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Effect of Epoetin Induced Normal Hb on Progression of Nephropathy in Insulin Treated Diabetic Patients with Renal Anaemia

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Abstract

Background and aims: Haemoglobin (Hb) decreases were associated with progression of nephropathy and it has been suggested that normal Hb levels may preserve renal function. Our aim was to investigate if this could be achieved by erythropoietin (EPO).

Methods: A prospective randomized study of treatment with erythropoietin (EPO) was carried out in insulin treated diabetic patients with albuminuria, morning urine albumin > 300 mg/l, and anaemia, Hb < 110 g/l. EPO was given in order to achieve Hb levels 115 g/l (low) or 130 g/l (normal). The patients were followed for one year and evaluated every third month with timed overnight urine collections, 24 hour blood pressure measurement and glomerular function (GFR) with iohexol clearance.

Results: Twelve patients achieved 115 g/l and seven patients 130 g/l. The median GFR before treatment was 26 (12-50) and 29 (15-53) ml/min/1.73m^2 (median and min-max, p=NS). The median decrease in GFR per month was faster in the patients with low Hb [- 1.0 (-2.89 to 0.25)] than in the normal Hb level group [-0.08 (-1.08 to 1.16)] ml/min/1.73m^2 per month (median and min-max, p= 0.015). Four patients were started on dialysis (n=2) or died (n=2), all in the low Hb group. Urine human complex (a-1-microglobulin) increased, i.e. proximal tubular function worsened, in the low Hb group with no change in the normal Hb group. Urine albumin, Immunoglobulin G, glycosaminoglycans, collagen IV non-collagenous part (NC1) and Tamm-Horsfall protein did not change in any of the groups. Glycated haemoglobin (HbA_{1c}) and blood pressure levels were unchanged in both groups.

Conclusions: The present study infers that EPO treatment may preserve both glomerular filtration rate and proximal tubular function. Further studies are needed to confirm our findings.

Keywords: Anaemia, a-1-Microglobulin, Diabetes, Nephropathy, Erythropoietin

Introduction

Diabetic patients may have a blunted erythropoietin (EPO) response to anaemia (1, 2), which is augmented by poor metabolic control (2). Renal insufficiency leads to decreased EPO production and anaemia. The latter may be corrected by subcutaneous administration of recombinant EPO (3, 4). The effect of this on renal function, retinopathy and the cardiovascular system have been very little studied in diabetic patients (5-8). With EPO therapy the rate of deterioration in the renal function may be attenuated (5) and diabetic retinopathy improve (6). Cardiovascular hemodynamics may improve in dialysis patients during treatment with

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EPO (7) and in predialytic stage of diabetic patients with heart failure renal function may stabilize (9).

Erythropoietin is a glycoprotein that controls the generation of erythrocytes from hematopoietic progenitor cells. Recent experimental reports using in situ hybridization (10), immunohistochemical characterization by light/electron microscopy (11) and transgenic mice have been published (12). Possible renal sites of EPO production are the peritubular cell (11), the fibroblast type 1 interstitial cell (12-14) and 5′-nucleotidase-positive fibroblast residents in the peritubular labyrinth (11, 13). Hence, the expansion of interstitial fibrosis in the tubulointerstitial damage may be linked to renal anaemia probably by a reduced number of cells producing EPO. Focal or generalized tubulointerstitial lesions are in fact frequently found in diabetic patients (15, 16). Furthermore, the diabetic kidney is particularly sensitive to hypoxia and is mostly on the border to hypoxia (17). Anaemia may be suspected to induce further hypoxia and be deleterious to the kidneys.

Anaemia may occur very early in diabetic nephropathy (18) and may be a significant predictor of decline in glomerular filtration rate (GFR) (19). In addition, diabetic patients with nephropathy have four times higher risk than other renal patients for being anaemic (20). Even mild anaemia in diabetic patients with nephropathy increases the risk for progression to end stage renal disease (21).

The aim of the present study was to investigate the effect of EPO treatment in order to achieve two different Hb levels, corresponding to slight anaemia, 115 g/l, and normal Hb 130 g/l, as previously suggested by Besarab and Aslam (22), on GFR and secondarily on urinary parameters, reflecting glomerular, proximal and distal tubular system.

