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# **Glycaemic and non-glycaemic effects of pioglitazone in triple oral therapy of patients with type 2 diabetes**

Triple oral therapy in secondary drug failure

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**Keywords: type 2 diabetes, thiazolidinediones, adiponectin, NT-proBNP, oral therapy**

Abbreviations: ALT, alanine aminotransferase; TZD, thiazolidinediones; Nt-proBNP, N terminal pro Brain Natriuretic Peptide; PPAR $\gamma$ , Peroxisome Proliferator-Activated Receptor  $\gamma$

## Abstract

**Objectives.** To examine pioglitazone as add-on to metformin and insulin secretagogues in patients with type 2 diabetes and inadequate glycaemic control and its effect on glycaemic control, surrogate measures of insulin sensitivity (adiponectin) and beta cell function (proinsulin/insulin) and fluid retention.

**Design and setting.** Prospective open-label study of 54 patients with type 2 diabetes and HbA1c > 6.5% admitted to outpatient unit at Malmö University Hospital. The patients received 30-45 mg pioglitazone daily during 26 weeks in addition to their existing antidiabetic medication. After 26 weeks, one third of patients were followed for three months without pioglitazone.

**Results.** HbA1c decreased ( $7.8 \pm 0.9$  to  $6.3 \pm 0.9\%$ ,  $p < 0.001$ ) with 61% of patients achieving levels < 6.5%. However, in the group followed for another three months HbA1c increased ( $6.1 \pm 0.73$  to  $7.1 \pm 0.9$ ,  $n = 18$ ,  $p < 0.001$ ) after pioglitazone withdrawal.

Adiponectin increased ( $6.1 \pm 2.8$  to  $13.2 \pm 5.8$   $\mu\text{g/ml}$ ,  $p < 0.001$ ) and the proinsulin to insulin ratio decreased ( $0.89 \pm 0.66$  to  $0.66 \pm 0.53$ ,  $p < 0.001$ ). Nt-proBNP increased from  $487.3 \pm 252.2$  pmol/l to  $657.8 \pm 392.1$  pmol/l ( $p < 0.001$ ).

**Conclusions.** Pioglitazone is effective in achieving glycaemic targets and reducing risk factors involved in atherosclerosis and improving beta cell function when used as part of triple oral therapy in patients with type 2 diabetes and secondary drug failure. Nt-proBNP increase with concomitant decrease in haemoglobin suggests a sub-clinical sign of fluid retention.

## Introduction

Type 2 diabetes (T2D) is a progressive disorder with a consistent and steady increase in HbA<sub>1c</sub> over time associated with enhanced risk of micro-and macrovascular complications (1-3). The deterioration of glycaemic control over time is independent of the mode of treatment with traditional oral antidiabetic drugs, meaning that most patients with T2D at some point will reach the point of secondary drug failure. In fact, in the United Kingdom Prospective Diabetes Study (UKPDS) adding insulin to oral therapy did not change the slope of this deterioration curve (4). Secondary drug failure is considered to be a consequence of deteriorating beta cell function more than of worsening of insulin resistance (5-8). The untoward effects of glucose (glucotoxicity) and fat (lipotoxicity) on beta cell function are most likely the causes of this secondary failure. Decreased plasma glucose levels can be achieved by insulin secretagogues (sulfonylureas and meglitinides), agents reducing hepatic glucose production (biguanides), insulin and insulin sensitizers (thiazolidinediones (TZDs)). The most common treatment strategy of T2D today is to start with metformin and add sulfonylurea (or vice versa) and when this combination therapy fails, to add or replace it with insulin. An alternative, which has become increasingly popular, is to add a thiazolidinedione.

