

**Effects of pravastatin or 12/15  
lipoyxygenase pathway inhibitors  
on indices of diabetic  
nephropathy in an experimental  
model of diabetic renal disease**

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# INTRODUCTION

# *Diabetic nephropathy (DN)*

- DN is defined as the appearance of persistent ‘clinical’ albuminuria in an individual with diabetes for more than 5 years and concomitant retinopathy, in the absence of urinary tract infection, other renal diseases and heart failure.
- People with both type 1 and type 2 diabetes are at risk.
- The risk is higher if blood-glucose levels are poorly controlled.

- Significant structural changes, particularly thickening of the glomerular basement membrane and mesangial expansion occur only after several years of diabetes.
- At this stage, the kidney may start allowing more serum albumin than normal in the urine (microalbuminuria).
- As DN progresses, increasing numbers of glomeruli are destroyed by nodular glomerulosclerosis. Now the amounts of albumin being excreted in the urine increases, and may be detected by ordinary urine analysis techniques.

**Therefore, DN is categorized into two stages:**

- a. Microalbuminuria: (urinary albumin excretion [UAE]  $>20 \mu\text{g}/\text{min}$  and  $\leq 199 \mu\text{g}/\text{min}$ )
- b. Macroalbuminuria: (UAE  $\geq 200 \mu\text{g}/\text{min}$ ).

# *Lipoxygenases (LOs)*

- LOs are a family of non-heme iron containing enzymes that insert molecular O<sub>2</sub> into polyunsaturated fatty acids.
- They are classified as 5-,8-,12-, and 15-LOs according to the carbon atom of arachidonic acid at which oxygen is inserted.
- Three major isoforms of 12-LO are:
  - Platelet-type
  - Macrophage- or leukocyte-type
  - Epidermal-type

- Human and rabbit 15-LOs, as well as leukocyte-type 12-LO have high homology, and are classified as 12/15 LOs.
- Studies have indicated the presence of leukocyte-type 12/15-LO in various cells, including vascular smooth muscle cells, brain, and kidney.

- Substantial evidence suggests that 12/15-LO play an important role in systemic homeostasis and renal-cardiovascular pathology, through mediating growth factor effects in vascular smooth muscle cells, fibroblasts, and mesangial cells, as well as responses to vascular injury.
- Deletion of the 12/15-LO gene is associated with reduced atherosclerosis in animal models.



- High glucose levels have been shown to directly increase 12/15-LO expression in cultured mesangial cells.
- Glomerular 12/15-LO is found to increase in streptozotocin (STZ)-induced diabetic rats associated with an early increase in glomerular activated nuclear transcription factor cyclic-AMP-responsive binding protein, thereby implicating the 12/15-LO pathway in the pathogenesis of the expanded mesangial matrix characteristic of DN.

- Moreover, 12/15-LO pathway has been shown to be a critical mediator of angiotensin II-induced mesangial cell hypertrophy and extracellular matrix accumulation.
- Therefore, 12/15-LO inhibition by NDGA may be useful as a therapeutic strategy for prevention or amelioration of DN

# *Nordihydroguaiaretic acid (NDGA)*

- ✓ NDGA is a lignan found in large amounts in the “*Larrea tridentate*” plant.
- ✓ NDGA is a potent LO inhibitor that specifically inhibits the leukocyte 12/15- LO pathway.
- ✓ Research on this compound and its natural and synthetic derivatives has shown them to be potentially useful in the treatment of cancer, diabetes, viral, and bacterial infections.

## *Three-HMG-CoA reductase inhibition*

- Three-hydroxy 3-methylglutaryl Co-enzyme A reductase inhibition also has been shown to provide protection against a variety of renal diseases characterized by inflammation or enhanced cellular proliferation.
- Many of the beneficial effects occur independently of cholesterol lowering effects because they can alter cellular proliferation/apoptosis, reduce reactive oxygen species generation, and inhibit neutrophil and macrophage recruitment.
- More important is their action in increasing expression and activity of endothelial nitric oxide synthase, even under hypoxic conditions.

# AIM OF THE STUDY

**In light of these findings, the aim of this study is to:**

- Evaluate the effects of early STZ-induced diabetes on renal functions.
- Attenuate the diabetic renal inflammatory and vasomotor injury through supplementation with either pravastatin or NDGA, which specifically inhibits the leukocyte 12/15-LO pathway.
- Elucidate the effect of 12/15-LO inhibition on key indices of DN and its relation to inflammatory mediators of renal injury (as VEGF).
- Gain insight into the possible beneficial effects of pravastatin and NDGA in regulation of renal function, and so their use as therapeutics for DN.

# MATERIALS & METHODS

- Eighty eight male Wistar rats were housed in a controlled environment with free access to water ad libitum.
- To induce diabetes, rats were injected with STZ 65 mg/kg intraperitoneally.
- The development of diabetes was confirmed by tail-vein blood glucose levels (glucometer) on the third day after STZ injection.
- Rats whose blood glucose levels were between 300-500 mg/dl were selected.



Urinary albumin and creatinine were measured in each sample for selection of normoalbuminuric and microalbuminuric rats that were divided according to the following categories:

1. Non-diabetic control rats with normal ACR. Those rats received neither STZ nor any medication.
  2. Normoalbuminuric diabetic rats with urinary ACR  $<30$   $\mu\text{g}/\text{mg}$ .
  3. Microalbuminuric diabetic rats with urinary ACR in the range of 30-299  $\mu\text{g}/\text{mg}$  in at least 2 urine samples.
- The microalbuminuric group was defined as a DN group.

Male rats were assigned into 3 groups:

**Group I:** included 8 control rats receiving vehicle.

**Group II:** (8 rats/subgroup): included normoalbuminuric diabetic rats receiving vehicle (IIa), NDGA (IIb), both NDGA and insulin (IIc), pravastatin (IId), or both pravastatin and insulin (IIe).

**Group III:** (8 rats/subgroup): included microalbuminuric diabetic rats receiving vehicle (IIIa), NDGA (IIIb), both NDGA and insulin (IIIc), pravastatin (IIId), or both pravastatin and insulin (IIIe).

All the drugs and chemicals were supplied from Sigma (St. Louis, MO, USA).

# *Drug Regimen*

- Long acting insulin (ultralente) was administered subcutaneously at a dose of 3-4 U/day immediately after induction of diabetes and continued up to the end of the study (for 4 months).
- NDGA was injected subcutaneously, 5 mg/kg, daily for 4 months.
- Pravastatin was gavaged at a dose of 0.4 mg/kg in dilution of normal saline daily for 4 months