Materials and Methods

It was a one year open-label single center study with active product with two levels of Hb as aim performed during 2004-2006. The patients were randomized in blocks of six patients, with blinded envelopes conaining three with Hb aim of 115 and three with 130, to either Hb levels 115 (110-120) or 130 (125-135) g/l. Inclusion criterias were: Insulin treated diabetic patients with diabetic nephropathy, defined as morning urine albumin (u-albumin) >300 mg/L, and Hb < 110 g/l. Exclusion criteria: 1) a history of a psychiatric disease or condition such as to interfere with the patient's ability to understand the requirements of the study. 2) A disease likely to interfere with the evolution of the patient's safety and the study outcome. 3) Anaemia caused by non-renal disease or uncontrolled hypertension or diastolic blood pressure above 100 mmHg. 4) Within the last month myocardial infarction or stroke, unstable angina or thromboembolic disease within the last six months. 5) Chronic liverinsufficiency or epilepsia. 6) Allergy toward bensolacid (the conserving medium). Informed consent was obtained from all patients. The study was approved by the local ethics committee.

Disease evaluation (efficacy criteria)

Definitions of renal, glomerular and tubular function. Renal function was evaluated by measurement of serum creatinine and glomerular filtration rate of iohexol. Iohexol clearance was measured by the plasma clearance of iohexol by analysis of a single exponent (23) within one month before start of treatment and during treatment every three months. Glomerular function was furthermore evaluated by measuring the urinary excretion of immunoglobulin G (u-IgG) and albumin. Proximal tubular function was evaluated by measuring urinary excretion of protein HC (Human Complex = a-1-microglobulin, u-HC) and distal tubular function by excretion of Tamm-Horsfall protein (u-THP). Worsening of glomerular and proximal tubular function was defined as an increase in any of the parameters studied, while worsening of distal tubular function was defined as a decrease in Tamm-Horsfall protein.

Urine sampling: The patients collected three timed overnight urines before treatment and thereafter one sample every three months. Urine was collected as 10 ml aliquots in polystyrene tubes and stored at 4°C for less than four days before analysis in the central laboratory. For enzyme linked immunosorbent assay (ELISA) the urine was stored at -20°C before analysis in the renal laboratory.

Analytical techniques: Glycated haemoglobin (HbA_{1c}) levels was analysed by fast liquid chromatography (normal value <5.3%). Total cholesterol, LDL, HDL and triglycerides, cobalamin, folate, transferrin, iron and ferritin were analysed on an automated analyser (Cobas Mira S, Roche, Basel, Switzerland) with techniques described by Roche. Urinary immunoglobulin G, and HC were analysed by turbidimetry with the automated analyser (Cobas Mira S, Roche, Basel, Switzerland), antibodies (rabbit anti-human) and techniques described by Dakopatts (Copenhagen, Denmark) with detection limit 5 mg/l. Urine- and serum creatinine levels were analysed by an enzymatic method (creatinine-hydrolase; EKTA chem-analyzer, Instrument KODAK, N.Y., USA). The above mentioned methods were available as routine analys at the central laboratory. Urine albumin concentration (24), collagen IV NC1 (25) (u-NC1) and total glycosaminoglycans (u-GAG) (26) and Tamm-Horsfall protein (27) were analysed by previous described methods at the renal research laboratory.

Dosage regimen & dose adjustment

The patients were examined for haemoglobin in the stools, deficiency of folate or cobalamines, s-iron levels and study parameters. Thereafter treatment was started with erythropoietin 2000 units/week (Epoietin beta, Neorecormone, Roche AB, Stockholm, Sweden). The drug was given subcutaneously in the thigh or abdomen. The dose was increased by 2000 units/week every month until the treatment goal was achived. Systolic and diastolic blood pressure was analysed by 24 hour measurements with the Spacelab model 902171 and was treated to normal levels if possible (daytime < 140/80 and nightime <130/80).

Concomitant treatment

There was no restrictions. Iron (Venofer, Iron (III) hydroxide/sackarose-Complex, Renapharma AB, Uppsala) was supplemented iv if there was an iron deficiency. Blood pressure was treated equally in both groups: with selective b blockers or ACE inhibitors during three months. If the aim of 140/80 mmHg (130/80 nighttime) was not achived the doses were increased or the drugs combined. If the aim was still not achieved furosemide and/ or Ca-blockers were added. If the ACE inhibitor was not tolerated an angiotensin II recptor blocker (ARB) was used. The cholesterol was lowered with statins to reach the goal of p-LDL of 2,0 mmol/l.

