

# Anaemia in Diabetes: An Emerging Complication of Microvascular Disease

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**Abstract:** Diabetes as the dominant cause of ESRD is also the major cause of renal anaemia. However, most patients with diabetic kidney disease will succumb to co-morbid vascular disease or heart failure before developing severe renal impairment. In these patients, anaemia is also common finding, with a 2-3 times greater prevalence and earlier onset than in patients with renal impairment from other causes. We have recently shown that at least one in five outpatients with type 1 or type 2 diabetes in tertiary referral clinics have anaemia, in whom it constitutes a significant additional burden. Impaired renal erythropoietin release in response to declining haemoglobin levels appears to be the major contributor to anaemia in diabetes. This may be due to the predominance of damage to cells and vascular architecture of the renal tubulointerstitium associated with diabetic nephropathy that may be apparent, like albuminuria, before demonstrable changes in renal function. In addition, systemic inflammation, autonomic neuropathy and reduce red cell survival may also compound anaemia in diabetes. While anaemia may be considered a marker of diabetic kidney disease, reduced haemoglobin levels, even within the normal range, identify diabetic patients with an increased risk of hospitalisation and mortality. Anaemia may also be significant in determining the outcome of heart failure and hypoxia-induced organ damage in patients with diabetes. Upcoming studies will determine whether correction of anaemia in diabetes will lead to improved outcomes in these patients.

**Keywords:** Anemia, diabetes, diabetic nephropathy, erythropoietin, haemoglobin, microvascular.

## BACKGROUND

Diabetes is the most common cause of chronic kidney disease (CKD) in the Western World, present in nearly two thirds of all patients with renal impairment [1]. Anaemia is a common complication of CKD, affecting over half of all patients [1,2]. Consequently, diabetes is also the most common cause of renal anaemia. However, over-and-above diabetes as simply a cause of renal disease, anaemia is also more common in patients with diabetes than those with renal disease of other causes. For example, the Third National Health and Nutrition Examination Survey (NHANES-III), found people in the general population with diabetes were nearly twice as likely to have anaemia, when compared to people without diabetes, but with a similar degree of renal impairment [3]. Anaemia also develops earlier in patients with diabetes than in patients with renal impairment from other causes [4]. Like many of the pathophysiological changes of diabetic nephropathy, such as albuminuria, anaemia may be apparent before demonstrable decline in renal function [5]. Indeed a normochromic, normocytic anaemia has been observed in diabetic patients without overt renal disease [5].

Anaemia has the potential to adversely affect the health of patients with diabetes in a variety of ways. It may have a major impact on sense of well-being, as well as impairing

their ability to work and affecting their social and sexual lives. Persons with anaemia generally have a lower quality of life compared with non-anaemic persons. In patients with renal impairment, anaemia is known to significantly contribute to morbidity, causing symptoms such as lack of energy, breathlessness, dizziness, poor appetite, reduced cognitive function, and decreased exercise tolerance [6,7]. Indeed, much of the impaired quality of life and morbidity previously suffered by patients with renal failure may have been as a consequence of renal anaemia. For patients with diabetes who have reduced exertional capacity, poor wound healing or co-morbid vascular disease regardless, anaemia constitutes an unwelcome additional burden. This review specifically examines this burden of anaemia in patients with diabetes.

## The Kidney in Haemopoiesis

The kidney has a pivotal role in the control of haemopoiesis, both in sensing small changes in tissue oxygenation and subsequently in stimulating haemopoietic precursors in the bone marrow through the production of erythropoietin. Erythropoietin is manufactured by peritubular interstitial fibroblasts of the renal cortex and outer medulla [8]. It is then secreted into peritubular capillary network from where it is delivered into the systemic circulation *via* the renal vein. The transcription and release of erythropoietin is regulated by feedback mechanisms related broadly to oxygen abundance in the kidney, such that production is increased when there is an imbalance between the supply of oxygen to the renal interstitium and oxygen consumption by the renal tubule. For example, a moderate reduction in

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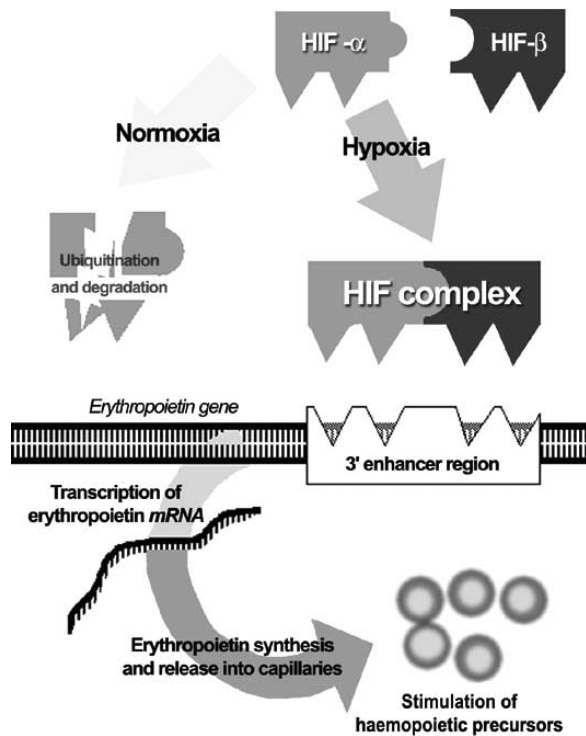


Fig. (1). The control of renal erythropoietin synthesis.

haemoglobin concentration (oxygen carrying capacity) resulting from blood loss of less than 500ml is sufficient to increase *erythropoietin* mRNA and active erythropoietin expression in renal cells [9]. Reduced arterial oxygen tension or haemoglobin-oxygen affinity will also stimulate erythropoiesis. For example, acidosis favors the dissociation of oxygen from haemoglobin. Consequently, the erythropoietin response to anaemia is lowered in acidotic and enhanced in alkalotic humans and animals [10]. In addition, a reduction in renal perfusion, such as that associated with congestive cardiac failure, lung disease or cirrhosis also results in the increased synthesis and release of erythropoietin [11,12]. However, it has been suggested that that decreasing oxygen supply to the kidney through reduction in renal blood flow (ischemic hypoxia) is overall less effective in increasing erythropoietin production than reducing the haemoglobin concentration (anaemic hypoxia) [13].

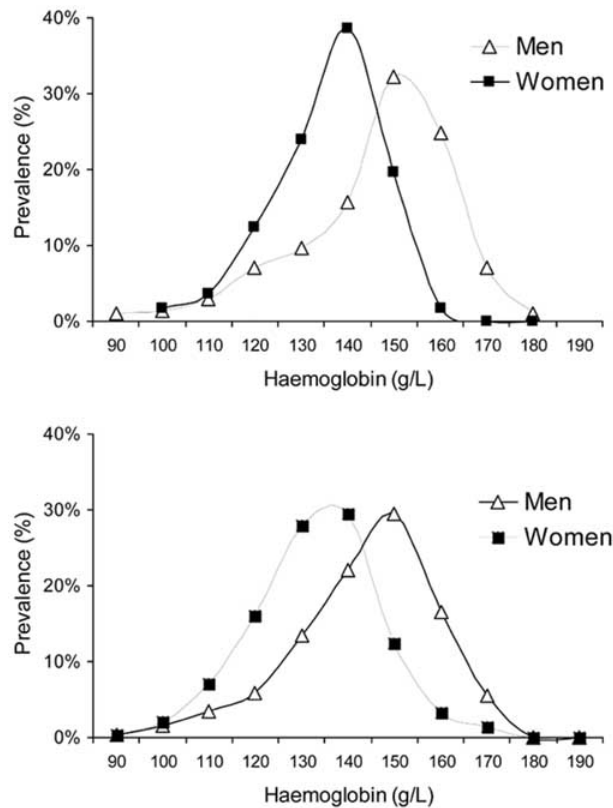
The stimulation of erythropoietin production associated with hypoxia is largely due to the transcriptional activation of the erythropoietin gene [14], although the prolongation of mRNA half-life may also have a significant role [15]. Hypoxia Inducible Factors (HIF) are crucial in sensing and integrating the response to hypoxia *via* the transcriptional activation of oxygen-sensitive genes including erythropoietin, vascular endothelial growth factor, glucose transporters, inducible Nitric Oxide synthase (NOS), heme oxygenase-1 and transferrin. HIF is a heterodimeric nonheme iron protein composed of alpha (HIF-1 or HIF-2) and beta (HIF-1 $\beta$ ) subunits [16]. HIF- $\beta$  is constitutively

expressed in the kidney at high levels. However, the expression of subunits is regulated by cellular oxygen concentrations [17,16,18]. Though HIF- $\alpha$  is continually synthesized, under non-hypoxic conditions it is rapidly degraded *via* the ubiquitin-proteasome pathway, meaning that protein levels are very low (Fig. 1) [19]. At oxygen concentrations less than 4-5% extracellular O<sub>2</sub> (25-35 mmHg) [20], ubiquitination and proteosomal degradation of HIF- $\alpha$  are inhibited [21] and the activity of the HIF- $\alpha$  trans-activation domains is increased [22], allowing HIF- $\alpha$  to dimerise with HIF- $\beta$ . The resulting active HIF-1 protein complex binds to the active site on the enhancer region in the 3' flanking region of the erythropoietin gene, leading to increased production of erythropoietin. Although it was originally thought that HIF-1 was the dominant signalling molecule for erythropoiesis, recent evidence suggests that it may be HIF-2 that specifically transactivates the erythropoietin gene [23]. The exquisite sensitivity of the renal erythropoietin system for the regulation of hemopoiesis is illustrated by the fact that renal erythropoietin are significantly increased within minutes of hypoxia, reaching a maximum after 6 hours [24]. In addition, HIF-1 and -2 can be induced under conditions of anaemia and not just severe hypoxia or conditions that result in a significant reduction in arterial saturation. For example, haemoglobin levels are normally held constant by the kidney despite the turnover of 1% of all red blood cells daily or intermittent losses with normal menstruation.

