Erythropoietin

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Erythropoietin (EPO) – a short history

- 1893-1977
  - hypoxia and bone marrow stimulation
- 1977
  - Miyake *et al* isolated erythropoietin from 2500 liters of urine from patients with aplastic anemia
- 1984
  - Lai *et al* characterized molecular structure

Erythropoietin (EPO) – a short history

- 1984 - human EPO gene cloned and expressed
- 1986-89
  - Clinical trials proved the rhEPO was effective in raising Hgb levels in HD, PD, predialysis and anephric patients
- July 1989 - FDA approved

- First administered in UK at Hammersmith

Before rhEPO

- Anaemia endemic in the dialysis and pre dialysis population
- Transfusion only consistent means of replacing blood

Before rhEPO

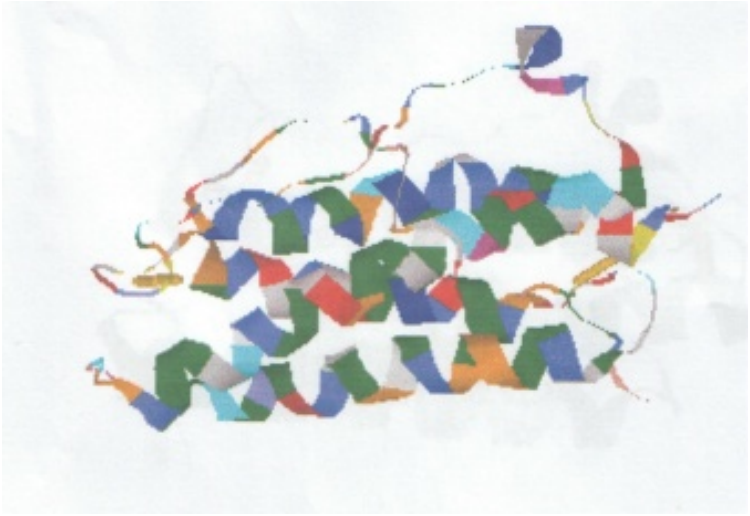
- Transfusion associated problems
  - Hepatitis B
  - Other blood borne viral infections
- Decreased transplant success
  - Sensitization of the patient to possible kidney transplants
- Iron Overload Syndromes
  - hemochromatosis

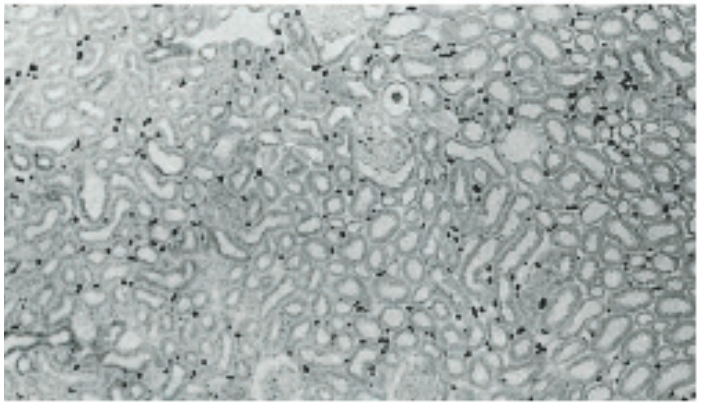
Erythropoietin (EPO)

- 30.4 kDalton glycoprotein
- 193 amino acids
- Growth Factor
- Stimulates erythrocytosis

Produced in the kidney (>90%) and liver (<10%)

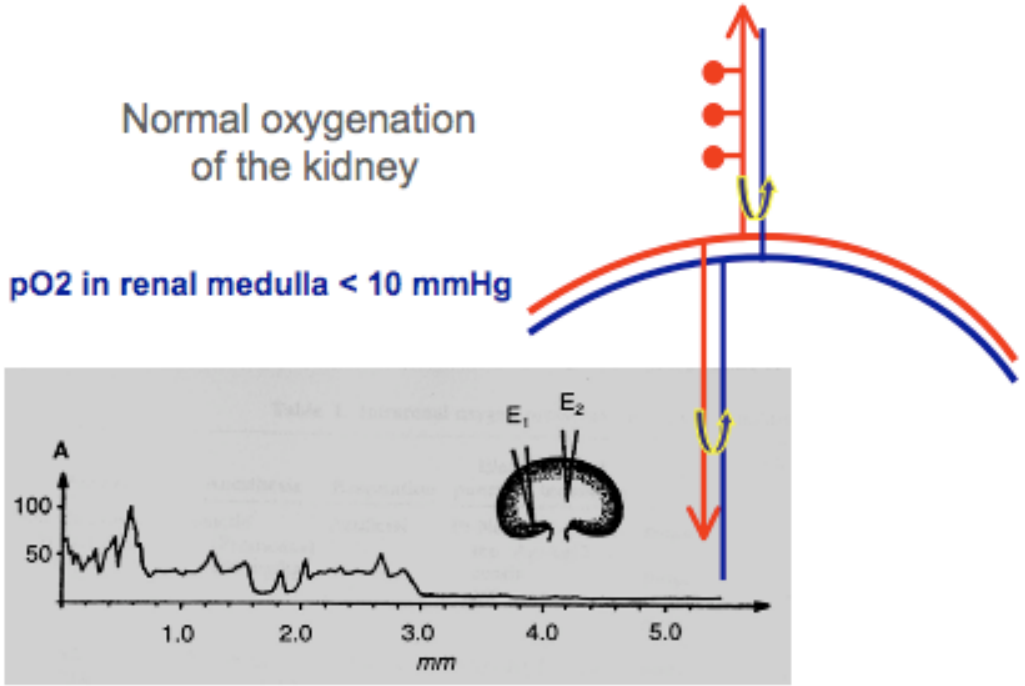
Made by fibroblasts in kidney and stellate cells in the liver





Erythropoietin (EPO)

- Why site control in the kidney?
  - Kidney distinguishes changes in renal blood flow from oxygenation
  - Steep oxygen gradient in the kidney



Regulation of Erythropoietin

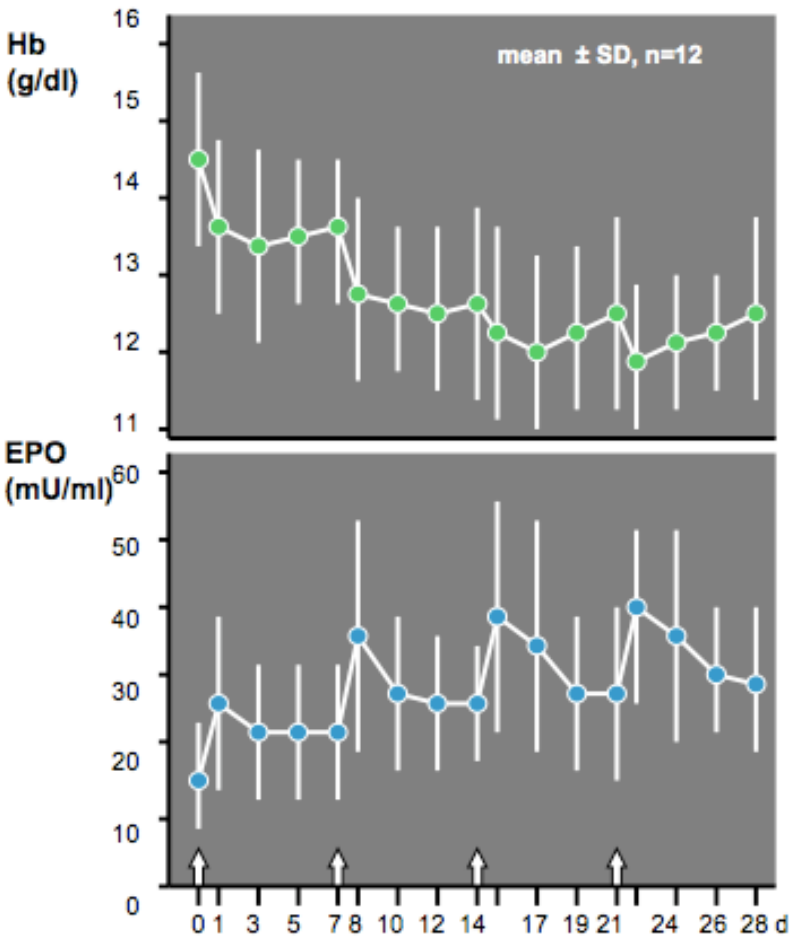
Oxygen sensing underlies the regulation of EPO

In a study the variations in EPO concentration during preoperative deposit of autologous blood

There is small and transient peak in plasma EPO after each donation.

A sustained elevation followed each peak.

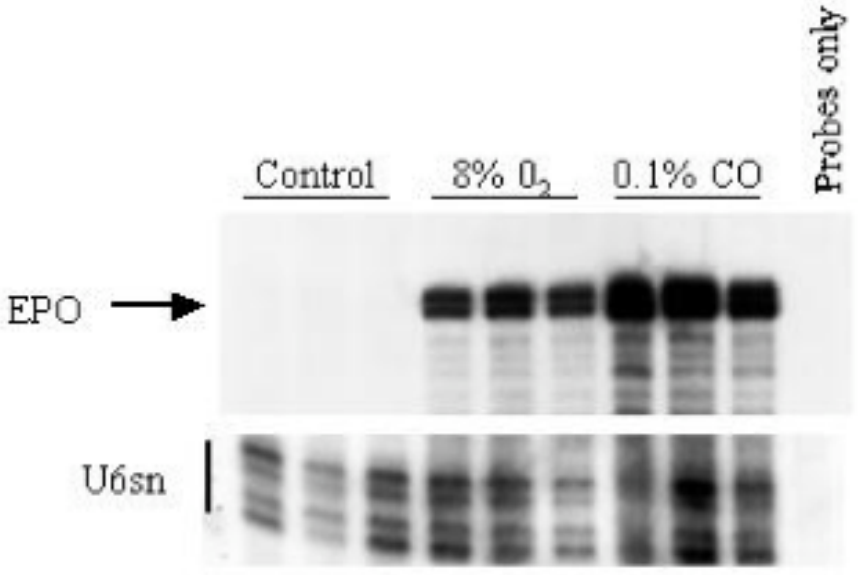
Anaemia stimulates EPO production and is a hypoxic stimulus



