Potential role of iron in CVD & diabetes

N.D. Vaziri, M.D., MACP
Division of Nephrology and Hypertension
University of California, Irvine

Disclosure of Interests

< Insert name of company/organization and category of financial relationship >

- Company A: Chemocentryx Inc. consultancy
- Company B: REATA Phrmaceutical Inc. research grant, consultancy,
- Company C: Keryx Pharmaceutical Inc. consultancy

The bright & dark sides of iron

As the major component of the oxygen transport molecules, the key component and cofactor of major enzymes and metalloproteins iron is an essential element for life and its deficiency has adverse consequences.

However, as a redox-active transition metal, by igniting and spreading oxidative stress high burden or improper packaging / handling of iron results in serious disorders.

For this reason elaborate biological mechanisms are in place to prevent iron overload and exposure to catalytically active iron.

High prevalence of iron overload in ESRD patients receiving standard treatment with ESA and IV iron preparations

A number of carefully conducted studies have documented a high prevalence of iron overload in hemodialysis patients receiving standard anemia treatment with ESAs and IV iron preparations:

I- Using a superconducting quantum interference device to measure non-heme hepatic iron content, Canavese et al found iron overload in 70% of their patients the majority of whom (70%) had serum ferritin values below 500 µg/L.

II- Ferrari et al showed a dramatic rise in liver iron content approaching those found in hemochromatosis in hemodialysis patients receiving regular IV iron therapy.

III-These observations were confirmed in carefully conducted studies by Rostoker et al

Taken together these findings illustrate that iron overload commonly occurs in ESRD patients treated with ESA and IV iron regimen in accordance with accepted guidelines.

Impact of IV versus oral delivery of iron

Iron absorbed from the gastrointestinal tract is safely transported by transferrin and delivered to the target tissues where it is safely stored as ferritin or incorporated in metalloproteins in a catalytically inactive state

Dialysis patients are commonly treated with 100-1000 mg of IV iron which far exceeds the iron carrying capacity of plasma (3-4 mg) and bypasses safeguards designed to protect against harmful actions of free or poorly liganded iron

This leads to a rise in plasma NTB iron, systemic oxidative stress, & flooding of the RE cells which are often overloaded due to the high hepcidin levels. Heavy influx of iron raises the intracellular labile pool of iron triggering oxidative stress, and inflammatory response in RE cells

Finally when the capacity of the RES is exceeded iron is diverted to parenchymal cells in vital organs causing insidious injury and dysfunction

Role of iron in the pathogenesis and complication of diabetes

Overt iron overload results in a high risk of type 2 diabetes which is marked by insulin deficiency & insulin resistance

Even modest elevation of body iron pool can promote insulin resistance, metabolic syndrome, and gestational diabetes

In contrast reduction of body iron pool with bloodletting or blood donation ameliorates insulin resistance and improves glycemic control in type 2 diabetics and reduces the risk of diabetes in normal subjects

Type 2 diabetes is the most common cause of CKD and preservation of the remaining insulin producing beta cells is essential for the well being of this vulnerable population

Mechanism of Iron-induced Type 2 diabetes

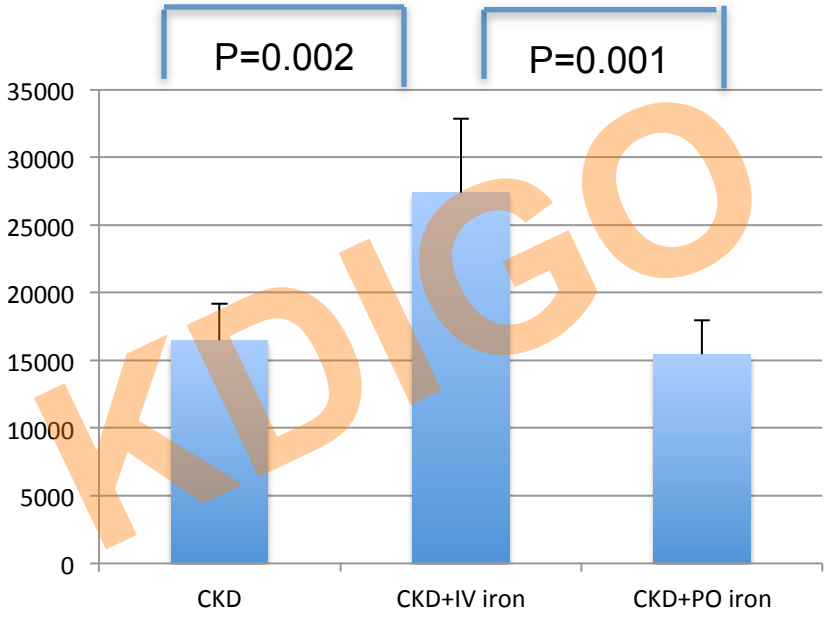
Due to their strict dependence on mitochondrial glucose metabolism and their limited antioxidant capacity, beta cells are exquisitely susceptible to oxidative stress.