Statistics

Calculations of number of patients were based on the assumption that treatment with erythropoietin would result in a 125% increase of the serum creatinine in patients with Hb levels 110-120 while no increase was expected in patients with Hb levels of 125-135 g/l. We would thus need 40 patients in the study (a=0.05 and b=0.20). However, as the study was a pilot study we reconsidered after inclusion of 18 patients and performed calculations on change in GFR which is a much better measure than change in creatinine. With a two sample T-test power analysis the power was 0.81.

Urine parameters are given as a ratio between the parameter studied and urine creatinine in order to correct for collection failures. The Mann-Whitney test was used for non-paired and the Wilcoxon test for paired differences. Differences in number of patients between groups were analysed by Chi-Square test. Correlations between variables were tested with Spearman´s test. The Statistic Package for Social Sciences (SPSS version 16.0, Chicago, USA) was used.

The significance level used was $p<0.05$.

Results

In total nineteen patients were included. Ten patients were randomised to normal Hb and nine to low Hb. Six of the patients allocated to normal Hb levels did not achieve the goal of an Hb above 125 g/l, while three of the patients randomised to low Hb achieved a normal Hb. It was difficult to foresee the effect on Hb levels of the above regime for dosing the EPO. No differences were found between the groups analysed by intention to treat. Consequently, we analysed the results not on basis of intention to treat but on the actual Hb level achieved.

All patients were treated for hypertension and all except one had diuretics. 13 patients had ACE inhibitors or ARBs (Table 1). Other medications were b, Ca and a-blockers. Seven patients, three in the normal Hb and four in the low Hb group (NS) had intravenous iron.

At baseline, there were no differences in type of diabetes, gender, age, duration of diabetes, retinopathy, Hb, HbA_{1c}, blood pressure levels, cholesterol, HDL or triglyceride levels or GFR or previous history of myocardial infarction or numbers treated with ACE inhibitors or cholesterol lowering drugs (statins) (Table 1). The LDL levels were higher in the group with normal Hb compared to low Hb ($p = 0.04$). During the treatment there were no differences between the groups and no change in the mean of

Table 1. Baseline data in patients who achieved low Hb or normal Hb levels

	Low Hb (115 g/l) N=12	Normal Hb (130 g/l) N=7
Type of diabetes (1/2)	1/11	3/4
Gender (m/f)	9/3	2/5
Age (years)	67 (51-83)	69 (41-71)
Diabetes duration (years)	23 (4-47)	36 (7-50)
Retinopathy (no/simplex/proliferative)	3/2/7	1/2/4
Hb (g/l)	103 (83-109)	105 (94-115)
HbA_{1c} (%)	7.0 (5.1-13)	7.5 (6.5-10.2)
SBP (mmHg)	147 (133-169)	138 (129-159)
DBP (mmHg)	74 (68-85)	66 (62-86)
GFR (ml/min/1.73m^2)	26 (12-50)	29 (15-53)
Cholesterol (mmol/l)	4.2 (3.1-7.3)	4.4 (3.3-10)
HDL (mmol/l)	1.2 (0.5-1.9)	1.4 (0.6-2.2)
LDL (mmol/l)	1.9 (1.5-4.8)	3.3 (2.2-7)*
Triglycerides (mmol/l)	1.7 (0.8-4.2)	1.7 (0.9-2.8)
Previous myocardial infarct	3	2
ACE/AII inhibitor (n)	8	5
Statins (n)	10	5

Data are given as median and range (min-max).

NS, Not statistical significant different.

* P = 0.04 *vs.* low Hb

Table 2. Median levels in patients who achieved low Hb or normal Hb levels

	Low Hb (115 g/l)	Normal Hb (130 g/l)
EPO (units/week)	4500 (1500-10000)	5000 (2000-10000)
Hb (g/l)	114 (102-124)	137 (127-167)*
HbA_{1c} (%)	7.1 (4.8-7.7)	7.9 (6.4-11.0)
SBP (mmHg)	152 (118-204)	150 (135-165)
DBP (mmHg)	75 (62-100)	73 (66-85)
SBP day (mmHg)	154 (120-205)	151 (140-167)
DBP day (mmHg)	77 (62-102)	74 (71-83)
SBP night (mmHg)	148 (118-204)	146 (132-159)
DBP night (mmHg)	72 (60-92)	67 (62-87)
ΔGFR (ml/min/1.73m^2/month)	-1.0 (-2.89-0.25)	-0.08 (-1.08-1.16)**
Started dialysis	2	0
Dead	2	0
Myocardial infarction	1	0

SBP, Systolic Blood Pressure; **DBP**, Diastolic Blood Pressure; **GFR**, Glomerular Filtration Rate; **DGFR**, Change in GFR per Month.