Regulation of Erythropoietin

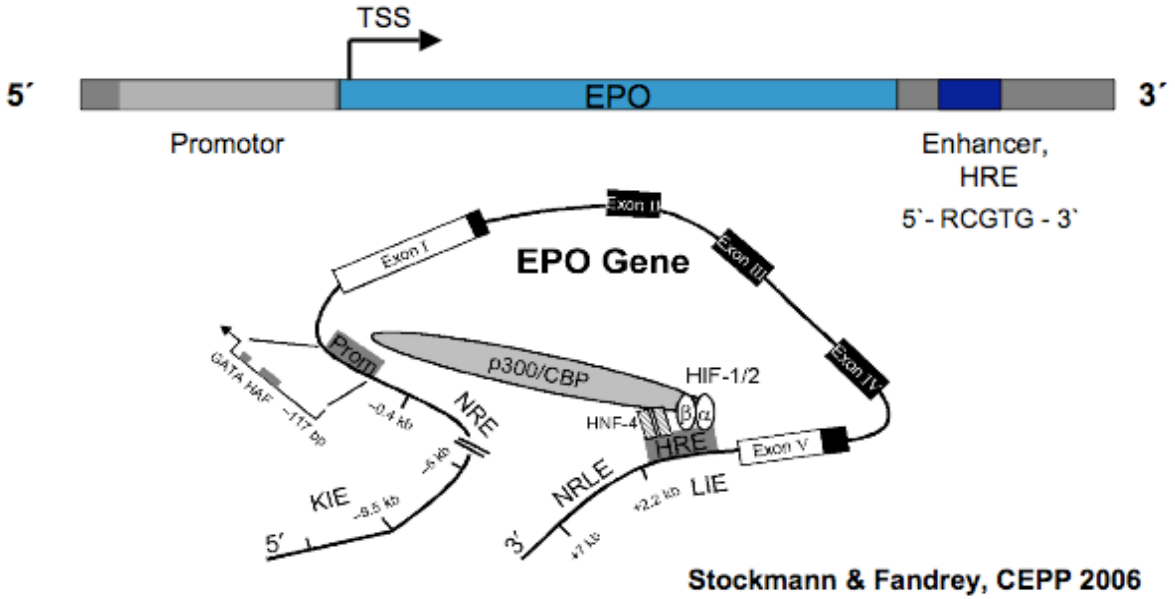
Oxygen sensing (or response to hypoxia) underlies the regulation of EPO

This RNAse protection assay looks for EPO mRNA in kidneys exposed to hypoxic stimuli.



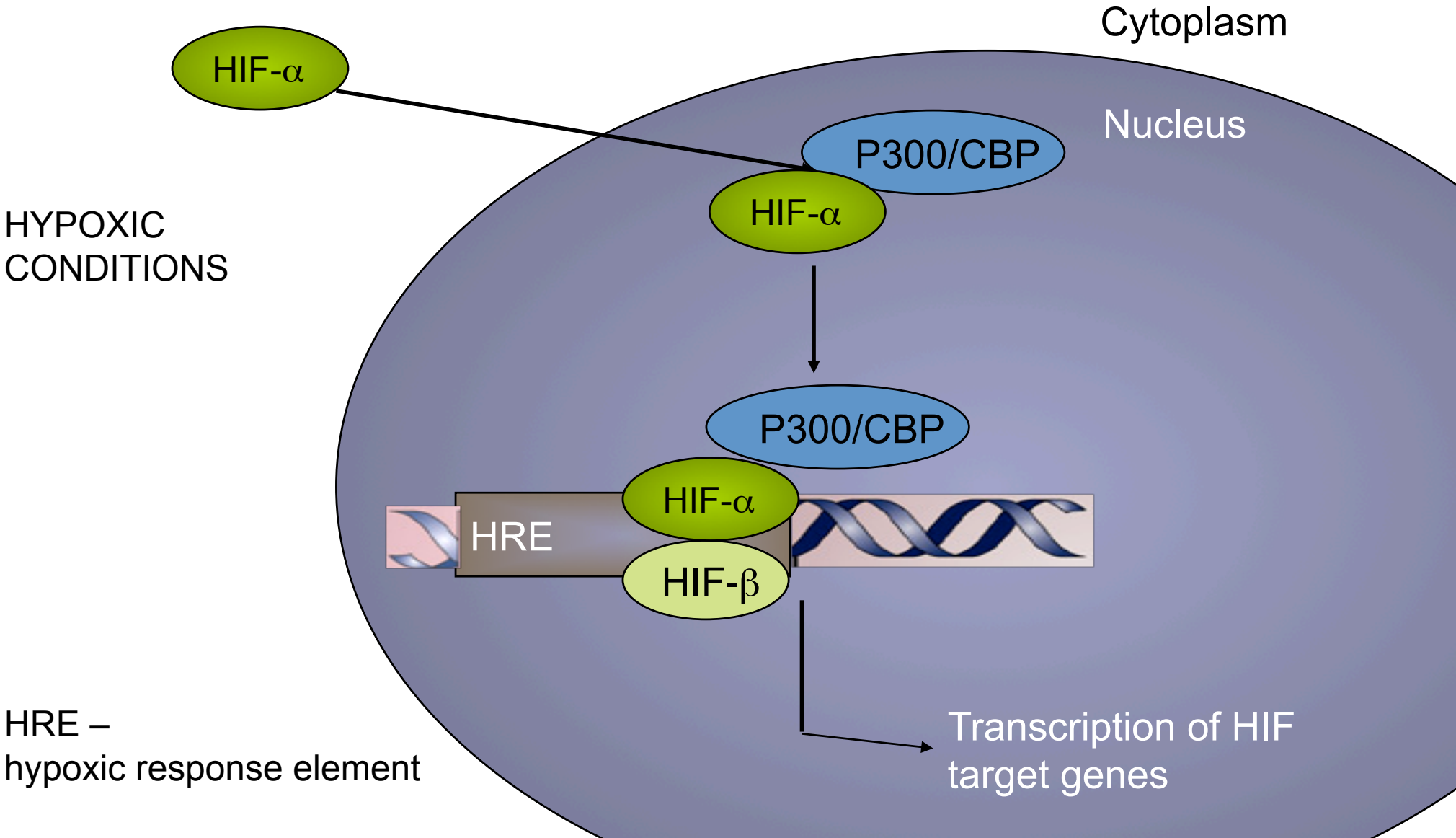
Oxygen sensing – Hypoxia Inducible Factor (HIF)

- HIF is a transcription factor
- It is constitutionally expressed
- EPO gene has a region called a HRE or hypoxic response element that binds HIF to upregulate EPO when activated

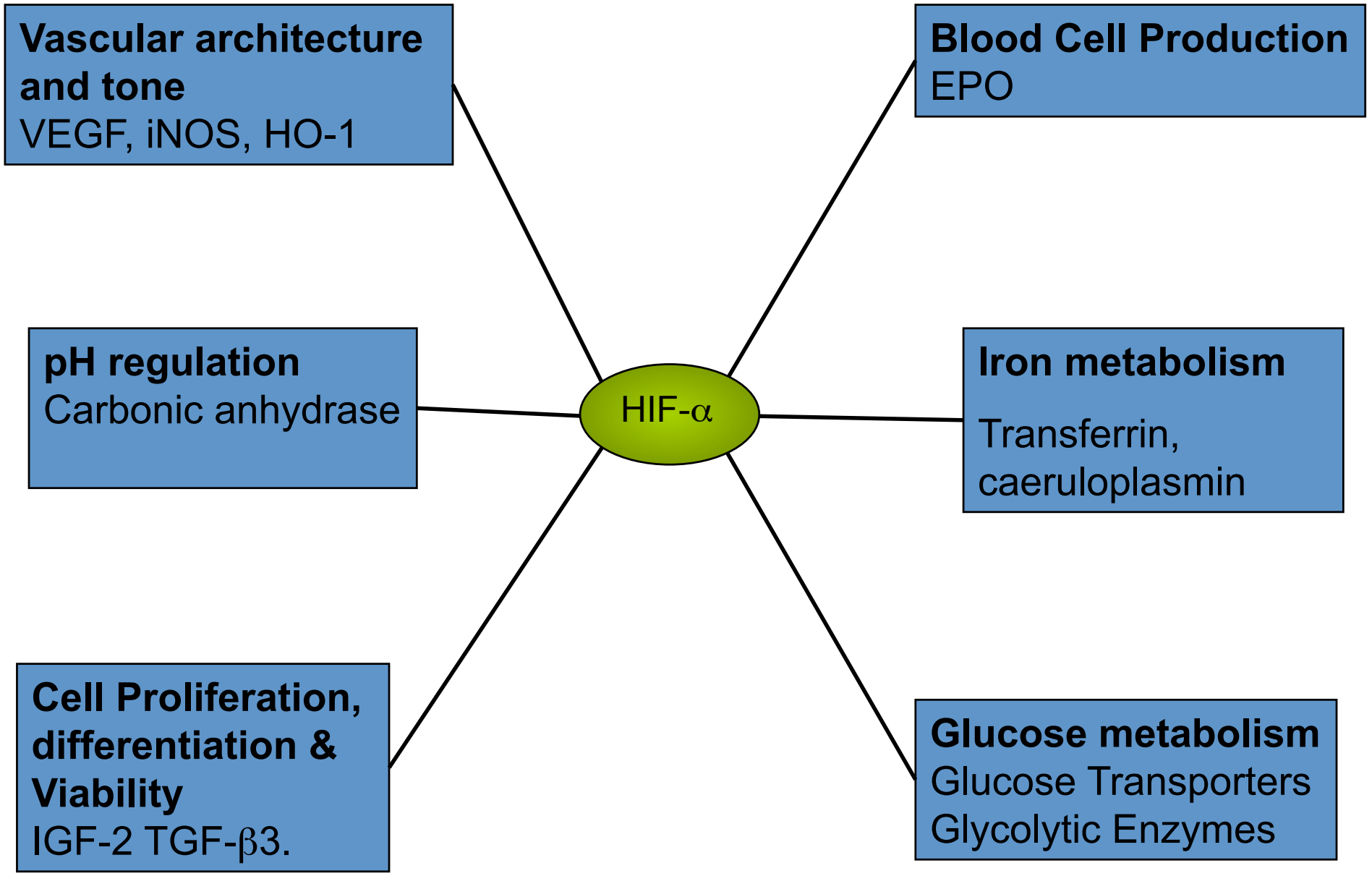


Stockmann & Fandrey, CEPP 2006

Oxygen sensing – Hypoxia Inducible Factor (HIF)