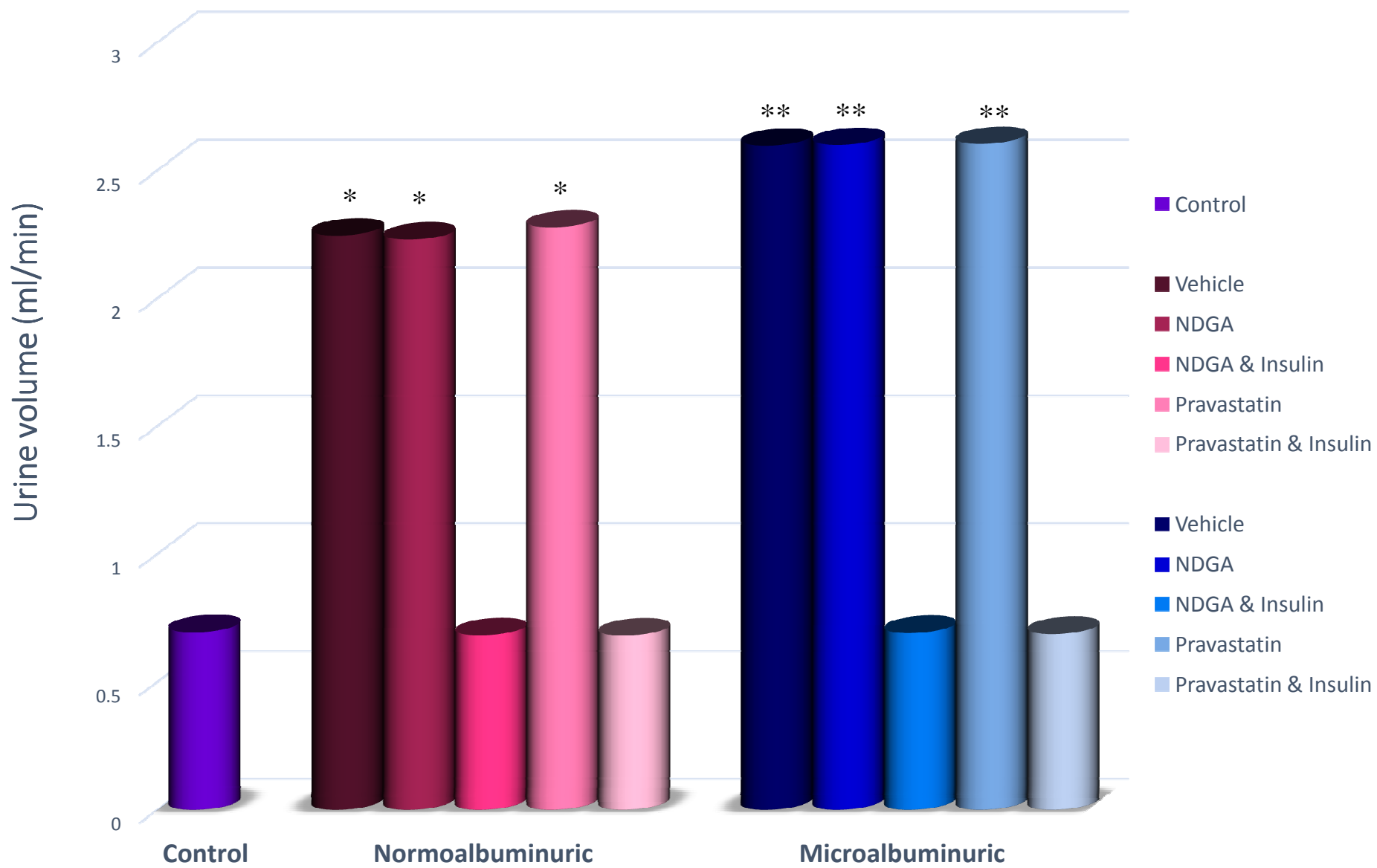
# *The measured parameters*

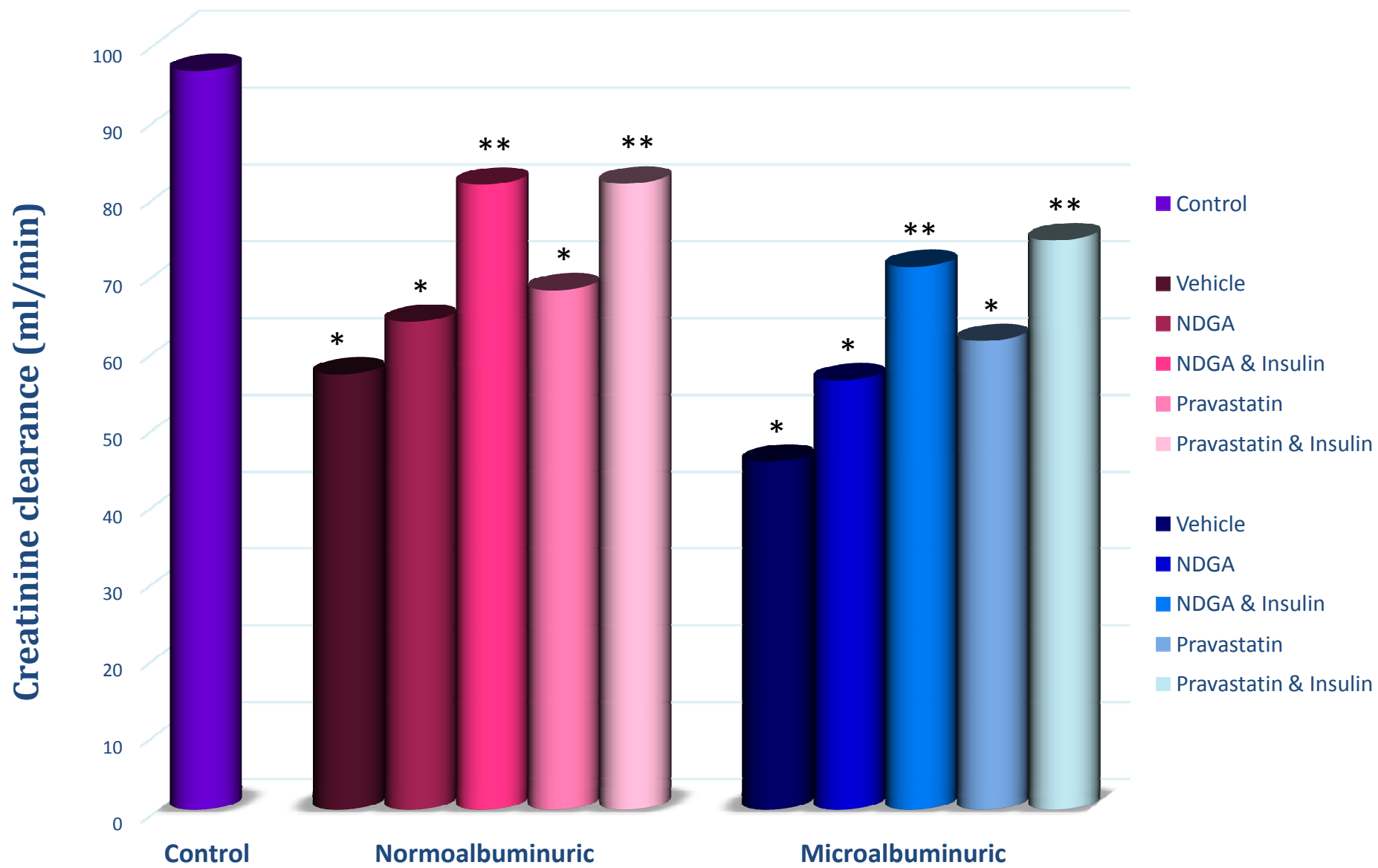
✿ Urine volume, creatinine clearance and urinary ACR were measured.

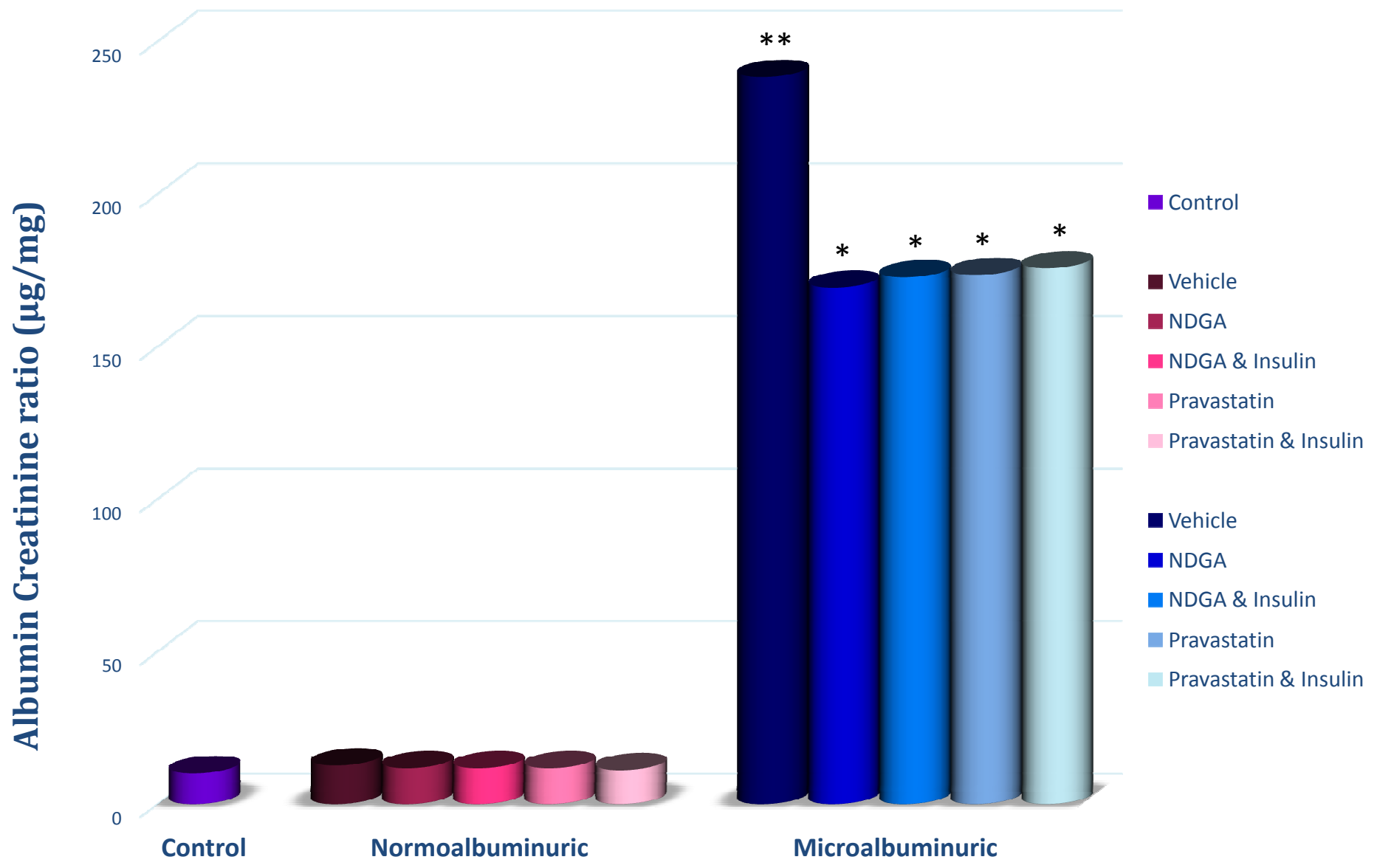
✿ Blood samples were analyzed for:

- Glycosylated hemoglobin (HbA1c)
- Cholesterol & triglycerides
- Total nitric oxide (NO) products as the sum of nitrite and nitrate
- Lipid peroxide
- Vascular endothelial growth factor (VEGF)
- Homocysteine (Hcy)

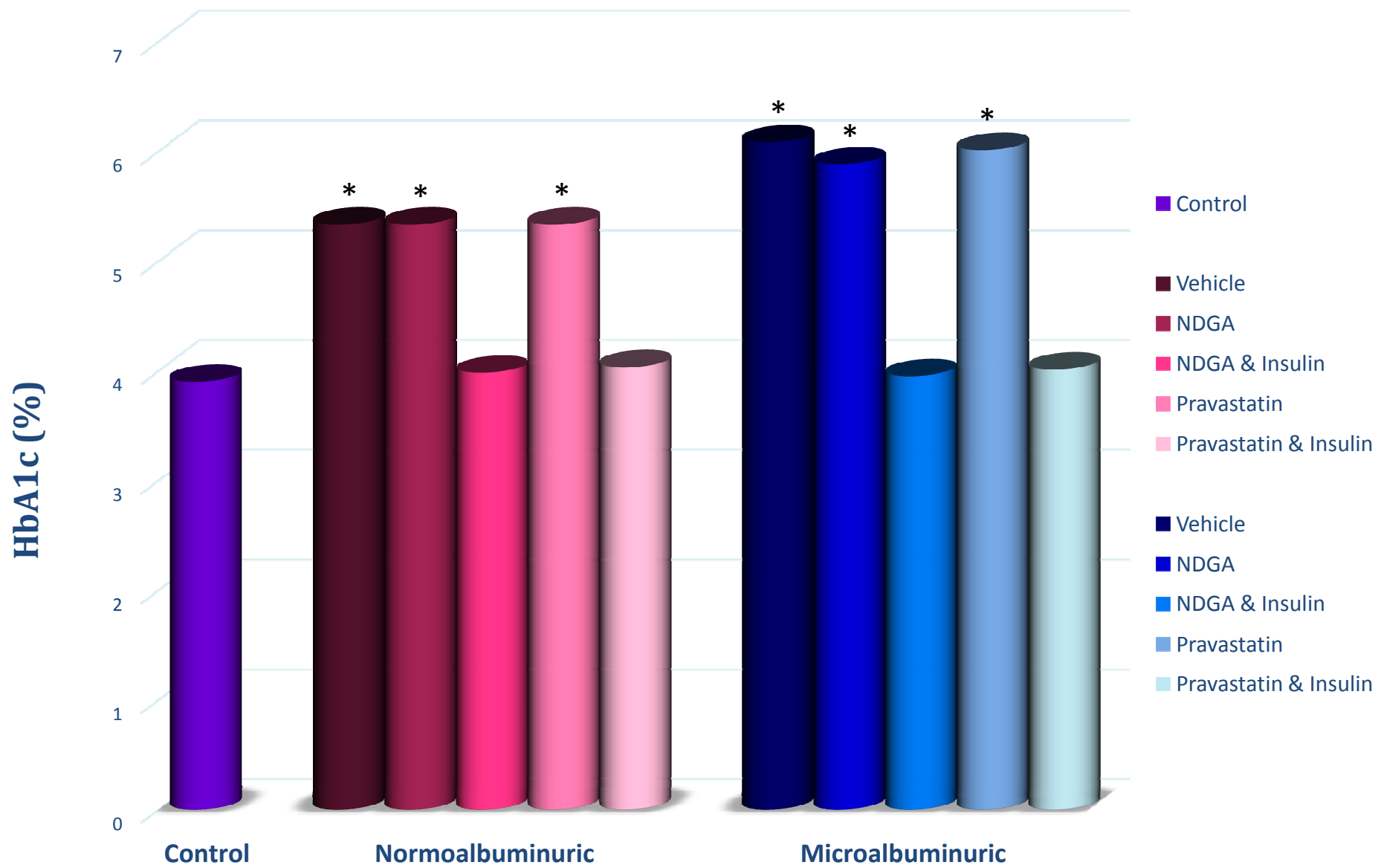