Beta cells avidly import non-transferrin-bound iron via DMT1. By catalyzing the Fenton reaction iron can lead to intra-cellular oxidative stress and death of beta cells (Beta cells are among rare non-hepatic cell types that express hepcidin)

These biological features render the beta cells vulnerable to the effects of iron overload or presence of catalytically active iron

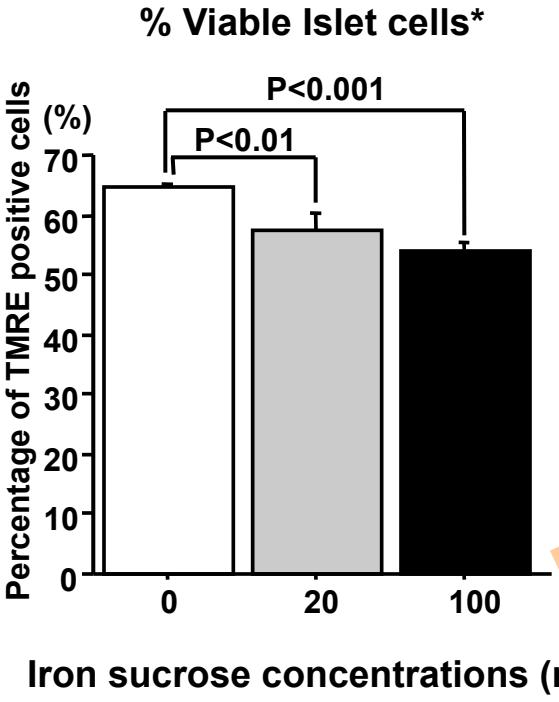
Effect of IV versus oral iron supplementation on glucose handling in animals with CKD

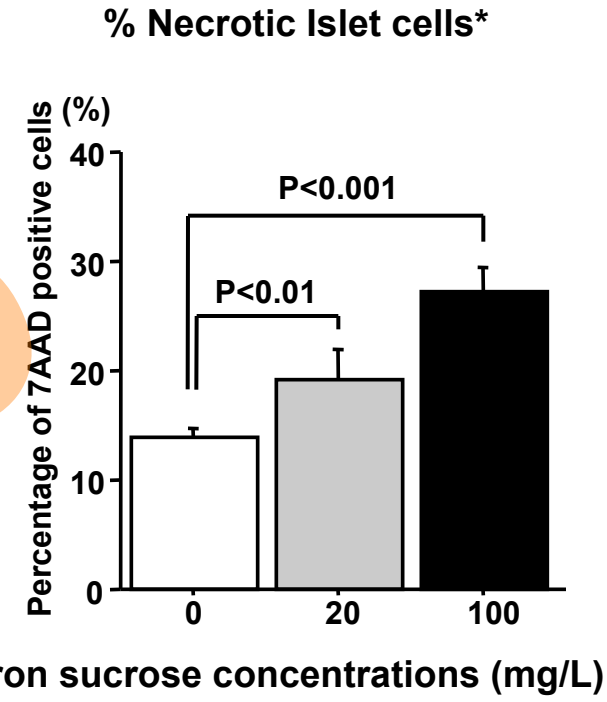
Intra-peritoneal glucose tolerance test (2g/kg bw)
Data represent the area Under Curve (AUC)



Rats with CKD (5/6 NX) were fed either a regular diet or iron fortified diet (400 ppm) for 4 weeks. A subgroup of CKD rats consuming regular diet was treated with two IV injections of iron sucrose (10 mg/kg) on weeks 2 and 3. IP GTT was performed at the end of week 4..