TZDs are selective ligands of the nuclear transcription factor peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and are currently approved for treatment of hyperglycaemia. These drugs have been demonstrated to improve glucose tolerance and to decrease insulin-resistance in patients with type 2 diabetes and to be effective in lowering plasma glucose levels both in monotherapy and in combination with SUs, metformin and insulin. These drugs have also been shown to have multiple beneficial effects not only on peripheral insulin sensitivity, hepatic glucose metabolism, and lipid metabolism, but also on endothelial function, atherogenesis, fibrinolysis and immune function (9). TZD treatment has

been associated with fluid retention, weight gain and peripheral oedema. This increase in body weight and oedema has been associated with heart failure (10). It is of interest to investigate whether it is possible to identify patients at risk of developing fluid retention associated with TZD-treatment. The concentration of Brain Natriuretic Peptide (BNP) increases with left ventricular overload and has been proposed as a possible indicator of fluid retention (11-13). Both BNP and Nt-proBNP are elevated in early left ventricular systolic as well as in diastolic dysfunction (14, 15). Nt-proBNP is a split product from the BNP that is more stable and should be preferred as a surrogate marker for left ventricular dysfunction..

The aim of this study was to evaluate the efficacy of adding pioglitazone to treatment with metformin and insulin secretagogues in patients with T2D and inadequate glycaemic control as well as the safety regarding the possible fluid retention with this regimen. Efficacy was defined as percentage of patients achieving treatment goals of HbA<sub>1c</sub> < 6.5%. As pioglitazone is supposed to primarily influence insulin sensitivity we used serum adiponectin concentrations as a surrogate measure of insulin sensitivity (16). Pro-insulin concentrations were measured to obtain insight into how therapy influenced beta cell function. Nt-pro BNP was measured as a cardiac marker, cystatin C to estimate GFR and haemoglobin in all together to assess a possible sub-clinical fluid retention.

## **Subjects and methods**

**Subjects:** Sixty-six patients with type 2 diabetes and secondary drug failure were screened; eight patients were ineligible due to either cardiac failure according to New York Heart Association III-IV (n= 2), severe renal disease that caused withdrawal of metformin (n =1), ongoing medication with Non-Steroid Anti Inflammatory Drugs (n=1), cancer (n=1),

proliferative retinopathy (n=1), severe hyperglycaemic symptoms and acute need of insulin therapy (n=2).

Secondary drug failure was defined as HbA1c > 6.5% (ref. 4-5.3%) measured by Swedish Mono-S (high performance ion-exchange liquid chromatography) method  $\cong$  > 7.3% NGSP (the National Glycohemoglobin Standardisation Program in the United States)(17) at the 2 latest measurements with at least 8 weeks in between during ongoing treatment with metformin > 1500 mg/day and glibenclamide > 7 mg/day or glipizide > 10 mg/day or glimepiride > 3 mg/day or repaglinide > 6 mg/day for at least 3 months. Other entry criteria were age between 30-75 years and BMI > 20. Local ethics committee approved the study and signed informed consent was obtained from all patients. Three patients were excluded during the study because of non-adherence to the protocol and one patient dropped out because of side effects (vertigo, weight loss). These four patients refused to be followed up at the hospital and there are no samples available on them after inclusion and thereby they are not included in the analyses. Fifty-four patients completed the study, all of them Caucasians (Fig.1). Only 50 samples were eligible for measurements of adiponectin and 45 samples for measurements of insulin, proinsulin, Nt-proBNP and cystatin C.

**Study design:** The study had an open-label prospective observational design with 26 weeks of follow up with intermediate visits at 8<sup>th</sup> and 16<sup>th</sup> weeks and pre-intervention sampling as control with routine blood samples, recording of body weight, cardiopulmonary symptoms and other side effects. Blood samples for measurement of HbA1c, haemoglobin blood lipids and ALT were drawn at each visit and for measurement of insulin, proinsulin, adiponectin, Nt-proBNP and cystatin C at base line and at the study end. The patients received a prescription of 30 mg pioglitazone daily in addition to their existing therapy with metformin and an insulin secretagogue. After 16 weeks the dose of pioglitazone was increased to 45



mg/day if HbA<sub>1c</sub> was not < 6.5% and the therapy was tolerated. At the end of the study one third of patients were randomized to be followed with HbA<sub>1c</sub> measurements for another three months after withdrawal of pioglitazone (but with unchanged previous medication)(Fig.1).

