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Pioglitazone in Addition to Metformin Improves Erythrocyte Deformability in Patients with Type 2 Diabetes mellitus.

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Abstract:

The aim of this study was to compare the effect of pioglitazone or glimepiride on erythrocyte deformability in T2DM.

The study covered 23 metformin treated T2DM patients with an HbA1c above 6.5%. Patients were randomized to receive either 15 mg Pioglitazone (PIO) bid. or 1 mg Glimepiride (GLIM) bid. in combination with 850 mg Metformin bid. for 6 months. Blood samples were taken for the measurement of fasting glucose, HbA1c, fasting insulin, intact proinsulin, adiponectin, and hematocrit (Hct). In addition, the erythrocyte elongation index (EI) was measured using laserdiffraction.

Both treatments significantly improved HbA1c levels (PIO $-0.9 \pm 1.1\%$; GLIM $-0.6 \pm 0.4\%$; $p < 0.05$ respectively) and end up in comparable HbA1c levels after 6 months (PIO 6.5 ± 1.2 ; GLIM 6.2 ± 0.4). Treatment with PIO reduced fasting insulin levels (-8.7 ± 15.8 mU/L; $p = 0.098$), intact proinsulin levels (-11.8 ± 9.5 pmol/L; $p < 0.05$), and Hct ($-1.3 \pm 2.3\%$; $p = 0.09$), while adiponectin levels increased (8.2 ± 4.9 $\mu\text{g/mL}$; $p < 0.05$). No significant change in these parameters could be observed during GLIM treatment. PIO improved the EI, resulting in a significant increase in EI at all physiological shear stress ranges (0.6 to 6.0 Pa; $p < 0.05$). The improvement in EI correlated with the increase in adiponectin levels ($r = 0.74$; $p < 0.001$), and inversely with intact proinsulin levels ($r = -0.47$; $p < 0.05$).

This is the first study showing an improvement in EI during treatment with pioglitazone, which was associated with an increase in adiponectin and a decrease in intact proinsulin levels.

Keywords: Pioglitazone, Diabetes mellitus Type 2, Erythrocyte Deformability, Adiponectin

Objective

Patients with type 2 diabetes mellitus suffer from an increased incidence of micro- and macrovascular complications. It is widely accepted that rheological properties of erythrocytes are of considerable significance in many circulatory disorders like atherosclerosis, essential hypertension and diabetes mellitus [1-3]. Several clinical studies document decreased erythrocyte deformability in diabetic patients [4-7], and even in children with short duration of diabetes, impaired erythrocyte deformability was observed [8]. Impaired tissue perfusion, as observed in diabetes mellitus, could be attributed to a reduction in erythrocyte deformability and an increase in blood viscosity [9;10]. It is crucial for the erythrocytes to pass through the capillaries in order to supply oxygen to the surrounding tissues, and decreased erythrocyte deformability contribute to an impaired oxygen and nutrition supply in diabetic microangiopathy [11;12].

Insulin resistance as observed in patients with diabetes mellitus type 2 is associated with endothelial dysfunction [13;14] and an increase in blood viscosity [15;16]. Pioglitazone was shown to improve insulin sensitivity [17], to improve endothelial function [18-21] and to improve the overall cardiovascular risk profile in diabetic and in non-diabetic subjects [22-25]. In contrast, there is increasing evidence, that antidiabetic treatment with sulfonylurea, especially in combination with metformin, might have a negative impact on the cardiovascular outcome in patients with diabetes mellitus type 2 [26-28]. The aim of this investigation was to compare the effect of adding pioglitazone or glimepiride on erythrocyte deformability in patients with type 2 diabetes mellitus already treated with metformin.

Methods and Results

Patients

This is a sub analysis of the patients from the PIOfix study, treated at the ikfe study site in Mainz, in whom erythrocyte deformability was measured immediately after venous blood draw. Patients had to be on a stable treatment with metformin monotherapy in a maximal individually tolerated dosage (850 – 2000 mg) within the last 12 weeks and with an HbA1c \geq 6.5 %. Subjects were excluded if they had been treated with insulin or other oral antidiabetic drugs with the exception of metformin within the last 12 weeks. All other concomitant treatments were kept stable during the study. Patients were randomised to pioglitazone 15 mg bid. or glimepiride 1 mg bid. in combination with 850 mg metformin bid.

