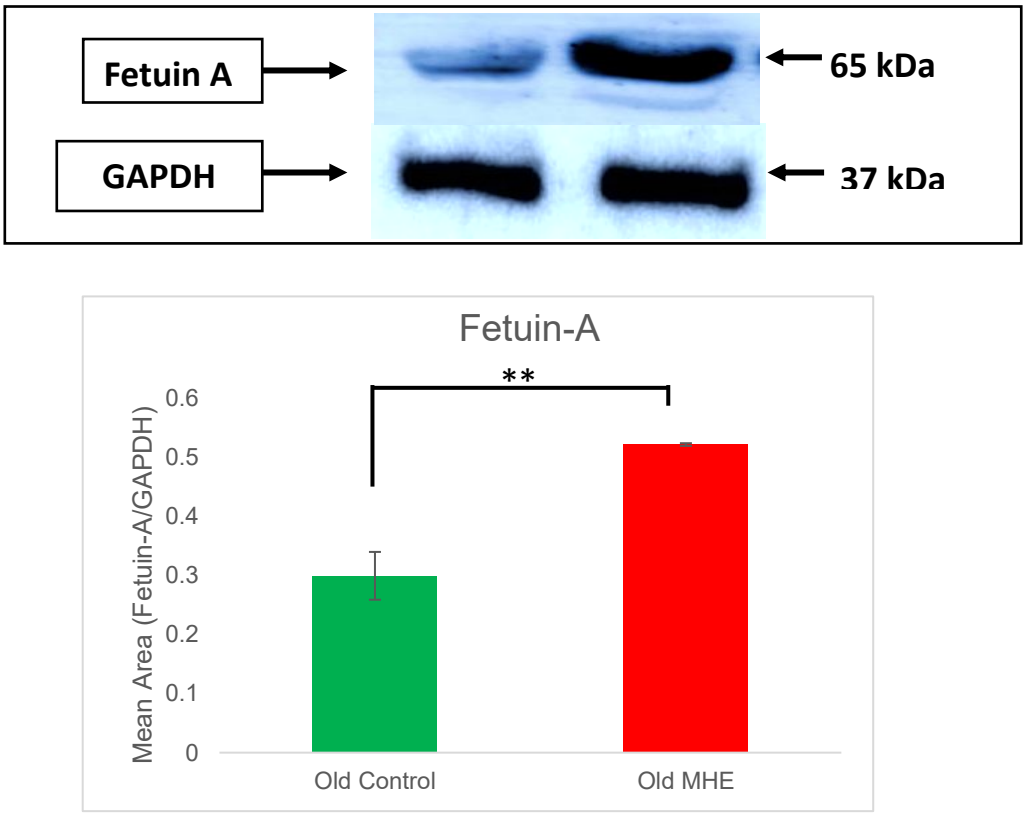
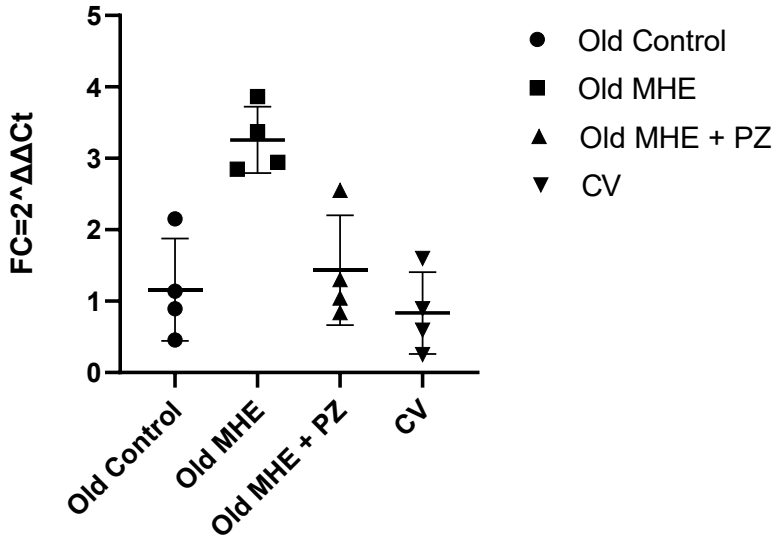
Supplementary Method

Real-time polymerase chain reaction (real-time PCR)

Total RNA was isolated using TRI reagent from the hippocampi of old control, old MHE, and Old MHE rats treated with pioglitazone and Control vehicle (n = 3 per group), and cDNA was synthesized using a cDNA synthesis kit. This was followed by qPCR to measure Fetuin-A gene expression using SYBR green-based real-time PCR on a Quant Studio™ 5 system. For primer details, see Supplementary Table 1. The analysis was performed using the 2−ΔΔCt method (Bustin et al. 2009). The Ct value of GAPDH, a housekeeping gene, was used as the internal control.



Supplementary Figure S1: Validation of Fetuin-A by western blotting in serum. Bar graph showing the fold change in the expression of Fetuin-A in the serum of the old MHE rats compared to the old control rats.



Supplementary Figure S2: Expression pattern of Fetuin-A by qPCR at the transcript level. Bar graph showing the fold change in the expression of the Fetuin-A mRNAs in respective experimental groups.

Supplementary Table 1

Primer pair	Fetuin-A primer Sequence
Forward Primer (5'->3')	AGTTATTCCAGACACAGCCCC
Reverse Primer (3'->5')	GTCCTACCACCACGGATTCA