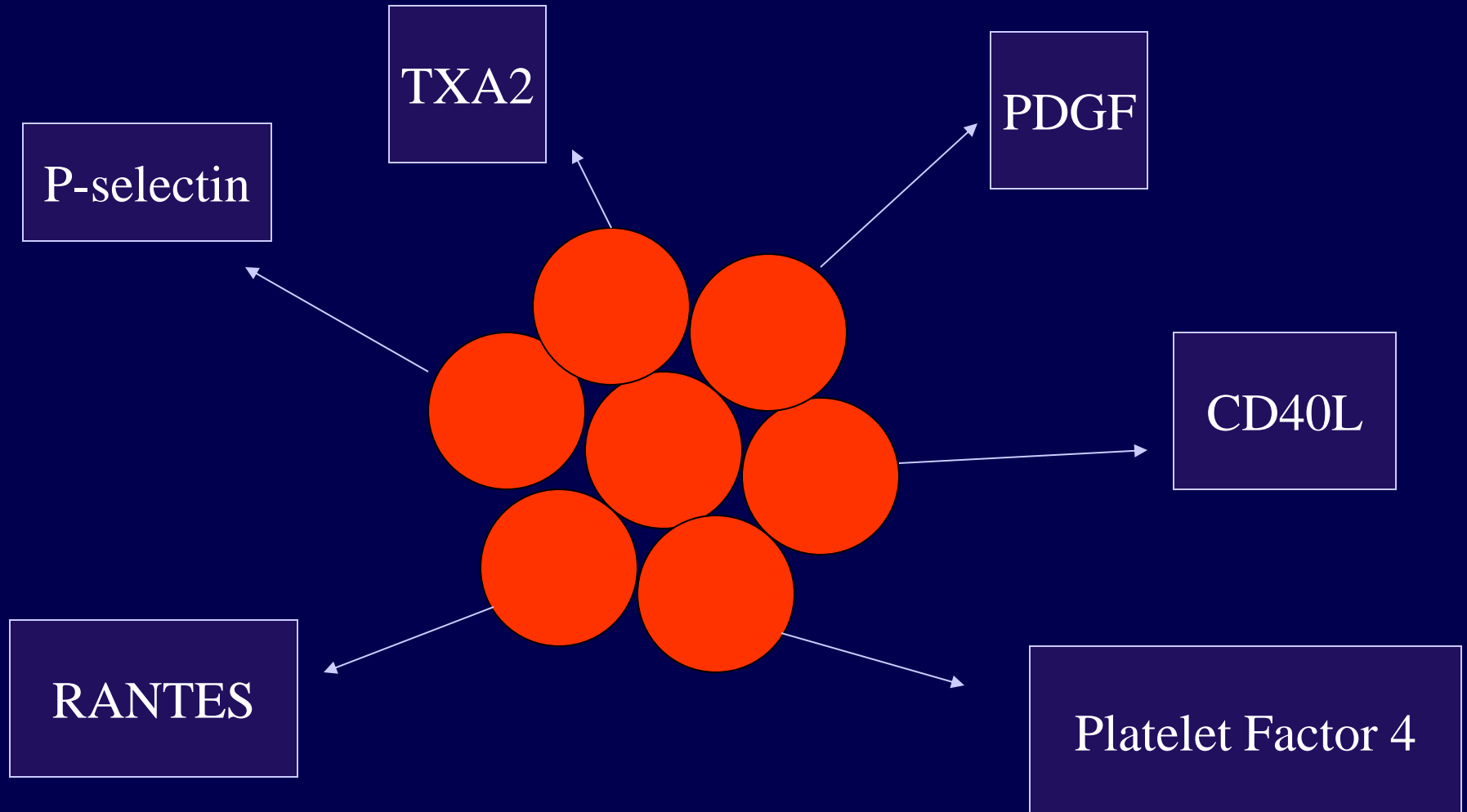


**Anti-platelet effects of Rosiglitazone:  
A direct consequence of  
PLATELET PPAR- $\gamma$  ACTIVATION**

Dr Manish Khanolkar,  
SpR in Diabetes & Endocrinology,  
University of Wales College of Medicine