Fasting blood samples were taken at baseline and after 24 weeks of treatment for the measurement of plasma glucose, HbA1c, insulin, intact proinsulin, adiponectin, haematocrit, and the assessment of erythrocyte deformability.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethical committee. All subjects gave written informed consent.

Measurement of Erythrocyte Deformability

Measurement of erythrocyte deformability was performed using laserdiffractoscopy from freshly obtained venous blood samples. Laserdiffractoscopy was performed using Rheodyn SSD shear stress diffractometer and corresponding Dextran FP-60 24 mPas (Myrenne GmbH, Roetgen, Germany). The method of laserdiffractoscopy has been described in detail previously [29]. In summary a He-Ne-Laser detects deformation of erythrocytes between two parallel glass discs, one of them rotating and executing defined shear stresses as per following formula:

$$\tau \text{ (Pa)} = 2\pi r * \eta / t * h$$

(τ = shear stress, r = laser beam distance from rotation center, η = viscosity, h = height of gap between discs, t = time)

Adjusting for equipment-specific values ($r = 25\text{mm}$, $h = 0,5\text{mm}$ und $\eta = 24\text{mPas}$) the formula is condensed to

$$\tau \text{ (Pa)} = 7,536 \text{ Pa} * \text{rpm (rpm = revolutions per minute)}$$

The applied shear stress range is electronically regulated and consists of 8 levels (0,3 Pa; 0,6 Pa; 1,2 Pa; 3 Pa; 6 Pa; 12 Pa; 30 Pa; 60 Pa).

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The erythrocyte deformability measurement detects scattered light intensities along orthogonal axes (A, B) of red blood cells within the laser diffraction light cone. The erythrocyte Elongation Index (EI) is calculated by the following equation:
$$EI (\%) = ((A-B)/(A+B))*100.$$

According to Baskurt et al. the maximal RBC elongation index (EI_{max}) and the shear stress required for half of this maximal deformation ($SS_{1/2}$) was calculated [30].

Additional Laboratory Measurements

Blood samples for all other laboratory measurements were centrifuged and kept at -20°C until analysis. Plasma glucose concentrations were determined by the glucose dehydrogenase method (Super GL, RLT, Möhnese-Delecke, Germany). Insulin and intact proinsulin was measured by a chemiluminescence assay (Invitron, Monmouth, UK), adiponectin was measured by an Sandwich- Assay (ELISA) (TECOmedical, Sissach, Switzerland). HbA1c was determined by high-performance liquid chromatography (Menarini Diagnostics, Neuss, Germany).

Subjects sample size considerations and statistical analysis

No clinical information was available about the effect of pioglitazone or glimepiride on erythrocyte deformability in patients with diabetes mellitus type 2. Therefore, no confirmatory study size calculation was possible and the obtained results were interpreted in an exploratory sense.

For the erythrocyte deformability two-sided p-values for within- and between-group treatment differences were calculated for the absolute change from baseline, considering an ANCOVA model with the fixed effect factors for treatment group and centre and with the baseline value as covariate. For all other within and between group comparisons, two-sided p-values resulting from the one- and two-sample t-tests are reported. Pearson correlation coefficients are calculated to assess the relationship between different variables. P-values < 0.05 were considered to be statistically significant.

Results:

Twenty three patients with type 2 diabetes were included in the analysis. Eleven patients were randomised to the pioglitazone group and twelve patients were randomised to the glimepiride group. The clinical characteristics of the patients are presented in table 1. At baseline, HbA1c,
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LDL and Triglycerides were lower in the glimepiride group compared with the pioglitazone group. No other relevant difference could be observed in between the two groups.

