

ORIGINAL ARTICLE

Low testosterone and anaemia in men with type 2 diabetes

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Summary

Objective Anaemia is frequently found in patients with diabetes, in whom it is associated with increased morbidity and mortality. Low testosterone levels are also common in men with type 2 diabetes. We hypothesized that low testosterone levels are also associated with anaemia in men with type 2 diabetes, over the effects of chronic kidney disease.

Design Cross-sectional cohort study, performed in 2005 in a tertiary diabetes clinic.

Patients 464 men with type 2 diabetes.

Main outcome measure Anaemia (haemoglobin (Hb) < 13.7 g/dl in men aged < 60, or < 13.2 g/dl in men aged 60 and older).

Results About 24% of study participants had anaemia, which was associated with the presence and severity of chronic kidney disease, systemic inflammation, increased age, and reduced iron availability. In addition, testosterone levels were independently associated with reduced Hb levels, determining between 6 and 8% of the total variability in raw Hb levels in this population after adjusting for these other factors. Individuals with total testosterone level < 10 nmol/l (43% of the cohort) were more likely to have anaemia (adjusted odds ratio 1.7; 95% CI 1.1–2.8). Similarly, anaemia was twice as common in individuals with a calculated free testosterone of < 0.23 nmol/l (adjusted odds ratio 2.0, 95% CI 1.2–3.1).

Conclusions These findings suggest that testosterone deficiency may contribute to the increased frequency of anaemia in men with type 2 diabetes. However, the appropriate clinical response to testosterone deficiency in anaemic patients remains to be established by prospective clinical trials.

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Introduction

Anaemia is a frequent finding in patients with diabetes.^{1,2} While some of this excess is explained by the high prevalence of chronic kidney disease (CKD) in this population, anaemia is more common and more severe when compared with patients with nondiabetic kidney disease, even with similar degrees of renal impairment.³ Low testosterone levels are also common in men with type 2 diabetes,^{4,5} and more common than in the age-matched general population.⁶ Because testosterone stimulates erythropoiesis,⁷ we hypothesized that low testosterone levels may also contribute to anaemia in men with type 2 diabetes.

Experimental subjects

We performed a cross-sectional study in 464 unselected men with type 2 diabetes attending diabetes outpatient clinics at Austin Health, a tertiary referral centre in Melbourne, Australia in 2005. Patients with established hypogonadism, testosterone replacement therapy, end-stage kidney disease or erythropoietin therapy were excluded from the analysis. In a randomly selected subgroup of 262 men with type 2 diabetes, testosterone determinations were repeated at their next routine clinic appointment.⁵ All men gave written informed consent, and the study was approved by the Human Research Ethics Committee, Austin Health, and conducted in accordance with the Declaration of Helsinki.

Materials and methods

Quantification of testosterone levels

Blood samples were drawn between 8 and 10 am after an overnight fast. Total testosterone (TT) was measured using the Access testosterone assay (Beckman Coulter, Inc., Fullerton, CA) with a minimum detection limit of 0.35 nmol/l. In our hands, the intra- and interassay coefficients of variation (CVs) assessed for two different concentrations (4.7 and 26.0 nmol/l) were 3.9, 4.8 and 5.7, 5.0, respectively. The lower limit of the normal range for this specific platform was 10 nmol/l, based on a reference panel of 124 healthy, reproductively normal young men (Ken Sikaris personal communication⁸).

In the blood of normal men, 44% of total T is bound to sex hormone binding globulin (SHBG), 2% is unbound [free testosterone (FT)], and 54% circulates bound to albumin and other proteins.⁹

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The US Endocrine Society position statement (2006) states that 'as a practical matter, FT often correlates better with the androgenic state of the patient than does TT'.¹⁰ Consequently, FT values were also calculated from TT, SHBG (Immolute 2000 analyser, Diagnostics Products Corporation, Los Angeles, CA) and serum albumin using Vermeulen's formula,^{11,12} which compares 'extraordinarily well' with FT¹⁰ measured by equilibrium dialysis. The lower limit for the Access testosterone/Immolute SHBG combination for calculated FT (cFT), based on the reference panel of 124 men, was 0.23 nmol/l (Ken Sikaris personal communication⁸), which is a similar threshold to that detailed elsewhere.^{10,12}