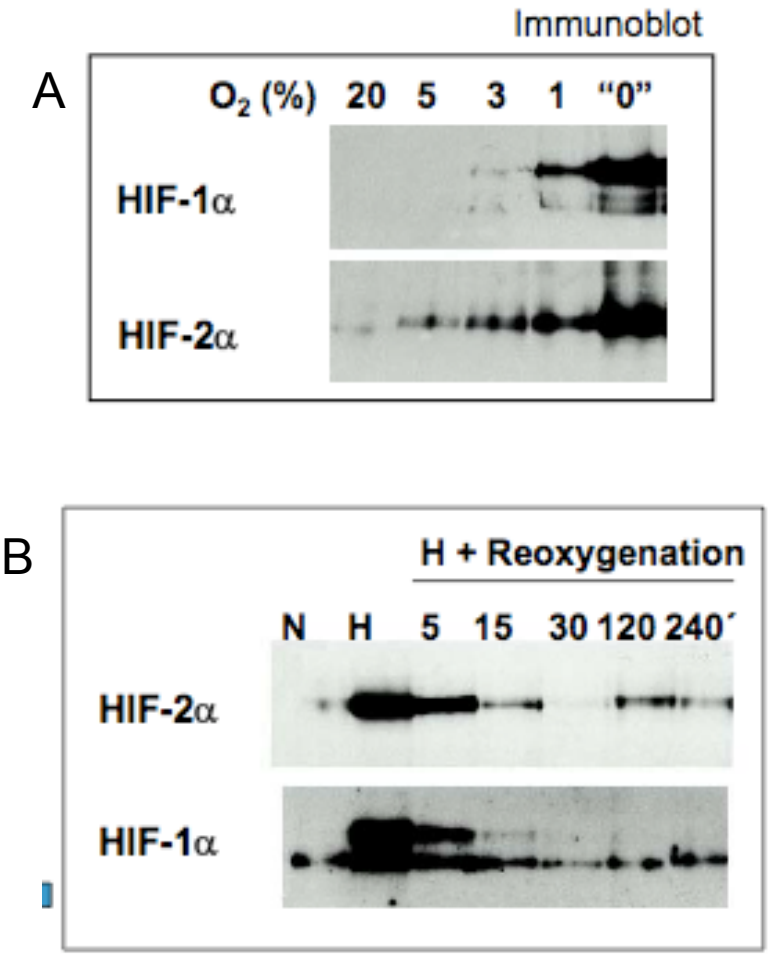


HRE –
hypoxic response element

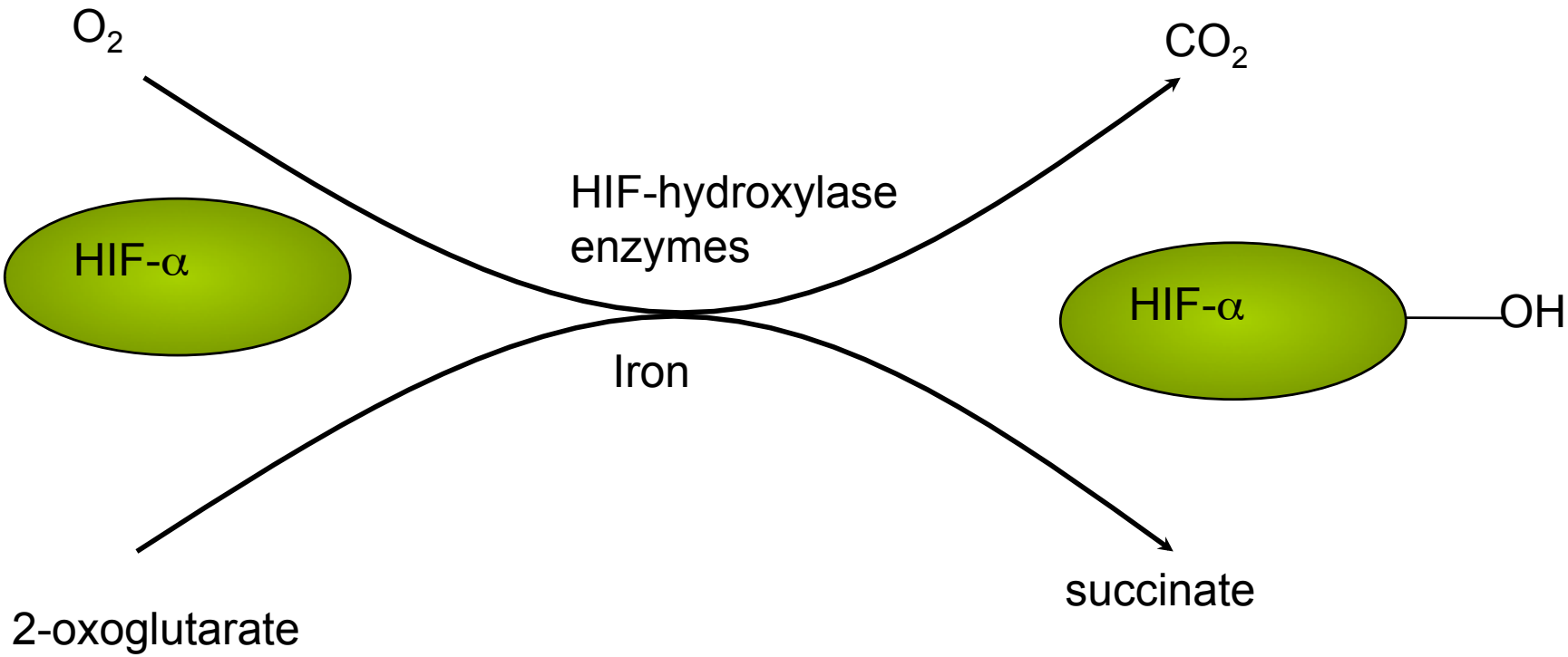

Vascular architecture and tone
VEGF, iNOS, HO-1
Blood Cell Production
EPO
pH regulation
Carbonic anhydrase
HIF-α
Iron metabolism
Transferrin, caeruloplasmin
Cell Proliferation, differentiation & Viability
IGF-2 TGF-β3.
Glucose metabolism
Glucose Transporters
Glycolytic Enzymes

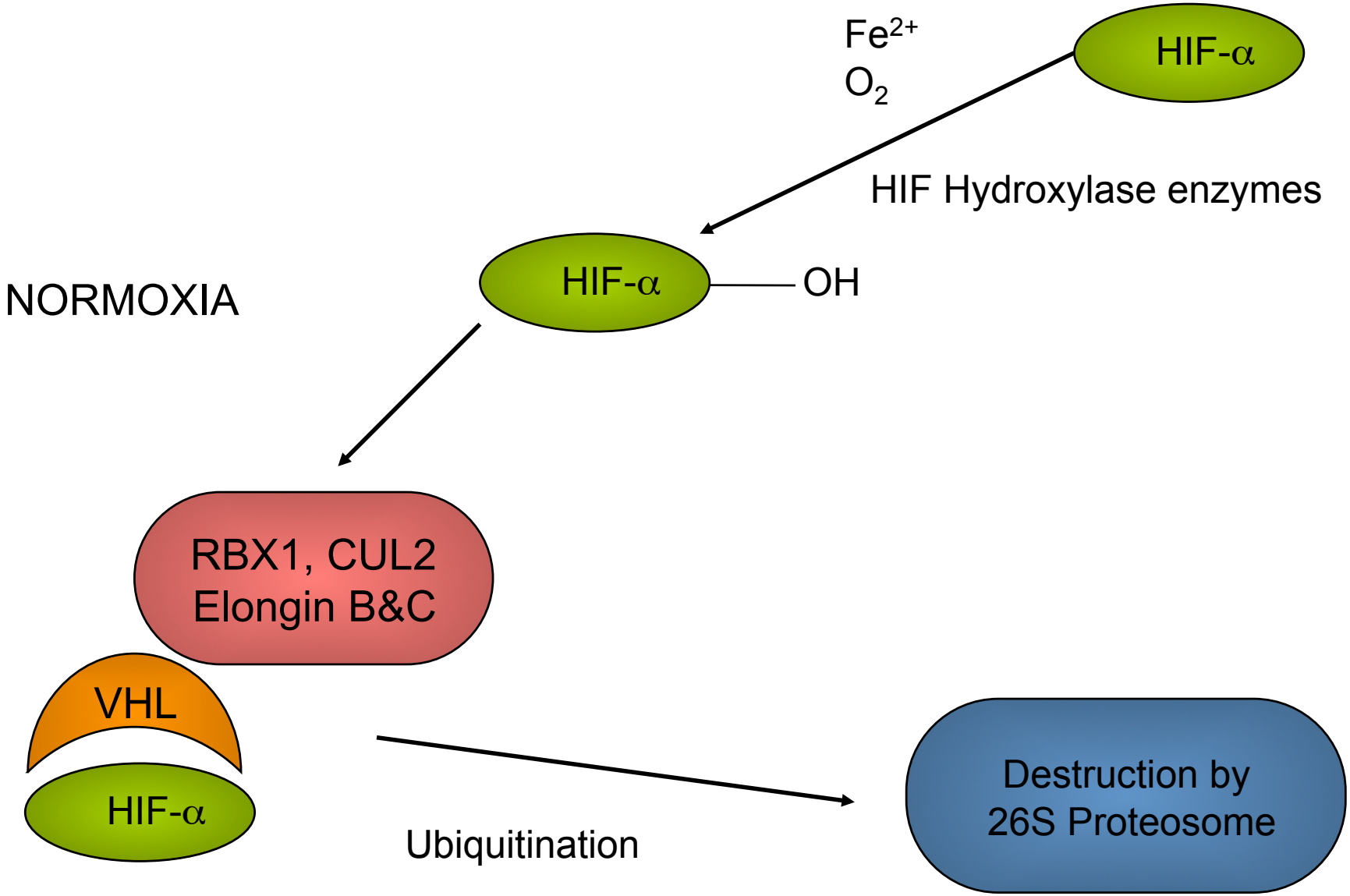
HIF degradation is oxygen dependent

- In this experiment cells are incubated at various oxygen concentrations
- Levels of HIF alpha subunits are detected using immunoblots
- As Oxygen levels fall a dose response curve is seen (A)
- In reoxygenation HIF protein detection falls (B)

