{+/+}$.

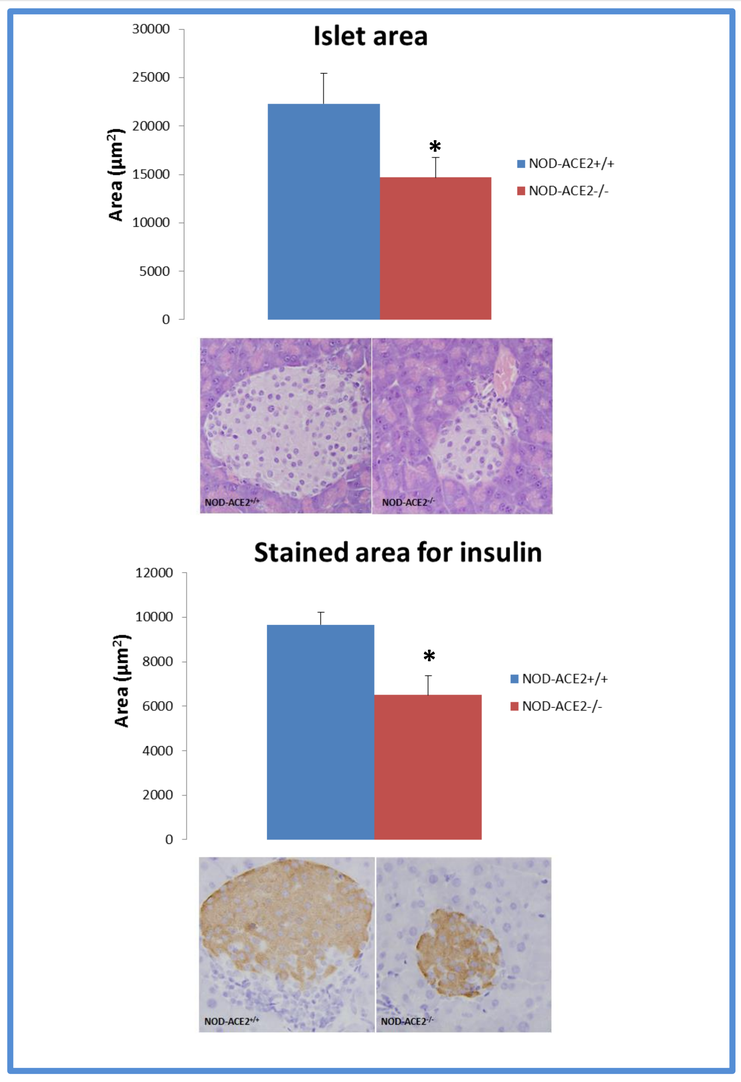


Figure 2: Islet area and stained area for insulin in NOD-ACE2$^{+/+}$ and NOD-ACE2$^{-/-}$ mice. NOD-ACE2$^{-/-}$ mice present lower islet area and less area stained for insulin in comparison with NOD-ACE2$^{+/+}$ mice. These results suggested that NOD-ACE$^{-/-}$ mice produce less insulin because of a smaller islet area. *p≤0.05 NOD-ECA2$^{-/-}$ vs NOD-ECA2$^{+/+}$.