*P<0.001

**Fisher's exact test: P=0.015

HbA_{1c} or blood pressure levels (Table 2). The EPO amount given was higher in the normal Hb group than in the low Hb group although not significant. "Normal" Hb included three patients with 140, 143 and 167 g/l. There was no statistical difference in serious complication like renal death, i.e. dialysis

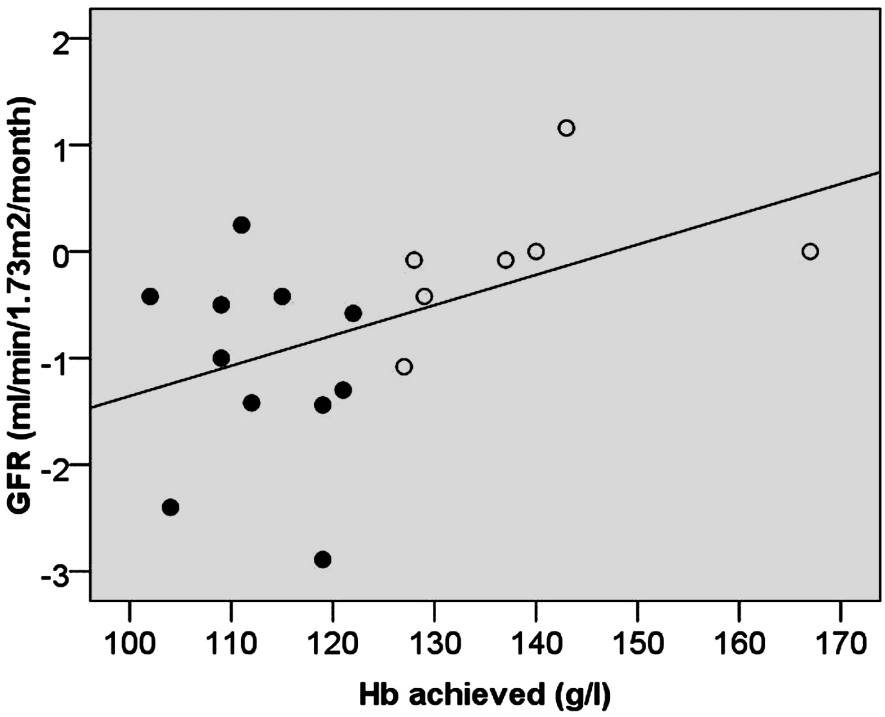

Figure 1. Change in GFR in relation to Hb level achieved during the treatment. Low Hb levels, filled circles and normal levels, open circles.

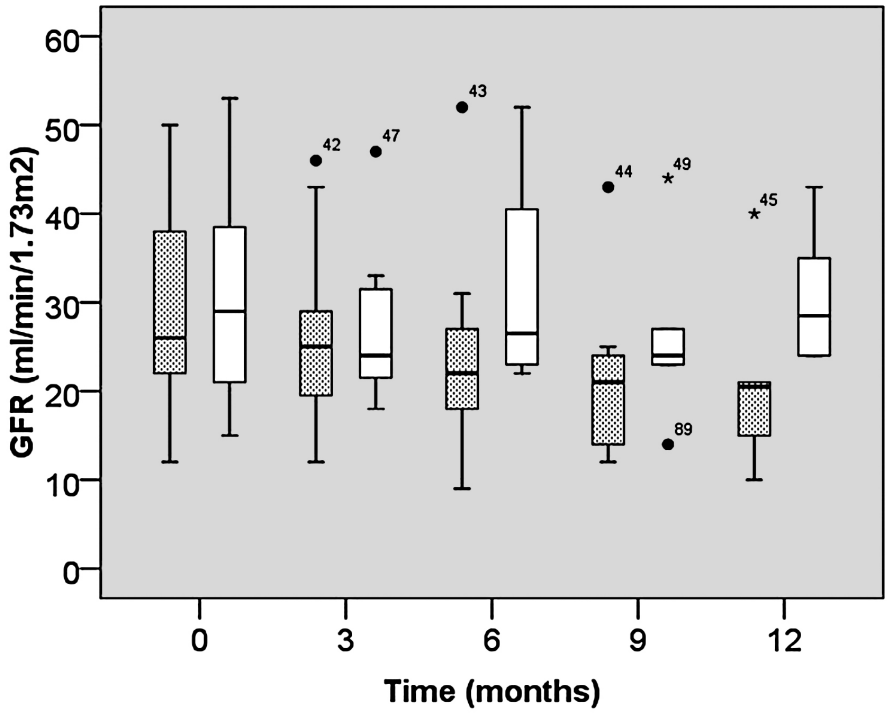

Figure 2. Box plot of GFR levels, every three months, in the two Hb groups, low (filled bars) versus normal Hb (open bars).

started or patient death or myocardial infarction between normal Hb and low Hb group. However, all four cases with such complications were found in the group who achieved low Hb (Table 2). The one patient with myocardial infarction died.