# RESULTS

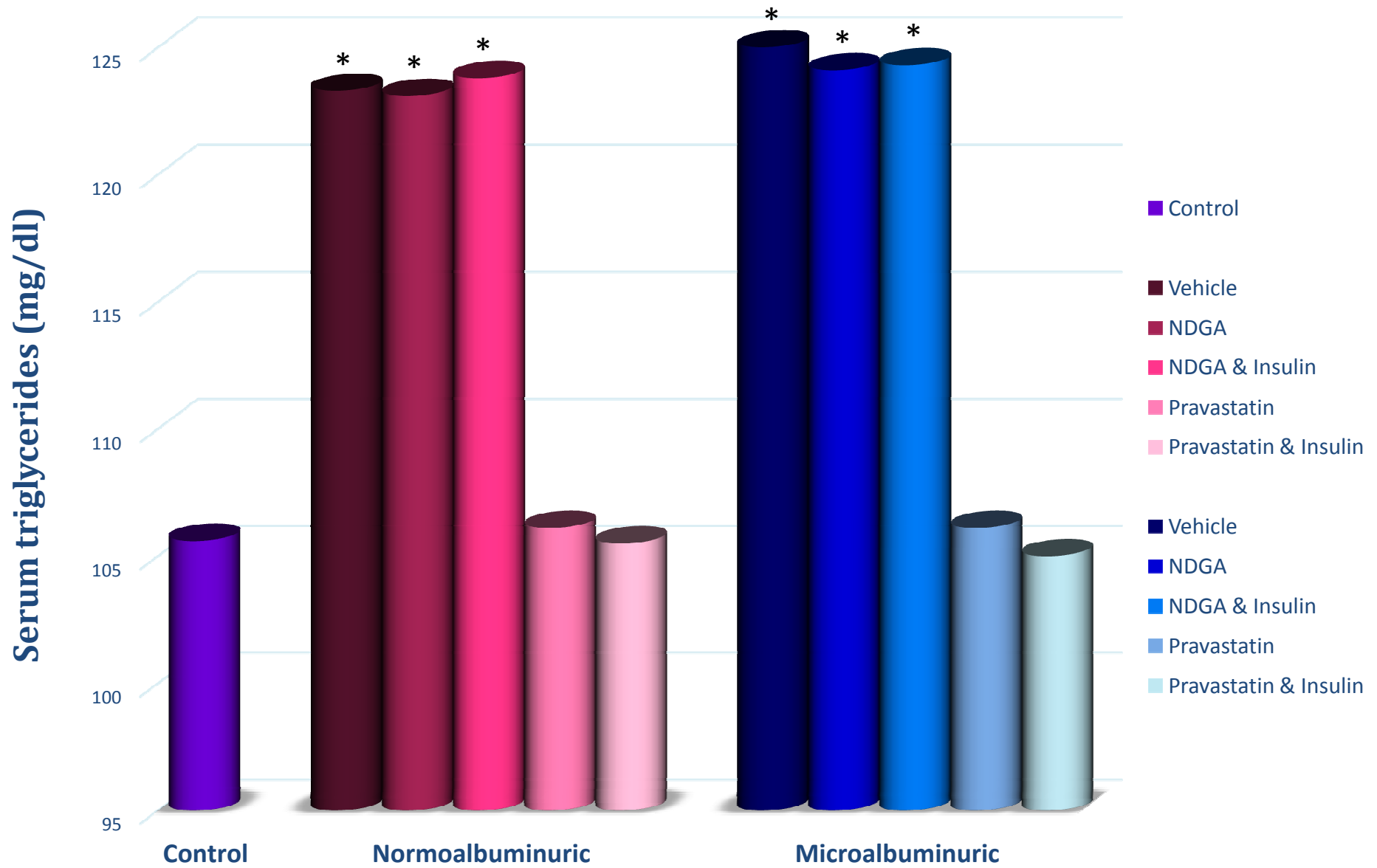


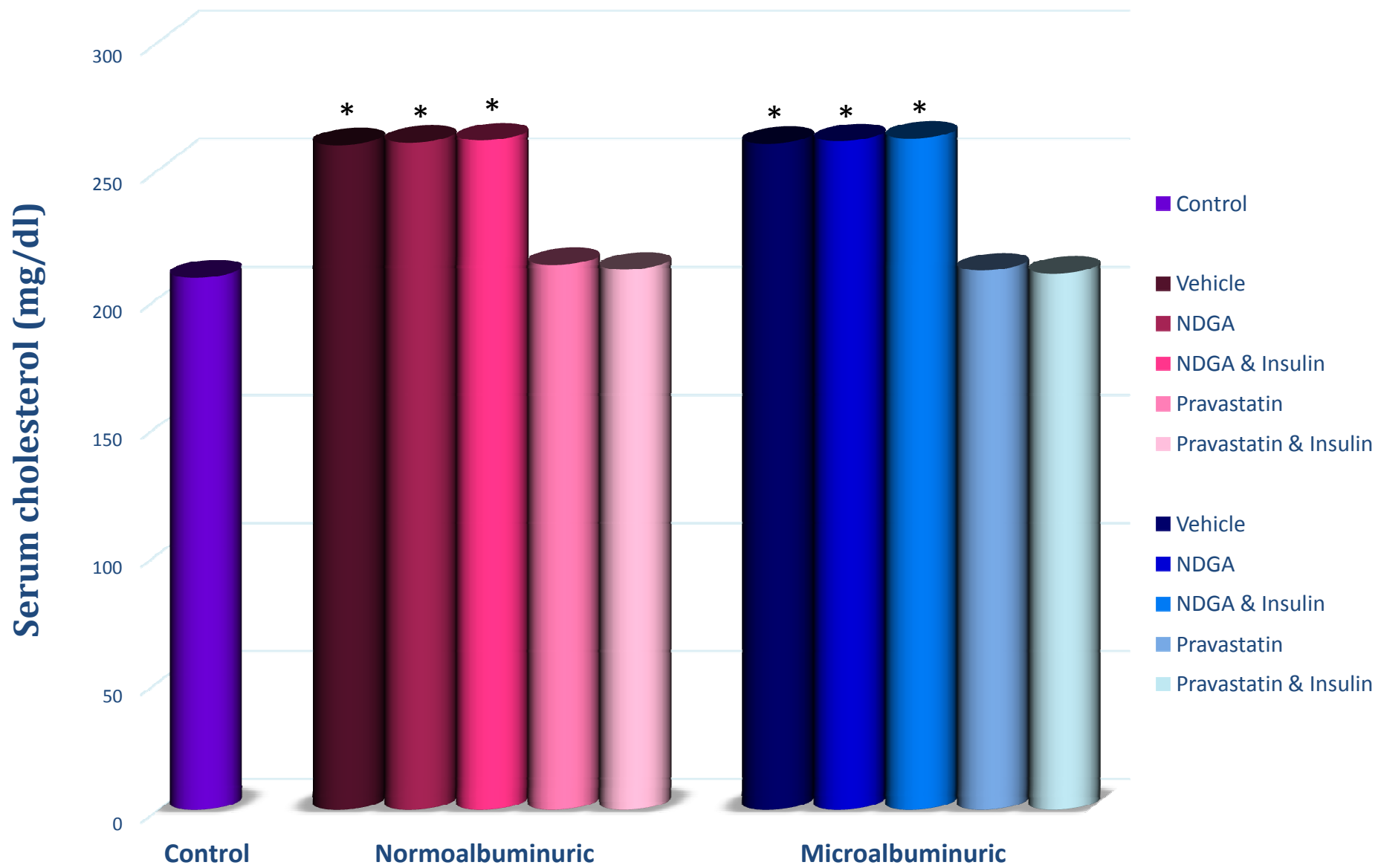


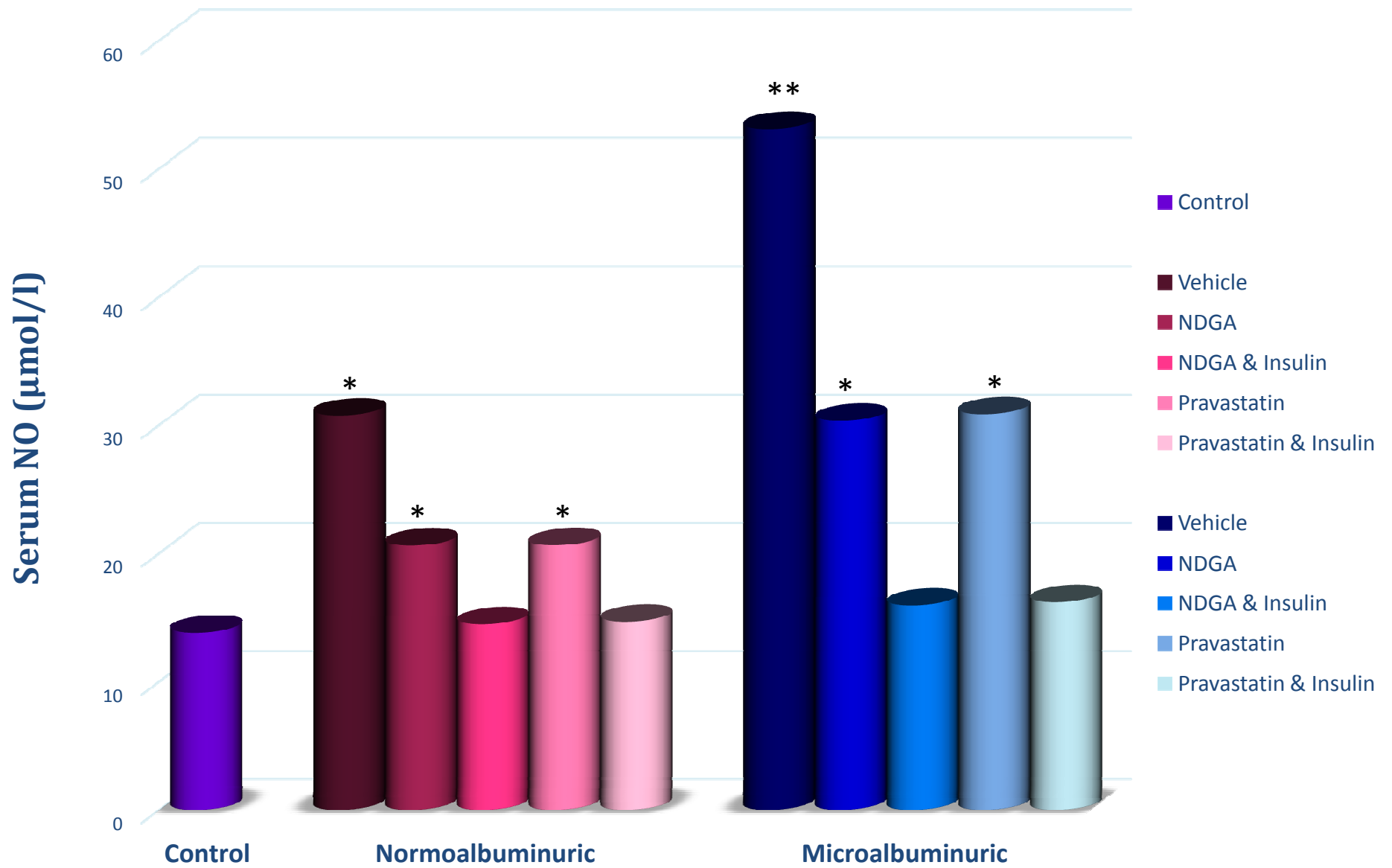




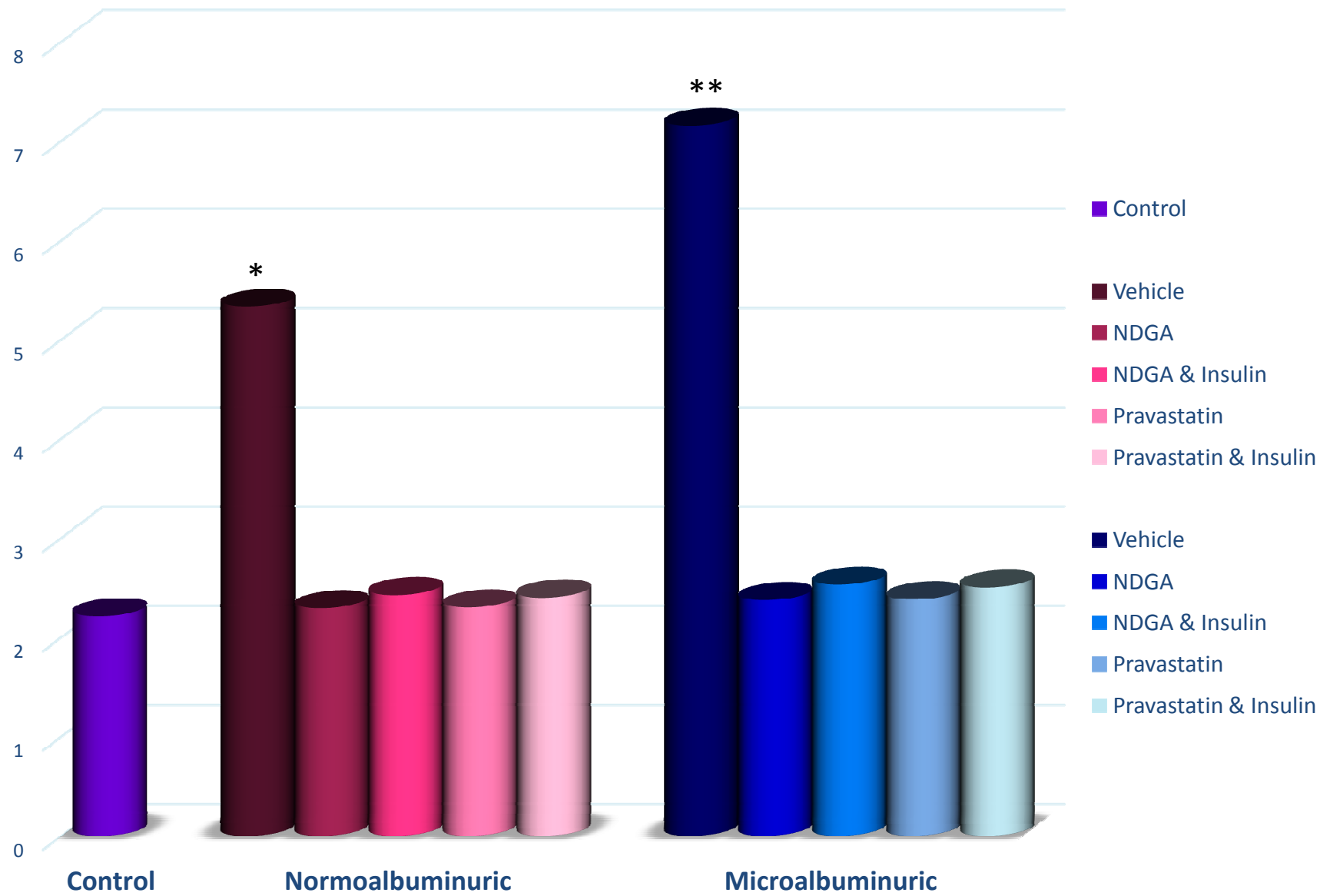


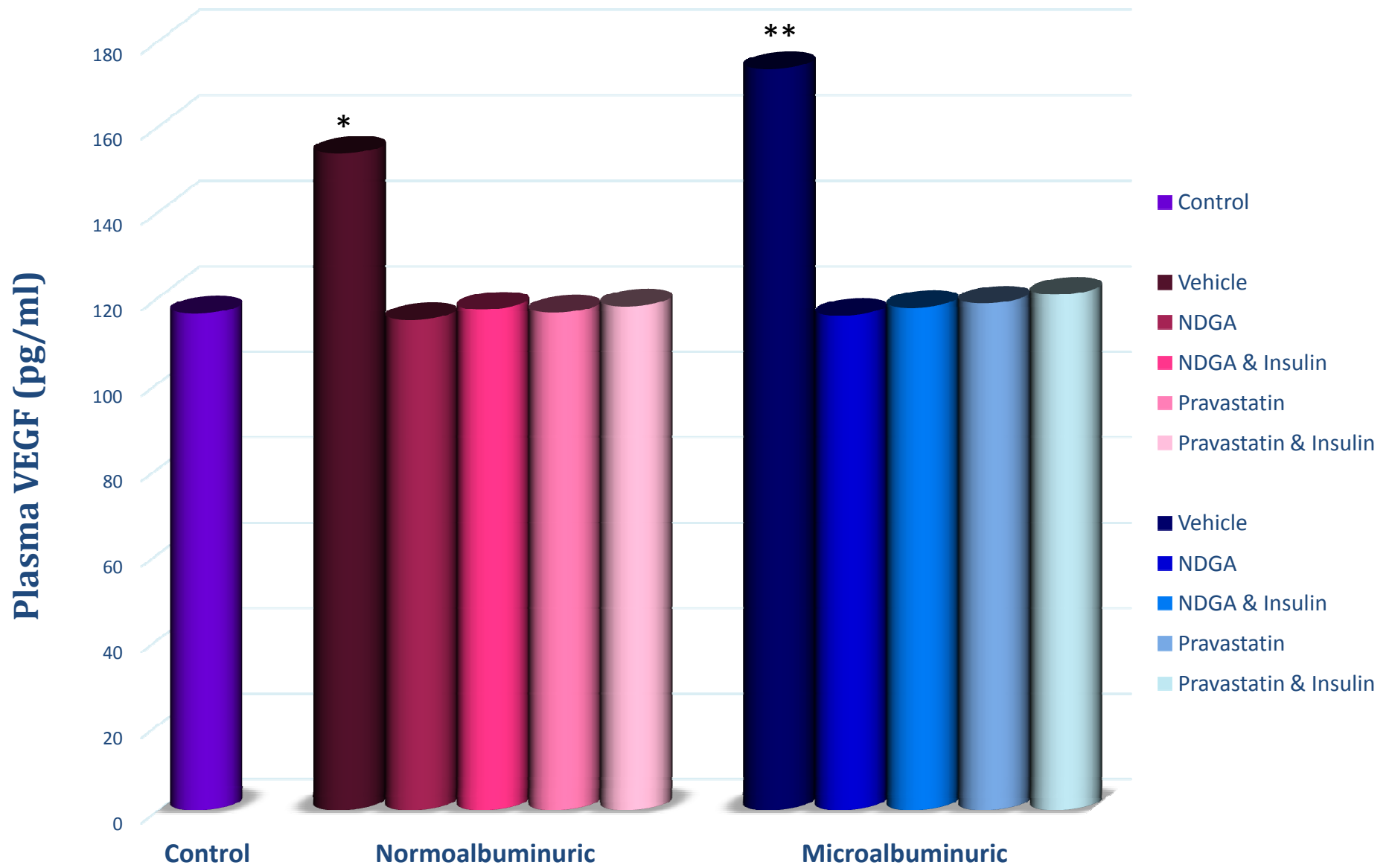