**Assays:** HbA<sub>1c</sub> was analyzed using the Variant II chromatographic method from Bio-Rad with a CV of 3.0% at HbA<sub>1c</sub> 4.4%- 8.8%. Plasma insulin concentrations were measured with a double antibody enzyme-linked immunosorbent assay (ELISA) (Dako, Cambridgeshire, U.K.) with an intra-assay and inter-assay CV of 7%. Plasma Proinsulin levels were measured with an ELISA assay (DakoCytomation Total Proinsulin) with an inter-assay CV of 7%. Serum concentrations of adiponectin were analyzed using a commercial radioimmunoassay (Linco Research, St. Charles, Missouri, USA). Nt-proBNP was analyzed using a competitive Enzyme Immuno Assay (Biomedica laboratories, Vienna, Austria) with an inter-assay CV of 6.5% and intra-assay CV of 4.4%. Plasma cystatin C was measured by an automated particle-enhanced turbidometric assay (Dade Behring) and a calibrator obtained from DakoCytomation (Glostrup, Denmark)(18, 19).

**Statistical analysis:** Data are expressed as means  $\pm$  SD. Differences over time of normally distributed variables were tested by paired t-test and of non-normally distributed variables by Wilcoxon rank test. Differences between group means were tested by unpaired-test or Mann-Whitney rank sum test, where appropriate. All tests are two-tailed and a P value of less than 0.05 was considered statistically significant. Efficacy was defined as percentage of patients achieving HbA<sub>1c</sub> < 6.5%. All analyses were carried out using the SPSS statistical software, version 12.02.

## Results

Patients' characteristics at base line and changes during therapy are summarized in table 1. There were no gender differences except in HbA<sub>1c</sub> changes, which was greater in women,  $p=0.029$ .

### **Pioglitazone induces weight gain but improves metabolic control**

After 26 weeks of treatment, HbA<sub>1c</sub> decreased from  $7.8 \pm 0.9$  to  $6.3 \pm 0.9$ ,  $p < 0.001$  (Fig.2A). While forty-three patients (80%) had at start HbA<sub>1c</sub>  $> 7\%$ , thirty-three (61%) reached the goal of HbA<sub>1c</sub>  $< 6.5\%$ . In the 18 patients, which were followed off-pioglitazone HbA<sub>1c</sub> increased after three months from  $6.1 \pm 0.73$  to  $7.1 \pm 0.9$ ,  $p < 0.001$ . During the study there was a significant weight gain from  $90 \pm 15$  to  $94 \pm 16$  kg ( $p < 0.001$ ) corresponding to an increase in BMI from  $31 \pm 4$  to  $32 \pm 4$  kg/m<sup>2</sup> ( $p < 0.001$ ) (Fig.2B). Despite the increase in BMI there was a significant decrease in waist-to-hip ratio (WHR) from  $1.03 \pm 0.08$  to  $1.00 \pm 0.06$  ( $p = 0.002$ ) (Fig.2C) that was due to smaller increase in waist ( $+1.6$  cm;  $p = 0.026$ ) than hip ( $+4.9$  cm;  $p < 0.001$ ) circumference. Also ALT decreased ( $0.5 \pm 0.3$  to  $0.4 \pm 0.1$   $\mu$ kat/l,  $p < 0.001$ ) (Fig.2D). Triple therapy was associated with an increase in HDL ( $1.06 \pm 0.23$  to  $1.11 \pm 0.29$  mmol/l;  $p = 0.029$ ) and decrease in triglycerides ( $1.9 \pm 0.9$  to  $1.6 \pm 0.8$  mmol/l;  $p = 0.008$ ) concentrations whereas total cholesterol concentrations remained unchanged.

### **Improvement of surrogate measures of insulin sensitivity and beta-cell function**

Adiponectin levels, which were used as surrogate markers of insulin sensitivity, increased more than two fold ( $6.1 \pm 2.8$  to  $13.2 \pm 5.8$   $\mu$ g/ml,  $p < 0.001$ ) (Fig.3 A) and the increase correlated with the decrease in HbA<sub>1c</sub> ( $r = -0.45$ ,  $p = 0.001$ ). There was also a highly significant decrease in proinsulin to insulin ratio ( $0.89 \pm 0.66$  to  $0.66 \pm 0.53$ ,  $p < 0.001$ ) without any significant changes in insulin levels (Fig.3 B)