After 24 weeks of study treatment both groups improved metabolic control, and at the end of the study, HbA1c and HDL levels were comparable in between both treatment groups (HbA1c: PIO 6.5 ± 1.2 vs. GLIM 6.2 ± 0.4 %, n.s.; HDL: PIO 46.2 ± 5.6 vs. GLIM 39.9 ± 6.8 mg/dl; n.s.). Triglyceride levels remained significantly higher in the pioglitazone compared with the glimepiride group (PIO 203.3 ± 36.5 vs. GLIM 158.1 ± 58.0 , $p < 0.05$).

In pioglitazone treated patients, fasting insulin levels tended to decrease from 18.0 ± 14.9 pmol/L to 9.3 ± 2.7 pmol/L ($p = 0.098$), and intact proinsulin levels declined from 21.4 ± 10.4 pmol/L to 9.7 ± 4.0 pmol/L ($p < 0.05$). Adiponectin levels increased from 4.1 ± 1.7 μ g/mL to 12.3 ± 5.7 μ g/mL ($p < 0.05$). No significant changes in these laboratory parameters could be observed during GLIM treatment (fasting insulin from 17.2 ± 8.4 to 18.5 ± 8.0 pmol/L; intact proinsulin from 16.2 ± 16.3 to 15.9 ± 8.9 pmol/L; adiponectin from 4.3 ± 2.5 to 4.7 ± 2.6 μ g/mL; n.s. respectively).

Treatment with pioglitazone increased erythrocyte deformability at all shear stress rates tested in our study protocol (figure 1A). In the physiological shear stress range of 0.6 to 6.0 Pa a significant improvement in erythrocyte deformability compared to baseline could be observed during treatment with pioglitazone. In contrast, glimepiride treatment showed a slight, albeit non-significant deterioration in erythrocyte deformability within our study (figure 1B). In the physiological shear stress range in between 0.6 Pa and 6.0 Pa, the change from baseline in the erythrocyte elongation index (EI) was significantly different in between the two treatment groups. During pioglitazone treatment, EI_{max} decreased from 82.1 ± 8.5 to 76.9 ± 17.8 % ($p < 0.01$), and the $SS_{1/2}$ decreased from 6.3 ± 1.4 to 5.2 ± 3.7 Pa ($p < 0.001$). No significant effect of glimepiride treatment on EI_{max} (from 87.9 ± 19.9 to 86.3 ± 15.1 %) or $SS_{1/2}$ (from 6.4 ± 3.2 to 6.7 ± 2.5) could be observed in our study.

During pioglitazone treatment Hct slightly decreased from 42.5 ± 2.8 % to 41.1 ± 3.8 % ($p = 0.09$), while a slight increase from 41.0 ± 2.4 % to 41.2 ± 2.6 % (n.s.) could be observed during treatment with glimepiride

A significant correlation could be observed in between the increase in adiponectin plasma levels and the increase in the EI ($r = 0.74$; $p < 0.001$; figure 2) while an inverse relationship could be observed in between the EI and intact proinsulin plasma levels ($r = -0.47$; $p < 0.05$;

figure 3). No association between erythrocyte deformability and fasting glucose, insulin, HbA1c levels, Hct, or lipids was found in our study.

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Conclusions

Diabetes mellitus type 2 is linked to numerous functional and morphological disturbances in micro- and macrovascular blood flow, merging in malperfusion and tissue damage. Since erythrocytes usually have a size of 8 μm , and the capillary lumen is in the range of 4 – 9 μm , the property of the erythrocyte to assimilate while passing the nutritive capillaries become a crucial factor for sufficient tissue perfusion. In patients with diabetes mellitus, increased capillary recruitment could only incompletely compensate for rigid diabetic red blood cells [31;32]. It has been suggested that reduced erythrocyte deformability, could at least in part account for the impaired tissue perfusion observed in several tissues in patients with diabetes mellitus [9;10].

Pioglitazone is used for the treatment of insulin resistance in patients diabetes mellitus type 2. Beyond improving blood glucose control, treatment with pioglitazone was shown to effect micro- and macrovascular blood flow, and to reduce the risk for cardiovascular complications in patients with type 2 diabetes [10;19;23;33-35]. Pioglitazone was shown to reduce inflammation [35], to improve endothelial function [21], to reduce the intima media layer of the carotid artery [24;36], and to decrease atherosclerotic plaques in the cardiovascular system [25]. In contrast to glimepiride, pioglitazone improved erythrocyte deformability at all physiological shear stress ranges. The improvement in erythrocyte deformability was found to be associated with an increase in plasma adiponectin concentrations and a decrease in intact proinsulin concentrations.