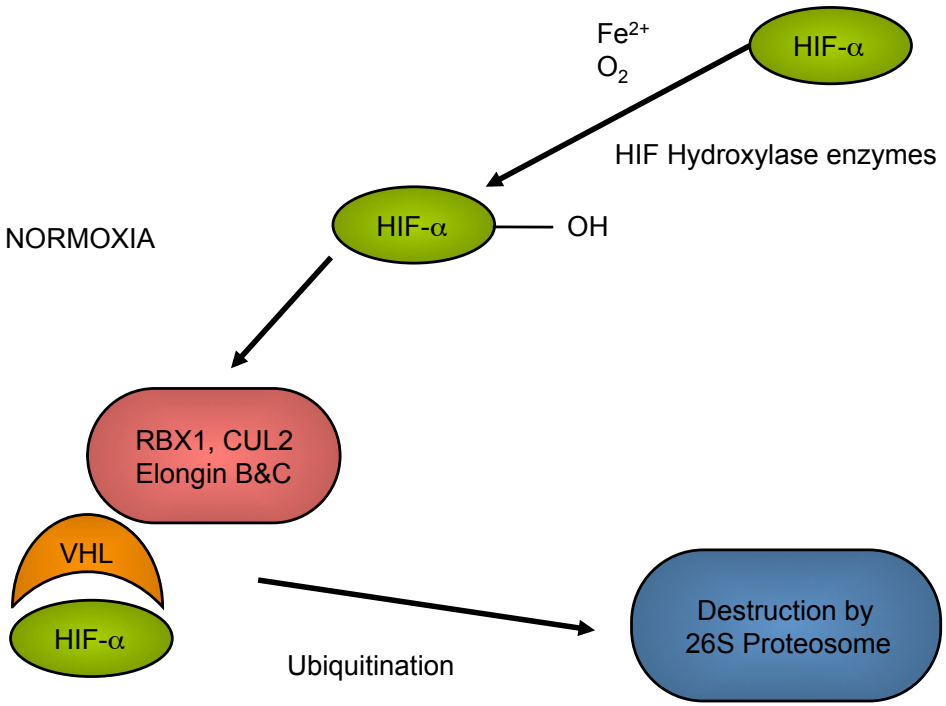


HIF degradation

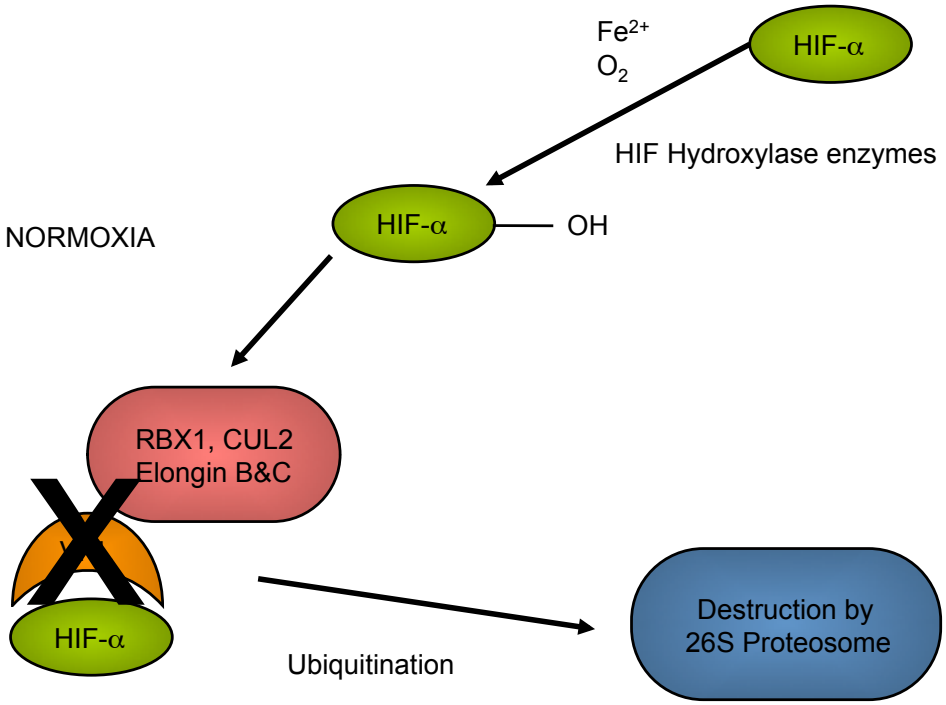



Fe^{2+}
O_2
HIF-α
HIF Hydroxylase enzymes
NORMOXIA
HIF-α
OH
RBX1, CUL2
Elongin B&C
VHL
HIF-α
Ubiquitination
Destruction by
26S Proteosome

What happens when the HIF axis is abnormal?



What happens when the HIF axis is abnormal? Loss of regulatory protein VHL



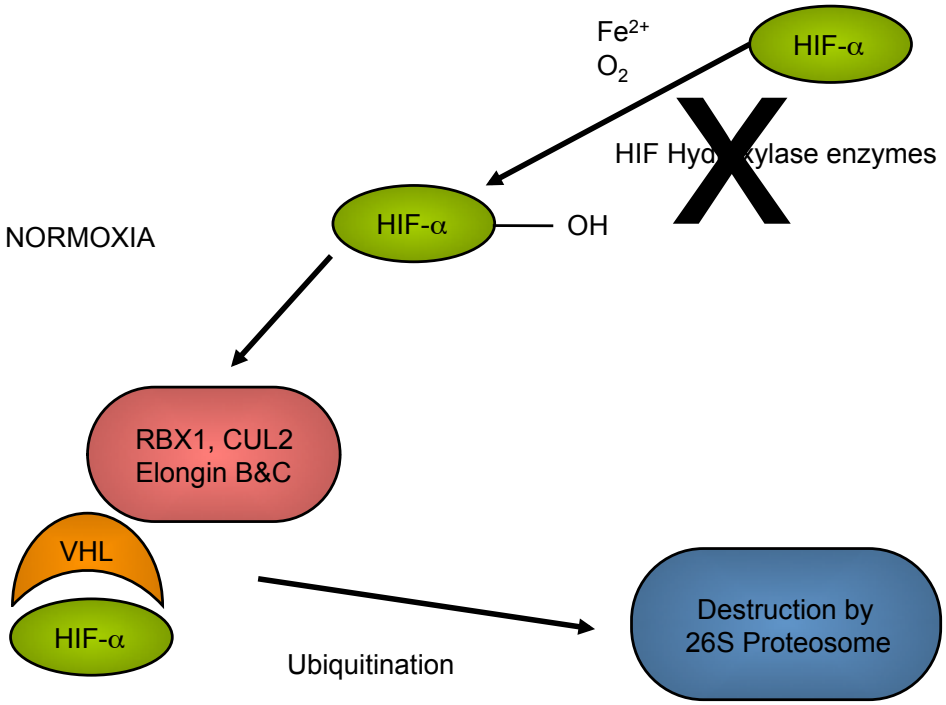
von Hippel Lindau Syndrome

Characterised by

Angiomatosis, haemangioblastomas, pheochromocytoma, renal cell carcinoma, pancreatic cysts and café au lait spots

What happens when the HIF axis is abnormal?

Loss of HIF hydroxylase enzymes.

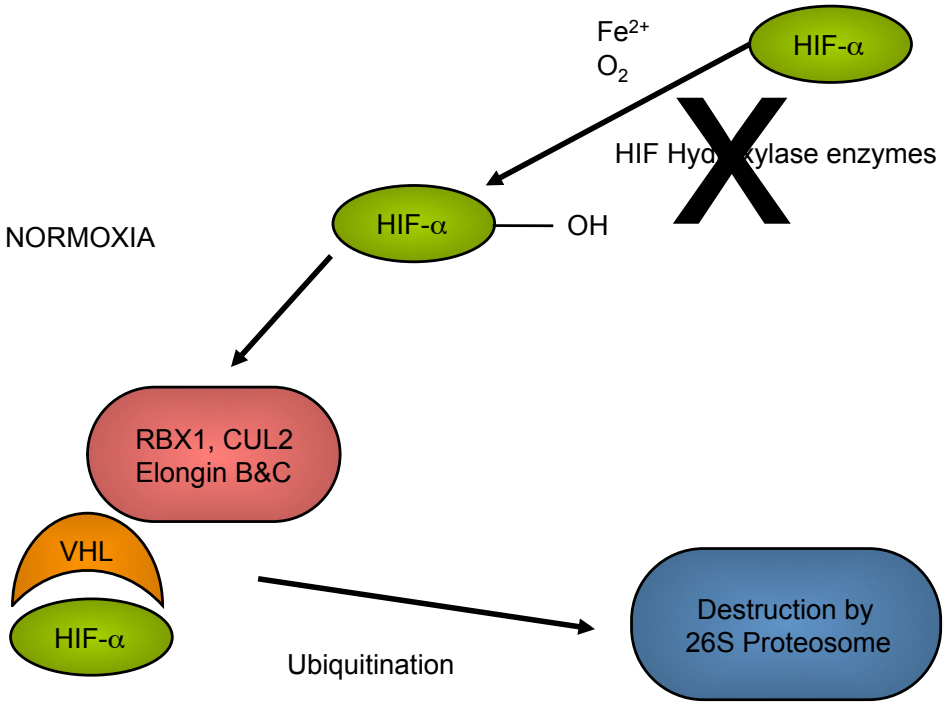


Loss of HIF Hydroxylase enzyme (Prolyl Hydroxylase (PHD) 2 leads to increased erythrocytosis

Chuvash Polycythaemia

What happens when the HIF axis is abnormal?

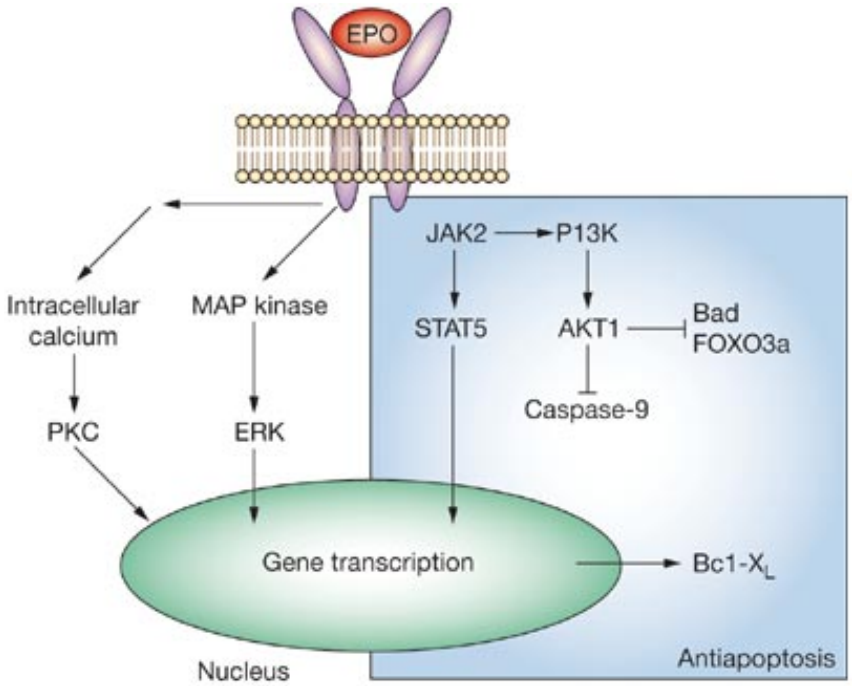
Abnormal HIF subunits.