### **Nt-proBNP increases during pioglitazone therapy**

Nt-proBNP levels increased significantly after 26 weeks of treatment with pioglitazone from  $487.3 \pm 252.2$  pmol/l to  $657.8 \pm 392.1$  pmol/l ( $p < 0.001$ ) (Fig.4A). The elevation in Nt-proBNP was still significant after exclusion of patients with known cardiovascular disease from the analysis, from  $447.2 \pm 223.6$  pmol/l to  $638.8 \pm 385.7$  pmol/l ( $p = 0.002$ ). Cystatin C increased from  $0.96 \pm 0.20$  g/l to  $1.02 \pm 0.21$  g/l, ( $p = 0.004$ ) which corresponded to a  $6.8 \pm 0.18\%$  change in GFR as calculated from the cystatin C values (20). The individual change in GFR and Nt-proBNP did not correlate with each other ( $r^2 = 0.02$ ). In the 37 patients without cardiac dysfunction the change in Nt-proBNP correlated negatively with the change in haemoglobin ( $R = -0.353$   $p = 0.032$ )

### **Side effects**

The most common side effect was oedema reported by ten patients (19%), in four (7%) of them transient. Diuretics were prescribed for the other six (11%) of them. Twelve patients (22%) experienced mild hypoglycaemia; none of them required assistance by third person but these patients had because of hypoglycaemic episodes to reduce the dose of sulfonylurea.

There was a significant decrease in haemoglobin concentrations from  $139 \pm 11$  g/l to  $131 \pm 13$  g/l ( $p < 0.001$ ) (Fig.4B).

### **Conclusions**

In this study we have shown that pioglitazone can achieve treatment targets of glycaemic control in more than 60% of patients with type 2 diabetes not responding to combined therapy with metformin and an insulin secretagogue. About half of the patients

achieved this goal with 30 mg of pioglitazone (n = 29, 54%) while 80 % of patients had HbA<sub>1c</sub> levels > 7 % at start.

This study had an open-label design with patients being their own control with their pre-intervention values. Even if the open study design without a comparator could miss controlling the effect of just being monitored in a study, the results of the withdrawal test at the end of the study confirms the additive effect of pioglitazone. The change in HbA<sub>1c</sub> levels was associated with a two-fold increase in a surrogate marker of insulin sensitivity, adiponectin (Fig.3A) that also correlated with improvement in HbA<sub>1c</sub>. In pioglitazone-treated patients there have been observations of a nearly 3-fold increase in adiponectin levels and strong association with a decrease in hepatic fat content and improvements in hepatic and peripheral insulin sensitivity (21, 22). Adiponectin has also been suggested to mediate improvements in hepatic insulin sensitivity during PPAR $\gamma$ -agonist therapy (23). Of note, treatment with metformin or glyburide does not enhance adiponectin levels despite a similar improvement of HbA<sub>1c</sub> (24). In our study there was also a continuous decrease in ALT (Fig.2D) that could reflect a redistribution of fat from liver adipose depots. Notably, we observed a significant decrease in WHR. However, in accordance with results from Shadid et al (25) this was due to a greater increase in hip than decrease in waist circumference reflecting the depot specific site of action of pioglitazone and supporting the view that TZDs promote redistribution of fat from abdominal to subcutaneous regions. The increase in HDL and decrease in triglycerides concentrations in our study whereas total cholesterol concentrations remained unchanged are in agreement with some previous studies (26, 27).

Changes in beta-cell function are both an early and critical component in the pathogenesis of the hyperglycaemia of type 2 diabetes (28). Beta cell dysfunction is the

hallmark of secondary drug failure and can manifest among other ways in altered conversion of proinsulin to insulin (29-34). The ratio of proinsulin to insulin is therefore a qualitative marker of beta-cell function (35). In animal models of obesity related diabetes TZD treatment demonstrated a preservation of pancreatic islet area and density as well as a reduction in rates of beta cell death (36) suggesting that methods reducing insulin demand may act to protect and spare pancreatic beta-cells. In our study we observed a highly significant decrease in the proinsulin to insulin ratio suggesting an alleviation of the demands on the beta cells and reduced beta-cell stress.