Identification of patients with anaemia

The presence of anaemia was defined on the basis of a full blood count by a haemoglobin (Hb) < 13.7 g/dl in men aged < 60, or < 13.2 g/dl in men aged 60 and older.¹³ These age-specific cut-offs have been shown to be better at identifying those individuals with a Hb level below the 'normal range' for their age than the traditional World Health Organisation (WHO) cut-off of 13.0 g/dl in men.¹³

Measurement of erythropoietin levels

Using serum samples obtained at the same time as the full blood count determination, circulating erythropoietin levels were determined using a solid phase chemiluminescent immunometric assay (Immolute 2000 (Diagnostics Products Corporation)). As previously described,¹⁴ erythropoietin levels in patients with type 2 diabetes, normal renal function, iron indices and a Hb level within the normal range were used to define the 'normal' erythropoietin range (16 ± 7 U/l). This was similar to a 97.5% upper limit of 28.5 IU/l observed for healthy volunteers.¹⁵ Patients with anaemia and erythropoietin levels inappropriately within the 'normal' range, were said to have an 'inappropriate renal response to anaemia'.¹⁴

Additional clinical parameters

All patients had taken a detailed clinical history, including anthropometric measurements, age, gender, body mass index (BMI), duration of diabetes, treatment modalities and the presence or absence of diabetic complications. Concurrent blood samples were taken for serum creatinine, C-reactive protein (CRP), lipid levels, HbA_{1c}, transferrin saturation (TSAT) and 24-h urine samples obtained for urinary albumin excretion rate (AER) measured using standard methodology. Estimated glomerular filtration rate (eGFR) was determined using the 4-variable Modification of Diet in Renal Disease (MDRD) equation, which is a better marker of kidney function than serum creatinine.¹⁶ Renal function was further stratified according to Kidney/Dialysis Quality Initiative (K/DOQI) guidelines for the management of diabetic kidney disease.¹⁷

Statistical analysis

Continuous variables were expressed as mean \pm SEM, unless otherwise indicated. Where appropriate, means and proportions were compared by using analysis of variance and chi-square tests, respectively.

To identify parameters independently associated with Hb levels and anaemia, we used linear and logistic regression analysis, respectively. All variables known to be associated with Hb were included in the final model, along with any variables associated with Hb in univariate analyses with a *P*-value of less than 0.01. A multivariate model was used to confirm the contribution of associations, and unrelated variables were removed from the model. Each variable was entered as continuous data or as their log derivative (AER, CRP, fasting plasma glucose). The final model variables were determined by sequential penalised likelihood (Akaike information criterion). The potential for multiple colinearity was tested using the variance inflation factor (VIF) and condition number (CN), where VIF < 10 and CN < 30 are desirable. Hockey-stick models were fit to the adjusted data to determine whether there was a change in the relationship between TT and anaemia at any specific level of TT. All analyses were performed using SPSS, version 11.5 (SPSS Inc, Chicago, IL).

Results

Cohort characteristics

Four hundred sixty-four men with type 2 diabetes were studied (their baseline characteristics are shown in Table 1). Their mean age was 64 years and median duration of diabetes was 10 years. About 51% of patients were obese (BMI > 30 kg/m²). Over half of the men in this cohort had chronic kidney disease. Forty-three per cent had an elevated urinary albumin excretion rate (AER > 20 μ g/min), including 15% with macroalbuminuria (AER > 200 μ g/min). Twenty-seven per cent of all patients had moderate to severe renal impairment (eGFR < 60 ml/min/1.73 m²).

Testosterone levels in men with type 2 diabetes

As previously described in this cohort,⁵ 43% of men had testosterone levels had TT < 10 nmol/l (*n* = 199), including one hundred men (21%) with a TT level below 8 nmol/l, the current threshold for subsidized treatment in Australian men with age-related hypogonadism. About 57% had cFT levels below the normal range (< 0.23 nmol/l).