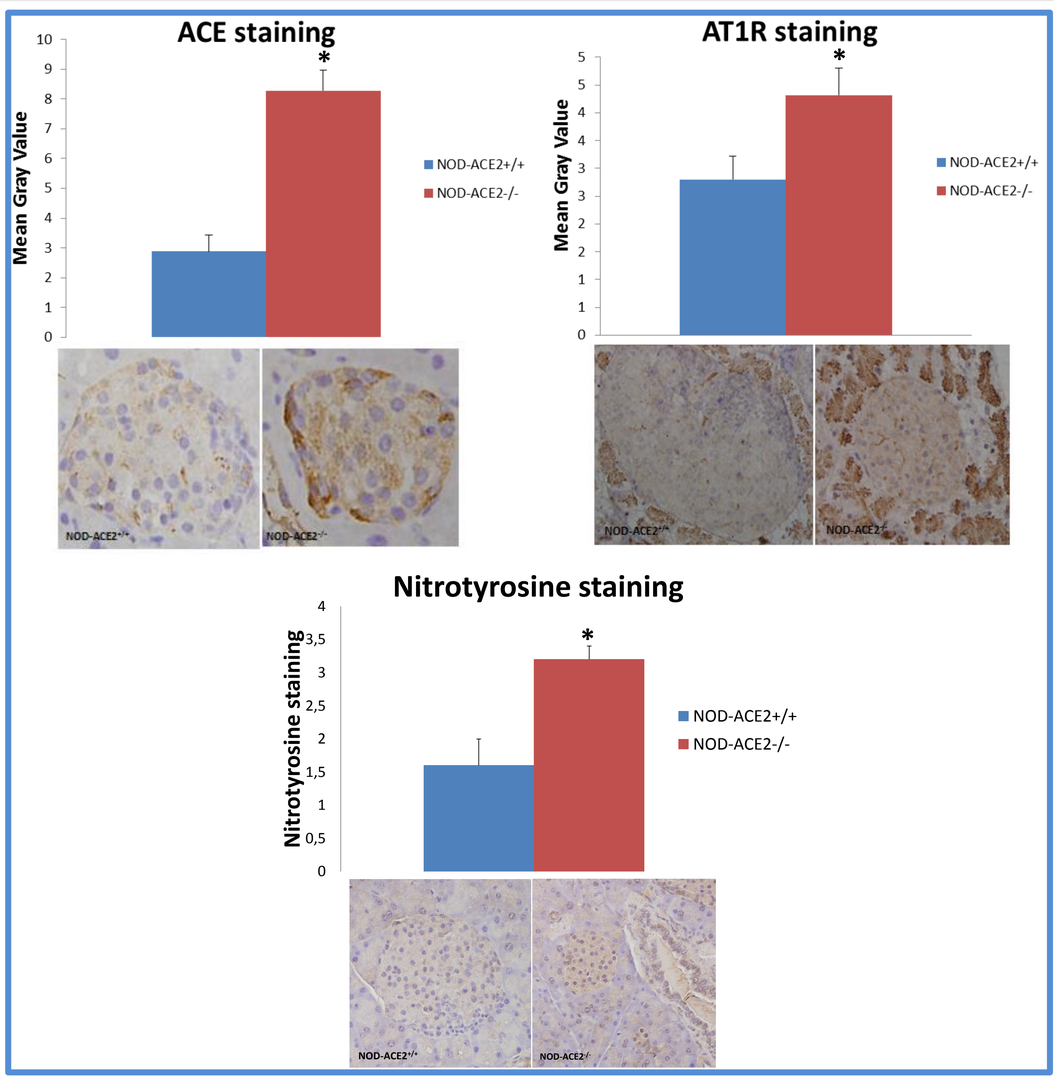


Figure 3: ACE, AT1R and nitrotyrosine staining in pancreas from NOD-ACE2$^{+/+}$ and NOD-ACE2$^{-/-}$ mice. NOD-ACE$^{-/-}$ mice show higher ACE, AT1R and nitrotyrosine protein levels in comparison to wild-type mice. Nitrotyrosine levels were analyzed in a semiquantitative manner (scale 0-4).
*p≤0,05 NOD-ECA2$^{-/-}$ vs NOD-ECA2$^{+/+}$.

CONCLUSIONS

- NOD-ACE2$^{-/-}$ mice present altered glucose tolerance, functional and morphological alterations at pancreatic level due to insulin synthesis and secretion as well as decreased islet size.
- ACE2 deletion leads to a worsening of glucose homeostasis in NOD mice accompanied to higher levels of oxidative stress.
- ACE2 deletion stimulates RAS pathways by increasing the expression of ACE and AT1R.

References

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Diabetes -Basic science
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DOI: 10.3252/pso.eu.54ERA.2017
ePosters supported by F. Hoffmann- La Roche Ltd.
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