### Anaemia in CKD

Anaemia is a common complication of CKD, affecting at least half of all patients [1,2]. A significant increase in the prevalence of anaemia can be demonstrated when the glomerular filtration rate falls below 70 mL/min among men and less than 50 mL/min in women [3]. At this level of renal function, serum creatinine levels may be only slightly elevated or, in some cases, still within the 'normal range'. This makes the diagnosis of renal anaemia difficult without the accurate assessment of renal function that includes urinalysis and appropriate estimation of glomerular filtration rate (GFR).

The anaemia of CKD is generally regarded as multifactorial. Occult blood losses, malnutrition, hyperparathyroidism, systemic inflammation, inhibitors of erythropoiesis and reduced survival of red blood all have an important role in reducing haemoglobin levels in uraemia. However, the striking response to exogenous erythropoietin indicates that these other factors are of lesser importance than the failure of the kidney to increase erythropoietin release in response to a declining haemoglobin level. It should be noted that erythropoietin levels in anaemic patients with CKD are in the 'normal range' rather than low, although they are lower than those seen in patients with a similar degree of anaemia from other (non-renal) causes. This suggests that erythropoietin levels could hypothetically be adequate to maintain a normal haemoglobin level, in the absence of requirements for increased haemopoiesis. However, in the setting of additional haemopoietic stressors associated with uraemia, such as a reduced red cell survival [25], the renal response is clearly inadequate.



**Fig. (2).** The distribution of haemoglobin levels in men and women with type 1 diabetes (top) and type 2 diabetes (bottom) at the AMC.

There is a direct relationship between the severity of the anaemia and the decline in renal function [26], causally linking the failing kidney with failing renal erythropoietin production. Ultimately, this may be seen regardless of the underlying disease, consistent with the common pattern of interstitial fibrosis and nephron drop out that characterizes advanced CKD. However, the inverse relationship between plasma erythropoietin activity and blood haemoglobin concentration is better maintained in chronic glomerulonephritis than in non-glomerular renal diseases [27]. For example, in one study patients with glomerulonephritis and normal renal function had increased serum erythropoietin levels and significantly higher red cell parameters than the patients from the same subgroup with tubulointerstitial nephropathies [28]. However, by the time patients developed severe renal failure, serum erythropoietin levels were inappropriately low for the degree of anaemia in both groups. This has led to the suggestion that regulation of erythropoietin production, and its loss in renal disease, is directly related to proximal tubular function [29].

The capacity to produce erythropoietin is not necessarily abolished in CKD, for renal anaemia to occur. Although most studies suggest that the renal response to hypoxia is lost in patients with ESRD [5], this is not true in all patients. For example, in one study, erythropoietin levels were measured in six hemodialysis patients at an altitude of 400 m above sea level, before being transported to an alpine research facility at 3450 m above sea level [30]. Notably, an adequate response to the acute drop tissue oxygenation could be

demonstrated. Increased serum erythropoietin levels have been observed in children with CKD during hypoxic episodes, even though erythropoietin levels were inappropriately low for the degree of anaemia in the stable state [31]. This has led to the suggestion that the tissue oxygenation-erythropoietin-hematocrit feedback mechanism may operate at a lower set-point in patients with CKD. This may be due to chronic renal hypoxia. By contrast to acute hypoxia, patients with chronic hypoxic stress, such as those with COPD, may have a *reduction* in renal erythropoietin synthesis [32]. Stagnation of blood flow in peritubular capillaries, tubulointerstitial injury and ultimately loss of peritubular capillaries [33,34] have the potential to disrupt the delicate interaction between interstitial fibroblasts, capillaries and tubular cells required for normal haemopoiesis. In addition, the formation of radical species associated with nephron loss can induce degradation of HIF1- $\alpha$ , negatively regulate erythropoietin gene expression and thereby alter the molecular adaptation of tubular cells to hypoxia [35]. Increased energy demands associated with salt retention in surviving nephrons, also serves to induce a 'functional hypoxia' in the tubulo-interstitium of damaged kidneys.

### The Prevalence of Anaemia in Diabetes

Anaemia is also a common complication of diabetes. The overall distribution of haemoglobin levels in our population is shown as Fig. 2, stratified for gender and type of diabetes.

The estimated prevalence of anaemia depends on essentially arbitrary criteria used to define the presence or absence of anaemia and the precise population in which is employed. For example, our studies indicate that ~7% of ambulatory patients with diabetes have a haemoglobin level less than 11 g/dL [36]. However, such criteria for anaemia do not take into account gender and age influences on haemoglobin. By contrast, the WHO guidelines, which are gender-specific, recommends investigation of anaemia when the haemoglobin concentration is less than 12 g/dL in women and less than 13 g/dL in men [37]. Using this definition, nearly 1 in 4 (23%) patients with diabetes in our clinic had anaemia warranting evaluation. More recently, the European Best Practice Guidelines for the Management of Anaemia in Patients with Chronic Renal Failure have defined anaemia when the haemoglobin concentration falls more than 2 standard deviations below the mean for the general population, adjusted for age and gender (i.e. <11.5 g/dl in adult female patients, <13.5 g/dl in adult male patients, <12.0 g/dl in adult male patients aged >70 years) [38]. Using this more functional definition, 21% of diabetic patients in our clinic had anaemia.

In selected populations, the prevalence of anaemia may be even higher. For example, in a survey of 25 patients with diabetes and proteinuria (>1000mg/day), it was suggested that over 25% of patients had unrecognised anaemia [39]. In another study, in patients with type 2 diabetes and proteinuria, over 28% had anaemia [40]. In contrast, the prevalence of anaemia appears to be similar in pre-dialysis patients both with and without diabetes [41]. However, this finding may simply be a 'catch-up phenomenon' and may be biased by the premature death of diabetic patients with early anaemia.

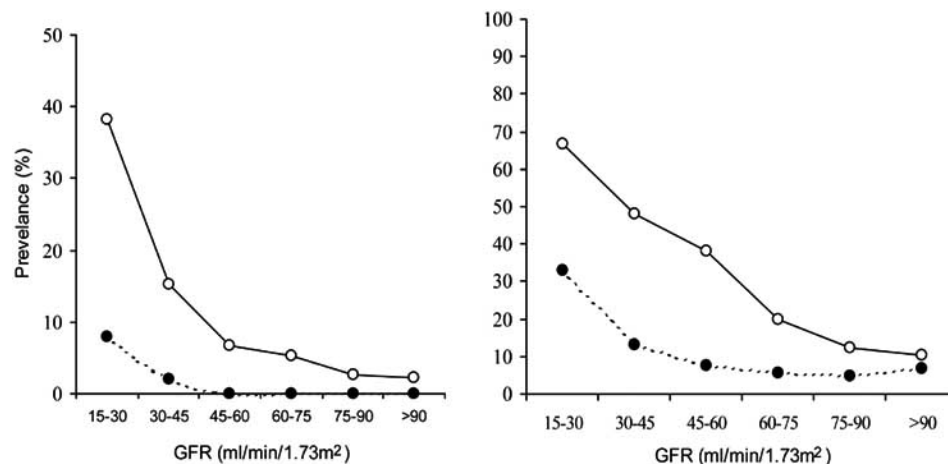
Although the high rates of anaemia in our surveys largely reflected the high rates of nephropathy, even after adjusting for renal function the risk of anaemia in patients with diabetes was approximately 2 times that seen in the general population (Fig. 3) [37]. This is consistent with the NHANES III survey showing an adjusted odds ratio of 1.7 for anaemia in patients with diabetes [3]. In addition, the effect of diabetes was not confined to patients with renal

impairment. Even in patients with so-called 'normal renal function' (GFR > 90ml/min/1.73<sup>2</sup>), the prevalence of anaemia was significantly increased by diabetes (Fig. 3).

### The Renal Erythropoietin Response in Diabetes

A number of mechanisms potentially contribute to the preferential development of anaemia in patients with diabetes. For example, the predominance of damage to cells and vascular architecture of the renal tubulo-interstitium, systemic inflammation, autonomic neuropathy and the induction of inhibitors of erythropoietin release have all been suggested as contributors to anaemia in diabetic nephropathy (table 1) [4,3]. However, it has recently become clear that the failure to increase circulating erythropoietin concentrations in response to falling haemoglobin levels is the dominant factor in the genesis of anaemia associated with diabetic nephropathy, as it is with CKD [39,40,42]. Moreover, the reduction in synthesis of erythropoietin in response to anaemia appears to be reduced beyond that seen in other renal (and particularly glomerular) diseases [5].