Adiponectin, an adipokine released from the visceral adipose tissue, was found to increase insulin sensitivity, to improve endothelial function and to evolve several anti-inflammatory and anti-oxidative effects [37;38]. Decreased adiponectin plasma levels contribute to the development of insulin resistance and type 2 diabetes mellitus, and a causal relationship between low adiponectin levels and the development of cardiovascular disease has been suggested [39;40]. Treatment with pioglitazone was shown to reduce intima media thickness of the carotid artery, which was found to be associated with an increase in adiponectin levels [41]. Erythrocyte deformability is highly dependent on the fluidity of the erythrocyte membrane [42;43]. In hypertensive patients it was shown that the membrane fluidity of erythrocytes was inversely related to plasma adiponectin levels, indicating that hypoadiponectinemia might be associated with decreased membrane fluidity and decreased

erythrocyte deformability [44]. It has been demonstrated that adiponectin stimulates the production of NO in endothelial cells [45]. Many studies demonstrated an interrelation between NO and erythrocyte deformability [46;47]. In our study, treatment with pioglitazone in type 2 diabetic patients increased plasma adiponectin levels and improved erythrocyte deformability over a wide range of physiological shear stress rates. Therefore it seems conceivable, that treatment with pioglitazone in type 2 diabetic patients improve erythrocyte deformability by increasing plasma adiponectin levels and subsequent stimulation of the endothelial NO release.

In type 2 diabetic patients, an increased haematocrit effects endothelial function [48], and is assumed to contribute to an increase in the risk coronary vascular disease [49]. In accordance with previous studies [50;51], our study confirmed a slight decrease in Hct during treatment with pioglitazone. Also, no association could be observed in between the improvement in erythrocyte flexibility and the reduction in Hct, interferences in between changes in Hct and erythrocyte deformability can not be excluded, due the limited study size.

In conclusion this is the first study to show an improvement in erythrocyte deformability during treatment with pioglitazone in patients with type 2 diabetes mellitus. In consideration with data from the literature, it could be assumed, that the increase in adiponectin levels caused by pioglitazone treatment might affect erythrocyte membrane fluidity and thereby improve erythrocyte deformability. Our results further confirm important effects of pioglitazone in the modification of the cardiovascular risk profile which can be observed beyond glucose control.

Study limitation

A potential limitation of our findings is that the data were obtained from a subgroup treated at one site within a large multi-centre study. Because erythrocyte deformability was a secondary endpoint and no confirmatory study size estimation for this parameter deemed possible, the findings should be interpreted in an exploratory sense. Even if no association was found in between erythrocyte deformability and HbA1c levels or lipid parameters in the study, insufficient statistical power do not allow to rule out such a relationship.

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	Pioglitazone	Glimepiride
n	11	12
Age (years)	56 ± 9	58 ± 12
Male / Female	9 / 2	7 / 5
Body Mass Index	33.5 ± 5.1	32.0 ± 3.6
Duration of Diabetes (years)	6.1 ± 3.9	6.4 ± 4.8
HbA1c (%)	7.5 ± 0.9	6.8 ± 0.3 *
Syst. Blood Pressure (mmHg)	128.6 ± 11.8	132.5 ± 16.9
Diast. Blood Pressure (mmHg)	82.0 ± 8.2	81.5 ± 9.7
HDL (mg/dl)	43.4 ± 4.9	39.3 ± 5.6
LDL (mg/dl)	105.9 ± 34.3	68.8 ± 30.5 *
Triglycerids	200.1 ± 31.3	149.3 ± 50.3 *

Table 1: Clinical characteristics of the investigated groups (Mean ± SD; * = p<0.05)