Men with TT levels < 10 nmol/l were older and more obese than individuals with TT levels in the normal range (Table 1). TT levels were also correlated with systemic inflammation (*P* < 0.01), with the higher CRP levels observed in patients with lower testosterone levels. In this study, TT or cFT levels were not associated with the HbA_{1c}, CKD or treatment modalities.

The frequency of anaemia in men with type 2 diabetes

Anaemia was common in this population. Overall, 24% of study participants (*n* = 108) had Hb levels below the age-standardised normal range,¹³ and 18% of men (*n* = 83) had a Hb < 13 g/dl. Almost all (96%) of those individuals with anaemia had both normocytic and normochromic red cell parameters. Fourteen per cent of all participants had reduced iron availability (TSAT < 16%), including 28% of those with anaemia. None had reduced vitamin B₁₂ or folate levels. As previously described in these anaemic patients¹ erythropoietin levels were not elevated but remained inappropriately

Table 1. Clinical characteristics of study participants, stratified by TT levels. Continuous data are expressed as mean \pm SEM, unless otherwise stated, categorical data are expressed as frequency (%)

	Total cohort (n = 464)	TT \geq 10 nm (n = 265)	TT < 10 nm (n = 199)
Haemoglobin (g/dl)	14.3 \pm 0.1	14.5 \pm 0.1	14.0 \pm 0.1*
Anaemia (%)	24%	20%	29%*
Age (years)	64 \pm 1	63 \pm 1	66 \pm 1*
Duration of diabetes (years)	10 \pm 1	10 \pm 1	11 \pm 1
Body mass index (g/m ²)	30.5 \pm 0.2	29.4 \pm 0.3	31.8 \pm 0.4*
HbA _{1c} (%)	7.5 \pm 0.1	7.4 \pm 0.1	7.6 \pm 0.1
Insulin therapy (%)	44%	44%	44%
Thiazolidinediones (%)	15%	15%	16%
Mean arterial BP (mmHg)	101 \pm 2	102 \pm 2	98 \pm 2
RAS blockade (%)	86%	86%	87%
β -blockade (%)	29%	29%	30%
Total cholesterol (mm)	4.4 \pm 0.1	4.3 \pm 0.1	4.4 \pm 0.1
Lipid lowering therapy (%)	65%	66%	65%
Median CRP (IU/ml)	3.0	2.7	3.5*
Transferrin saturation percentage	26 \pm 1	27 \pm 1	24 \pm 1*
Erythropoietin (IU/ml)	20 \pm 1	21 \pm 1	19 \pm 1
Normoalbuminuria (%)	57%	58%	56%
Microalbuminuria (%)	28%	28%	28%
Macroalbuminuria (%)	15%	14%	16%
eGFR (ml/min/1.73 m ²)	75 \pm 2	76 \pm 2	75 \pm 2
eGFR < 60 ml/min/1.73 m ² (%)	27%	26%	28%

*Univariate $P < 0.05$ between TT < 10 nm and TT \geq 10 nm.
RAS, renin-angiotensin system; TT, total testosterone.

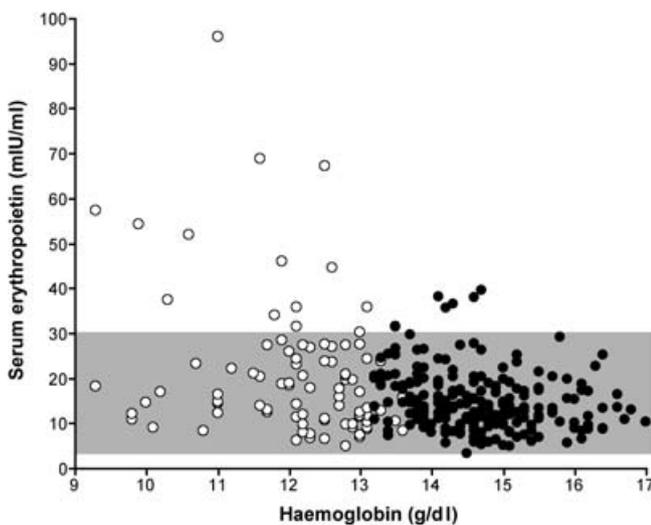


Fig. 1 The relationship between Hb and erythropoietin levels in men with type 2 diabetes. Shaded area denotes the 'normal range' derived from men with type 2 diabetes, a Hb in the normal range and normal iron stores. White circles denote individuals with anaemia. Black circles denote individuals with a Hb levels in the normal range for their age.

in the normal range in 83% of anaemic patients (Fig. 1), denoting an inappropriate renal response to anaemia. This is consistent with the finding that most of these individuals (75/91) also had chronic kidney disease. Of the remaining 17 patients with anaemia and

erythropoietin levels (appropriately) elevated beyond the normal range, 10 had reduced iron availability (TSAT < 16%).