Effect of pharmacologically relevant concentrations of a commonly used IV iron product on isolated pancreatic islet cells


% Viable Islet cells*
Percentage of TMRE positive cells
(%)
P<0.001
P<0.01
70
60
50
40
30
20
10
0
0
20
100
Iron sucrose concentrations (mg/L)


% Necrotic Islet cells*
Percentage of 7AAD positive cells
(%)
P<0.001
P<0.01
40
30
20
10
0
0
20
100
Iron sucrose concentrations (mg/L)

β-cells as % of total islet cell count

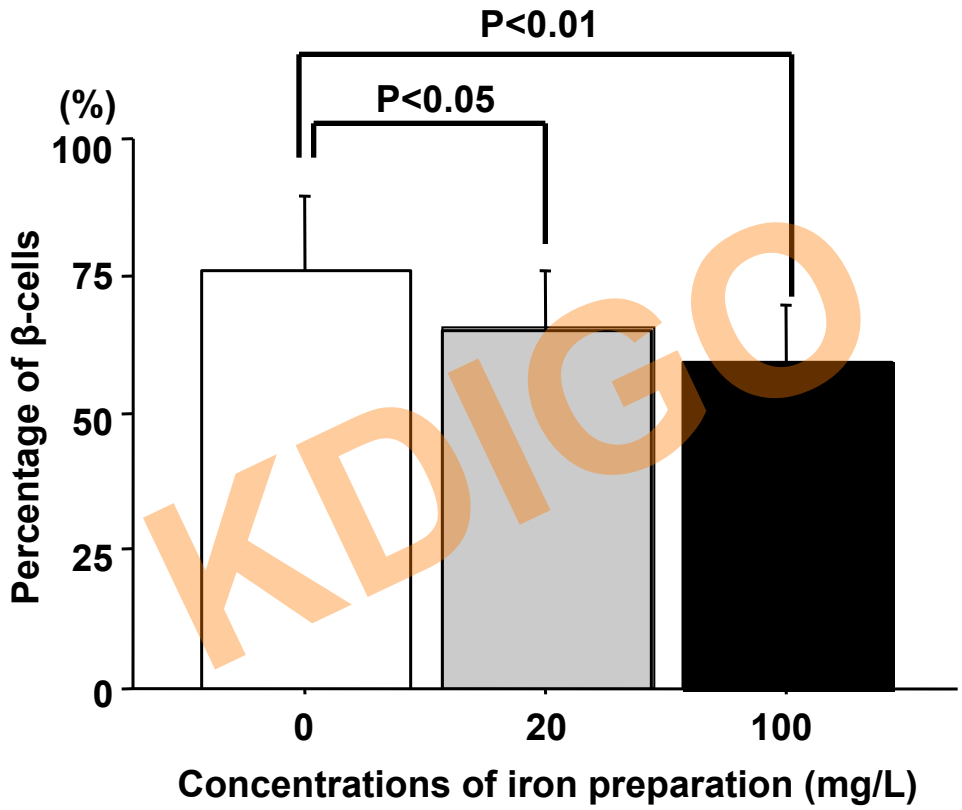
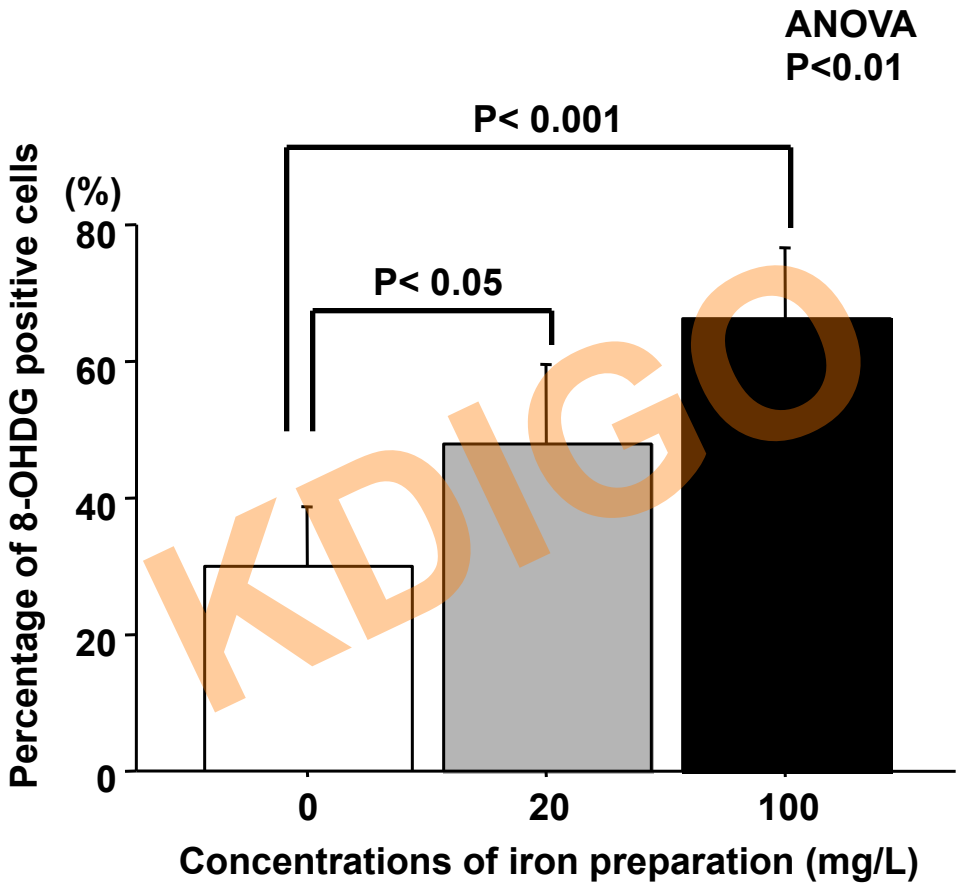