Mutation in HIF2 alpha subunit increased erythrocytosis (Hb 22) with pulmonary hypertension

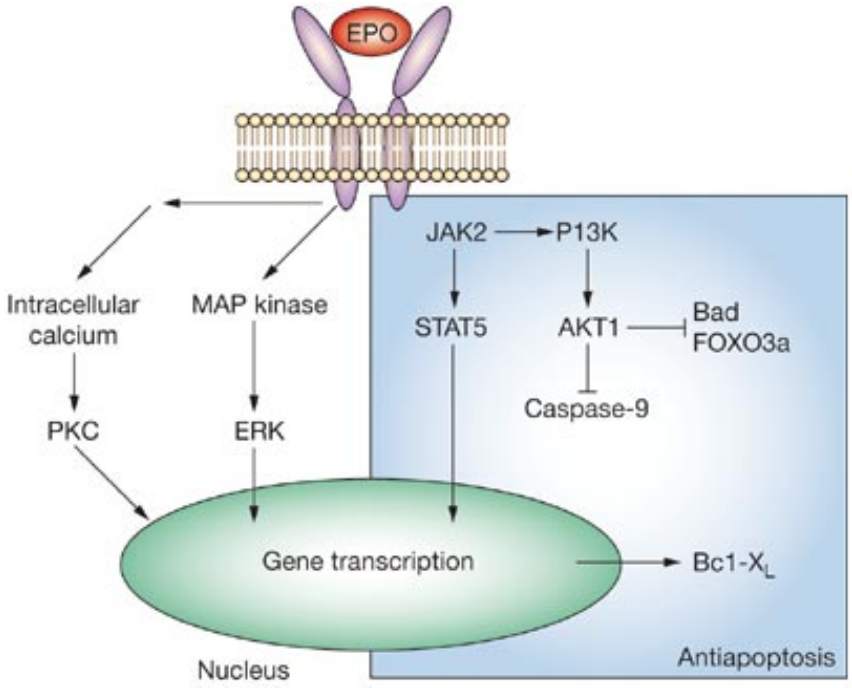
EPO effects

- Binds the erythropoietin receptor (EpoR) on the red cell surface and activates a JAK2 cascade.
- Primary role in red cell blood line to promotes red blood cell survival by protecting these cells from apoptosis.
- Other actions include vasoconstriction-dependent hypertension, stimulating angiogenesis, proliferation of smooth muscle fibers.



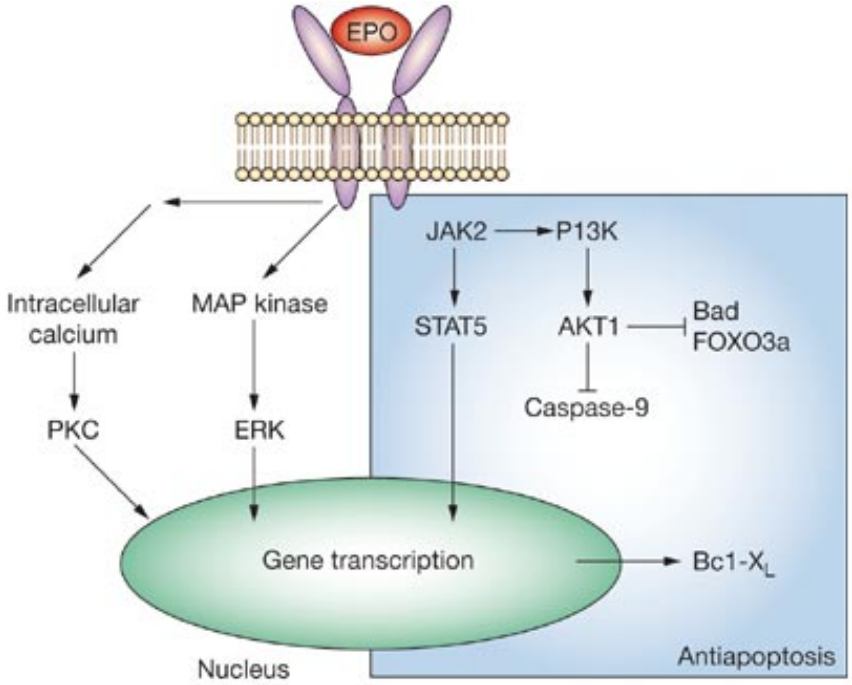
EPO effects

- This pathway can also be abnormal in disease.
- A rare genetic cause of polycythaemia is due to a mutated EPO-receptor.
- Uncontrolled haemoglobin production.



EPO effects

- This pathway can also be abnormal in disease.
- A rare genetic cause of polycythaemia is due to a mutated EPO-receptor.
- Uncontrolled haemoglobin production.
- But it did have benefit for one Finnish Cross Country Skier
- Eero Mäntyranta. He won seven medals in four Winter Olympics, he is one of the most successful Finnish skiers.



Therapeutic Uses

- Anaemia –
  - Associated with renal failure. Due to loss of renal function and loss of renal mass there is a loss of epo producing cells.
  - Associated with cancer and HIV. Low EPO levels are often detected.

- Pleotropic effects
  - Novel uses in cardiovascular disease
  - Potentially helpful in ischaemia reperfusion injury

Anaemia in renal disease

- As GFR falls below Hb levels fall.
- NHANES study –
- Low Hb (<12 g/dl in men, <11g/dl in women
  9% when GFR 30, 50% when GFR 15.

Anaemia in renal disease – why correct?

- 83% improvements in energy and physical function with improvement in cognition and mental acuity.
- Reduction in left ventricular mass (178 to 147g/m^2)
-

Anaemia in renal disease – why correct?

- Survival benefit

  In 4866 incident ESRF patients. In patients treated with EPO there was a survival benefit compared to EPO naïve patients (RR=0.8)

- Reduced the need for transfusions
  - Iron overload
  - Blood born viruses

Anaemia in renal disease – why correct?

- Slows progression of renal failure

In CKD patients with diabetes if Hb <13.8 progression of renal failure to dialysis was twice as fast compared to those with a normal Hb.

What drugs are available?

Erythropoietin – recombinant human EPO (rhEPO)

- First administered to 25 haemodialysis patients in Seattle and improved haemoglobin and resulted in transfusion independence
- Erythropoeitin alpha, beta and delta have been developed

What drugs are available?

Developed longer acting drugs that stimulate the EPO receptor and activate erythropoiesis –

eg Darbepoeitin (Aranesp)

Micera (Methoxy polyethylene glycol-epoetin beta)

And newer orally available EPO agents

Collectively these drugs are called ESA's or erythropoeitin stimulating agents

Correcting anaemia with ESA's

Should we normalizing haemoglobin?

UK current targets are between 10.5 and 12.5g/dl

Why are these targets below physiological ranges?

Correcting anaemia with ESA's

- Certainly no benefit in three randomised control studies called CHOIR, CREATE and TREAT
- Randomised patients to 'normal' Hb (~13) and 'target' Hb (~11)

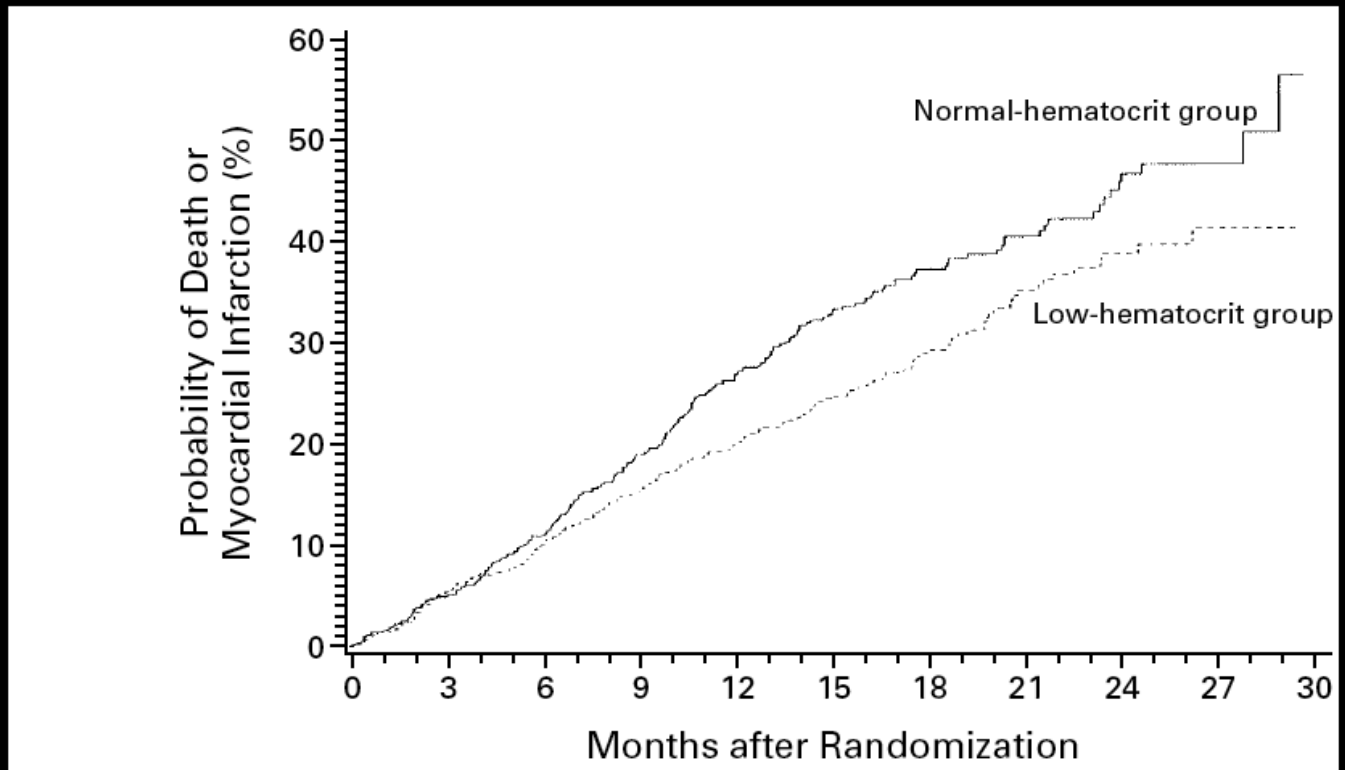
Normalizing Hct

- Besarab *et al* NEJM 1998;339:584
- 1223 patients with CHF or IHD
  - On dialysis
  - Group 1 - Hct of 42 Group 2 - Hct of 30
- Primary endpoints - death, non fatal MI
- Study halted at 29 mo, median duration 14 mo
- Supported by Amgen

Normalizing Hct

- Besarab *et al* NEJM 1998;339:584
- Group 1 (high): 183 deaths, 19 nonfatal MI
- Group 2: 150 deaths, 14 nonfatal MI

- Risk ratio Group 1 v Group 2 was 1.3 with confidence intervals of 0.9 - 1.9



No. at Risk										
Normal hematocrit	618	540	476	415	353	259	186	124	69	26
Low hematocrit	615	537	485	434	391	292	216	131	80	20

Correcting anaemia with ESA's

- CHOIR study- Found increased cardiovascular risk in 'normal' group

The CHOIR Study
Correction of Hemoglobin and Outcomes in Renal Insufficiency (funded by Ortho Biotech)

- Hypothesis – stable high Hgb level will decrease the risk of cardiovascular outcomes when compared to a lower Hgb level
- Open label, randomized trial
- 130 centers in the United States
- 1432 patients with CKD
  - 715 randomized to target Hgb of 13.5 g/dl
  - 717 randomized to target Hgb of 11.3 g/dl
- Eligibility
  - Age>18 years old
  - eGFR of 15 to 50 ml/min

NEJM 355: 2085-2098, 2006

Primary Outcomes

- 222 composite events occurred
- 125 events in the high Hgb group
- 97 events among the low Hgb group
- p=0.03
- Hazard ratio 1.34 with a 95% CI

NEJM 355: 2085-2098, 2006

Primary Outcomes

- Higher rates of composite events in the high Hgb group was explained by a combination of
  - Higher death rate
    - 48% higher in high Hgb group (p=0.07)
  - Higher rate of CHF hospitalization
    - 41% higher in high Hgb group (p=0.07)
- Improvement in QOL in both groups without statistical significance

NEJM 355: 2085-2098, 2006

Correcting anaemia with ESA's

- CREATE study– Found no benefit in outcomes when Hb treated to normal range.