Predictors of anaemia in men with type 2 diabetes

Low testosterone levels were more common in individuals with anaemia. About 53% of anaemic patients had a TT of < 10 nmol/l (vs. 41% in nonanaemic patients; $P < 0.001$). About 71% of patients with anaemia had a cFT of < 0.23 nmol/l (vs. 51% in nonanaemic patients; $P < 0.001$). Overall, individuals with type 2 diabetes and a TT < 10 nmol/l were more likely to have anaemia (Odds ratio 1.7; 95% CI 1.1–2.8) (Table 2), after adjusting for other variables associated with Hb levels (Fig. 2). A similar association was observed with cFT, where individuals with type 2 diabetes and a cFT of < 0.23 nmol/l were twice as likely to have anaemia (odds ratio 2.0; 95% CI 1.2–3.1) after adjusting for other variables (Fig. 3). Notably, this association was independent of the presence and severity of diabetic kidney disease, iron availability, systemic inflammation and age (Figs 2 and 3). However, there was no significant interaction between testosterone levels and these other variables in determining anaemia.

When modelled as a continuous variable, there was a gradual increase in the prevalence of anaemia with falling TT or cFT levels, after adjusting for other associated variables. Hockey-stick analyses of the adjusted regression models did not improve the curve fits, suggesting there were no 'thresholds' in the association. In addition, there was no significant interaction between testosterone levels and renal impairment in determining the frequency of anaemia, as the

Table 2. Clinical characteristics of study participants, stratified for the presence of anaemia

	Normal Hb (n = 356)	Anaemic (n = 108)
Haemoglobin (g/dl)	14.9 ± 0.1	12.2 ± 0.1*
Age (years)	65 ± 1	69 ± 1*
Duration of diabetes (years)	10 ± 1	13 ± 1*
Body mass index (g/m ²)	30.5 ± 0.3	30.2 ± 0.6
HbA _{1c} (%)	7.6 ± 0.1	7.4 ± 0.1
Insulin therapy (%)	42%	46%
Thiazolidinediones (%)	13%	16%
Mean arterial BP (mmHg)	101 ± 1	99 ± 4
RAS blockade (%)	83%	88%
β-blockade (%)	28%	32%
Total cholesterol (mm)	4.4 ± 0.1	4.1 ± 0.1*
Lipid lowering therapy (%)	60%	69%
CRP (IU/ml)	7%	18%*
TSAT < 16% (%)	8%	28%*
Erythropoietin (IU/ml)	16 ± 1	23 ± 2*
Normoalbuminuria (%)	60%	53%
Microalbuminuria (%)	26%	29%
Macroalbuminuria (%)	14%	18%
eGFR (ml/min/1.73 m ²)	79 ± 2	66 ± 4*
eGFR < 60 ml/min/1.73 m ² (%)	21%	47%*
Total testosterone (nmol/l)	11.5 ± 0.2	9.5 ± 0.4*
SHBG (nmol/l)	37 ± 1	38 ± 1
Calculated free testosterone (nmol/l)	0.23 ± 0.01	0.19 ± 0.01*

*Univariate $P < 0.05$ between vs. patients with a Hb in the normal age-specific range.

impact of testosterone levels was statistically similar in patients with and without renal impairment.

Predictors of haemoglobin levels in men with type 2 diabetes

When modelled as a continuous variable, Hb levels were independently associated with iron availability, eGFR, logCRP, triglyceride and erythropoietin levels and the HbA_{1c} (Table 3). In addition, TT and cFT were continuously correlated with Hb levels after adjusting for other associated variables in a multiple regression analysis ($P < 0.001$), contributing 6 and 8% of the variability in Hb levels, respectively, in this cohort. SHBG levels were not associated with Hb levels or anaemia, after adjusting to testosterone levels.