Renal function deteriorated significantly in patients on low levels of Hb ($p = 0.004$) and faster than in the patients with normal Hb (Table 2, Fig 1 and 2). In the latter, there was actually no significant change.

There were no differences in urinary glomerular or tubular parameters at entry or at end and no change in the groups except for an increase, i.e. worsening of proximal tubular function, of u-HC (Table 3).

Discussion

A higher dose should probably be used earlier than we did in order to achieve a normal Hb. Some patients however, responded very promptly to a low dose of EPO. Thus, not surprisingly as the number of patients was low we found no differences between the groups analysed by intention to treat. Consequently, we analysed the results not on basis of intention to treat but on the actual Hb level achieved.

The present study showed that GFR was preserved in patients treated with EPO who experienced an increase of Hb to normal levels. We further studied whether this was accompanied by similar improvement in the glomerular and tubular system. We found that proximal tubular dysfunction worsened in patients with low Hb compared to normal Hb levels achieved during treatment with EPO. This indicates that normal Hb levels preserves even proximal tubular function. As the amount of EPO used to achieve the goal was statistically similar the effect can probably not be explained by an effect of EPO per se. The distal tubular function, THP, was decreased in all patients and did not improve with EPO treatment. This may indicate that the patients had an irreversible stage of interstitial

Table 3. Urinary parameters in overnight collections and effect of treatment with EPO in relationship to Hb achieved, low or normal

	Low HB (115g/l)		Normal (130 g/l)	
	entry	End	entry	end
u-albumin (mg/mmol)	113 (10-455)	155 (5.4-429)	87 (1.9-140)	171 (2.6-507)
u-IgG (mg/mmol)	27 (2-86)	14.2 (3-69)	16.9 (0-38)	24 (1-100)
u-HC (mg/mmol)	8 (0.8-20.5)	14.8 (5.4-32)*	5.8 (3.8-6.6)	5 (2.7-11.3)**
u-THP (mg/mmol)	4.1 (0.1-21)	4.8 (1.2-8.9)	2.7 (0.7-5.4)	2.3 (0.1-4.7)
u-NC1 (µg/mmol)	0.3 (0.2-1.1)	0.4 (0.03-1.6)	0.3 (0.02-0.9)	0.2 (0.1-0.4)
u-GAG (mg/mmol)	1.1 (0.1-2.9)	1.4 (0.1-4.8)	1.8 (0-6)	2 (0.01-2.7)

All urinary parameters are given as the ratio per u-creatinine, Median and range (min-max).

* P=0.018 *vs* entry

** P=0.03 *vs* low Hb

fibrosis. At entry all patients had signs of both proximal, i.e. high HC (normally not detectable with the method used) and distal tubular dysfunction, i.e. low levels of THP.

In a recent survey of the literature (28) it was suggested that treatment with EPO may improve exercise tolerance and cognitive and sexual function, stabilise ventricular hypertrophy, reduce retinal exudates and macular oedema. It was considered unknown whether such effects was a result of correction of anaemia or of pleiotropic actions of EPO on the physiological response to hypoxia and oxidative stress. The present study with two groups of Hb but similar doses of EPO indicates that the effect of EPO on glomerular and tubular function is dependent on Hb levels.