# Platelet Activation

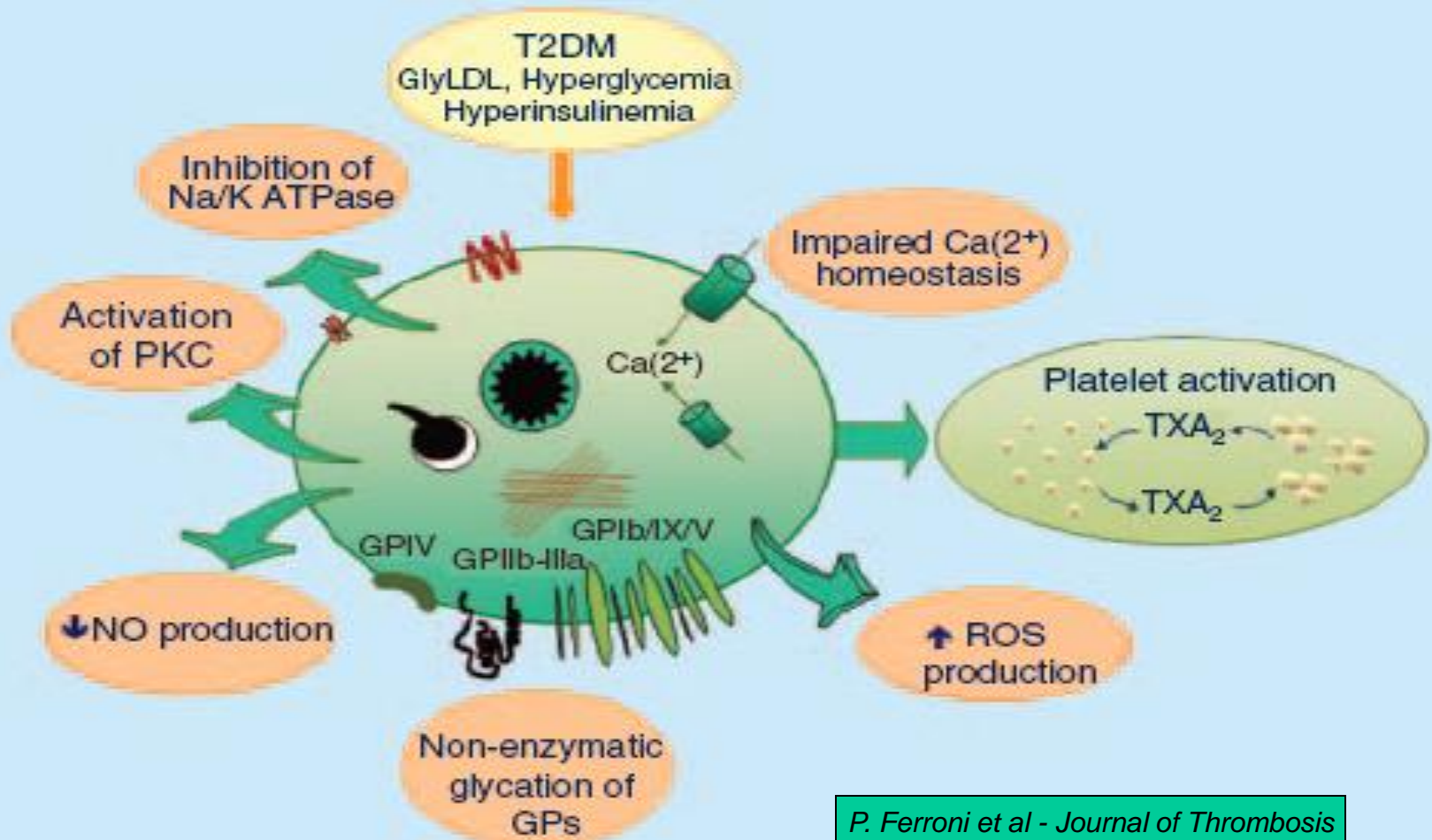
## Atherothrombosis-Atherogenesis



# Type 2 Diabetes

- 1.8 million people with diabetes in the UK
- Up to a million people with undiagnosed type 2 diabetes in the UK
- 80 percent of people with diabetes die from cardiovascular disease

# Platelets in Type 2 Diabetes



*P. Ferroni et al - Journal of Thrombosis & Haemostasis: 1284*

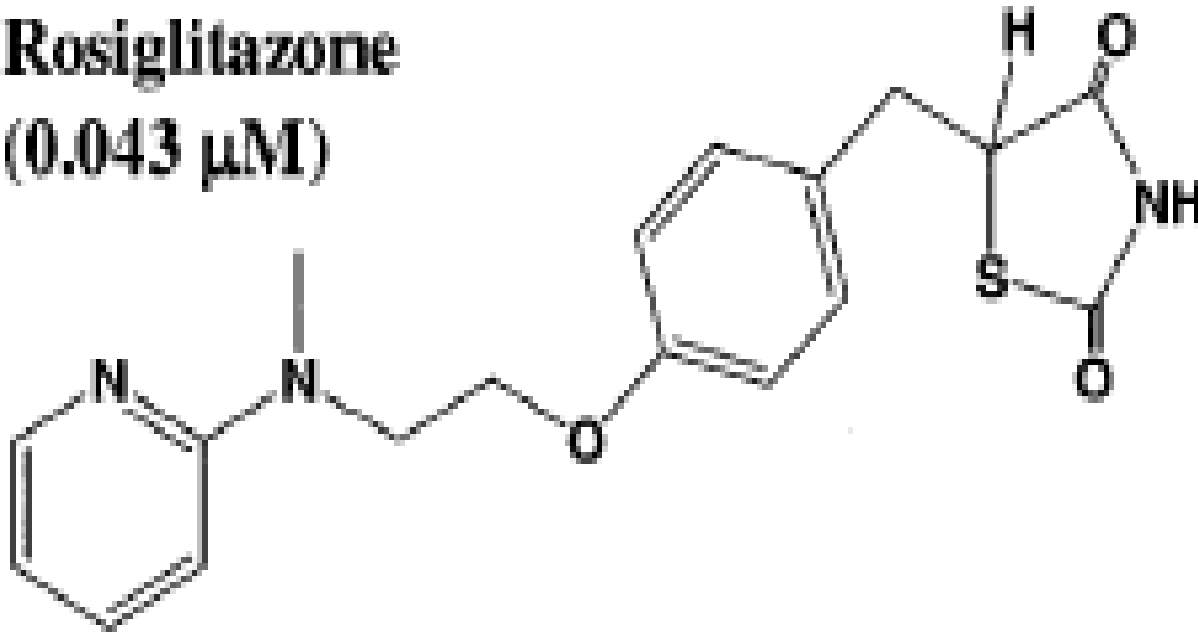
# **Are Platelets the Site of Insulin Resistance?**