There has been a general concern that some of the patients that receive glitazone treatment do retain fluid (10) and that this could be of potential hazard. Patients in our study did tolerate the treatment well but did gain weight. In our study 73% of the patients showed an increase in Nt-proBNP in response to pioglitazone. This could be either due to a direct cardiac effect, an effect on GFR or an increased fluid load. In contrast, glitazones have also been reported to increase cardiac performance in patients with diabetes and heart failure (37). Interestingly, the increase in Nt-proBNP levels was still significant after excluding patients with known cardiovascular disease. Taken together, the findings that Hb levels decrease in response to pioglitazone treatment and that weight increases would imply that the Nt-proBNP increase rather would be a result of increased haemodynamic load on the heart to explain the increased levels of Nt-proBNP than as an impairment of cardiac function per se. The decreases in Hb are generally attributed to fluid retention. There is some recent evidence that the fluid retention may be due to stimulation of ENaC-mediated renal salt absorption (38). The precise mechanism of TZD-induced oedema is not known but Nikolaidis et al argue for peripheral mechanisms which are unrelated to central haemodynamics (39). Insulin also promotes oedema (40) and it is therefore possible that enhanced insulin action in the kidney

or vessels may be involved. Basu et al. have recently reported that pioglitazone increases total body water, thereby accounting for the majority of weight gain (41).

There was a significant increase in cystatin C that by the recent established estimation of GFR would be equivalent to less than 10% decrease in GFR (20). The nature of this decrease is not known and need further studies to be explained. However the change of GFR didn't correlate to the changes in Nt-proBNP.

In our material we could not reproduce the results from Ogawa et al, in which the presence of a high baseline BNP predicted an increase in BNP during pioglitazone therapy (11). Of note, both study designs and patient populations differed between the studies. Also, Ogawa et al measured BNP whereas we measured Nt-proBNP.

The treatment was well tolerated and the most common side effects were weight gain, oedema and hypoglycaemia. These types of side effects are frequently encountered with treatment with other glucose lowering agents. However, the fluid retention seems to be class specific (26, 42, 43) and weight gain more prominent when treating with TZD. Although haematocrit has been shown to be a risk factor for ischaemic heart disease (44) it is not known whether small decrease in haematocrit achieved by TZD has an impact on macrovascular disease. Low doses of diuretics easily reversed oedema

The open study design without a comparator limits some of the interpretation of the results. However, the effect on HbA1c seems though to be specific to treatment with pioglitazone as HbA1c significantly increased after withdrawal of the drug. The most logical comparator after failure on two oral agents would be insulin, which is not easy to use in a double-blinded fashion.

In conclusion, our study indicates that pioglitazone is effective in reaching treatment goals in patients with secondary drug failure but this is achieved at a price of fluid retention, which in the majority of cases was mild and easily reversible, by the use of diuretics. The treatment seems to also have additional positive effects on insulin sensitivity, beta cell stress, lipid profile and body fat distribution. These changes can be translated into a decrease in macrovascular complications as shown by the PROactive study (45).

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## Figure legends

**Fig. 1.** Flow chart of the study design

**Fig. 2.** (A) There is a steady decrease in HbA<sub>1c</sub> during 26 weeks of treatment with pioglitazone ( $p < 0.001$ ) in spite of (B) an increase in BMI ( $p = 0.002$ ). These changes are accompanied by (C) decrease in WHR ( $p < 0.001$ ) due to a larger increase in hip ratio and (D) a steady decrease in ALT ( $p < 0.001$ ) indicating redistribution of fat from hepatic depots. Error bars present 95% confidence interval for mean. P values are calculated between weeks 0 and 26.