Decreased Hb levels may occur even in diabetic patients without clinical albuminuria and may be a significant predictor of subsequent decline in GFR (19). Retrospective studies showed that EPO treatment may slow the rate of renal function decline (29, 30) in mostly non-diabetic patients. Anaemia per se may be a factor and reversal of anaemia by EPO may retard the progression of renal failure (half of the patients had diabetes), treatment period 28 (18-36) months (31). Rate of deterioration in the renal function evaluated by change in creatinine and blood urea nitrogen concentration seemed to be attenuated after EPO therapy in eleven type 2 diabetic patients (5). These, previous studies were retrospective and/or performed by following p-creatinine or creatinine clearance. Our study was prospective, randomized and used clearance of iohexol for GFR estimation. Although EPO treatment in order to achieve normal Hb (hematocrit) was not recommended by Besarab *et al* (32), they actually showed decreased mortality in myocardial infarction in haemodialysis patients as a result of increased Hb levels. Thus, it was not surprising that Furuland *et al* (33) found no increased mortality with EPO treatment to normal Hb. Worsening of hypertension was not a clinical problem and the amount of antihypertensive drugs did not need to be increased. In the present study there was no increased mortality in patients with normal Hb, on the contrary serious endpoints were found only among the patients with low Hb levels. When we used patients who achieved higher Hb levels we may have selected patients who potentially have better renal function. This might have been detected if we had done GFR regularly before the study. Furthermore, in contrast, the patients who worsened in their primary disease actually may not have been able to maintain normal Hb. It may be of importance that there were more type 1 diabetic patients with longer duration of diabetes and lower blood pressure levels in the patients

with normal Hb. Thus, a larger randomized study will be needed.

In the present study we evaluated the function of the glomerular filter by analysing urine albumin and IgG. The proximal tubular function was evaluated by analysing HC and distal function by THP. The turnover of the NC1 part of collagen IV was taken as a measure for remodelling of glomerular and tubular tissue as it reflects turnover of both glomerular and tubular collagen IV. The excretion of GAG reflects the turnover of total GAG and reflects the membranous production and breakdown of GAG. We found that EPO had no effect on these parameters except for preservation of proximal tubular function.

The tubular function is affected by renal blood flow and O_2 levels. It is on the border of hypoxia in diabetic patients (17). However, patients with diabetes and chronic renal failure had lower renal blood flow than but similar renal O_2 extracting capacity and consumption as patients without diabetes (34). The part of the kidney particularly predisposed to hypoxia is the outer medulla (35). In this area we have a large part of the system for production of HC and THP. A decreased Hb level may be suspected to lead to renal hypoxia. EPO treatment in the rat has been shown to prevent ischemic reperfusion injury in the kidneys by reducing glomerular dysfunction and tubular injury (both by biochemical and histological assessment) (36). By increasing the Hb level more O_2 is carried to the medulla of the kidney. This should be able to improve tubular function. Indeed the proximal tubular function was preserved in the patients with normal Hb but worsened in the patients with low levels.

The renal function did not improve but was preserved in patients with normal while it decreased in the patients with low levels of Hb. The patients with normal Hb levels had no complications of renal or patient death or myocardial infarction. However, the patient number is too few to statistically evaluate the hard endpoints. As the amount of EPO given was higher but not significantly so in order to achieve a normal Hb, analyzing by intention to treat in a larger study may be of significance. The hyporesponsiveness to EPO was overcome as suggested (37) by supplementing with iron.

The present European guidelines recommend to start EPO when Hb < 110 g/l and target Hb above 110 g/l (38). The present study confirms this suggestion and infers that the Hb level treatment to Hb around 110 g/l with normal 130 g/l with however, different results. The CHOIR study found increased cardiovascular risk with normal Hb and no improved Quality of Life (QL), whereas, the CREATE study found improved QL and no increased risk (39, 40) . The studies found no differences in progression of renal failure measured with creatinine estimated GFR. This is in contrast to the findings in the present study using Iohexol GFR. The study by Rossert *et al* also used Iohexol but the study was terminated prematurely within 8 months and the difference in GFR decrease did not have time to achieve statistical significance (41). Whereas the study by Gouva *et al* with the same drug continued for 22.5 months and achieved highly significant difference in renal deterioration measured as doubling of serum creatinine, dialysis or death (42). These studies was however a mixture of very different types of renal disease with completely different progression rates. The study by Kuriyama *et al* (31) found that reversal of anaemia could retard the progression of renal failure in non-diabetic patients but not for the type 2 diabetic patients. Our study indicate that this may be caused by a need for the latter to reach a higher Hb level than the non-diabetic pateints. Therefore a subanalysis of the CHOIR and CREATE study would be of interest.

The present study showed no statistically significant difference between the two groups by intention to treat analysis. As this study was pilot study in order to prepare for a larger randomized, double blind study examining the effect of normal versus low Hb levels induced by EPO treatment the statistical analysis of these different Hb levels achieved was analysed and showed encouraging results for starting a new larger study.

Conclusions

The present study infers that normal Hb levels may have a renal protective effect. GFR and proximal tubular function was preserved during one year of treatment. This was not caused by EPO per se and was found independently of HbA_{1c} and blood pressure levels. Further studies are needed to confirm our findings.

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Conflict of interest

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