# Thiazolidinediones: PPAR- $\gamma$ Agonists

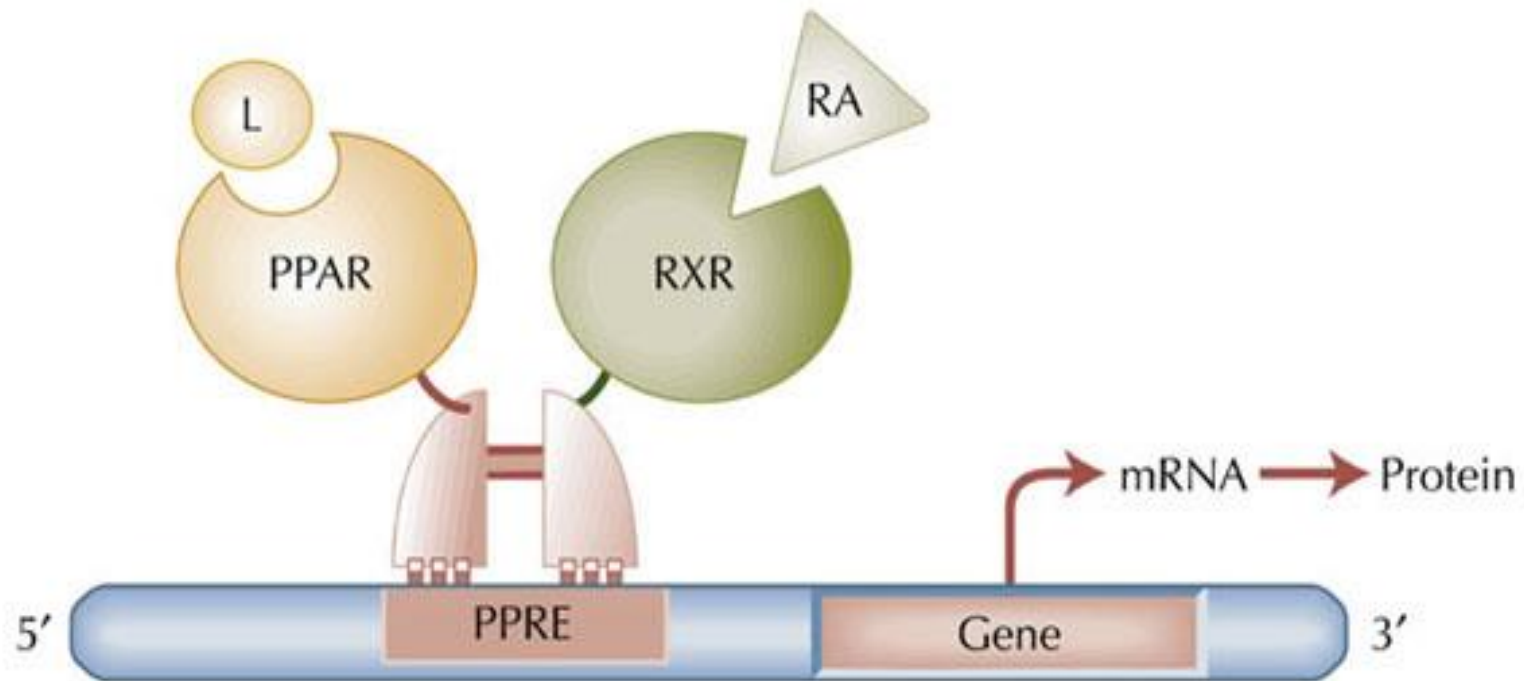
- TZD's counter insulin resistance – associated with increased platelet activity
- Pleiotropic effects
- Anti-inflammatory and Anti-oxidant properties

# Rosiglitazone

**Rosiglitazone**  
(0.043  $\mu\text{M}$ )



# PPAR- $\gamma$ : a nuclear receptor



PPAR response element (PPRE)