Although erythropoietin levels are inappropriately low in patients with diabetes and anaemia, like patients with CKD of other causes, some diabetic patients may remain able to mount an erythropoietin appropriate response to acute hypoxia [43]. This observation contrasts with that seen in most patients with ESRD where, not only is the erythropoietin production inappropriately low, but its production in response to hypoxic stress is also blunted. This suggests that the cells which produce erythropoietin are not simply lost in diabetes. This observation also fits with the occurrence of anaemia in early diabetic kidney disease, before the onset of irreversible tubular atrophy and nephron dropout. It seems likely that the *anaemia-sensing* (rather than erythropoietin secretory) mechanisms are dysfunctional at a local level in the anaemia of diabetes, which can still be overcome with sufficient stimulation. This is phenomenologically similar to impaired glucose sensing in diabetic islets, which may respond normally to acute stimulation with arginine or tolbutamide but inappropriately to a physiological glucose stimulus [44].



**Fig. (3).** The prevalence of anaemia stratified according to GFR. Anemia is defined as Hb <11g/dl (left) and according to the WHO definition (right). Patients from the AMC are compared to the Caucasians participants in the NHANES III survey [36].

**Table 1. Possible mechanisms of anaemia in diabetes.**

Reduced Production of Erythropoietin	Other Contributors
Microvascular damage	Reduced red cell half survival
Chronic hypoxia	Haematinic deficiency
Oxidative stress	Reduced iron availability
Systemic inflammation	Occult blood loss
Autonomic neuropathy	Systemic inflammation
Increase salt reabsorption	Celiac disease (type 1)
Hyperfiltration	Drug therapy
Urinary erythropoietin loss	

The precise mechanisms by which diabetes impairs the renal erythropoietin response to reduced haemoglobin levels remains to be established. Like anaemia, early tubulointerstitial damage, occurs independently and in advance of comparatively late changes of a declining glomerular filtration in diabetes. For example, thickening and reduplication of the tubular and epithelial basement membrane can be readily quantitated in the early diabetic kidney, even among normoalbuminuric patients [45]. It is conceivable that these changes in the basement membranes are capable of disrupting the delicate intercalation between tubule, peritubular fibroblast and endothelium required for normal haemopoietic function in the kidney. To this end, endogenous erythropoietin production has been suggested as a useful marker of the severity of tubulointerstitial dysfunction in diabetes [42].

Chronic renal hypoxia may also be an important mediator of anaemia in diabetes. In the diabetic kidney, vasoconstriction of tubular arterioles acts to decrease post-glomerular peritubular capillary blood flow and contributes to chronic hypoxia in the tubulo-interstitium. Tubular cells in their growth phase also release vasoactive factors, which can impair tubular capillary blood flow to local renal vascular beds and therein contribute to hypoxia. Factors such as activation of the renin angiotensin system can therefore affect the ability of the diabetic kidney to manufacture erythropoietin before the appearance of histological changes or renal impairment. Ultimately, the loss of peritubular capillaries associated with interstitial fibrosis further reduces blood delivery, which in turn exacerbates chronic tubular hypoxia.

Increased metabolic demand in tubular cells can also contribute to a functional hypoxia in the diabetic kidney. Oxygen consumption and therefore tissue oxygenation in the kidney is mainly determined by the fractional sodium reabsorption, a highly energy-consuming transport process. Among the many effects of high glucose in the proximal tubule is the stimulation of net sodium reabsorption across the brush border membrane *via* sodium-glucose co-transport [46]. This is thought to be the result of activation of tubuloglomerular feedback, hyperfiltration and tubular hypertrophy associated with early diabetes [47]. Certainly, erythropoietin levels are correlated with fractional sodium reabsorption in the context of diabetic nephropathy [48], and blockade of proximal tubular reabsorption by acetazolamide results in a drop of erythropoietin levels in normal individuals [29]. Decreased haemoglobin and increased sodium reabsorption

might therefore represent opposing stimuli for erythropoietin production in the diabetic nephron, potentially resulting in "normal" erythropoietin levels at the expense of anaemia.

In early diabetes, renal blood flow is increased leading to hyperfiltration. It is therefore conceivable that increased renal oxygen delivery (blood flow) may act to suppress erythropoietin production in the diabetic kidney. To support this hypothesis, blockade of the renin angiotensin system in experimental animals results in both an increase in renal blood flow and anaemia [49]. However, independent of renal blood flow, activation of the RAS in diabetes may also contribute to impaired erythropoietin release. For example, blockade of the RAS raises pO<sub>2</sub> in the interstitial microvascular compartment of the normal rat kidney [50]. However, inhibitors of the RAS may also have direct effects on the bone marrow [51], complicating the interpretation of these studies.

It has also been suggested that erythropoietin deficiency in patients with diabetes may result from autonomic dysfunction. Previous studies have found a strong correlation between polyneuropathy and the development of anaemia in diabetes [43]. However, polyneuropathy may also be closely correlated with other diabetic complications, including nephropathy, making it difficult to separate cause from effect. Nonetheless, this hypothesis is supported by observations that patients with primary autonomic failure also suffer an erythropoietin deficiency and anaemia [39]. In addition, experimental studies confirm that splanchnic denervation, as occurs in diabetes, is associated with blunted production of erythropoietin in response to hypoxia [52]. The hypothesis gains further support from the observation that patients with diabetes and abnormal diurnal blood pressure variation (as a surrogate of autonomic dysfunction) have lower erythropoietin levels than those with normal diurnal blood pressure rhythms, despite having similar haemoglobin levels [53]. Against this, denervated kidneys, as used for renal transplantation, appear to synthesise and release erythropoietin normally. Indeed, erythropoietin production may even be increased, resulting in post-transplant erythrocytosis [54].

At a local level, a number of factors may also inhibit the release of erythropoietin in the diabetic kidney. For example, inflammatory cytokines, such as IL-1 and tumour necrosis factor-alpha (TNF- $\alpha$ ) suppress hypoxia-induced erythropoietin production in isolated perfused rat kidneys [55]. However, the renal lesion associated with diabetes is

not generally considered to be driven by inflammation. In addition, some studies have shown that systemic inflammation has little effect on erythropoietin synthesis, as the response to hypoxia was found to be the same in rats with acute inflammation and anaemia and control animals with a comparable degree of anaemia [56]. Nonetheless, systemic inflammation associated with diabetic microvascular disease is able to reduce the activity of erythropoietin on the proliferation of erythroid precursor cells [57,58,59].

Other compounds that accumulate in the diabetic kidney may act to suppress erythropoietin production. Oxidised nucleic acids, endogenous polyamines [60] and cobalt [61] all inhibit the cellular release of erythropoietin *in vitro*, and are increased in diabetes. We have recently shown that advanced glycation end products (AGEs) that are formed in diabetes as a result of chronic hyperglycaemia and oxidative stress, are also linked to haemoglobin levels [62]. In addition, recent evidence suggests that kynurenines and other tryptophan metabolites are correlated both with degree of the renal insufficiency and anaemia. Certainly, tryptophan availability is rate limiting for protein biosynthesis in erythropoiesis and its accelerated oxidation in diabetes [63] may contribute to reduced hematopoiesis. However, both 3-OH kynurenine and quinolinic acid have been shown to directly inhibit the release of erythropoietin *in vitro*, possibly through the induction of oxidative stress [64,65].

Finally, proteinuria in patients with diabetes results in the urinary loss of endogenous erythropoietin. Certainly, patients with heavy proteinuria have substantial loss of erythropoietin in their urine, which results in reduced plasma levels of erythropoietin [66,67,68]. Our studies have demonstrated that patients with proteinuria have an increased prevalence of anaemia, independent to renal function [36]. However, neither the urinary excretion of erythropoietin nor its fractional excretion appears to be significantly increased in diabetes in the absence of heavy protein losses [69].

### Red Cell Abnormalities in Diabetes

Patients with diabetes have been reported to have several metabolic and functional abnormalities of their red blood cells [70,71]. Erythrocyte properties are significantly modified by hyperglycaemia, including altered membrane lipid composition [72], impaired filterability [73], altered red cell deformability [74], and increased adhesion [75]. This is thought to arise from a decrease in the activity of red cell Na/K-ATPase [76] as well as modifications in protein structure due to oxidation [77] and the accumulation of AGEs on the red cell membrane [78]. In addition, hyperglycaemia induces the exposure of the aminophospholipid phosphatidylserine on the red cell surface, as occurs in ageing cells, leading to red cell (senescence) recognition and removal by the reticulo-endothelial system [78,79]. In experimental models, this may be manifested by impaired red cell survival. For example, Manodori and colleagues found that after 30 days of study, approximately 50% of labeled red cells survive in control animals, while only 29% of the red blood cells of diabetic mice still survived [80]. The life span of red blood cells is also reported to be reduced in patients with diabetes [81,82].