The CREATE Study

Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (Funded by F Hoffman-LaRoche)

- 603 patients, 3 year follow up
- Patient characteristics
  - Mean GFR 25 ml/min (range 15 to 35) calculated by the Cockcroft-Gault and MDRD equations
  - Baseline Hgb had to be 11 to 12.5 g/dl
- Groups were targeted for Hgb 13.5 g/dl vs. Hgb 11.5 g/dl
- Echocardiography was performed at baseline and then annually or at initiation of hemodialysis

NEJM 355: 2071-2084, 2006

Control of Blood Pressure

- Control of blood pressure
  - Mean blood pressures did not differ between groups
  - Incidence of hypertension was higher in the high Hgb group (P=0.005)
  - Higher use of beta blockers in group 1 (high Hgb)
  - In all groups the number of antihypertensive drugs increased over the time of the study

NEJM 355: 2071-2084, 2006

Cardiovascular Events

- A total of 105 patients had cardiovascular events
- No significant difference (hazard ratio 0.78; 95% CI; P=0.20)
- Censoring data by start of dialytic therapy did not change the hazard ratio

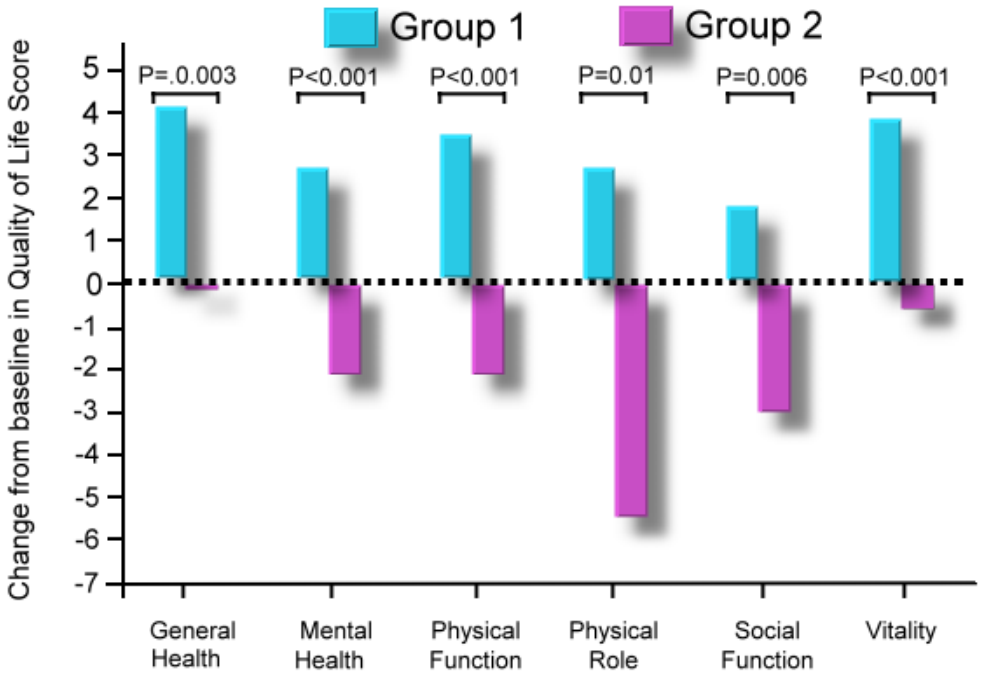
- Group 1 (High Hgb)
  - 58 events
  - 10% deaths
  - 4% deaths from cardiac cause
  - 7% cardiovascular intervention
  - 61% hospital admission
  - 33 days duration of hospital stay

- Group 2 (Low Hgb)
  - 47 events
  - 21 deaths (7%)
  - 3% deaths from cardiac cause
  - 6% cardiovascular intervention
  - 59% hospital admission
  - 28.3 days duration of hospital stay

NEJM 355: 2071-2084, 2006

Quality of Life

- Measured by SF-36
- Statistically significantly better in Group 1 in year 1
- Differences between groups may not be clinically significant
- By year two the difference was maintained for
  - general health (P=0.008) and
  - vitality (P=0.01)



NEJM 355: 2071-2084, 2006

Correcting anaemia with ESA's

- TREAT study– increased risk of Stroke

  – Use of Aranesp doubled stroke risk
    - Patients with Type 2 DM, CKD, moderate anemia
    - N = 4038
    - Strokes in 101 receiving aranesp and 53 receiving placebo

Why do high targets cause a problem?

- Thrombosis
- Hypertension
- ? Oncogenic potential
- There are now FDA and European guidelines targeting a lower haemoglobin range

Are there other problems?

- Red cell aplasia
  - Cross reactivity with EPO to generate antibodies to EPO-Receptor
  - EPO-receptor can not be stimulated
  - Patients become transfusion dependent

Are there other problems?

- Oncogenic potential
  - Danish Study showed more aggressive cancer disease in patients treated with an ESA

EPO – in cancer and HIV

Various clinical show a benefit of ESAs in raising hemoglobin levels and decreasing transfusion requirements in a substantial number of patients with cancer-related and chemotherapy-induced anaemia.

But increased risk of thromboembolic events, inferior survival, and worse cancer outcomes.

Worse outcome when anaemia is unrelated to chemotherapy and in those receiving myelosuppressive chemotherapy with the intent of cure.

Remains a controversial area.

Are there other problems?

- Cost
- £2000/pt/year
- But this is cheaper and cost effective compared to the alternative – transfusion and hospitalization

Approaching an anaemic renal patient.

1) Erythropoetin deficiency - Give ESA

2) EPO uses iron as a co-factor so need to ensure adequate stores

Correct iron stores – use iron supplements. Iv iron works best.

Correct ferritin to 100-800ng/ml. (normal range is 50-100)

Approaching an anaemic renal patient.

Why do we need to give so much iron?

Iron is not available for use in the renal patient.

It is kept in the enterocyte by the ‘uraemic’ environment.

Under control of further factor Hepcidin

Other uses of EPO/ESA

Ischaemia reperfusion injury

Cardiac

Stroke

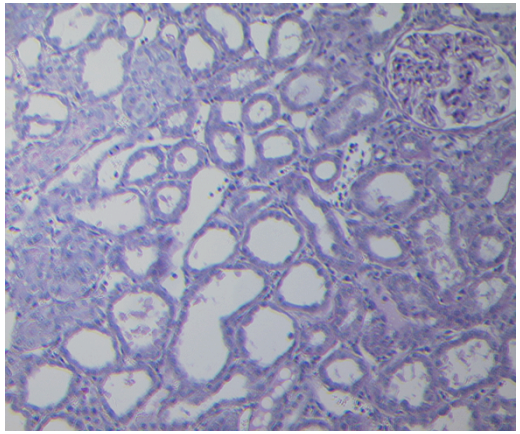
Renal Transplantation

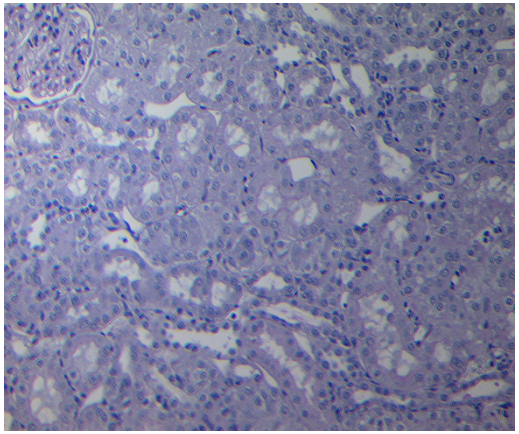
Other uses of EPO/ESA

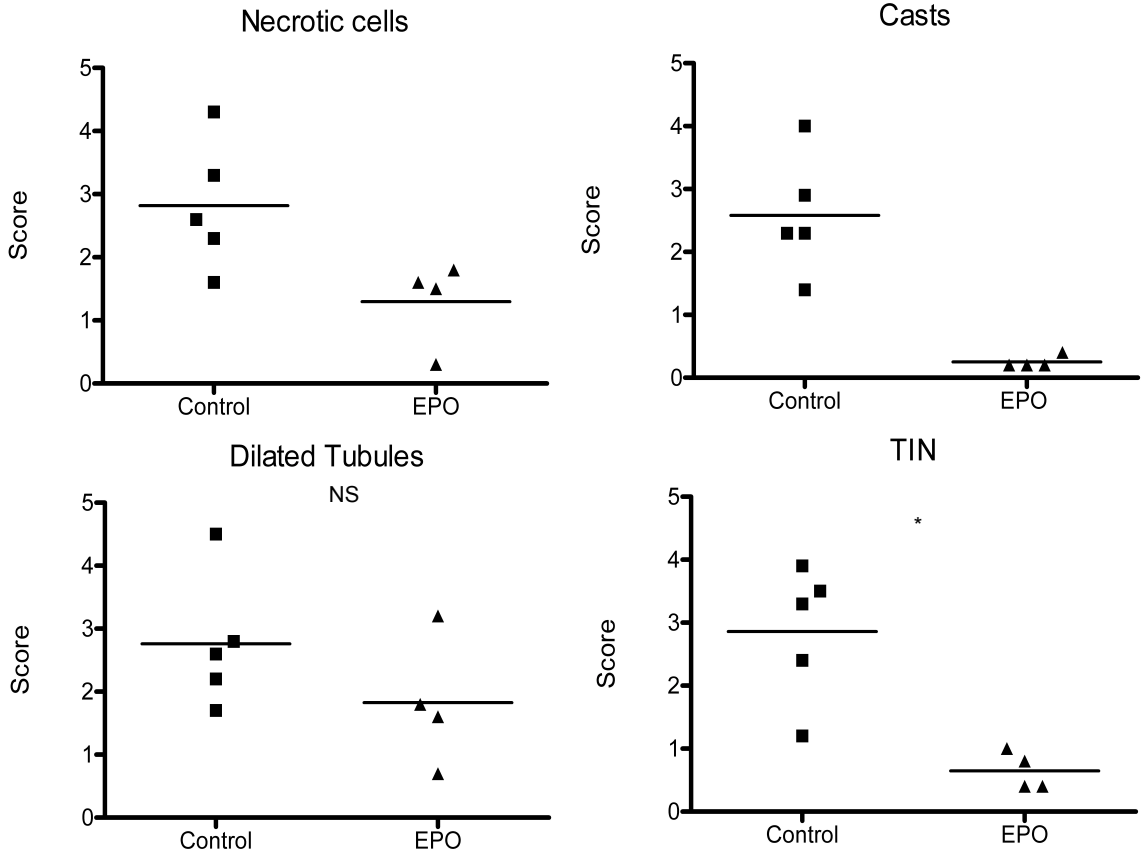
Pleotropic effects of EPO/ESA

- Cardiac protection –
- Stroke protection
- Renal Ischaemic injury and transplantation