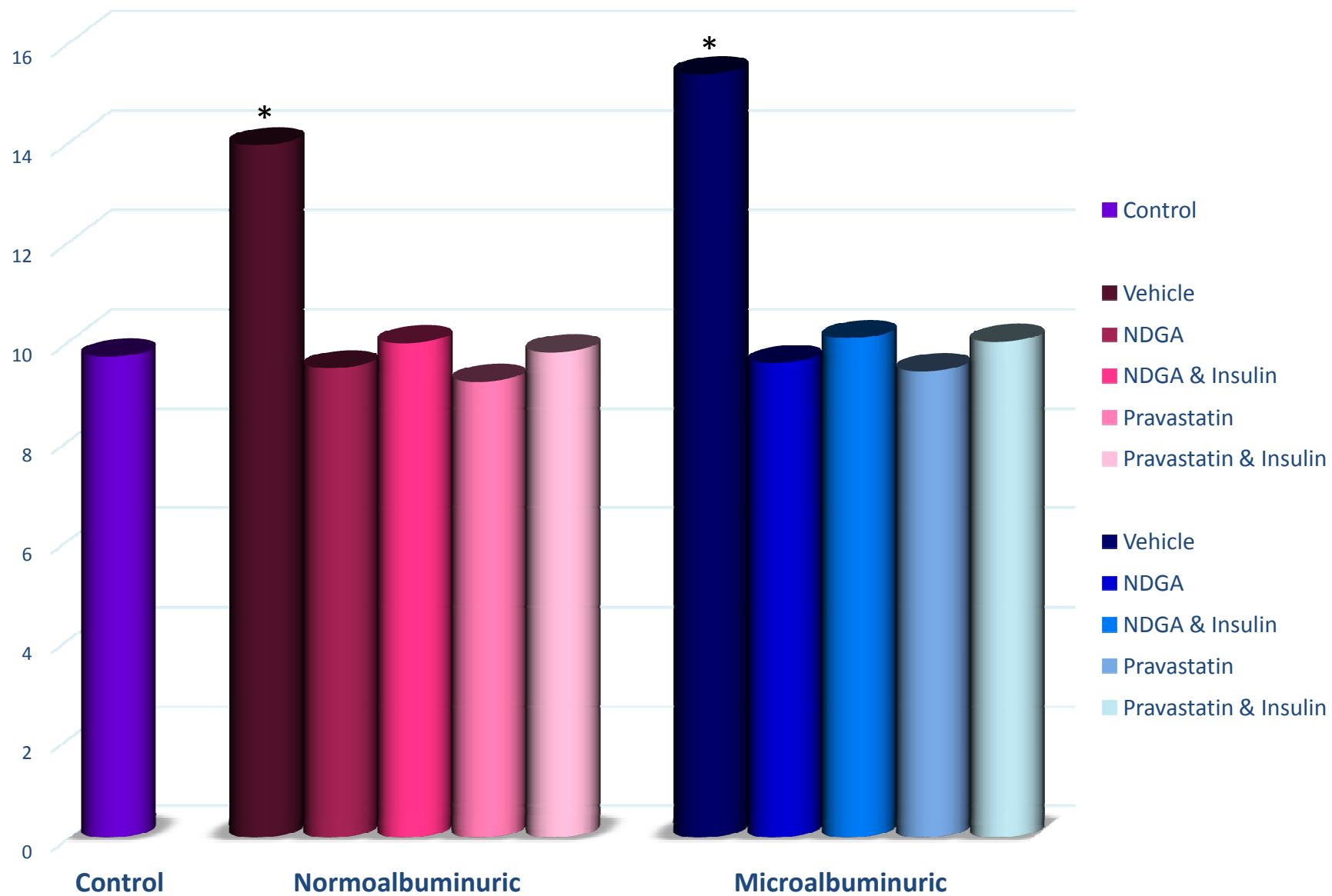


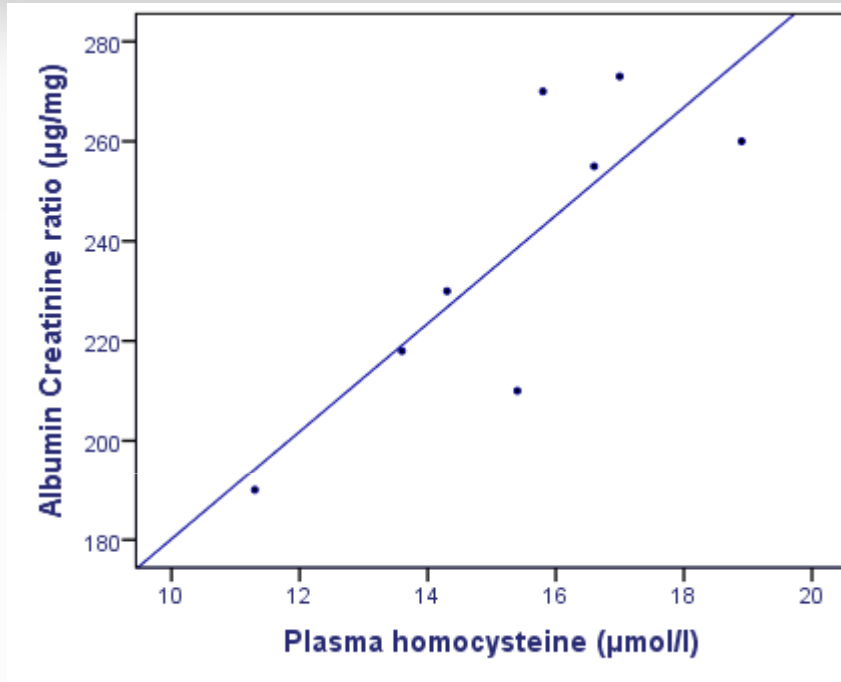
Serum lipid peroxide (nmol/l)



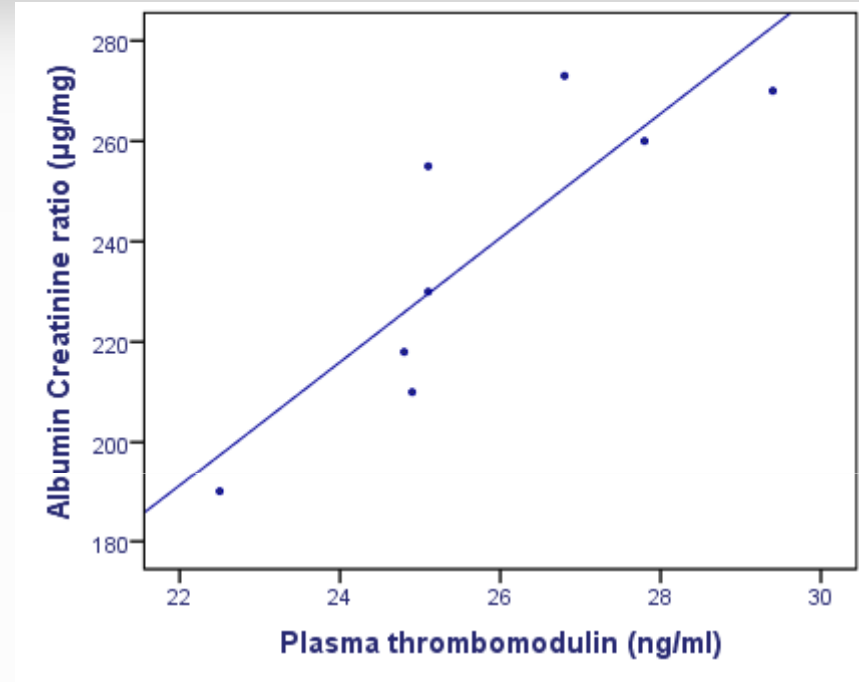


Plasma homocysteine ( $\mu\text{mol/l}$ )