Persistently low testosterone levels and anaemia

As a single low testosterone level is generally considered inadequate for making the diagnosis of hypogonadism, testosterone levels were repeated in a randomly selected subgroup of 262 men with type 2 diabetes.⁵ The clinical characteristics of this subgroup were not significantly different to those of the total cohort of men with type 2 diabetes. As previously described,⁵ 73% of individuals with TT level < 10 nmol/l on the first estimation continued to have a TT < 10 nmol/l on repeat testing. The frequency of anaemia in

Table 3. Model summary of variability in Hb levels in patients with type 2 diabetes

Model	R	R ²	R ² change	Sig for change
1	0.358(a)	0.128	0.128	0.000
2	0.486(b)	0.237	0.108	0.000
3	0.537(c)	0.288	0.052	0.000
4	0.581(d)	0.338	0.049	0.000
5	0.602(e)	0.362	0.024	0.002
6	0.627(f)	0.393	0.031	0.000
7	0.636(g)	0.408	0.015	0.004
8	0.648(h)	0.420	0.012	0.024

a Predictors: (Constant), TSAT.

b Predictors: (Constant), TSAT, eGFR.

c Predictors: (Constant), TSAT, eGFR, TT.

d Predictors: (Constant), TSAT, eGFR, TT, triglycerides.

e Predictors: (Constant), TSAT, eGFR, TT, triglycerides, logAER.

f Predictors: (Constant), TSAT, eGFR, TT, triglycerides, logAER, logCRP.

g Predictors: (Constant), TSAT, eGFR, TT, triglycerides, logAER, logCRP, erythropoietin.

h Predictors: (Constant), TSAT, eGFR, TT, triglycerides, logAER, logCRP, erythropoietin, HbA_{1c}.

TSAT, transferrin saturation; eGFR, estimated glomerular filtration rate; TT, total testosterone; AGR, albumin excretion rate; CRP, C-reactive protein.

individuals with TT persistently below 10 nmol/l was twice that observed in individuals with TT persistently in the normal range (36 vs. 19%, $P < 0.01$). Individuals with TT < 10 nmol/l on only one determination (either the first or second time-point) also had higher rates of anaemia (35%) than individuals with TT persistently in the normal range ($P < 0.01$).

Discussion

Anaemia is more common in type 2 diabetes than in the general population, even after adjusting for the presence and severity of renal impairment.³ The reasons for this excess remain to be established. However, low testosterone levels are also common in men with type 2 diabetes⁴⁵ and have been shown to increase the risk of anaemia in older men and women without diabetes.¹⁸ In this study, we demonstrate that low testosterone levels are independently associated with anaemia in men with type 2 diabetes. These data confirm the previous association between androgens and haemopoiesis in men with type 2 diabetes.¹⁹ In addition, we show that testosterone levels significantly contribute to Hb variability and anaemia, independent and additive to the to the actions of chronic kidney disease, iron storage and systemic inflammation, potentially confounding factors also associated with hypogonadism and anaemia.

Because of the cross-sectional design of our study, we cannot definitively establish whether testosterone deficiency directly contributes to the increased frequency of anaemia in men with type 2 diabetes. Nonetheless, there is strong clinical and experimental data that testosterone is able to influence haemopoiesis on a number of levels. Certainly, hypogonadism and the use of antiandrogens is associated with normocytic normochromic anaemia.^{20,21} Testosterone is known to stimulate the proliferation of erythroid progenitors, largely