EPO protects transplants from ischaemic injury





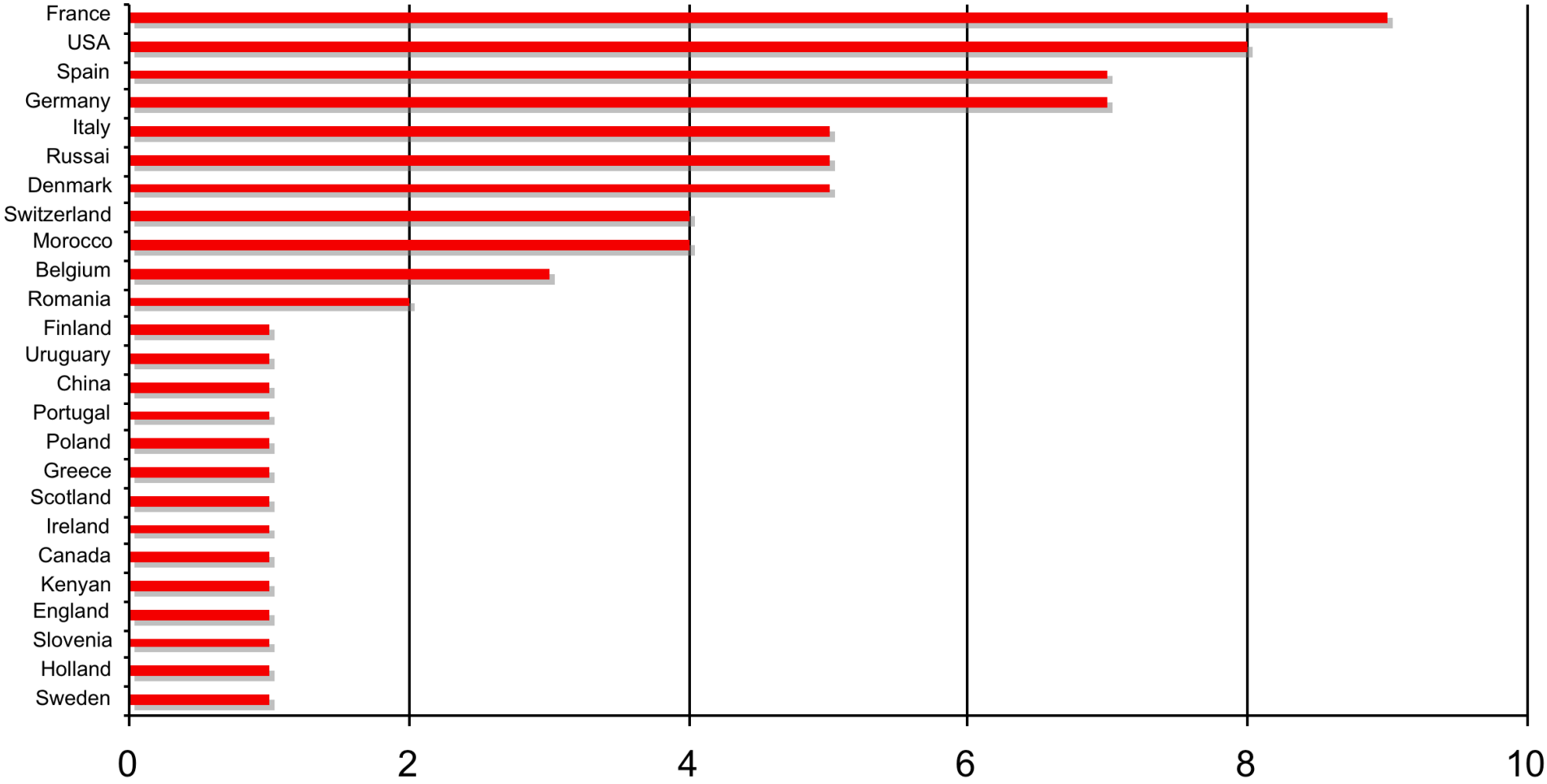


EPO in sport

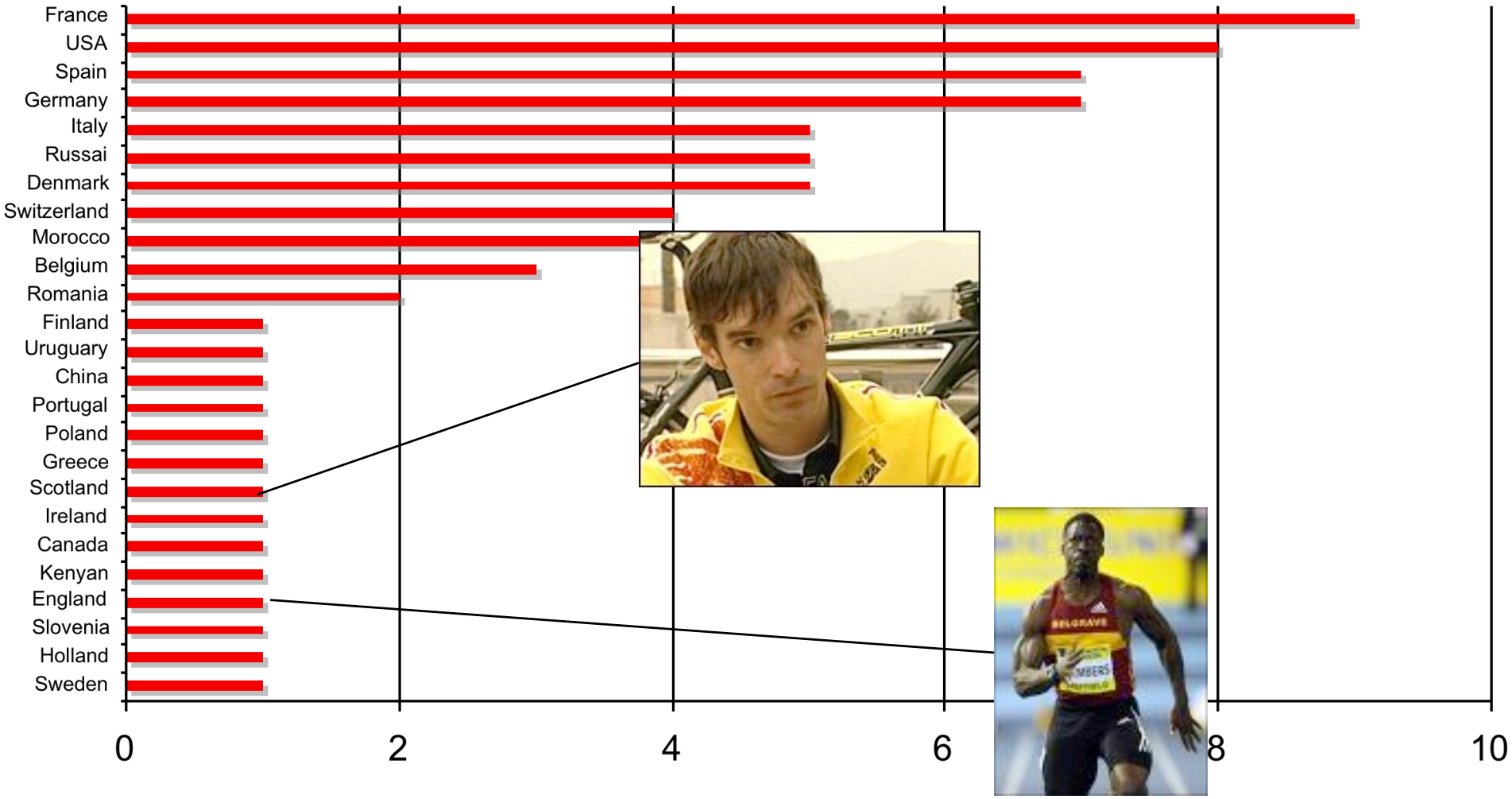
- Used in sports to improve endurance

- Detect differences in recombinant (post -translational modifications) and human endogenous EPO in urine

EPO Cheats (pre USPS team admissions)