Fig. 3


ANOVA
P<0.01
P< 0.001
P< 0.05
(%)
80
60
40
20
0
Percentage of 8-OHDG positive cells
0
20
100
Concentrations of iron preparation (mg/L)

Iron overload amplifies complications of diabetes

- **Iron facilitates protein glycation which is a critical mediator of renal & vascular complications of diabetes. In fact iron chelation therapy lowers glycated hemoglobin levels in diabetic animals and humans**

- **Glycated proteins avidly bind iron forming complexes in which iron retains its catalytic activity . Thus iron facilitates formation of glycated proteins and glycated proteins sustain its catalytic activities, events that contribute to oxidative stress, inflammation & renal and cardiovascular complications**

- **Plasma non-transferrin-bound iron is commonly elevated in diabetic patients and contributes to the pathogenesis of CV complications**

- **Preliminary studies have revealed significant reduction in proteinuria with iron chelation therapy in patients with diabetic nephropathy**

- **Thus caution should be exercised to avoid iron overload in diabetic patients**

Role of iron in cardiovascular disease (human data)

- Carotid artery lesions in humans contain large amounts of iron which strongly correlates with the plaque's cholesterol and oxidized protein contents.
- In patients with carotid atherosclerosis serum ferritin level correlates with the level of low molecular weight iron compounds and lipid peroxidation products in the carotid endarterectomy specimens.
- Carotid artery thickness in ESRD patients is directly related to annual IV iron dosage and serum ferritin level
- Interaction of iron and lipoproteins in the plaque promotes plaque instability by inducing foam cell apoptosis
- The randomized trial of mild iron reduction therapy (phlebotomy Q 6 months) in elderly patients with peripheral vascular disease (the "FeAST" trial) showed if initiated early Fe reduction can saely reduce CV and overall M & M