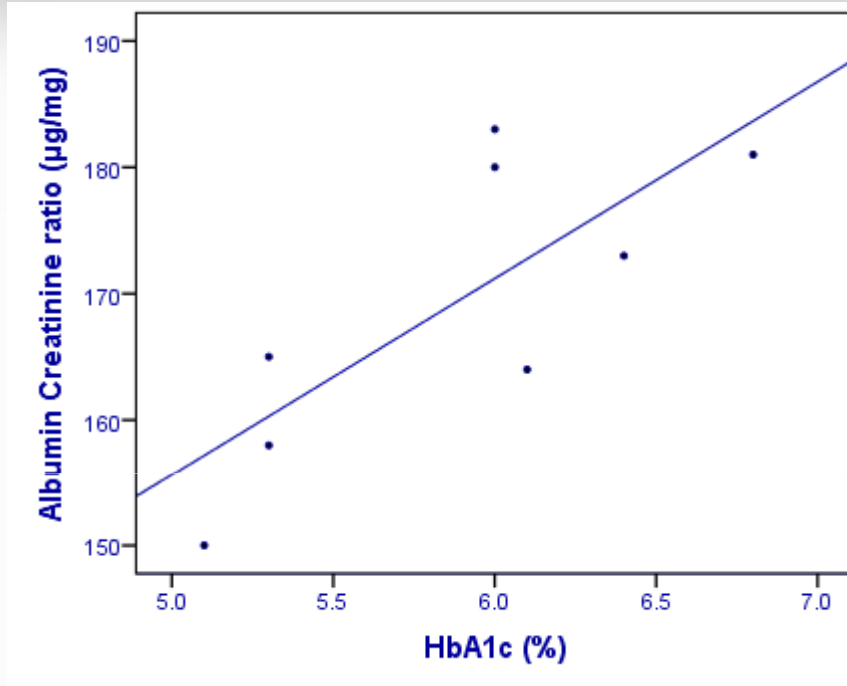
R= 0.820, P= 0.013\*\*



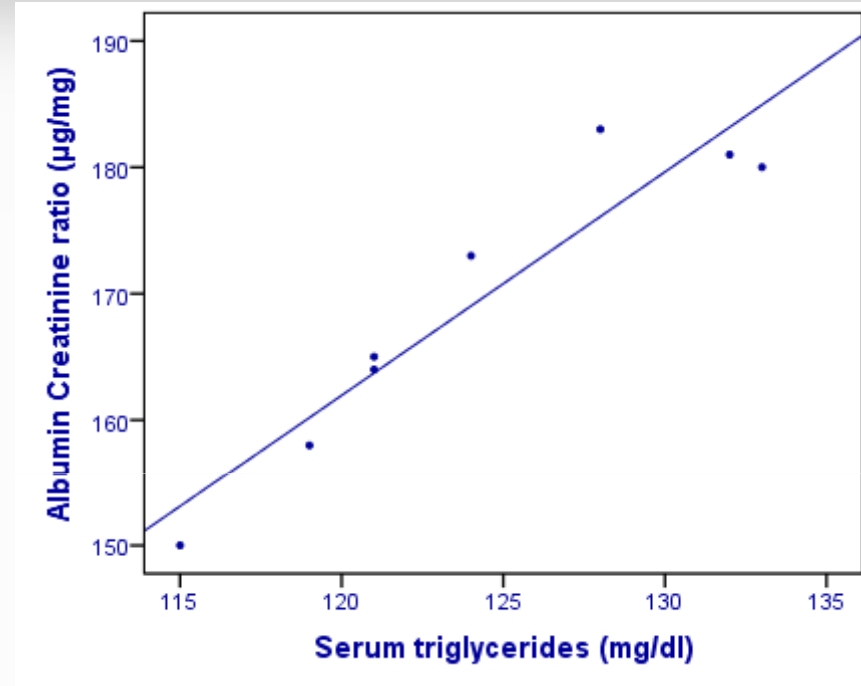
R= 0.858, P= 0.006\*\*

Correlations between ACR and each of plasma homocysteine and thrombomodulin in gp IIIa (non treated microalbuminuric rats)