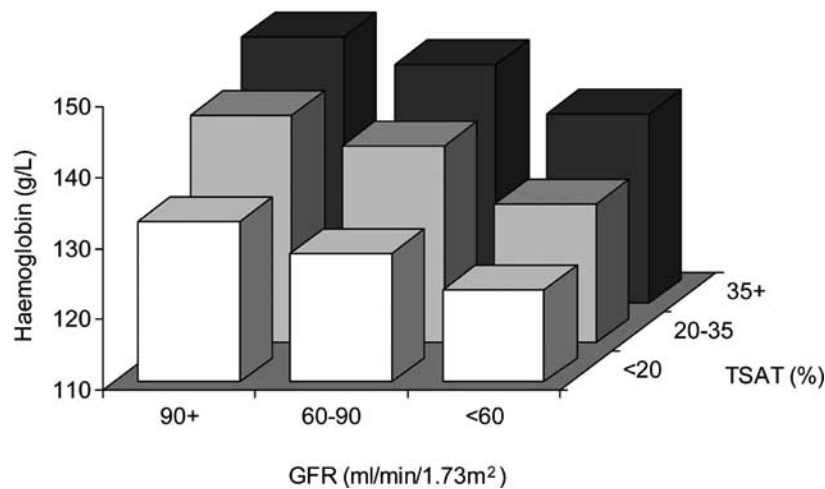
However, other studies in patients with poorly controlled diabetes have found no reduction in red cell survival [83]. Although red cell abnormalities certainly occur in diabetes, their clinical significance, and more particularly their contribution to premature anaemia in diabetic patients, remains to be established. Nonetheless, it is conceivable that minor changes in red cell turnover, when in the setting of impaired renal erythropoietin responsiveness, may contribute to a progressive fall in haemoglobin levels and ultimately anaemia. In addition, it is important to note that fragmentation haemolysis may be observed in patients with severe diabetic angiopathy [84] and in the setting of accelerated hypertension.

### Diabetes and Occult Blood Loss

Diabetes is also associated with a number of potential sources of occult blood loss. In particular, autoimmune gastritis in type 1 diabetes has been associated with iron deficiency [85]. Infection with *Helicobacter pylori*, the major risk factor for atrophic gastritis, may be more common in patients with diabetes and more often associated with the presence of endoscopic lesions and chronic gastritis [86]. Diabetes may also alter the risk of developing a variety of cancers, that may contribute to anemia. For example, it has been suggested that slower bowel transit among diabetics resulting in increased exposure to toxic substances, increased production of carcinogenic bile acids, and higher insulin levels may contribute to increased risk of colon cancer [87]. However, a definitive association is currently only available for cancer of the pancreas and liver [88,89]. Occult haematuria is present in patients with microalbuminuria, and further increased in patients with macroalbuminuria [90]. Notably, the urinary concentration of albumin and haemoglobinuria appear to be closely correlated in patients with both type 1 and type 2 diabetes [91]. Patients with diabetes may also be faced with a large number of blood tests as part of their routine management and regular self-assessment of glycaemic control may contribute to blood loss [4]. However, in our studies the haemoglobin level in patients with diabetes was independent to the cumulative amount of blood drawn by venipuncture [36]. Again each of these potential causes of anaemia should be associated with elevated erythropoietin levels if they were the primary aetiological factor. Given that this is not the case in most patients with anaemia and diabetes, the contribution of occult blood loss to the overall prevalence of anaemia is, at best, adjunctive.

### Hematinic Deficiency in Diabetes

Iron stores are a key determinant of the haemoglobin level in both health and disease. For example, much of the gender difference in haemoglobin levels may be explained by differences in iron stores between men and women. The effects of iron stores as measured by transferrin saturation (TSAT) were clearly additive to the effects of renal function in determining haemoglobin levels (Fig. 4). However the relationship between iron stores and haemoglobin was attenuated in patients with severe renal impairment, reflecting the predominant role of erythropoietin deficiency in the aetiology of anaemia in these patients.



**Fig. (4).** Haemoglobin levels in patients with diabetes, stratified for renal function and iron stores.

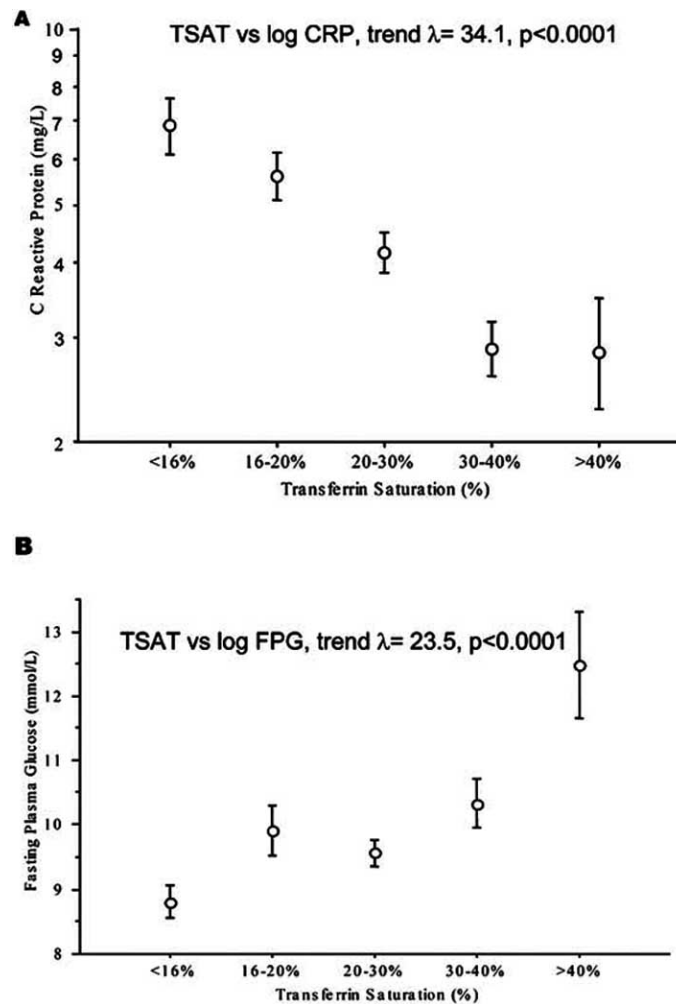
Significant fluctuations in iron stores may occur in patients with diabetes. On average, transferrin saturation and ferritin are significantly higher in diabetic subjects than in the general population [92]. However, patients with elevated iron stores are over three times more likely to develop type 2 diabetes [93], leading to an over-representation in cross-sectional surveys. Urinary iron excretion is also increased early in the course of diabetic renal disease [94,95] and iron losses continue to escalate with the development and progression of proteinuria. Nutritional regimens associated with reduced protein and increased fibre and carbohydrate [96,97], as well as the periodic analysis of blood chemistry, may also contribute to iron depletion. Ultimately, failing renal function sees the transference of body iron from circulating erythrocytes and the progressive accumulation of tissue iron [98].

In our patients with diabetes, iron deficiency (TSAT <16%) occurred 27% percent of post-menopausal women, 40% of women aged less than 50-years and 13% of men (gender difference  $p < 0.001$ ) [92]. Nearly half of all women had inadequate iron stores to support effective erythropoiesis (TSAT <20%) compared to 29% of men (gender difference  $p < 0.001$ ). However, the overall prevalence of iron deficiency in patients with diabetes was not significantly different from that seen in the age-matched adult Australian population in both males and females. Despite the association between iron deficiency and anaemia, greater than 85% of patients with iron deficiency were normocytic (mean cell volume 80-100 fl). In addition, there was no difference in mean cell volume between men and women with diabetes despite significant differences in iron stores and haemoglobin levels. Consequently, mean cell volume is an inadequate way to judge iron stores in patients with diabetes and anaemia, or the aetiology of anaemia.

The main predictors for iron stores in patients with diabetes were gender, duration of diabetes, glycaemic control and inflammatory status [92]. In general, a low transferrin saturation was associated with female gender, high CRP, low fasting plasma glucose and a prolonged duration of diabetes. Notably, these variables were equally predictive in patients

with both type 1 and type 2 diabetes, despite the differences between these disorders. Overall, transferrin saturation levels were only weakly associated with GFR. However, in subgroup analysis, patients with diabetes and moderate to severe renal insufficiency (<60 ml/min/1.73m<sup>2</sup>) appeared to have a significant and premature increase in the risk of iron deficiency [36]. This association cannot be explained by iron losses due to transferrinuria, as TSAT levels were not correlated with the albumin excretion rate ( $R = -0.05$ ,  $p = 0.17$ ) and did not vary significantly across macro-, micro- and normoalbuminuric cohorts. Iron absorption, nutritional status and dietary changes associated with failing renal function may contribute. Certainly, patients with diabetes are more likely to be malnourished when reaching ESRD than patients with renal failure from other causes, possibly because uraemic symptoms start earlier and may be more severe in patients with diabetes [99]. Against this, the presence or absence of diabetes has been shown to have limited impact on gastrointestinal symptoms of uraemia [100]. However, the excess of iron deficiency in patients with renal impairment was eliminated after adjusting for CRP. This suggests the early influence of declining renal function on iron indices may also be linked to systemic inflammation [101,102]. Chronic inflammation that results in the accumulation of iron in the reticulo-endothelial system is known to directly reduce TSAT and increase ferritin levels. In both men and women, an elevated CRP was significantly associated with a reduced TSAT (Fig. 5a).

Glycaemic control among patients with diabetes was also correlated with iron indices (Fig. 5b). In our study, patients with iron deficiency appeared to have the best glycaemic control. We used fasting blood glucose as our marker of glycaemic control as interpretation of HbA<sub>1c</sub> may be influenced by reduced erythropoiesis in patients with low iron markers. For example, some studies have documented that iron-deficiency anaemia is associated with a rise in Hb A<sub>1c</sub>, which decreased significantly after repletion of iron [103]. While the association between iron stores and glycaemic control may be partially explained by poor nutrition or dietary over-vigilance, a direct role for iron is



**Fig. (5).** The relationship between iron stores and systemic inflammation as measured by C-reactive protein (a) and glycaemic control, as measured by fasting blood glucose (b).

further supported by the finding that improvement in glycaemic control can be achieved by iron reduction in patients with diabetes who have elevated iron indices [104]. It is also possible that the apparent association with glycaemic control is confounded by the interaction between hyperinsulinaemia or advanced glycation end products and iron indices. Indeed, iron stores may be directly correlated with impairment of insulin sensitivity and subsequently improved by chelation or phlebotomy [105].