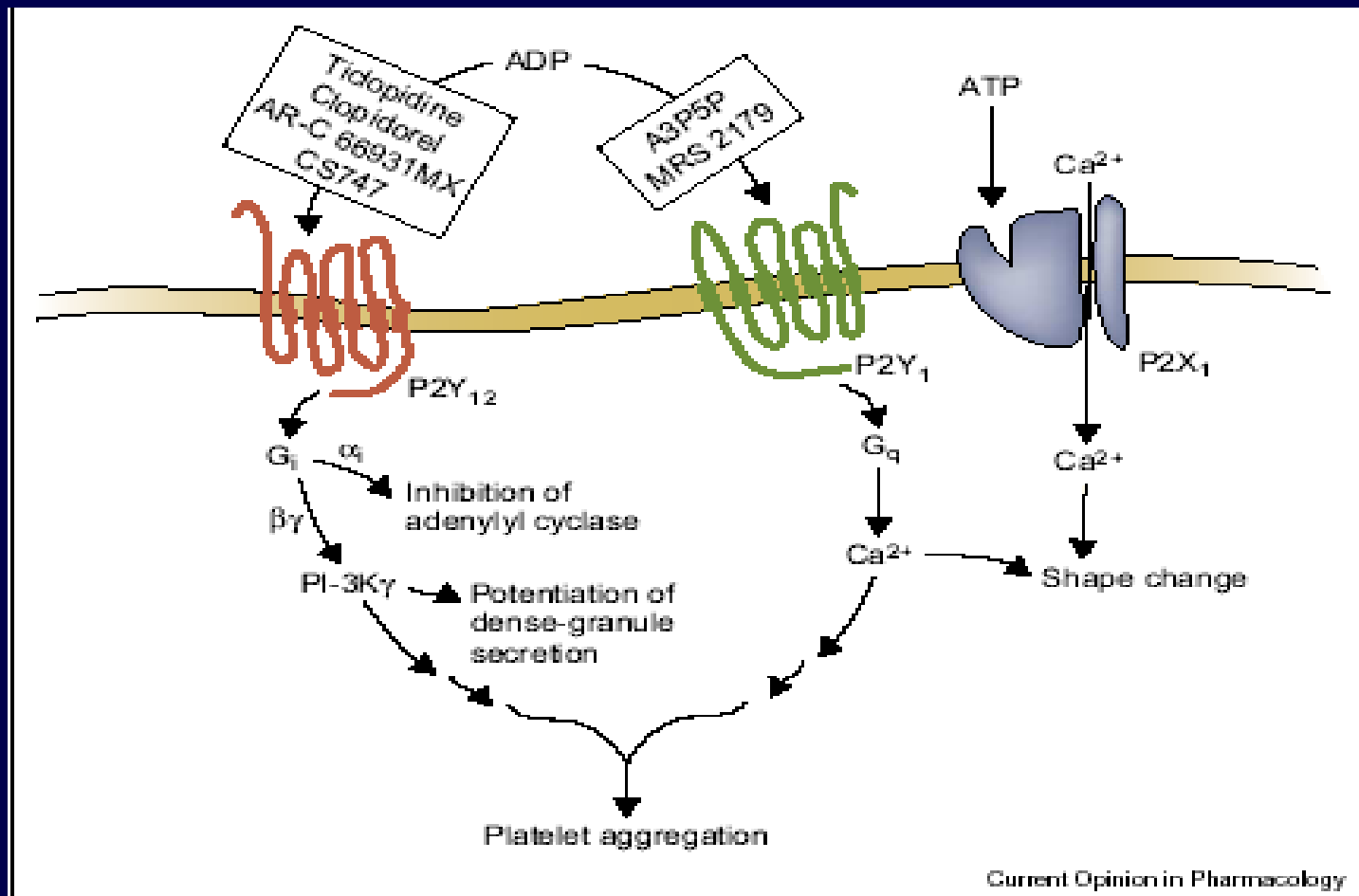
- Direct repeat 1 (DR-1)
- AGGTCA n AGGTCA