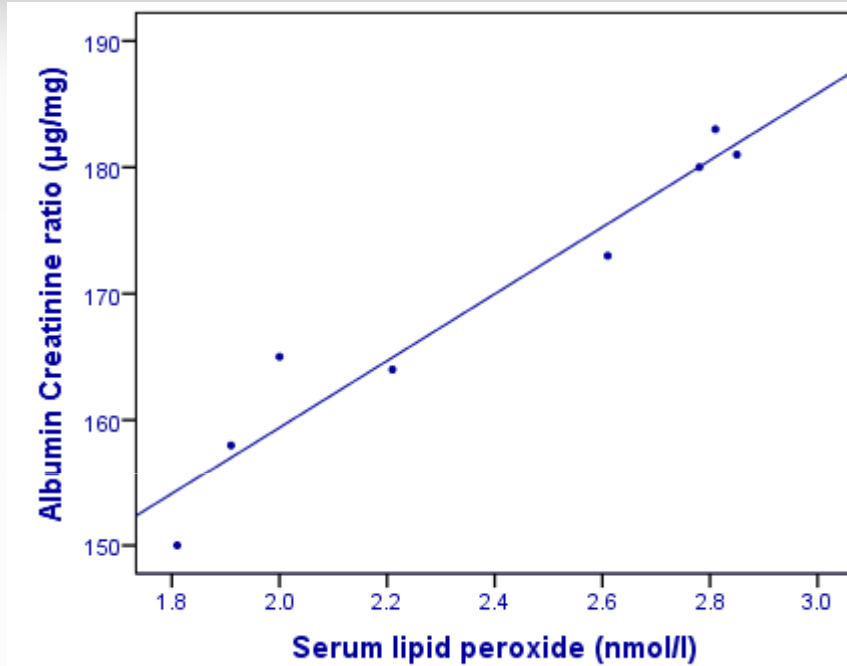


R= 0.775, P= 0.024\*

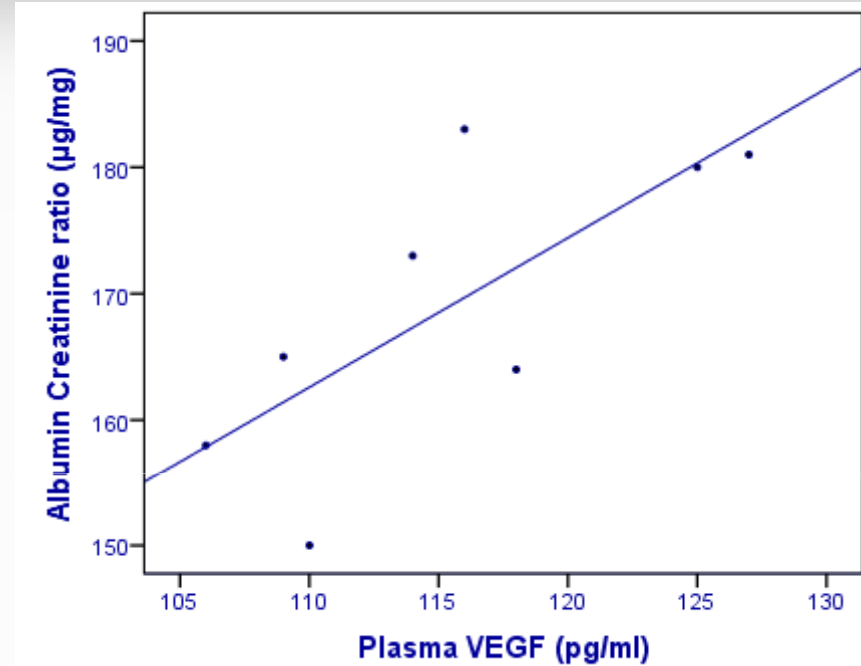


R= 0.944, P= 0.000\*\*

Correlations between ACR and each of HbA1c and triglyceride in gp IIIb & IIIc (NDGA and pravastatin treated microalbuminuric rats)

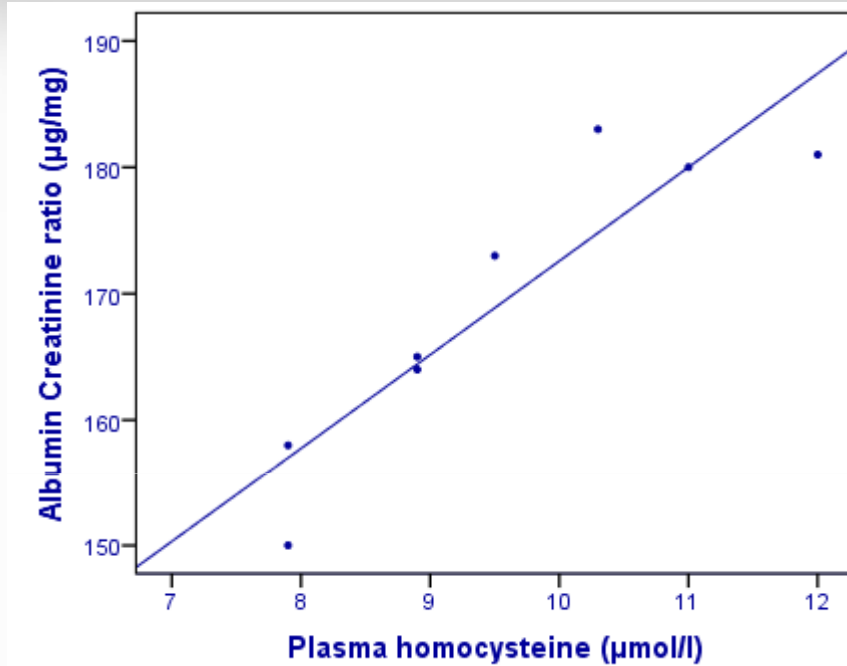


R= 0.967, P=0.000 \*\*

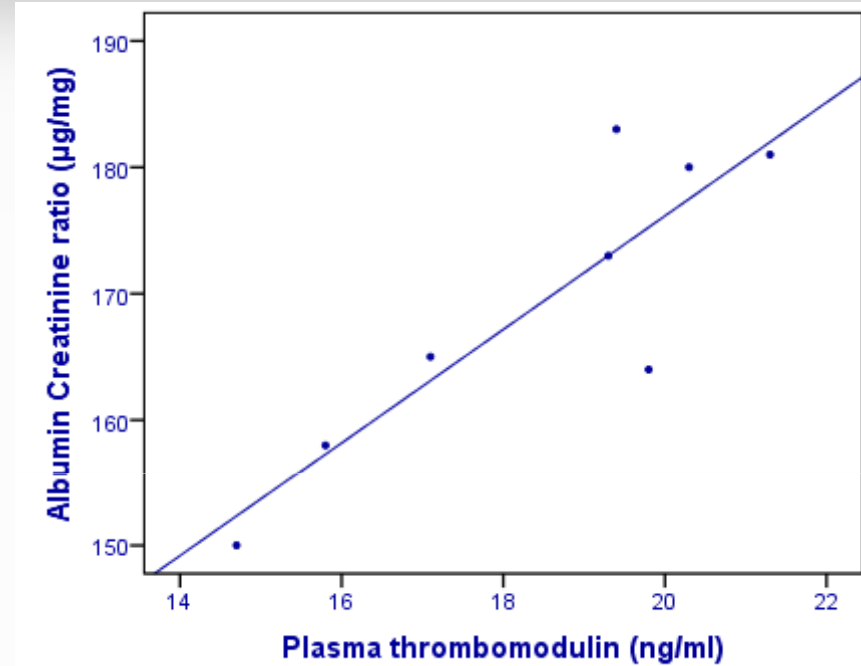


R=0.742, P=0.035\*\*

Correlations between ACR and each of lipid peroxide and plasma VEGF in gp IIIb & IIIc (NDGA and pravastatin treated microalbuminuric rats)