Patients with diabetes also have an increased incidence of vitamin deficiency, which may contribute to anaemia in some cases. Patients with type 1 diabetes are at increased risk of developing latent pernicious anaemia [85], especially if they also have autoimmune thyroid disease [106]. Patients taking biguanides such as metformin on a continuous basis occasionally develop vitamin B<sub>12</sub> deficient megaloblastic anaemia [107]. Type 1 diabetes is also strongly associated with celiac disease, which may produce anaemia through malabsorption of iron, folate, vitamin B<sub>12</sub> or trace elements due to loss of surface area. Between 3-16% of children and adolescents with type 1 diabetes mellitus have identifiable celiac disease [108], compared to less than 1% of the general

population [109]. Both diseases have common immunology and genetic characters including associated with associated with HLA DR3-DQ2 and DR4-DQ8 [110]. The risk of having both diseases is highest in children diagnosed with type 1 diabetes under the age of 4 years and girls appear to have a higher risk of having both diseases than boys [111]. Usually celiac disease is silent or minimally symptomatic in patients with diabetes. Iron or folic acid deficiency with or without anaemia are the most common laboratory abnormalities seen in patients with both disorders. It is controversial whether all patients with type 1 diabetes should be screened for celiac disease [112]. However, the workup of unexplained anaemia in a patient with type 1 diabetes should certainly incorporate serological testing for celiac disease. In contrast to type 1 diabetes, there is no evidence that the risk of celiac disease in type 2 diabetes mellitus is increased compared with the population at large.

#### Drug Therapy and Anaemia

There is good evidence that many drugs used to treat diabetes may exacerbate anaemia associated with diabetes. In particular, it has been suggested that the widespread use of

**Table 2. Multivariate associations with haemoglobin levels in patients with diabetes.**

Variable	Adjusted p-value	Delta R
Transferrin Saturation	<0.001	0.47
Gender	<0.001	
Glomerular Filtration Rate	<0.001	0.31
HbA <sub>1c</sub>	<0.001	-0.16
CRP	0.003	-0.11
Diabetes Duration	0.001	-0.11
Albumin Excretion Rate	0.008	0.09

ACE inhibitors may contribute to a reduction in haemoglobin in patients with diabetes [4]. Certainly, blockade of the RAS with angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor antagonists is associated with a dose-dependent reduction in hematocrit [113], seen within the first month therapy [114]. The effect is most marked in patients with erythrocytosis (eg post renal transplantation, chronically hypoxemic patients, renovascular hypertension), with treatment reducing haemoglobin levels to within the normal range in these conditions. It is thought that activation of the RAS in these conditions, possibly through a reduction in renal blood flow in the setting of impaired autoregulation, inappropriately activates secretion of erythropoietin despite elevated haemoglobin levels. Both ACE inhibitors and angiotensin receptor antagonists may also have direct effects on erythroblast proliferation. While blockade of the RAS may also exacerbate anaemia in patients with CKD, recent studies have found no significant link between ACE inhibitor use and haemoglobin levels [115]. In support of these findings,

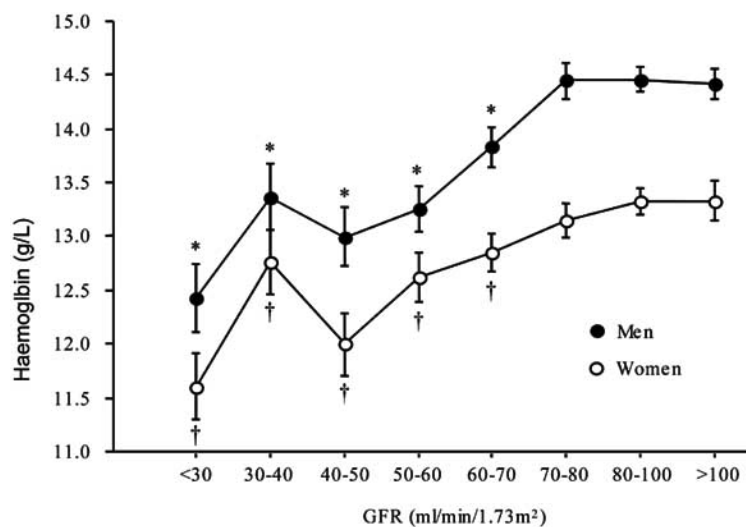
we were unable to detect any association between ACE inhibitor use and haemoglobin levels, after correcting for differences in GFR [36].

Thiazolidinediones, a class of insulin-sensitizing compounds, have also been associated with anaemia [116]. It is thought that volume expansion associated with these agents results in an increased incidence of peripheral oedema, increased body weight, heart failure and hemodilution. Blockade of the alpha peroxisome proliferator activator receptor (PPAR) with fibrates, used for the management of dyslipidaemia, has also been associated with anaemia [117].

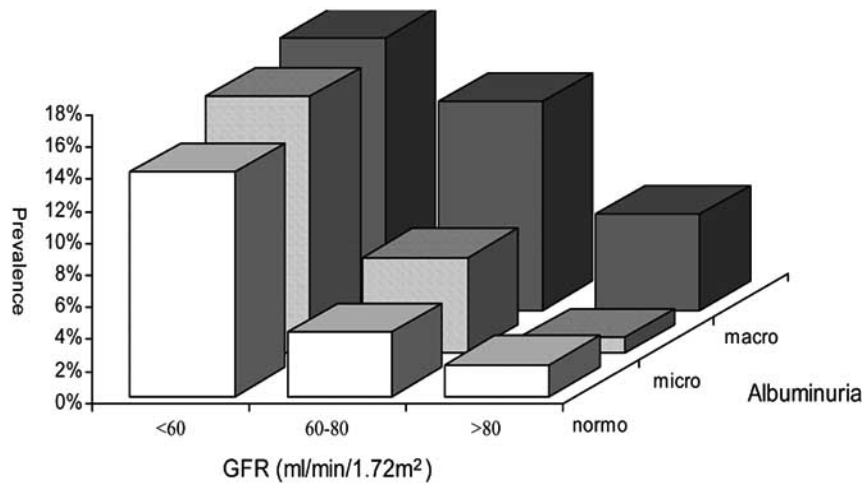
### Predictors of Anaemia in Patients with Diabetes

In our studies, the strongest predictors of haemoglobin levels are renal function, gender and iron stores [36]. The distribution curves for men and women are similar in shape (Fig. 2), with the female curve displaced to the left by ~1g/dl, as per the gender-specific reference range for haemoglobin in this population [118]. This suggests that the impact of other factors on the haemoglobin level in diabetes is independent to gender. Other variables associated with haemoglobin levels in patients with diabetes are shown in Table 2. Together these variables together explained approximately 46% of the haemoglobin variance in our clinic population. The multivariate variables associated with raw haemoglobin level remained independent predictors when analysis was restricted to patients with moderate renal impairment (n=568, GFR <60ml/min/1.73m<sup>2</sup>), although the effect of iron indices was markedly attenuated. Only GFR was no longer significantly associated with haemoglobin levels at levels of normal renal function (GFR >90 ml/min/1.73m<sup>2</sup>).

Patients at greatest risk of anaemia could be readily identified by the presence of diabetic renal disease, manifested as impaired renal function and/or elevated albuminuria, suggesting that the predominant cause of anaemia is renal in origin. As renal function fell, the



**Fig. (6).** The association between haemoglobin and declining GFR in patients with diabetes \*vs GFR >80 ml/min/1.73m<sup>2</sup>, p<0.05.



**Fig. (7).** The prevalence of anaemia in patients with diabetes, stratified for GFR and level of albuminuria.

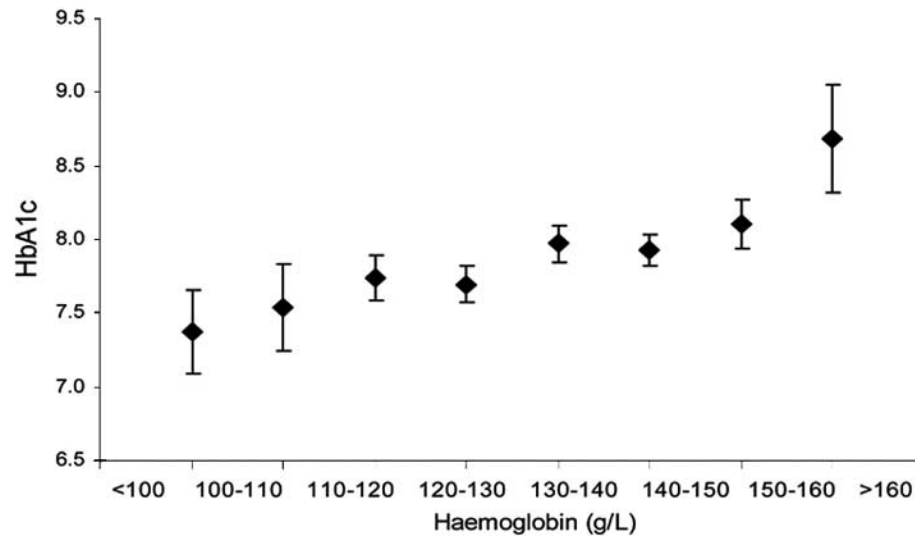
prevalence of anaemia increased exponentially. The association between GFR and haemoglobin was essentially continuous at lower levels of GFR. Stratification by gender revealed parallel trends in men and women; with a statistically significant decrease in haemoglobin was apparent in both men and women below a GFR of 80 ml/min/1.73m<sup>2</sup> (Fig. 6). However, the influence of renal function on haemoglobin levels was not confined to patients with elevated serum creatinine levels. Notably, even in patients within the so-called 'normal range' of serum creatinine (<110μmol/L), GFR was significantly associated with the haemoglobin level. This finding emphasises the importance of estimated GFR in the management of patients with diabetes, particularly in the elderly where reduced muscle mass can lead to 'pseudonormalisation' of serum creatinine levels. More importantly, the patients with diabetes most likely to have anaemia can be easily recognised. In our population, 60% of patients with anaemia warranting investigation had a GFR < 60 ml/min/1.73m<sup>2</sup> and nearly half (46%) of patients with macroalbuminuria had anaemia.