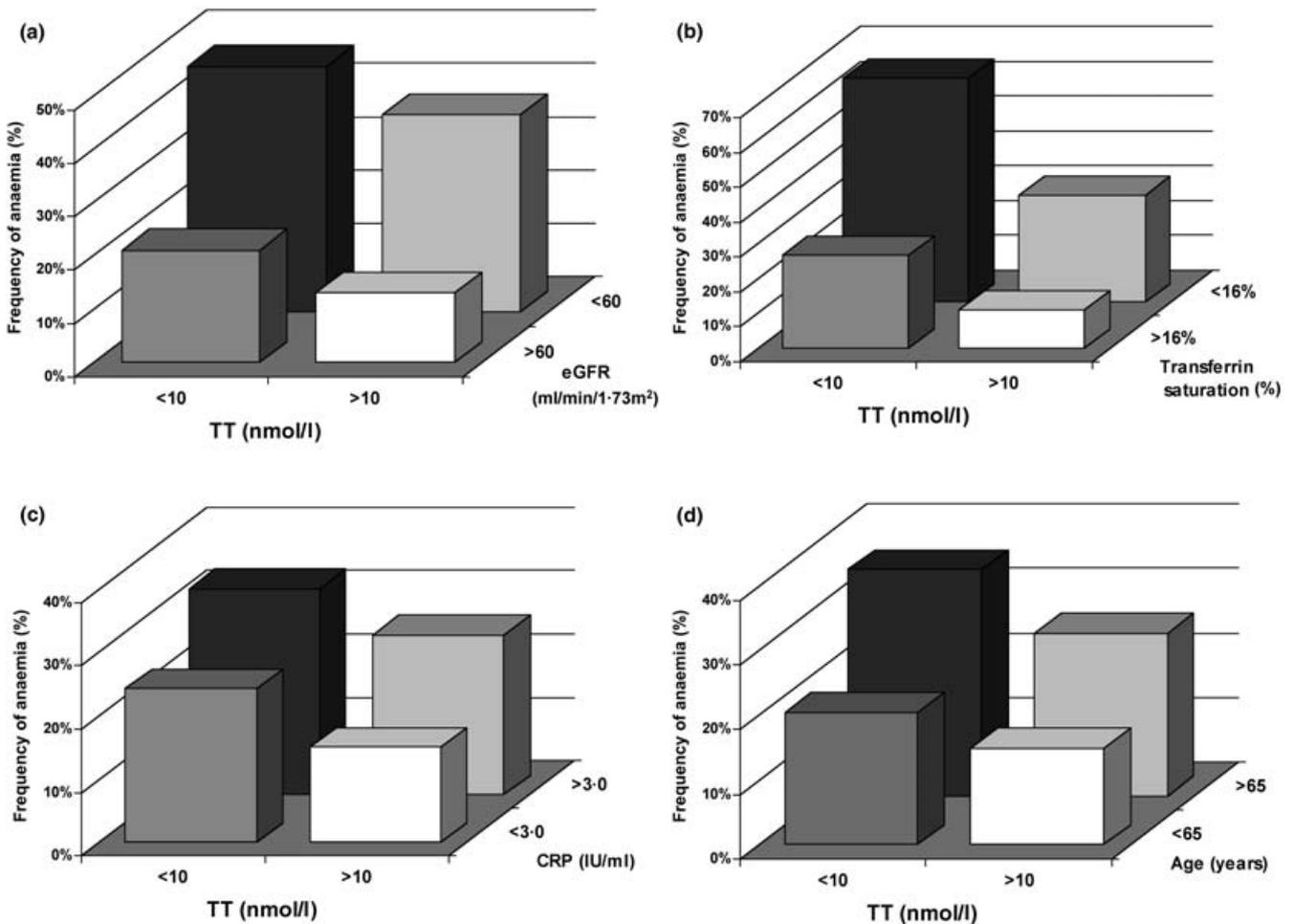


Fig. 2 Anaemia is more common in patients with total testosterone (TT) < 10 nmol/l, when adjusted for renal impairment (eGFR < 60 ml/min 1.73 m² a), reduced iron stores (TSAT < 16%, b), C-reactive protein (CRP) (above and below cohort median (3.0 IU/ml) (c) and age (above and below cohort median (65 years) (d)).

by erythropoietin-independent mechanisms.⁷ This function may be particularly important in the setting of diabetes, where the uncoupling of erythropoietin synthesis from Hb levels (Fig. 1) may require the integrity of such pathways to maintain Hb levels.² Testosterone may also be important in the response to reduced iron availability. Interestingly, in our study, anaemia was significantly more common in the setting of reduced iron availability when testosterone levels were also low (Figs 2d and 3d). This difference is also reflected in gender differences in Hb, which are partly androgen dependent.¹⁸