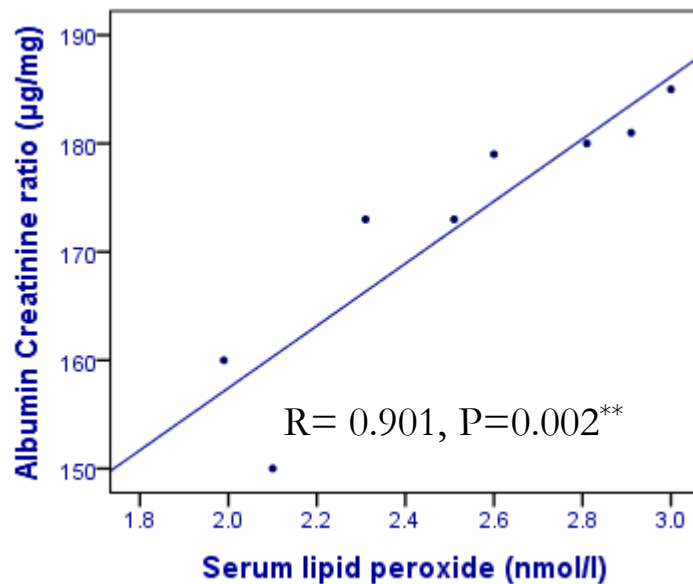
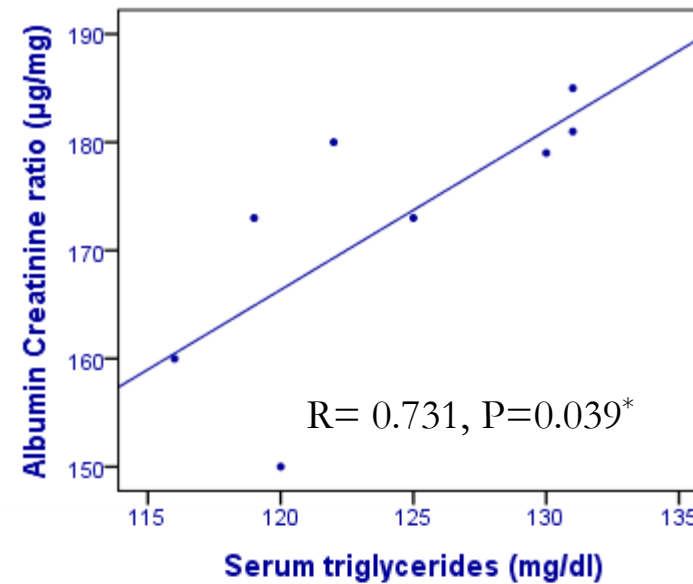
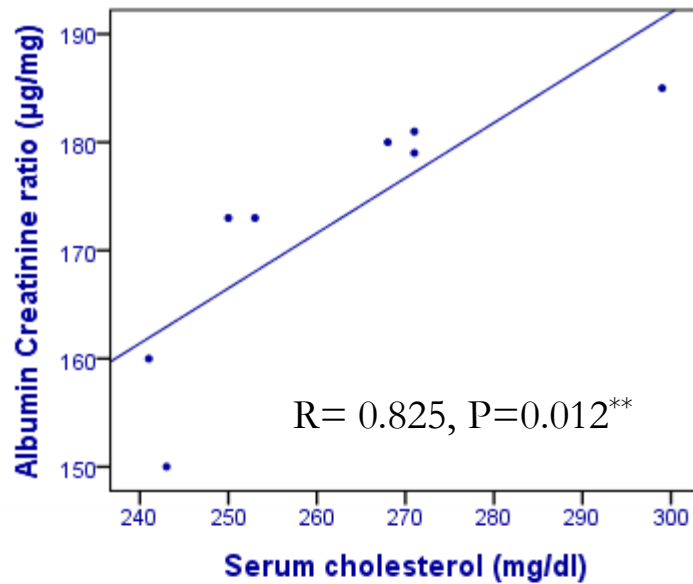


R= 0.875, P=0.004\*\*

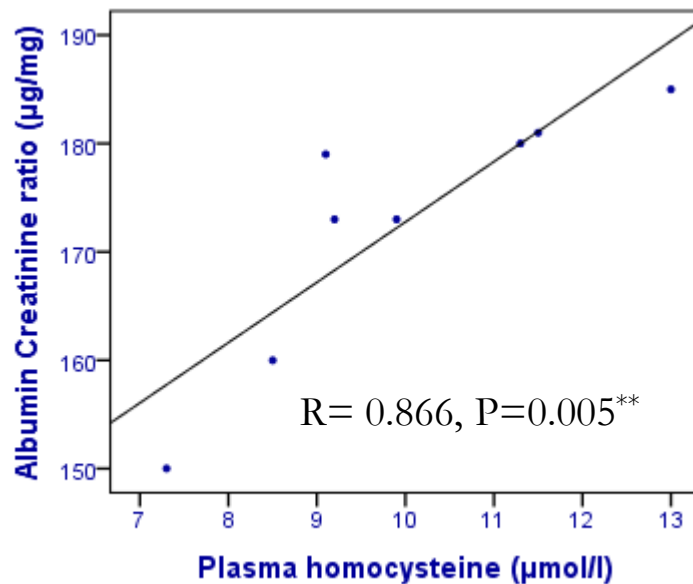
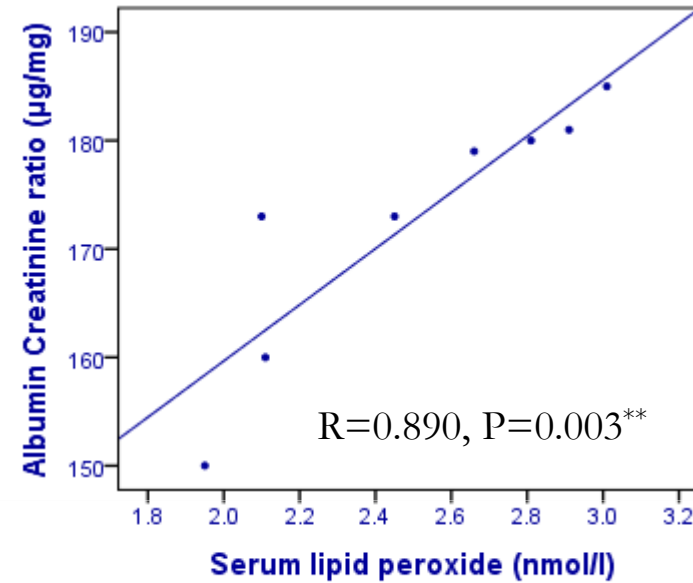
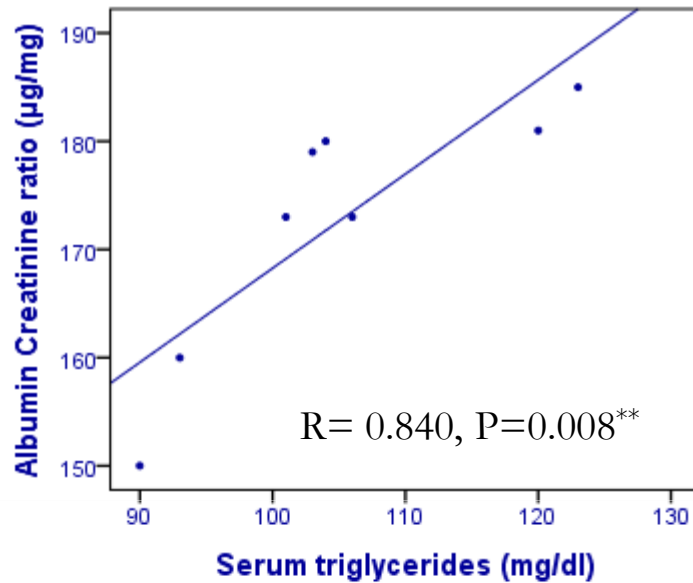


R= 0.908, P= 0.002\*\*

Correlations between ACR and each of plasma Hcy and thrombomodulin in gp IIIb & IIIc (NDGA and pravastatin treated microalbuminuric rats)



Correlations between ACR and each of serum cholesterol, TG and lipid peroxide in gp IIIc (NDGA+insulin treated microalbuminuric rats)



Correlations between ACR and each of serum TG, lipid peroxide and Homocysteine in gp IIIe (pravastatin+insulin treated microalbuminuric rats)

CONCLUSION

- Pravastatin and the 12/15 LO inhibitor, NDGA, have beneficial effects on STZ induced DN by improvement of renal function, decreasing urinary ACR, and indices of DN in microalbuminuric rats.
- A strategy of 12/15 LO inhibition with a drug regimen capable of more sustained 12/15 LO inhibition may improve albuminuria in DN.
- These findings may provide insight into the feasibility of clinical use of pravastatin or 12/15-LO pathway inhibitors as a complementary therapy for the prevention/treatment of DN.
- Despite the possible theoretic benefit, long-term, prospective clinical trials are needed before clarifying the medical and, perhaps, economic benefits of statins or 12/15-LO inhibitors for DN.