Multiple regression analysis revealed that albuminuria was independently associated with haemoglobin levels after adjusting for renal function, iron stores and gender ( $p < 0.008$ ). In both men and women, haemoglobin levels were lowest in the patients with macroalbuminuria, regardless of GFR (Fig. 7). When patients were stratified according to GFR and the level of albuminuria subgroups, after adjusting for iron stores, logistic regression revealed that, compared to normoalbuminuric patients with a GFR >80 ml/min/1.73m<sup>2</sup>, patients with persistent microalbuminuria ( $n=222$ ) had four times the risk of anaemia (OR = 4.0, 95% CI=1.1 to 15.9,  $p < 0.05$ ) [36]. Patients with persistent macroalbuminuria ( $n=94$ ) had more than 12 times the risk of anaemia (OR = 12.9, 95% CI=3.3 to 51.3,  $p < 0.001$ ). Subjects with normoalbuminuria but impaired renal function (GFR <60 ml/min/1.73m<sup>2</sup>,  $n=222$ ) had almost 11 times the risk of anaemia (OR = 10.9, 95% CI=2.4 to 44.2,  $p < 0.001$ ), i.e., a similar risk to patients with macroalbuminuria.

Mechanistically, an association between albuminuria and anaemia is not surprising. Albuminuria is known to be a marker of low grade microvascular inflammation and damage, both in the kidney as well as in extra-renal sites, as described in the STENO hypothesis [119]. Indeed, albuminuria is associated with tubulointerstitial injury that occurs independently and in advance of declining GFR in diabetic nephropathy [120,121]. Proteinuria itself does not appear to be the causal factor, as patients with persistent proteinuria from non-diabetic aetiology have less anaemia than their diabetic counterparts [4].

Over and above the effect of renal disease, the longer the duration of diabetes the more likely it is for the patient to have anaemia. Patients with diabetes of over ten years duration were over three times more likely to have anaemia than patients with a shorter duration of disease. Patients with prolonged duration of diabetes (>10 years) had significantly lower haemoglobin levels than those with a shorter duration of disease ( $p < 0.001$ ). This effect was independent of renal impairment and the presence of other diabetic complications. Anaemia was more common with advancing age in patients with diabetes. Approximately 70% of patients with diabetes and anaemia in each centre were over the age of 65-years (compared to 50% of those patients without anaemia). However, this observation was largely confounded by the increased prevalence of renal disease (both in terms of low GFR and albuminuria) in elderly patients. Although the so-called 'anaemia of ageing' may contribute to this phenomenon, this association was eliminated after adjusting for the increased risk of renal impairment and albuminuria associated with advancing age.

A weak independent association between HbA<sub>1c</sub> and haemoglobin levels was identified (explaining ~3% of the variance in haemoglobin levels in our population). Nonetheless, there was a clear trend for HbA<sub>1c</sub> levels to fall as haemoglobin levels also fell, independent of declining renal function (Fig. 8). It is conceivable that poor diabetic control may be associated with chronic fluid retention and haemodilution. HbA<sub>1c</sub> may also be spuriously elevated in patients with anaemia because deficiency of erythropoietin



**Fig. (8).** The relationship of HbA<sub>1c</sub> and Hb levels in patients with diabetes (Mean  $\pm$  SEM, trend  $p < 0.001$ ).

results in tardy access of newly produced erythrocytes into the circulation, increasing their exposure to a hyperglycaemic environment. HbA<sub>1c</sub> levels may also parallel serum and tissue levels of advanced glycation products that may act to alter erythropoietin responsiveness through glycation of erythropoietin or its receptor or *via* induction of suppressive inflammatory cytokines [62].

An association between serum triglyceride and haemoglobin was also identified. In general triglyceride levels correlate with the severity of renal disease and proteinuria, although our study suggested that the effect of hypertriglyceridaemia on haemoglobin levels in diabetes may be independent of albuminuria. It is possible that the known effects of serum triglycerides on red cell aggregation and membrane fluidity influence the shortening of red cell half-life seen in diabetes. However, the relationship between fasting triglycerides and haemoglobin levels may be confounded by the influence of nutritional status. Notably, serum triglyceride levels may parallel the clinical level of nutrition, with both triglyceride and haemoglobin levels falling in malnourished patients. One marker of nutritional status, serum albumin has also previously been shown to be a strong predictor of haemoglobin levels. Serum albumin was not used in our study, as it had already been incorporated into the formula for estimating GFR [122]. However, if albumin was additionally incorporated as an independent variable, the association between lipids and haemoglobin was attenuated.

### Anaemia and Quality of Life

Untreated anaemia significantly contributes to the reduced quality of life seen in patients with CKD. Following correction of renal anaemia, patients report feeling much better and their quality of life indices are often markedly improved [123]. Patients show improvements in many areas including enhancement of physical performance, cognitive function, sleep, appetite, sexual function, social activities, and employment [124]. Relief from a variety of uraemic

signs and symptoms, such as pruritus [125] and impaired carbohydrate and cortisol metabolism have also been documented [126].

Physical activity is one of the most important therapies available for reducing morbidity from diabetes and maintaining quality of life [127] and inactivity is possibly the best predictor of adverse outcomes [128,129]. Recent studies have shown that adults with diabetes undertake less physical activity than non-diabetic individuals [130,131]. Although physical activity is encouraged, up to one third of adults with diabetes remain completely sedentary, and only a third regularly taking some form of physical activity [132,133]. The reasons behind this level of inactivity are many and various. However, lack of confidence in ability to perform exercise appears to be one of the most important barriers to performing exercise, associated with feelings of tiredness and low mood [133]. Given that fatigability is one of the cardinal symptoms of anaemia, it is likely that anaemia also contributes to inactivity in diabetes. In a recent survey over a half of all patients with diabetes experienced tiredness to some extent, and this was increased to 74% in those with anaemia [134]. Furthermore, fatigability was associated with significant functional morbidity, anxiety, and depression and poor quality of life. There is little doubt that treatment with erythropoietin is 'performance enhancing' leading to its ban in competitive athletes. However, there are many patients with diabetes in whom a little 'performance enhancing' may go a long way to restore physical activity, independence and improve quality of life.

### Anaemia and the Progression of Diabetic Renal Disease

Anaemia has been reported to be an important risk factor for progression to end stage renal disease in patients with CKD, with or without diabetes [135,136,137,138,139,140,141,142,143]. However, no large-scale, long-term study has addressed the issue of whether patients with anaemia simply have more severe renal disease. As anaemia may be considered a manifestation of renal injury [42], it is easy to

imagine that damaged kidneys may be subject to more aggressive renal disease. It is important to note that anaemia *per se* does not result in with renal disease. However, there are a number of physiological arguments as to why anaemia might have an independent effect on the progression of established kidney diseases.

Firstly, anaemia is associated with tissue hypoxia, particularly in the kidney that has low oxygen saturation and high oxygen consumption [144]. Hypoxia has a variety of mitogenic and fibrogenic effects on the kidney, associated with expression of multiple growth factors, hormones, vasoactive reagents and enzymes [145]. HIF-1 regulates genes involved in angiogenesis (such as the prosclerotic mitogen, VEGF), vasomotor response (inducible nitric oxide synthase (iNOS), heme oxygenase-1 and endothelins), glycolysis (the glucose transporter GLUT-1 and glycolytic enzymes), matrix metabolism (TGF-1, collagens, matrix metalloproteinases) and cell survival [146], all pathways implicated in the pathogenesis of progressive renal disease. In addition, tissue hypoxia may be associated with elevated sympathetic activity, leading to renal vasoconstriction, activation of the intra-renal RAS and release of antidiuretic hormone [145] that contribute to fluid retention and hypertension associated with progressive nephropathy [147]. Tissue hypoxia may also directly promote proteinuria in diabetes, possibly enhancing tubular protein load through differential control of pre-glomerular and post-glomerular resistance, following activation of the sympathetic nervous system [146].

Anaemia is also closely correlated with oxidative stress, as erythrocytes represent a major antioxidant component of the blood [148]. Renal anaemia is associated with a reduction in both the number of red blood cells and antioxidant potential of erythrocytes [149]. In addition, renal anaemia is associated with increased production of free radicals [150]. On the other hand, erythropoietin is able to enhance the expression of red cell antioxidants [151], including superoxide dismutase, catalase and glutathione, as well as cellular proteins that bind with reactive oxygen species, such as vitamin E or coenzyme Q. It is conceivable that the combination of increased oxidative stress and tissue hypoxia associated with anaemia may act to stimulate the production of extracellular matrix, increasing interstitial fibrosis and tubular apoptosis [152], and leading to tubular atrophy associated with progressive renal disease.