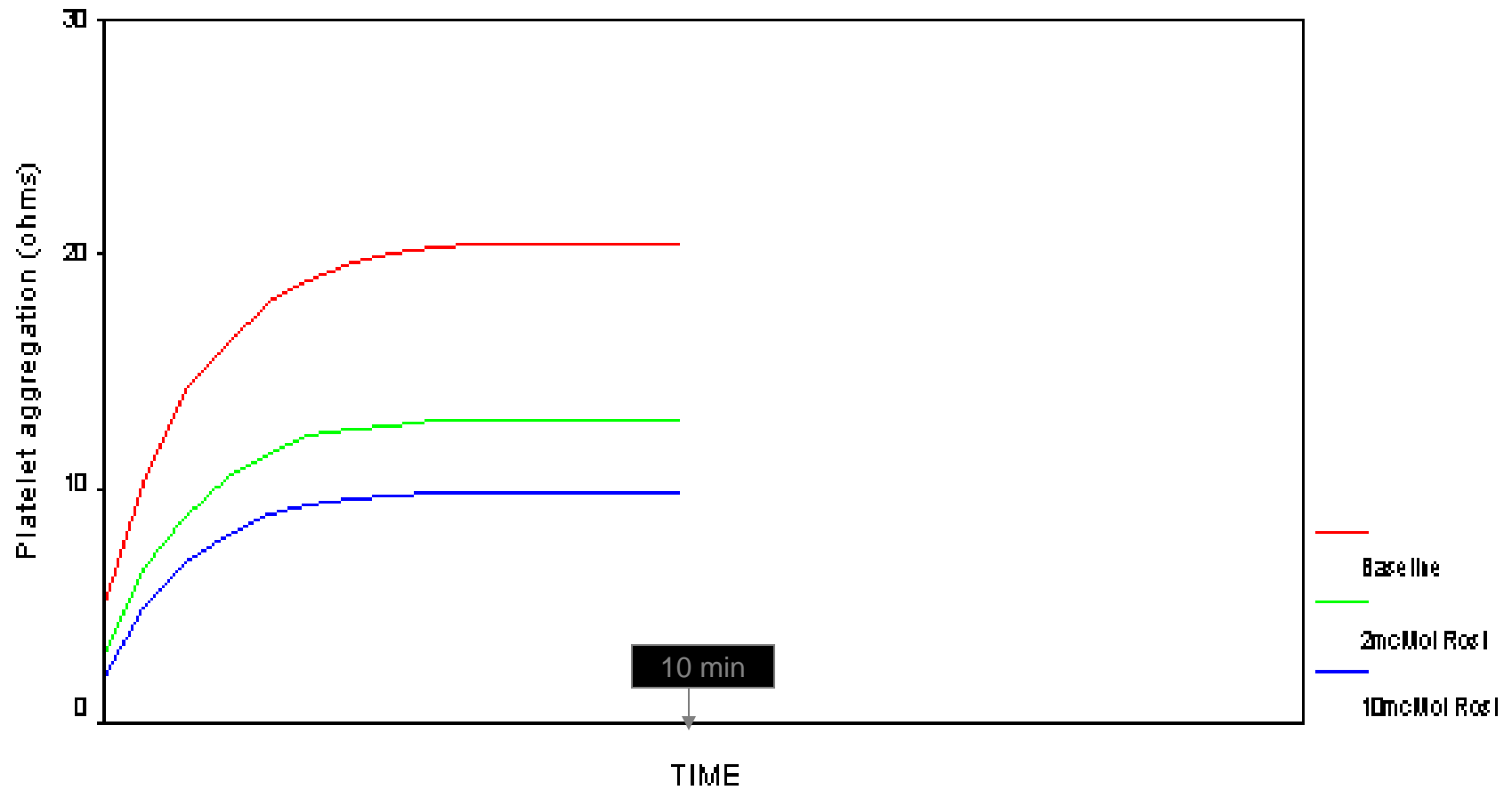
# In-vitro Study

- N=7, healthy, fasting volunteers, not on any medications especially NSAID's
- Effects of rosiglitazone (2 and 10  $\mu\text{M}$ ) on platelet aggregation

# ADP induced Platelet Aggregation



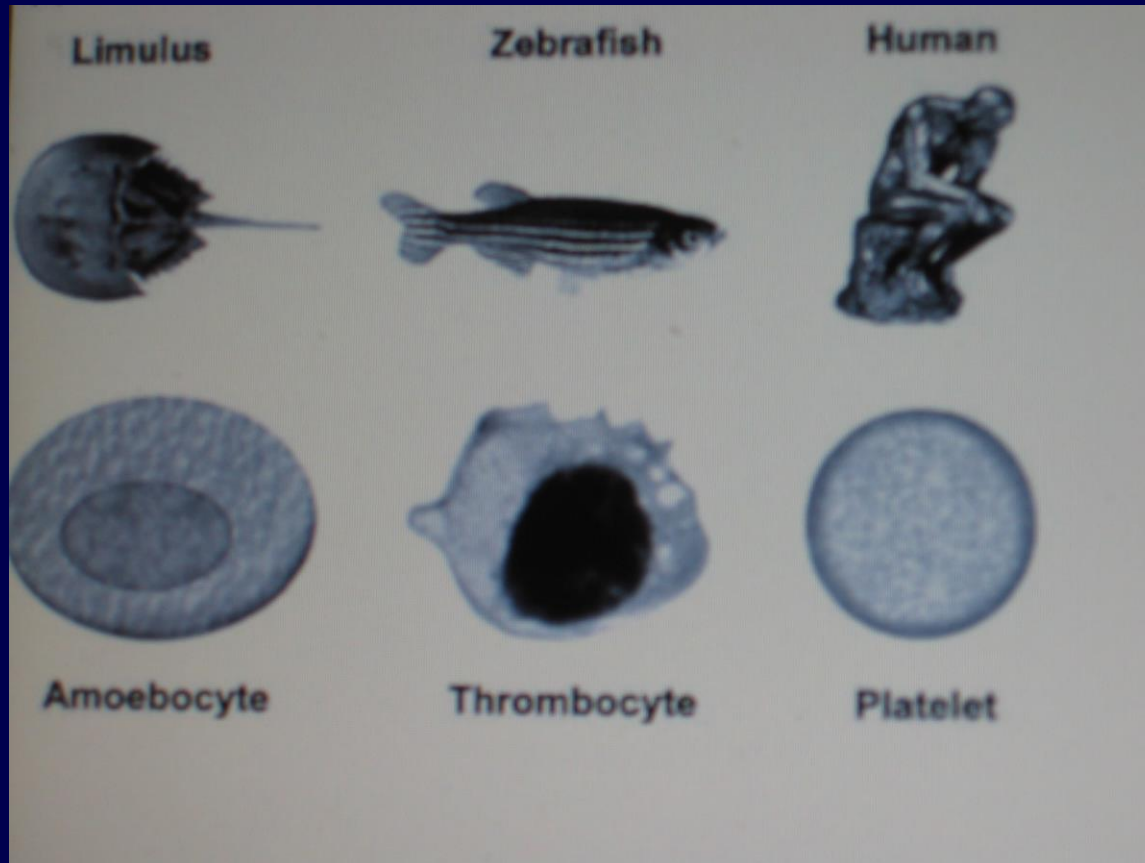
# Results



## Results (Contd)

- This study demonstrates direct anti-platelet effects of rosiglitazone in a dose dependant manner.
- A recent study demonstrated functional PPAR- $\gamma$  receptors in human platelets (Filiz Akbiyik et al, Blood: May 2004).