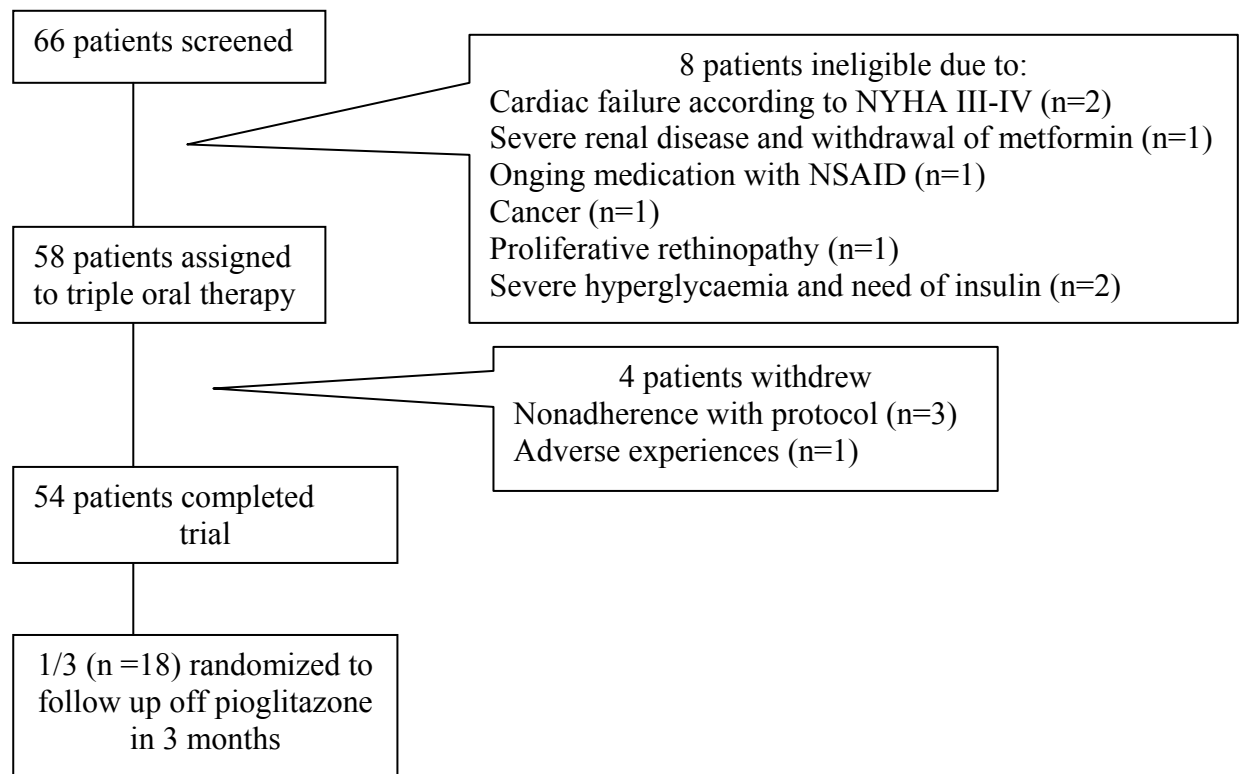
**Fig. 3.** (A) Changes in adiponectin levels ( $p < 0.001$ ) and (B) proinsulin to insulin ratio ( $p < 0.001$ ) during 26 weeks of treatment with pioglitazone indicating increased insulin sensitivity and reduced load on beta cells.

**Fig. 4.** (A) Increase in Nt-proBNP is associated with (B) decrease in haemoglobin suggesting haemodilution to contribute to increased pressure in left ventricle leading to increased levels of Nt-proBNP.  $P < 0.001$  between week 0 and 26.