Anaemia may also be a marker for 'functional erythropoietin deficiency'. Certainly, erythropoietin has been shown to protect endothelial cells against apoptosis [153,154]. Proximal tubular cells also express the erythropoietin receptor, whose activation may be important in tubular growth and development. Erythropoietin also has pro-angiogenic effects [155]. In theory, 'erythropoietin deficiency' might accelerate the progression of renal failure, not only through anaemia but also by way of direct effects of erythropoietin depletion on renal function and response to injury.

There have been at least three prospective clinical studies in patients with advanced nephropathy to show delayed deterioration of renal function in patients in whom anaemia was corrected with exogenous erythropoietin. In the first

study, 73 patients with anaemia and severe renal failure (mean creatinine clearance 18.2 ml/min) were randomly assigned to receive erythropoietin or no intervention [156]. During the 36-week follow-up period, creatinine doubled in 52% of patients in the erythropoietin-treated group, and in more than 90% of patients in the control group ( $p < 0.001$ ). Furthermore, 64% of patients in the control group required dialysis, compared to 33% of those in the erythropoietin-group ( $p < 0.01$ ).

In a second study, 83 patients with severely impaired renal function (mean GFR  $\sim 10$  ml/min) and severe anaemia, were randomly assigned to receive erythropoietin or no intervention [157]. In this study, the rate of GFR decline was three times slower in the erythropoietin-treated group than in the control group ( $-0.13 \pm 0.35$  ml/min/month versus  $-0.39 \pm 0.65$  ml/min/month,  $P = 0.05$ ).

In the most recent study [158], 88 patients with mild to moderate anaemia (9.0 to 11.6 g/dL) and moderate renal impairment (serum creatinine 20-60mg/dl) were randomized to early treatment of their anaemia, or deferred treatment, initiated only when the haemoglobin fell below 9 g/dL. Patients with systemic diseases including diabetes were excluded from this study. After two years of follow-up, 13 patients from the early treatment arm reached the primary endpoint, defined as doubling of creatinine, a creatinine of  $> 8$  mg/dL, initiation of renal replacement or death from any cause. By comparison, 23 patients from the deferred intervention arm reached the primary end point. Notably, the early initiation of EPO appeared equally beneficial in patients with different degrees of baseline renal impairment, haemoglobin level and degree of proteinuria.

It is notable that these studies were in patients with advanced renal disease. The potential utility of correction of anaemia in patients with early renal injury and specifically in patients with diabetes has not been formally tested.

### Anaemia and Cardiovascular Disease

The risk of cardiovascular disease in patients with moderate to severe renal impairment can be attributed, in part, to the high burden of traditional risk factors (such as diabetes, hypertension, and dyslipidaemia) in this population. However, recent evidence suggests that anaemia may also represent a significant additional risk for cardiovascular disease in patients with CKD. Certainly, anaemia in CKD identifies patients at increased risk for hospitalisation and premature death. The addition of anaemia to diagnoses of CKD approximately doubles the risk of increased the risk of death on dialysis [159]. Among middle-aged, community-based persons, the combination of chronic kidney disease and anaemia is independently associated with an increased risk of stroke [160]. Anaemia is also an important predictor of adverse outcomes in patients with heart failure [161] and following myocardial infarction [162], coronary surgery [163] or angioplasty [164]. Patients with ischaemic heart disease and anaemia are also more likely to have more advanced degree of cardiac ischaemia, heart failure, rhythm disturbance and higher mortality rate than those with a haemoglobin level in the normal range [165].

The additional burden of renal anaemia is also significant in determining the outcome of the hypoxia-induced organ damage in patients with diabetes [166]. Patients with diabetes may be considered more vulnerable to the effects of anaemia because many also have established macrovascular disease. As anaemia is closely correlated with the presence of nephropathy, an established risk factor for cardiovascular morbidity and mortality, it is difficult to determine the true role of anaemia in this setting. Nonetheless, there is also some evidence that anaemia may independently contribute to the irreversible changes in cardiac and vascular function characteristic of patients with diabetes.

A correlation between anaemia and angina is well described. In so far as the tissue injury associated with atherosclerotic disease is ischaemic in origin, reduced oxygen carriage associated with anaemia may aggravate existing tissue hypoxia. In addition, increased cardiac output, volume overload, increased heart rate and activation of the sympathetic activity associated with anaemia all act to increase myocardial oxygen demand. In diabetes, cardiac ischaemia is often silent, so that the impact of chronic myocardial hypoxia may be difficult to establish without careful investigation. Consequently, the potential contribution of anaemia to myocyte loss and progressive fibrosis associated with the diabetic heart is also difficult to assess. Nonetheless, recent studies point to anaemia as an important risk factor in patients with ischemic cardiac disease. In our population, the risk of ischaemic cerebrovascular disease or peripheral vascular disease was significantly higher in patients with anaemia [36]. This is clearly confounded by the presence of other vascular risk factors in these patients, including renal impairment and albuminuria. However, it is possible that these factors act influence the heart at least partly through anaemia.

Left ventricular hypertrophy (LVH) is present in over 70% of all patients reaching ESRD, and in over 90% in patients with diabetes. There is a significant association between anaemia and LVH in patients with early CKD [167]. A modest decrease in haemoglobin (1g/dl) may be independently associated with a 33% increase in LVH [168]. At least a partial regression of LVH may be possible after the correction of anaemia in CKD [169,170]. In the long term, these haemodynamic alterations lead to gradual development of cardiac enlargement and LVH. The LVH is eccentric, characterized by increased LV internal dimensions and a normal ratio of wall thickness to cavity diameter, as occurs in other forms of volume overload. When anaemia-related LVH develops in an otherwise 'healthy' humoral environment, the lesions are reversible and the type of LVH is primarily physiological and is not associated with impaired diastolic function. However, in a heart already stiff as a result of diabetes and chronic microvascular ischaemia, the effect may be compounded and potentially irreversible.

Diabetes is commonly associated with heart failure [171]. Between 20% and 40% of all patients with heart failure have diabetes [172]. In addition, the prognosis of heart failure is significantly worse in patients with diabetes than in non-diabetic patients [173]. Anaemia may contribute to this risk, as part of the cardio-renal-anaemia syndrome [174]. In a study of survival of patients with heart failure, the

haemoglobin level at the time of initial diagnosis of heart failure was a significant predictor of survival [175]. As anaemia is more common in patients with diabetes, it is possible to speculate that anaemia may also contribute to increased adverse outcomes in patients with diabetes and heart failure. The prevalence of diabetes in resistant heart failure patients with anaemia also appears to be higher than is normally found in patients with heart failure [176,177] and may be related to the tendency for anaemia to present early in diabetes. Several studies in diabetic patients with moderate kidney impairment also demonstrate improved cardiac function with regression of left ventricular mass after erythropoietin treatment [178,174]. In addition, correction of anaemia may also play a significant role in maintaining patients with heart failure in a community setting and improving patient wellbeing [179].

It is important to note that some studies have suggested that limited reductions in the haematocrit may have a protective influence on cardiovascular mortality. For example, cardiovascular survival is improved in women and blood donors [180]. Anaemia is associated with decreased after-load is due to vasodilatation and reduced vascular resistance as a consequence of lower blood viscosity, hypoxia-induced vasodilatation, and enhanced nitric oxide activity. Correction of anaemia on the other hand, may be associated with increased peripheral vascular resistance. Some retrospective studies have noted an increase in peripheral vascular events in diabetic dialysis patients receiving erythropoietin. In addition, increased haemoglobin levels in dialysis patients may be associated with an increase in macrovascular events [181].

### Anaemia and Blood Pressure Regulation

Erythropoietin and anaemia also have important interactions with blood pressure control in both health and disease. In patients with essential hypertension, endogenous erythropoietin levels are positively correlated with blood pressure levels and total peripheral resistance, independent of haemoglobin levels [182]. Human recombinant erythropoietin, administered for the correction of anaemia in patients with ESRD, results in an increased arterial blood pressure in 25–30% of patients [7,183]. In our patients, there was a weak association between blood pressure (especially systolic blood pressure) and haemoglobin level either before or after adjustment for estimated GFR (*vs* systolic blood pressure:  $R = -0.15$ ,  $p < 0.01$ ) [36]. It is conceivable that a reduction in erythropoietin synthesis may act to partly counterbalance blood pressure elevation associated with fluid retention in diabetes and CKD.

Erythropoietin is known to have direct vasoconstrictive actions and diminishes the vasodilator effects of nitric oxide on the vascular smooth muscles [184,185]. Erythropoietin also activates the renal RAS and synthesis of endothelin-1 and serotonin [186] and stimulates net tubular sodium reabsorption, and reduces urine volume and urinary excretion of sodium and potassium in anesthetized rabbits without renal failure. The non-steroidal antiinflammatory drug indomethacin prevents erythropoietin-induced hypertension [187], suggesting changes in calcium influx

and prostaglandin metabolism may contribute to arteriolar vasoconstriction during erythropoietin therapy.