# Platelet Evolution



# Clinical Study

# Study Design

- Randomised double blind study
- 50 T2D subjects with suboptimal control (HbA1c>6.5%) on metformin monotherapy
- Exclusion criteria:  
Smokers, anti-platelet medications  
Female subjects likely to get pregnant
- Randomised to receive either rosiglitazone (4mg) or gliclazide (80mg) for 6 months

# Measurements at 0 and 6 months

- Anthropometrics, HbA1c, HOMA-R, Lipid profile
- Platelet function: Platelet aggregation (Chronolog) and sCD40L (R&D)
- Endothelial activation and inflammation: sICAM-1, s-Eselectin (R&D) and hs-cRP
- Oxidative stress: Protein Carbonyls (Colorimetric assay) and TBARS (Fluorimetric method)



# Baseline Characteristics

	<b>Rosiglitazone</b>	<b>Gliclazide</b>
<b>No:</b>	<b>25 (14 Male)</b>	<b>25 (15 Male)</b>
<b>Age (y)</b>	<b>59 (40-71)</b>	<b>56 (36-67)</b>
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	<b>34.55 ± 6.83</b>	<b>33.66 ± 7.09</b>
<b>Waist Hip Ratio</b>	<b>0.92 ± 0.07</b>	<b>0.89 ± 0.08</b>
<b>HbA1c (%)</b>	<b>7.33 ± 1.01</b>	<b>7.08 ± 0.69</b>
<b>HOMA-R</b>	<b>2.25 ± 1.21</b>	<b>2.24 ± 1.24</b>

# Effect of Treatment

	Rosiglitazone Baseline	Rosiglitazone 24 weeks	Gliclazide Baseline	Gliclazide 24 weeks
BMI (Kg/m <sup>2</sup> )	34.55 ± 6.83	34.90 ± 7.12	33.66 ± 7.09	33.61 ± 6.92
HOMA-R	2.25 ± 1.21	1.19 ± 0.47*†	2.24 ± 1.24	1.79 ± 1.01
Mean BP (mm Hg)	88.9 ± 5.9	83.5 ± 4.5 *†	88.3 ± 3.9	90.3 ± 7.8
T Cholesterol (mmol/l)	4.76 ± 0.65	4.92 ± 0.89	5.37 ± 1.01	5.03 ± 0.87
LDL-C (mmol/l)	2.58±0.51	2.71±0.67	3.01 ± 0.51	2.99 ± 0.67
HDL-C (mmol/l)	1.19 ± 0.24	1.27 ± 0.26	1.04 ± 0.20	1.03 ± 0.24
Total Chol / HDL ratio	4.01 ± 0.44	3.87 ± 0.37	5.16 ± 0.59	4.88 ± 0.56
Triglycerides (mmol/L)	1.8 (1.2-2.75)	2.04 (1.42-2.62)	2.35 (1.53-3.9)	1.9 (1.53-3.28)

\* Denotes p < 0.05 Post treatment compared with baseline

† Denotes p < 0.05 For rosiglitazone therapy compared with Gliclazide

# Effect of Treatment (Contd)

	Rosiglitazone Baseline	Rosiglitazone 24 weeks	Gliclazide Baseline	Gliclazide 24 weeks
Platelet aggregation( $\Omega$ )	<b>15.07 <math>\pm</math> 4.18</b>	<b>10.56 <math>\pm</math> 2.90*<math>\dagger</math></b>	14.44 $\pm$ 4.99	12.85 $\pm$ 2.76
sCD40L (ng/ml)	<b>23.43 <math>\pm</math> 6.14</b>	<b>12.72 <math>\pm</math> 4.11*<math>\dagger</math></b>	21.16 $\pm$ 5.87	17.93 $\pm$ 4.76
sE-selectin ( $\mu$ g/L)	63.8 $\pm$ 20.1	47.5 $\pm$ 14.8	60.7 $\pm$ 18.4	54.2 $\pm$ 19.2
sICAM-1 ( $\mu$ g/L)	382 $\pm$ 54.3	347 $\pm$ 48.1	398 $\pm$ 48.9	366 $\pm$ 52.3
TBARS (nmoles/ml)	<b>0.98 <math>\pm</math> 0.24</b>	<b>0.71 <math>\pm</math> 0.16*<math>\dagger</math></b>	0.97 $\pm$ 0.21	0.88 $\pm$ 0.26
HbA1c (%)	<b>7.33 <math>\pm</math> 1.01</b>	<b>6.14 <math>\pm</math> 0.58*</b>	<b>7.08 <math>\pm</math> 0.69</b>	<b>6.08 <math>\pm</math> 0.66*</b>
hs-CRP (mg/L)	<b>0.77 <math>\pm</math> 0.70</b>	<b>0.49 <math>\pm</math> 0.18*<math>\dagger</math></b>	0.66 $\pm$ 0.39	0.54 $\pm$ 0.41