Shear stress (PA)	0.30	0.60	1.20	3.00	6.00	12.00	30.00	60.00
GLIM (EI; %)	-0.4±1.7	-0.5±1.1	-1.1±2.5	-1.5±3.1	-1.4±2.9	-1.3±2.9	-1.3±3.5	-1.3±3.9
PIO (EI; %)	1.3±2.1	2.4±1.3 ^{*,§}	3.2±2.2 ^{*,§}	3.3±2.8 ^{*,§}	3.1±2.9 [*]	2.7±2.8	2.5±2.6	2.7±2.6

Table 2: Change in erythrocyte elongation index (delta EI) from baseline (* p<0.05 vs. baseline; § p<0.05 vs. GLIM)

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Figure 1 A. Erythrocyte Elongation Index (EI) at baseline and 6 months after treatment with pioglitazone (mean \pm SD); \square baseline, \blacksquare 6 months treatment with pioglitazone; * = $p < 0.05$ baseline vs. 6 months)

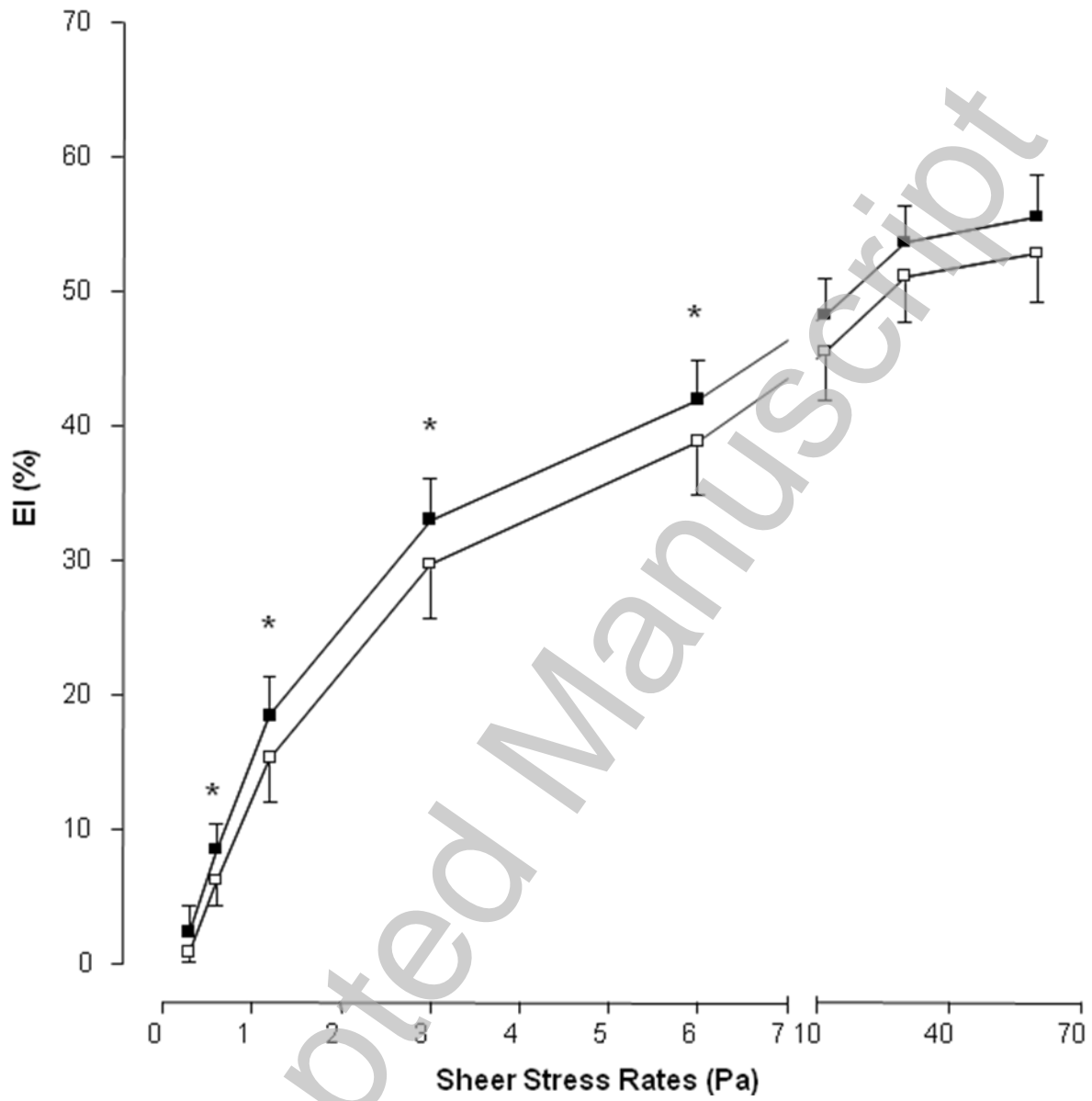
Figure 1 B. Erythrocyte Elongation Index (EI) at baseline and 6 months after treatment with sulfonylurea (mean \pm SD; \circ baseline, \bullet 6 months treatment with glimepiride)