The importance of these blood pressure effects is illustrated by findings from studies in sub-totally nephrectomised rats [188]. In these initial reports it was clear that erythropoietin treatment resulted in exaggerated glomerular hypertension, proteinuria and sclerosis. However, a subsequent study in the same model found no evidence for accelerated renal injury once hypertension induced by erythropoietin was controlled [189].

### **Anaemia and Diabetic Retinopathy**

Anaemia may be associated with an increased risk of background and proliferative retinopathy in patients with diabetes [190,191]. For example, in one cross-sectional study involving 1,691 diabetic patients, anaemia was associated with a two-fold increased risk of background diabetic retinopathy and a fivefold increased risk of pre-proliferative or proliferative retinopathy, compared with those with higher haemoglobin levels [191]. Given that renal disease and retinopathy are closely associated, it is perhaps not surprising to see an increased prevalence of anaemia in patients with more aggressive microvascular disease. However, anaemia may also have a direct effect on the development and progression of diabetic retinal disease. It is now well established that diabetic retinopathy is associated with tissue hypoxia, as a result of impaired autoregulation of the microvasculature and capillary occlusion [192,193]. It is thought that retinal hypoxia and resulting oxidative stress promotes the synthesis of vascular endothelial growth factor, a potent stimulant of neovascularization that also enhances capillary permeability resulting in tissue oedema and retinal exudates [194-196]. It is conceivable that anaemia may act to exacerbate retinal hypoxia in patients with diabetes. Consistent with this hypothesis, a few small studies have demonstrated that correction of anaemia in patients with diabetes may be associated with a reduction in macular hard exudates [197,198] and oedema [199]. Larger studies are required to confirm the utility of erythropoietin in this setting, especially given the real risk of hypertension associated with this therapy.

### **Anaemia and Diabetic Neuropathy**

Polyneuropathy occurs in over half of all patients with prolonged hyperglycemia [200], leading to paresthesia, pain, and neuropathic injury. Endoneural hypoxia, due to decreased microvascular blood flow and altered vascular permeability, is observed early in the course of diabetes and the resultant ischemia plays a role in the progressive diabetic neuropathy. Factors which exacerbate hypoxia are known to accelerate nerve injury in diabetes. For example, in patients with asymmetrical peripheral vascular disease, there is greater impairment of nerve function in the more ischaemic leg [201]. While anaemia is more common in patients with severe neuropathy [5], a clear role for anaemia in the exacerbation of endoneural hypoxia and oxidative stress remains to be established. Nonetheless, there is clear evidence that erythropoietin has both neuroprotective and neurotrophic effects in hypoxic neurons. In experimental models of diabetes, treatment with erythropoietin has been

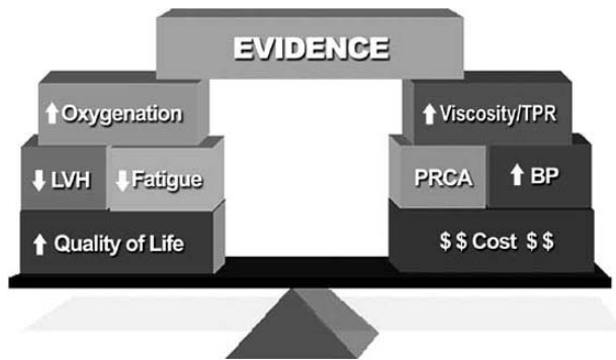
shown to protect against the development of neuropathy, as well as reverse established disease [202]. In pre-dialytic patients with polyneuropathy and anaemia, treatment with subcutaneous erythropoietin significantly improves motor nerve conduction velocity and compound muscle action potentials in both diabetic and non-diabetic individuals [203]. However, the improvement in non-diabetic patients was better than in diabetic patients. In addition, no significant correlation was found between the increase in haemoglobin and the improvement in nerve function, suggesting a direct action on peripheral neuronal cells rather than a benefit arising from correction of anaemia. It is important to note that deficiency of vitamin B<sub>12</sub> may also result in anaemia and neuropathy, and may be readily confused with the microvascular complications of diabetes.

### **Anaemia, Wound Healing and Infection**

Diabetic foot ulcers occur in ~15% of patients [204], contributing to 20% of the hospital admissions of diabetic patients and amputation in 6 out of every thousand patients [205]. One important component to foot ulceration is impaired wound healing activity in patients with diabetes [206]. This is thought to be due to a variety of abnormalities including an increased risk of wound infection, delayed cellular infiltration and granulation tissue formation, reduced neo-vascularisation, abnormal matrix synthesis and degradation [207]. It is known that wound collagen deposition and tensile strength is proportional to perfusion and wound oxygen tension. In diabetes, local ischemia prompts the production of reactive oxygen metabolites that impair normal wounds by damaging keratinocytes, endothelial cells, and collagen metabolism. Indeed one of the strongest risk factors for development of diabetic foot ulcers is impaired cutaneous oxygenation. The key importance of tissue oxygenation in the diabetic foot ulcer is further illustrated by the success of hyperbaric oxygen in augmenting wound healing in diabetes [208].

The circulating haemoglobin level may also significantly influence the wound healing in patients with diabetes. In healthy individuals, reduced haemoglobin levels can be compensated by increased peripheral perfusion [209], increased vaso-reactivity [210] and elevated erythropoietin levels that stimulate endothelial cell mitosis and motility important in neovascularization. The means that the clinical effects of mild or moderate normovolaemic anaemia on wound healing in healthy individuals are marginal. However, such compensatory responses are significantly impaired in patients with diabetes, particularly in those with microvascular complications of their disease. Diabetic foot wounds will not heal without adjuvant surgical treatment unless the trans-cutaneous pO<sub>2</sub> is greater than 30 mmHg [211]. Despite an increase in calculated total limb vascular resistance, correction of anaemia is associated with an improvement in skin oxygenation that may be beneficial in patients with poor-healing ulcers. In addition, recombinant human erythropoietin may have direct effects on angiogenesis in granulation tissue during wound healing [212,213].

Diabetes is also associated with an increased susceptibility to infection, largely due to macrophage



**Fig. (9).** The balance of risks and benefits in treating anaemia in patients with diabetes.

dysfunction and inhibition of cell-mediated immunity [214]. Anaemia may also have significant effects on the immune system. Erythropoietin is not only a hemopoietic factor but also an immuno-modulatory cytokine [215]. Components of the immune system, particularly delayed-type hypersensitivity, may be improved in haemodialysis patients by normalization of haemoglobin through the administration of increased doses of erythropoietin [216].

### Correction of Anaemia in Diabetes

Although there is a clear rationale for the use of supplemental erythropoietin in diabetic patients with 'functional erythropoietin deficiency', there is no conclusive evidence that correcting anaemia significantly improves clinical outcomes in patients with CKD, apart from quality of life indices [123]. Nonetheless, from the patient's perspective an intervention that reproducibly improves quality of life, exercise tolerance, cognitive and sexual function is not insignificant. Consequently, current international guidelines recommend the use of erythropoietin in patients with CKD when the haemoglobin level falls below 11g/L, when other causes of anaemia have been excluded [217]. However, many countries limit the use of erythropoietin to patients with severe renal impairment or ESRD. The applicability of such guidelines to patients with diabetes is questionable. Most patients with diabetic nephropathy do not have severe renal impairment and will not develop it. Approximately four out of every five patients who develop diabetic renal disease do not survive long enough to necessitate renal replacement therapies, succumbing instead to the vascular complications of diabetes before meeting criteria for referral to a nephrologist. In addition, few patients develop anaemia severe enough to qualify for therapy with erythropoietin under most current guidelines, though there is evidence that lesser reductions in haemoglobin are associated with adverse outcomes.

However, potential benefits need to be carefully balanced against the significant financial cost involved in treating patients with anaemia (Fig. 9). In addition, although exogenous erythropoietin or erythropoietin analogues are generally well tolerated, there is also a potential for significant adverse events, including raised blood pressure and pure red cell aplasia [7,218,183]. The impact of hypertension, increased blood viscosity and peripheral

resistance may offset any benefit arising from correction of anaemia [189]. Increasing the haemoglobin level to the high normal range may also be associated with increased mortality [181]. Correcting anaemia *via* other means such as transfusion also carries associated risks including HLA sensitisation, which may render a patient ineligible for transplantation. Blood transfusion in the setting of acute coronary syndromes is also associated with higher mortality [219]. Repletion of iron may also act to promote intracellular oxidative stress and impair insulin sensitivity.

### CONCLUSIONS

It is likely that the epidemic of diabetic nephropathy will generate a surfeit of patients with anaemia. Already diabetes as the most common cause of CKD in the Western World is the most common cause of renal anaemia. In addition, anaemia is twice as common in patients with diabetes. Patients at greatest risk can be readily identified by the presence of renal disease, in whom anaemia represents an important manifestation of renal microvascular dysfunction. On average, three quarters of anaemic patients in our centre had evidence of nephropathy. The majority of the remaining patients with anaemia and neither albuminuria nor renal impairment had a prolonged duration of diabetes. A reduced haemoglobin level identifies those at greatest risk for adverse outcomes. In addition, there is evidence to suggest that anaemia also independently contributes to the progression of microvascular complications. The majority of diabetic patients with anaemia have functional erythropoietin deficiency, providing a rationale for its supplementation in selected patients. It is only to be hoped that upcoming randomised, double-blind controlled studies (TREAT, CREATE, ACORD) will clarify the role of correction of anaemia in patients with diabetes and CKD [220,221,222].

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