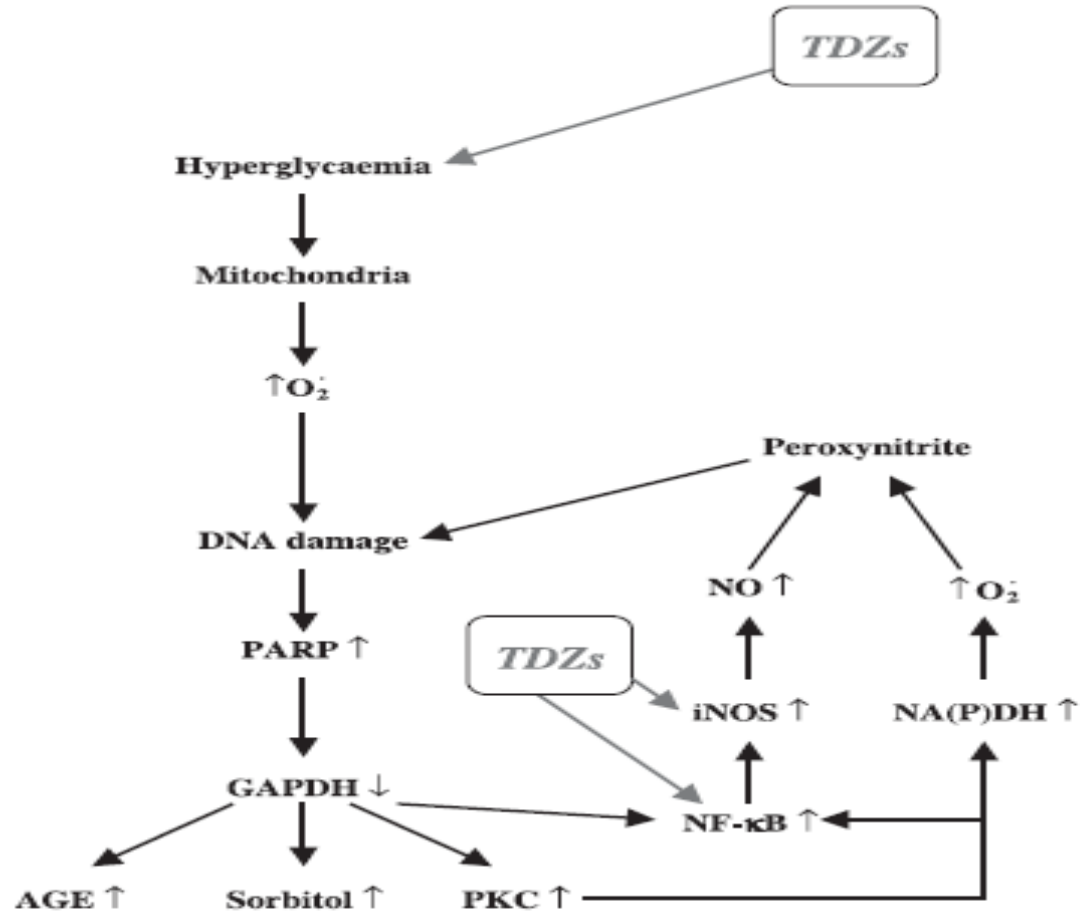
\* Denotes  $p < 0.05$  Post treatment compared with baseline

$\dagger$  Denotes  $p < 0.05$  For rosiglitazone therapy compared with Gliclazide

# Correlation Statistics

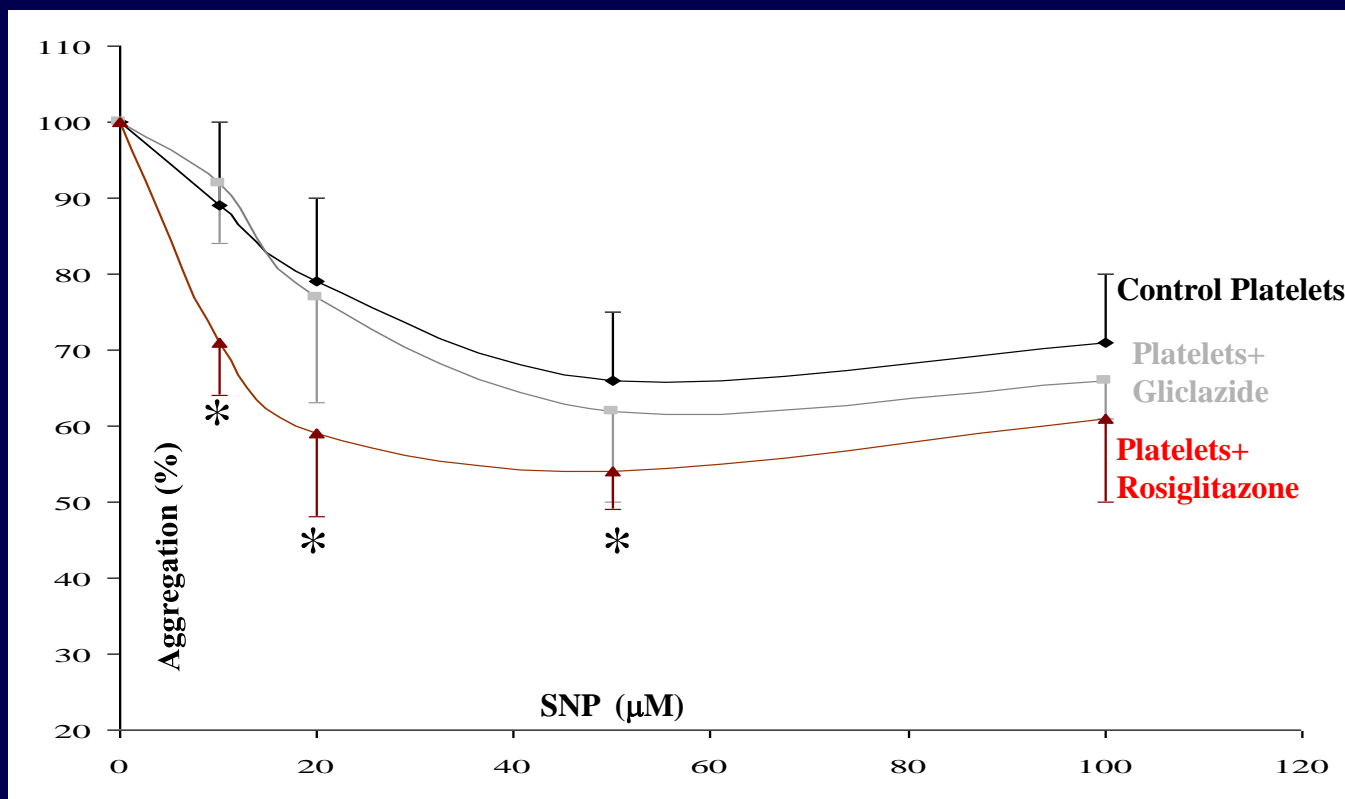
- Baseline platelet aggregation correlated with insulin resistance (HOMA-R) ( $r = 0.39$ ,  $p < 0.05$ ).
- In the rosiglitazone treated group, reduction in platelet activation correlated with the reduction in oxidative stress (TBARS) ( $r = 0.32$ ,  $p < 0.05$ ).