Figure 2: Linear correlation between the increase in adiponectin levels and the increase in erythrocyte elongation index (EI) at a physiological shear stress rate of 1.2 Pa

Figure 3: Linear correlation between the decrease in intact proinsulin level and the increase in erythrocyte elongation index (EI) at a physiological shear stress rate of 1.2 Pa

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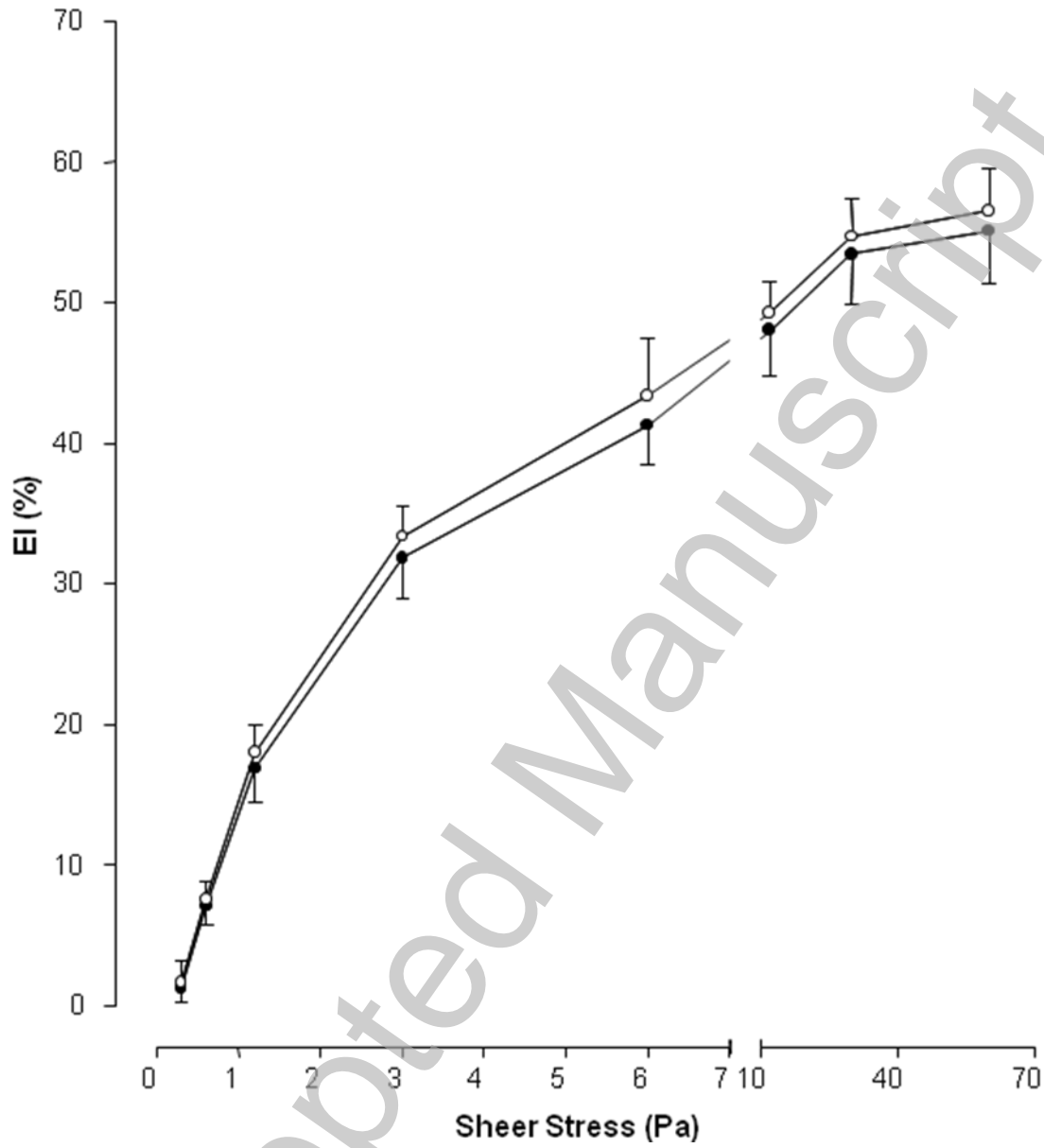
Figure 1 A:



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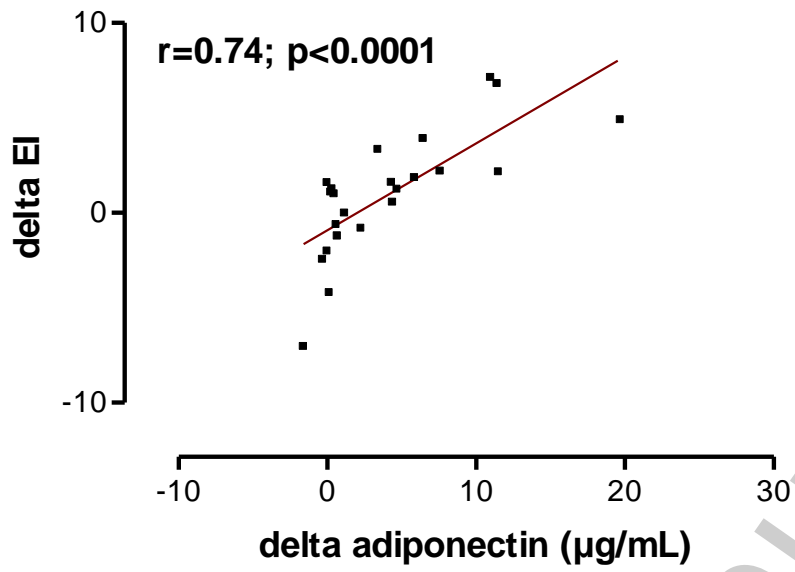
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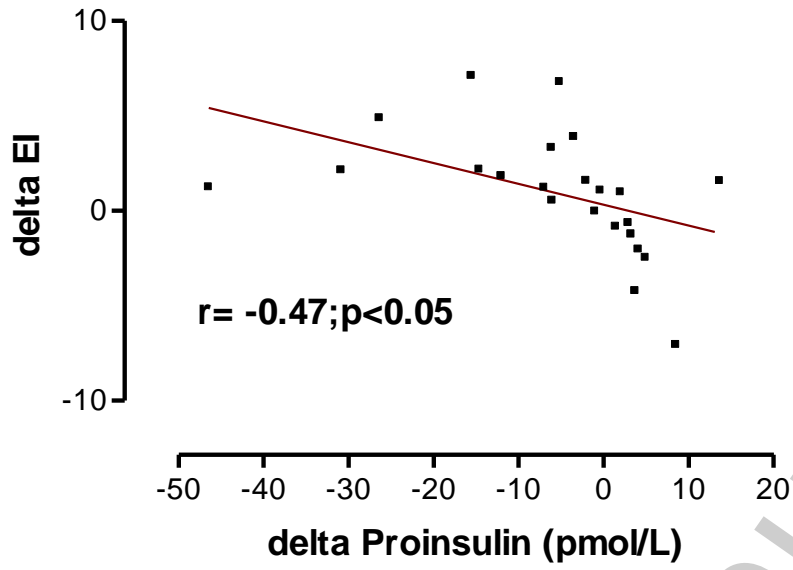
Figure 2:



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Figure 3:



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