EPO Cheats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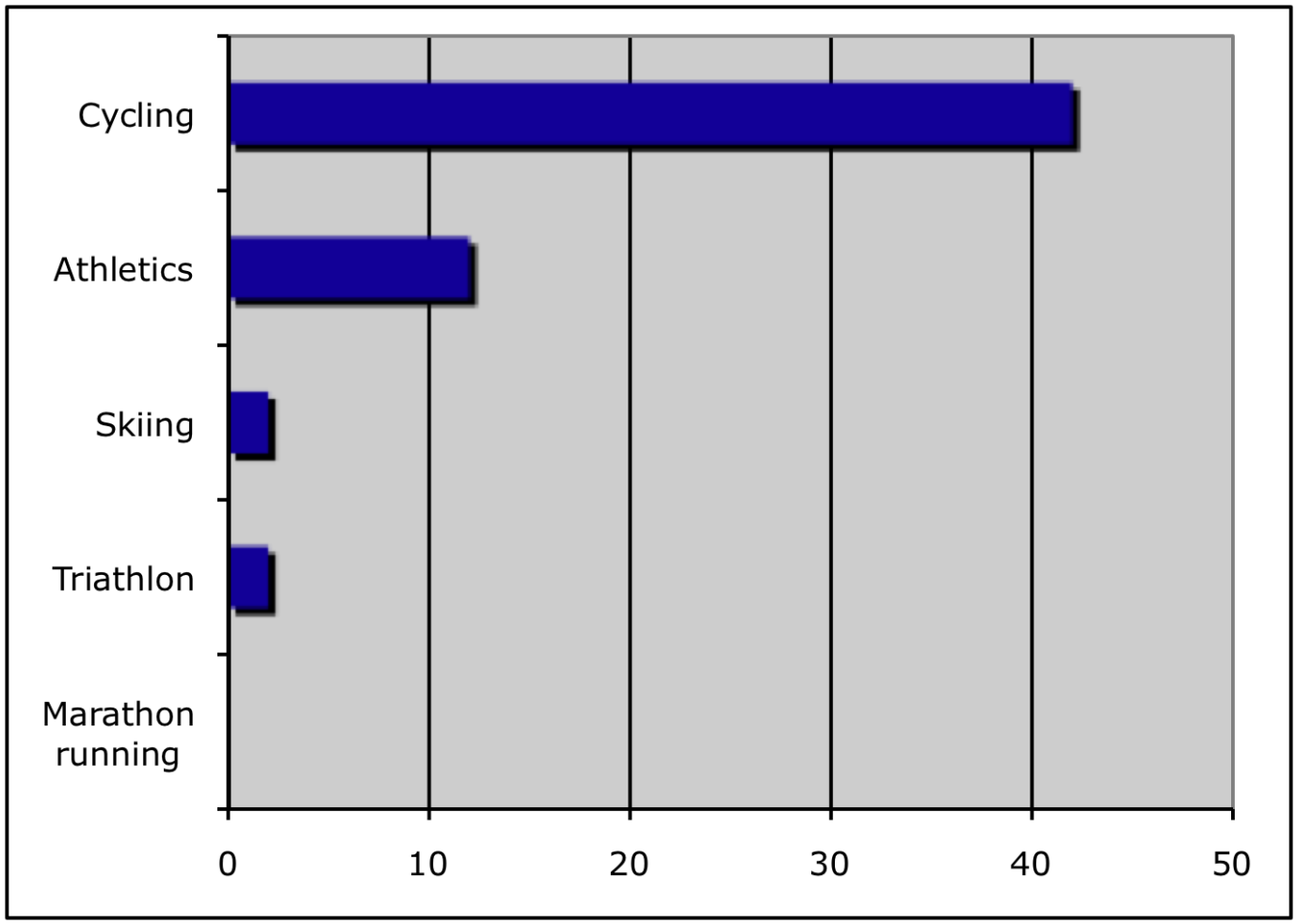
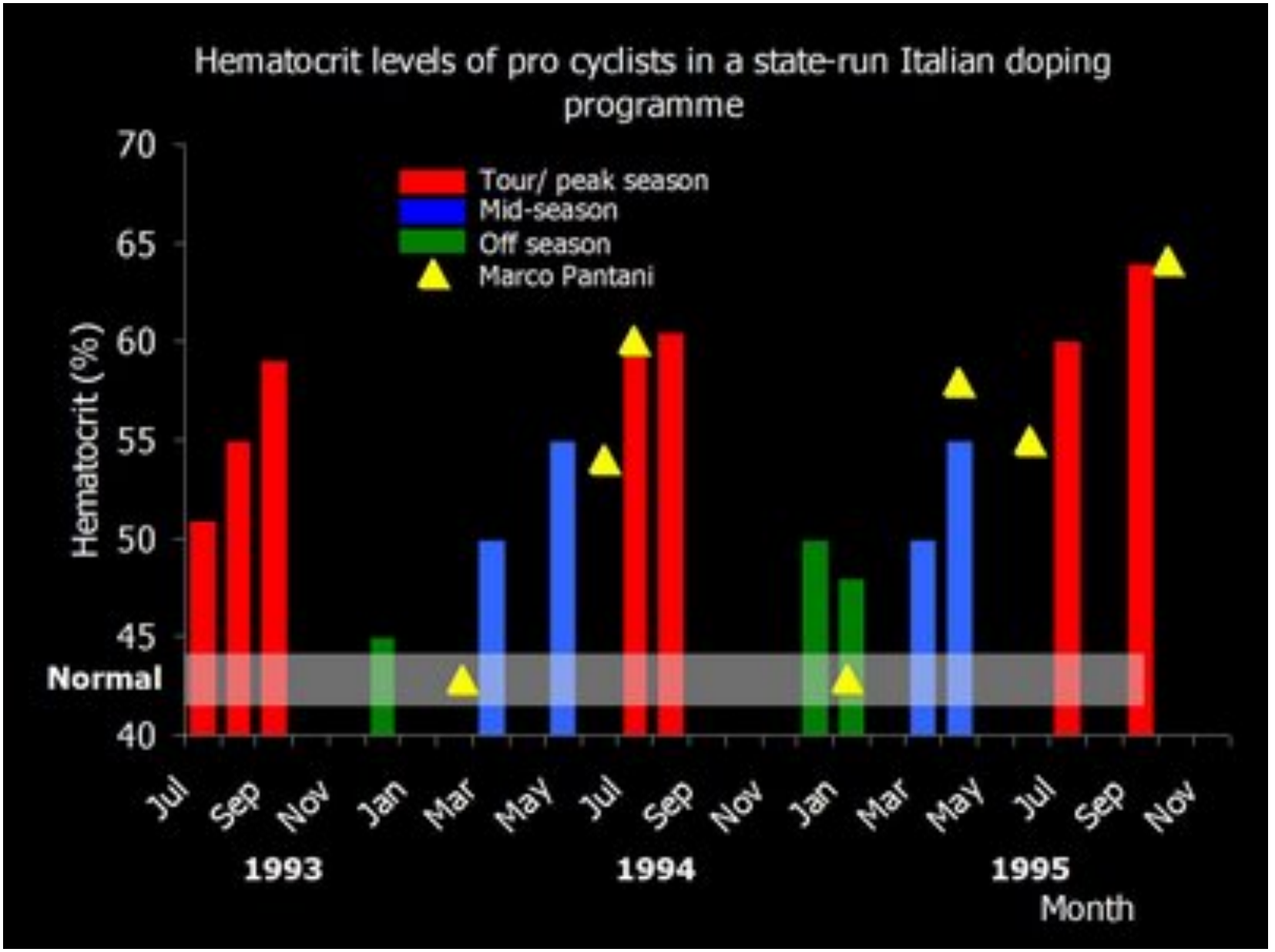
10

EPO Cheats

EPO Cheats by sport




Hematocrit levels of pro cyclists in a state-run Italian doping programme
Tour/ peak season
Mid-season
Off season
Marco Pantani
Hematocrit (%)
70
65
60
55
50
45
40
Normal
Jul
Sep
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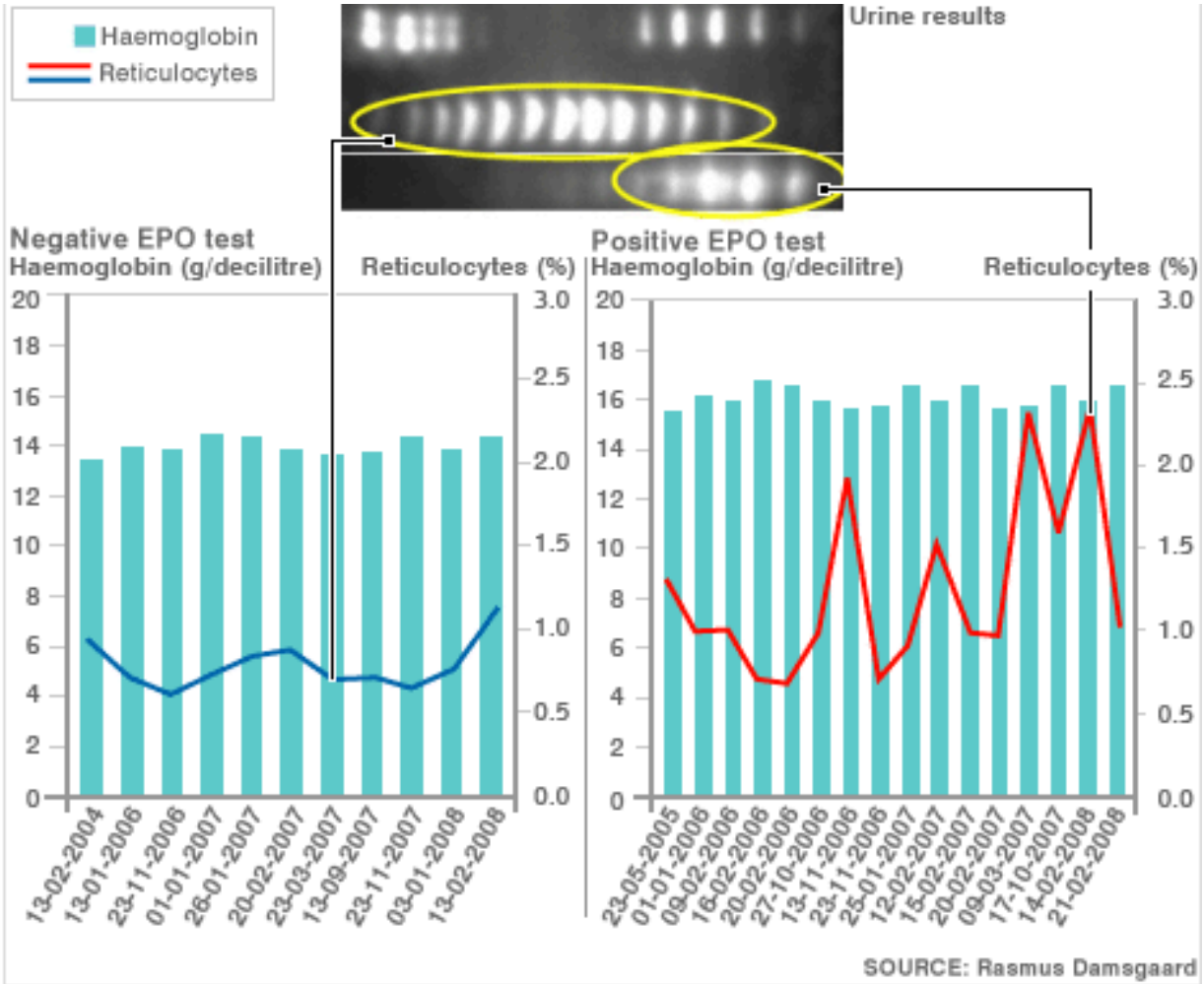
1995
Month



- The left hand graph shows the blood profile of an athlete who has not taken EPO. The urine test shows a grouping of white markers which represent a normal level of EPO in the blood.

- The right hand graph shows the blood profile of an athlete who has taken EPO. It shows a higher-than-normal proportion of reticulocytes, the young red blood cells produced by the bone marrow, due to stimulation by EPO.

- The positive urine test shows a lack of normal EPO - indicated by fewer white markers - suggesting the body has stopped producing its own EPO.

Conclusions

EPO is protein produced in the kidney hypoxic regulation that controls erythrocytosis

Under the regulation of HIF

Useful drug in the management of renal anaemia

High doses cause thromboembolism and increased risk of CV death

Has novel pleotrophic effects which are becoming increasingly important clinically

Further reading

Fliser et al, Mechanisms of disease: erythropoeitin – an old hormone with a new mission. Nature Clinical Practice Cardiovascula medicine 2006 10 563-572

Maxwell HIF-1: an oxygen response system with special relevance to the kidney JASN 2003 14 2712-2722.

www.renal.org/pages/pages/guidelines/current/complications.php - haemoglobin targets in the UK in renal disease.