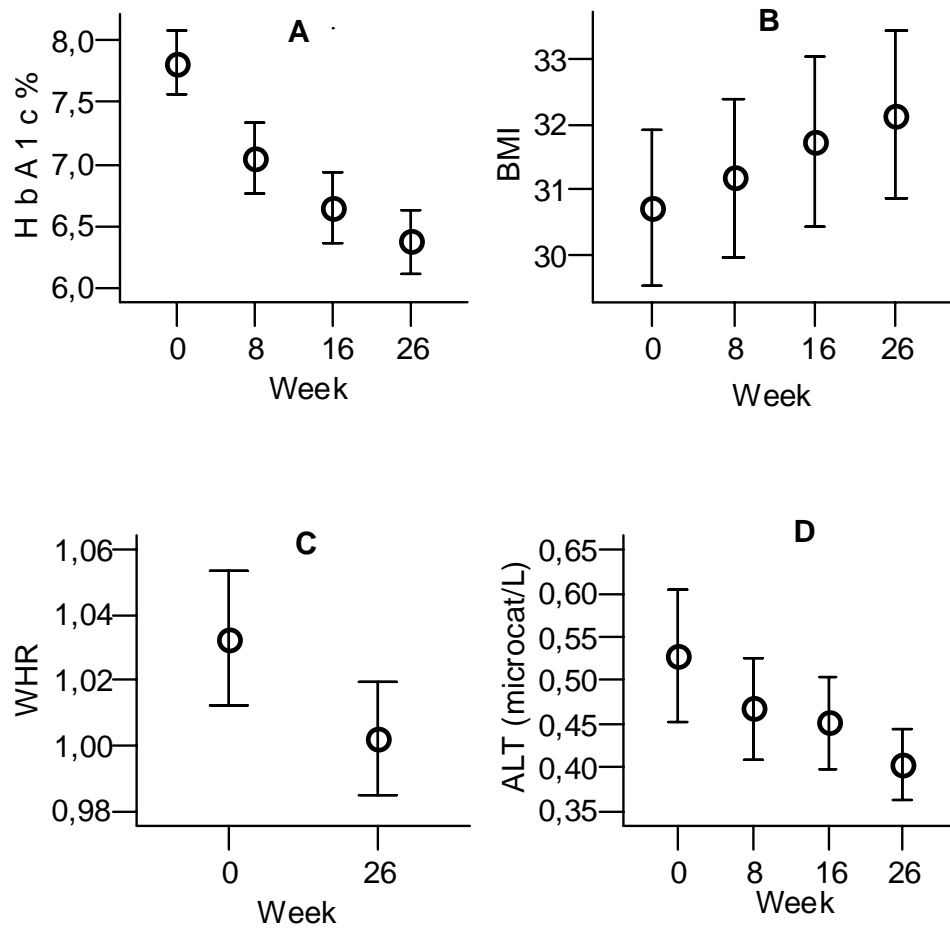
**Table 1.** Subjects' characteristics at base line and study end

	N	Baseline	Study end	P
Age (years)		60.6+7.8		
Sex (male/female)		(31/23)		
Diabetes duration (years)		11.3+6		
HbA1c (%) in all subjects	54	7.8+0.9	6.3+0.9	<0.001
Male	31	7.8+0.8	6.6+1	
Female	23	7.8+1.1	6.1+0.8	
Weight (kg)	54	89.6+15	93.7+15.6	<0.001
BMI (kg/m <sup>2</sup> )	54	30.9+4	32.4+4.3	<0.001
WHR	54	1.03+0.08	1.00+0.06	0.002
Haemoglobin (g/l)	54	139+11	132+13	<0.001
Cholesterol (mmol/l)	54	4.7+0.8	4.7+0.8	0.965
HDL (mmol/l)	54	1.06+0.2	1.11+0.3	0.029
LDL (mmol/l)	53	2.8+0.7	2.8+0.7	0.626
TG (mmol/l)	54	1.9+0.9	1.6+0.8	0.008
ALT (μkat/l)	54	0.5+0.3	0.4+0.18	<0.001
Adiponectin (μg/ml)	50	6.1+2.8	13.2+5.8	<0.001
Insulin (pmol/l)	47	51.2+31.3	57.4+47.3	0.218
Proinsulin (pmol/l)	46	45.7+38.7	30.9+27.2	0.001
Proinsulin/Insulin	44	0.89+0.66	0.66+0.53	<0.001
Nt-proBNP (pmol/l)	45	487+252	658+392	<0.001
Cystatin C (g/l)	45	0.96+0.20	1.02+0.21	0.004