It is possible that the association between anaemia and low testosterone levels observed in our study may be biased by residual confounding by factors not measured in the study, but potentially related to both factors. For example, oxidative stress, insulin resistance,²² endothelial (dys)function²³ and the accumulation of advanced glycation end products (AGEs)²⁴ in diabetic patients may influence both haemopoiesis and gonadal function. In addition, systemic inflammation may also be a potentially confounding factor. For example, in our study, and others,^{19,25} testosterone levels were inversely associated with markers of systemic inflammation, including CRP. As systemic inflammation also significantly impacts on haemopoiesis, this association may also partly contribute to the

increased prevalence of anaemia in men with reduced testosterone levels. However, testosterone replacement in patients with diabetes does not normalize inflammatory markers in the short term,²⁵ although it is able to increase Hb levels.²⁶ In addition, in our study, the anaemia observed in patients with low testosterone levels appeared to be independent to inflammation. As patients with anaemia¹² and those with low testosterone levels both have higher rates of macrovascular disease, it is possible that the elevated levels of CRP also reflect their underlying vascular burden.

The strengths of this study include the large number subjects, measurement of serum testosterone levels at the appropriate time of day (early morning), and accurate assays for total and free testosterone levels. However, it remains to be established whether the biochemical testosterone deficiency observed in this study represents a true hypogonadal state. Recent US Endocrine Society guidelines recommend that a diagnosis of hypogonadism be made 'only in men with consistent signs and symptoms and unequivocally low serum testosterone levels.'²⁷ Interpretation of our study is therefore limited because we did not obtain a detailed record of symptomatology history. This said, generalised symptomatology in individuals with anaemia is almost impossible to distinguish from those of hypogonadism.