# Oxidative Stress



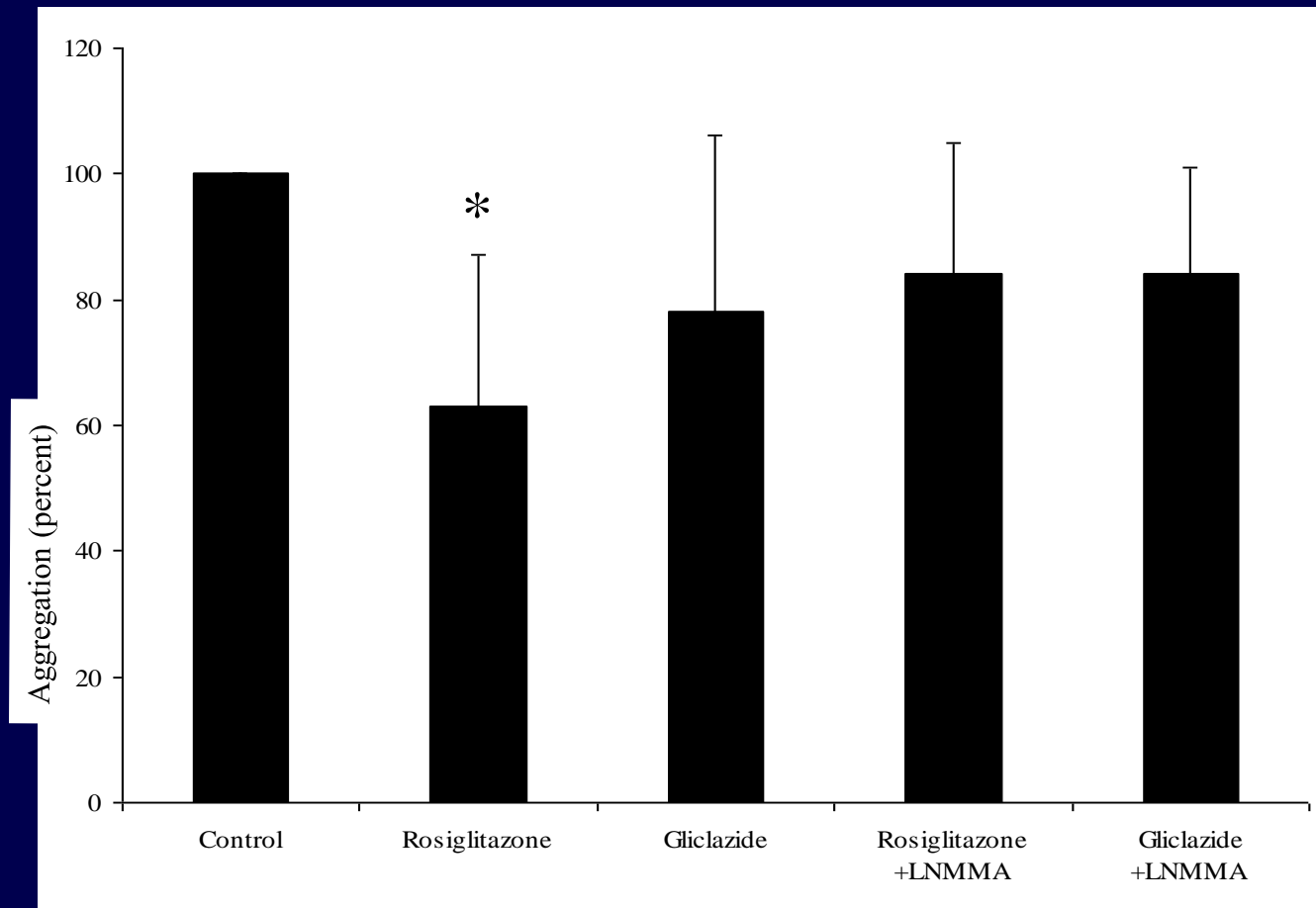
**Figure 1** Thiazolidinediones are a new class of insulin-sensitizing drugs that reduce plasma glucose levels and glucose production. Their beneficial effects are not limited to hypoglycaemic action; binding to the PPAR $\gamma$  receptor inhibits the expression of iNOS and antagonizes the activity of NF- $\kappa$ B resulting in oxidative stress reduction.

# In-vitro effects of SNP (NO Donor)



\*  $p < 0.05$  cf control at 10, 20 and 50  $\mu\text{M}$  SNP

# In-vitro effects of LNMMA (NOS Inhibitor)



\*  $p < 0.05$  cf control

# Conclusions

- Rosi + Met comb<sup>n</sup> therapy exerts greater anti-platelet effects compared to Glic + Met comb<sup>n</sup> therapy.
- This effect of rosiglitazone appears to be independent of its hypoglycaemic effect.
- Role of oxidative stress, nitric oxide and platelet PPAR- $\gamma$  receptors.



# Acknowledgements

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