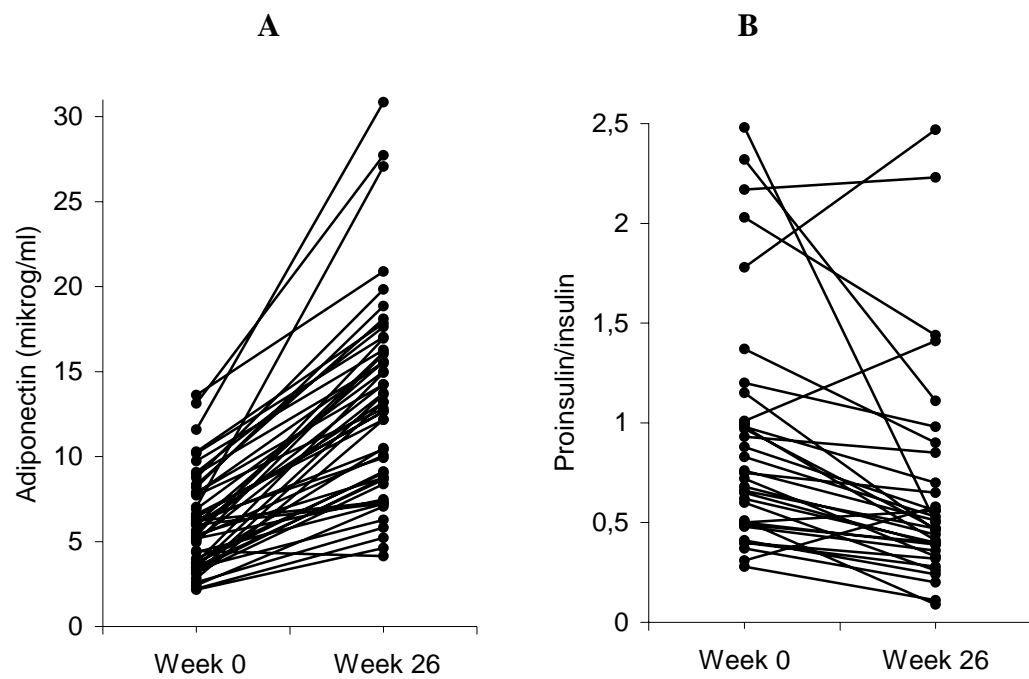
Data are expressed as mean + SD, (95% Confidence interval)



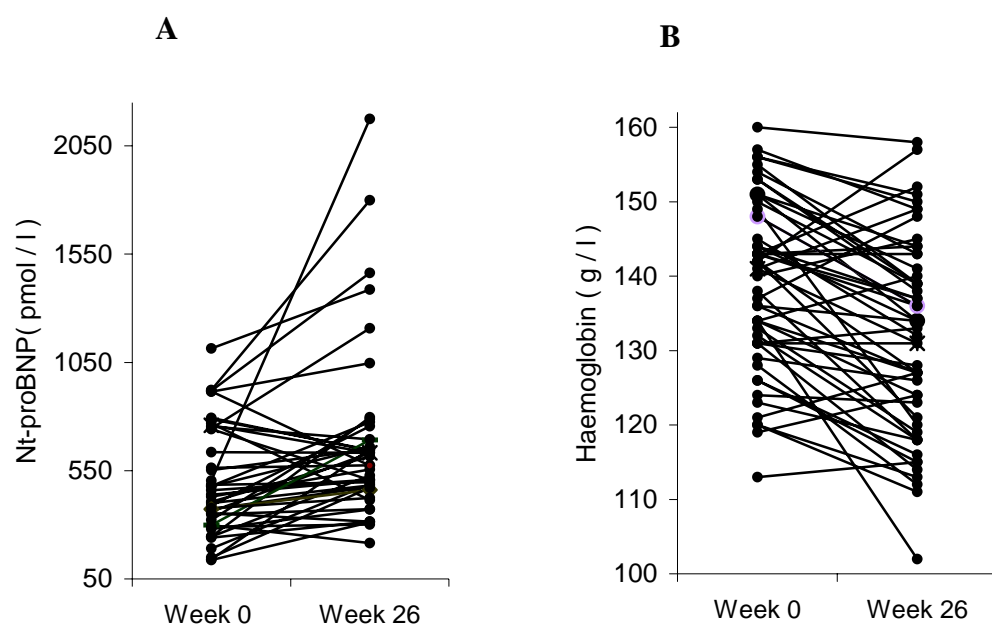
**Fig.1**



**Fig.2**



**Fig. 3**



**Fig.4**