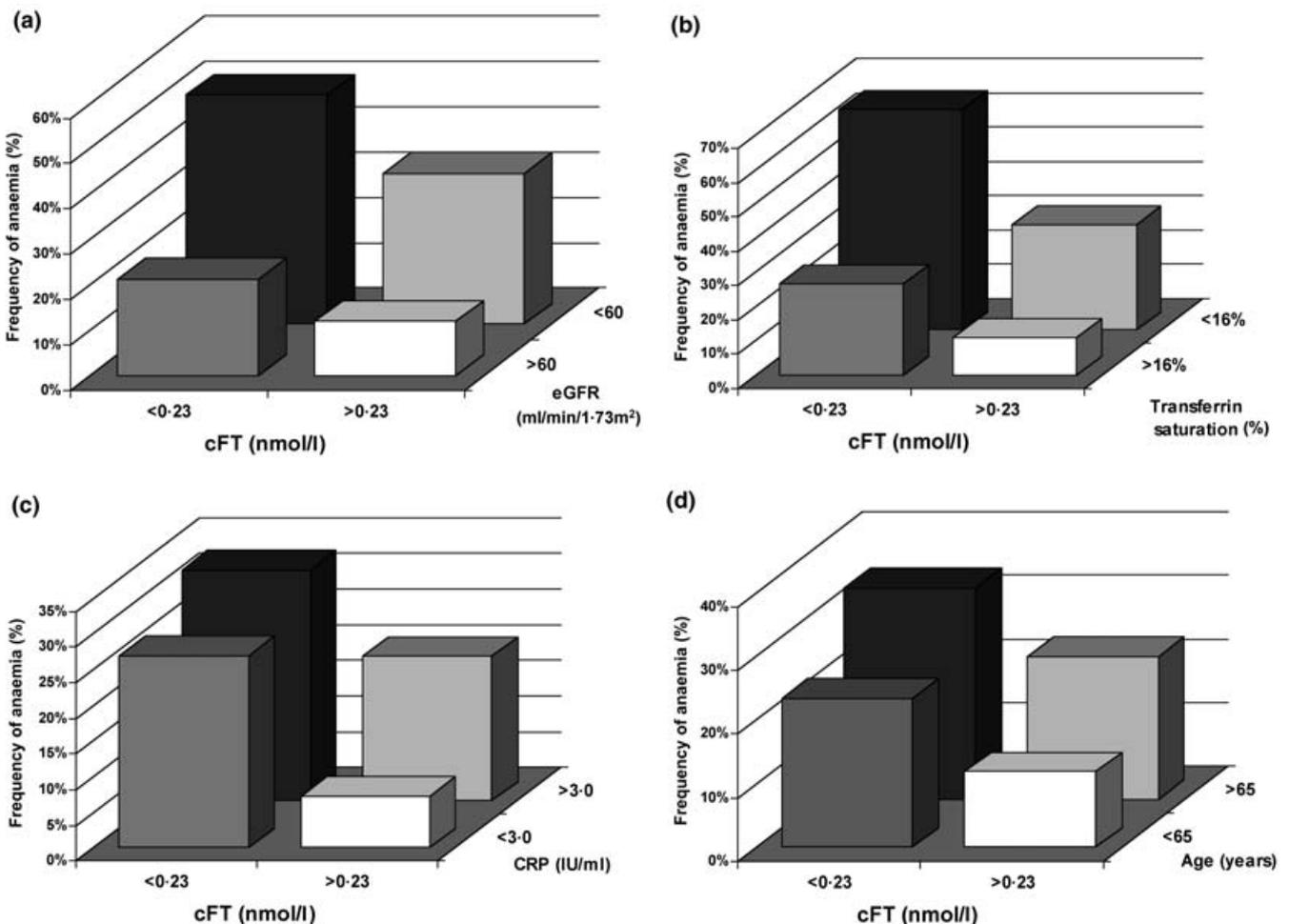


Fig. 3 Anaemia is more common in patients with cFT < 0.230 nmol/l, when adjusted for renal impairment (eGFR < 60 ml/min 1.73 m² a), reduced iron stores (TSAT < 16%, b), CRP (above and below cohort median (3.0 IU/ml) (c) and age (above and below cohort median (65 years) (d).

A single low testosterone level is generally inadequate for making the diagnosis of hypogonadism, given the variability in serum testosterone levels that can result from circadian rhythms, the pulsatile nature of its secretion, use of concomitant medications and measurement variations.^{10,27} Consequently, we repeated testosterone determinations in a randomly subset individuals with type 2 diabetes, finding that between 73% of individuals with testosterone levels below the 'normal range' subsequently continue to have low levels when retested.⁵ This is consistent with reports in nondiabetic men, where approximately 70% of men will continue to have abnormal levels when repeated.²⁶ However, the frequency of anaemia appeared to be increased in individuals with any finding of TT < 10 nmol/l, whether or not TT remained below the normal range on repeat testing. This finding is probably an artefact of the cut-off used for TT, as the frequency of anaemia was continuously associated with Hb levels, and most individuals with only one reading < 10 nmol/l had low-normal values recorded in the other.⁵

Testosterone deficiency may contribute to impaired performance, mood, libido, sleep and cognition in individuals with diabetes.²⁸ Given the overlapping symptomatology with anaemia, this suggests those presenting with anaemia should also be screened for testosterone

deficiency and *vice versa*. However, the appropriate clinical response to testosterone deficiency in anaemic patients with type 2 diabetes remains to be established. Certainly, testosterone replacement can improve performance, mood, and cognition in diabetic men with hypogonadism, partly by increasing Hb and reducing circulating adipokines.²⁹ However, testosterone may have deleterious actions on prostate disease and sleep apnoea,³⁰ and balance of benefits and risks from replacement therapy are still to be defined by large and long-term clinical trials. Moreover, the clinical utility of correcting anaemia per se remains problematic, with two recent studies, including (but not specifically in) patients with diabetes (CREATE and CHOIR) showing no or limited clinical benefit.^{31,32} Erythropoietin may also have complex effects on angiogenesis,³² blood pressure³³ and atherosclerosis.³⁴ The potential utility of correction of anaemia specifically in diabetic patients will be tested in the ongoing TREAT study.³⁵ At the present time, the identification of anaemia in a diabetic patient should serve to identify individuals at increased risk of a range of adverse clinical outcomes, among which hypogonadism should be considered. Equally, in patients with low testosterone levels, the potential contributing role of anaemia to symptomatology should also be considered.

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