Role of iron in cardiovascular disease (animal & in vitro data)

- **Iron accumulates in and contributes to atherosclerosis plaques formation in ApoE deficient. Iron supplementation accelerates whereas iron chelation therapy retards plaque formation in these animals**

- **Administration of iron chelator, deferoxamine, significantly inhibits intimal thickening and VSMC proliferation in the carotid balloon injury model in hypercholesterolemic rabbits pointing to the role of iron in arterial remodeling**

- **In vitro addition of iron compounds results in upregulation of adhesion molecules and monocyte adhesion in cultured human endothelial cells, these events can be reversed or prevented by iron chelator**

HAEC were grown in 6-well plates in EBM-2 growth media for 2 days to attain about 80% confluency. Cells were treated with various concentrations of iron sucrose (10–100 mg/L) for 4 h. Morphological changes in HAEC were examined by phase contrast microscopy using Nikon Eclipse 300 inverted microscope (! 20 magnification).

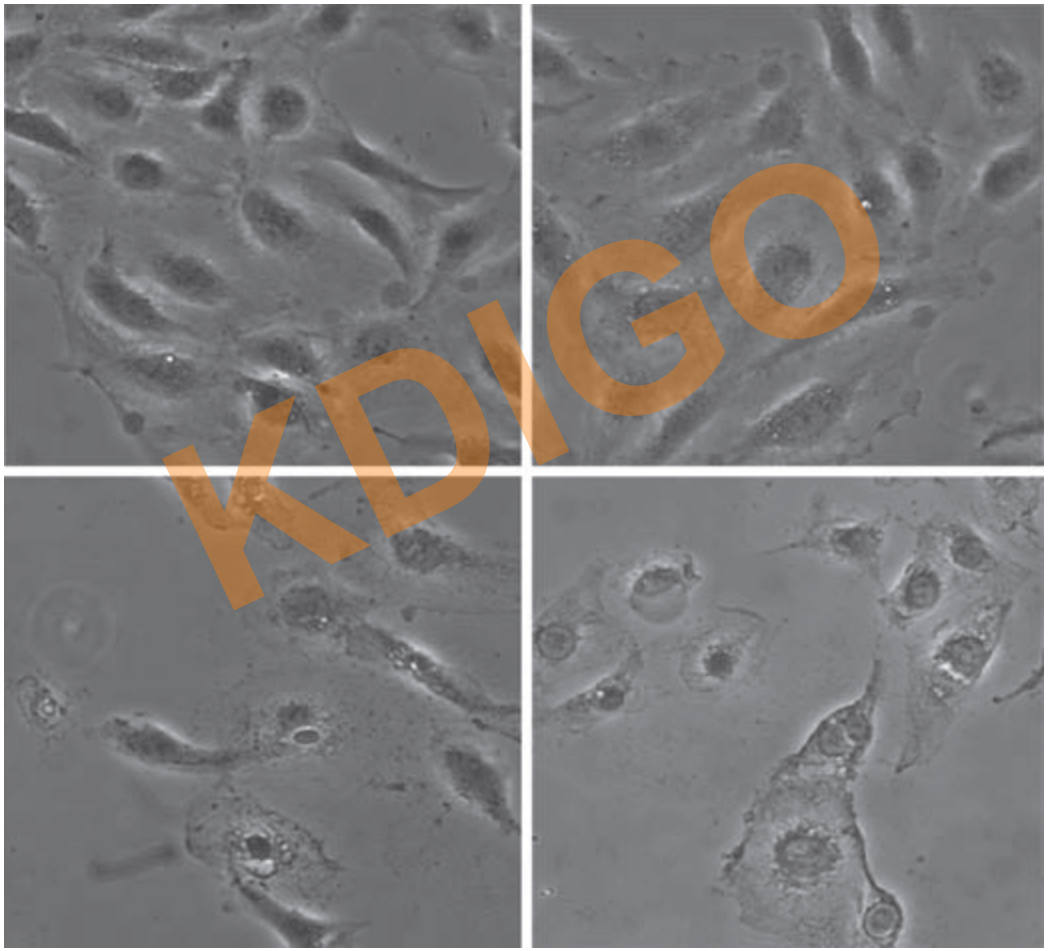
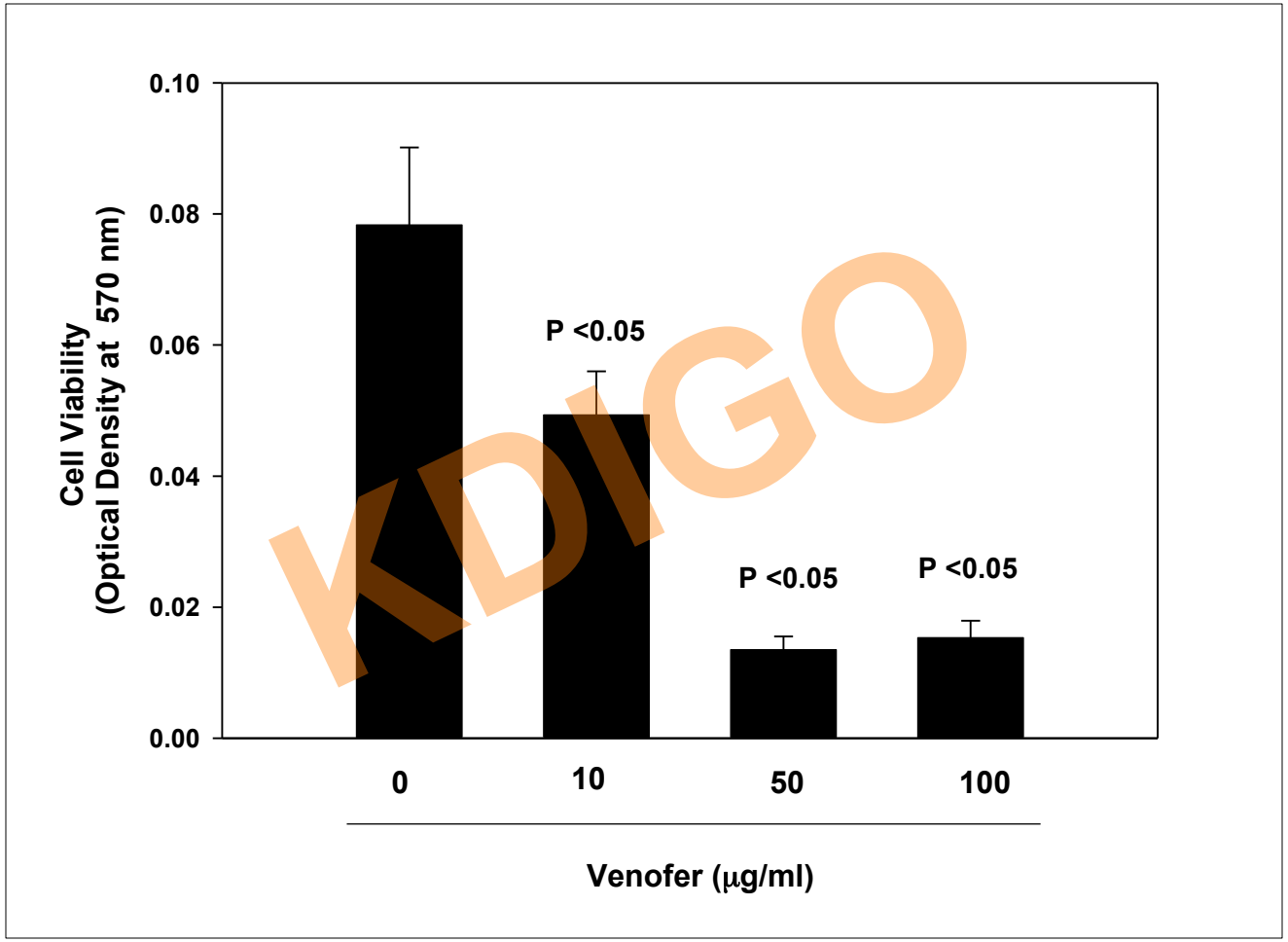
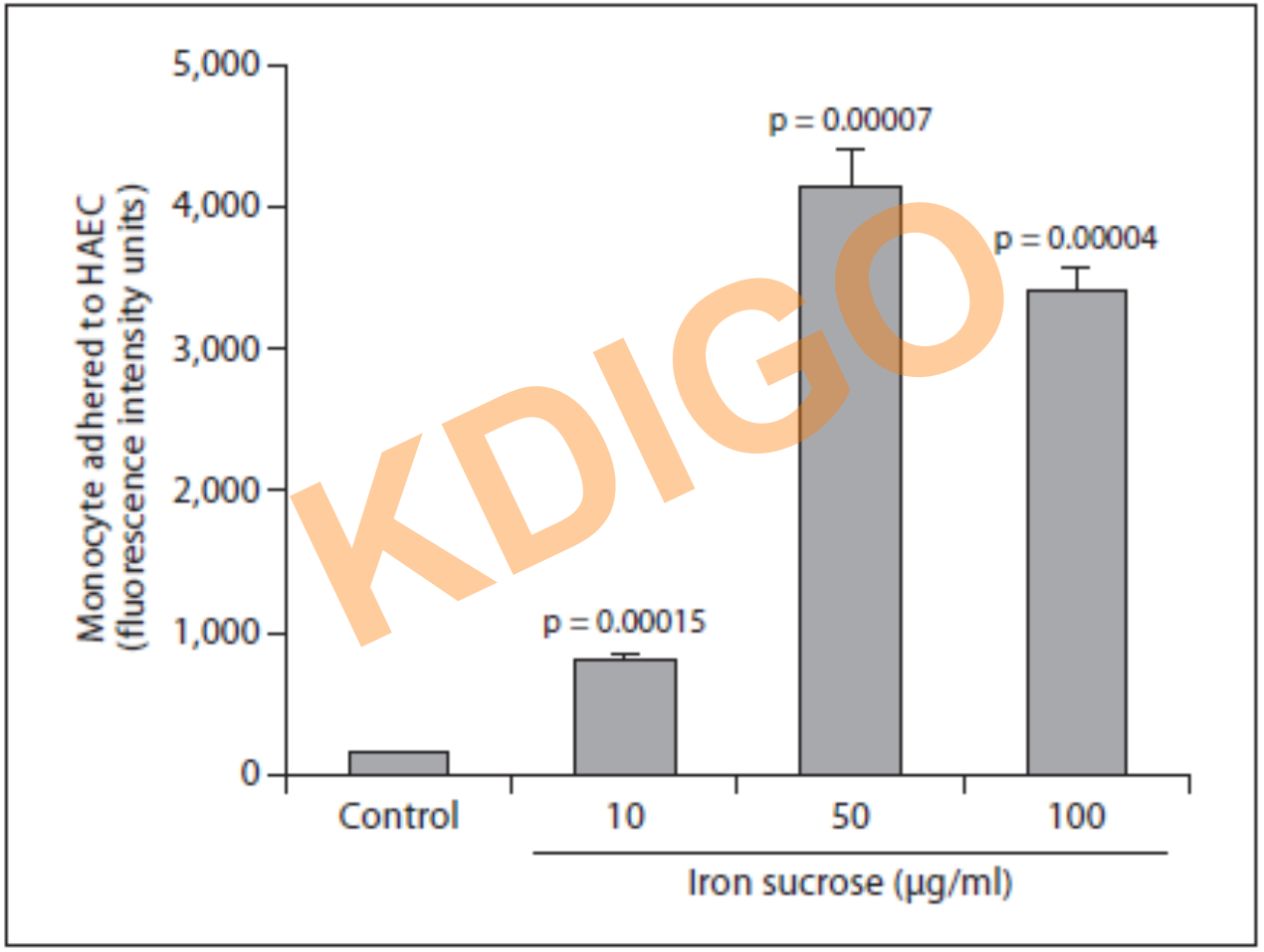
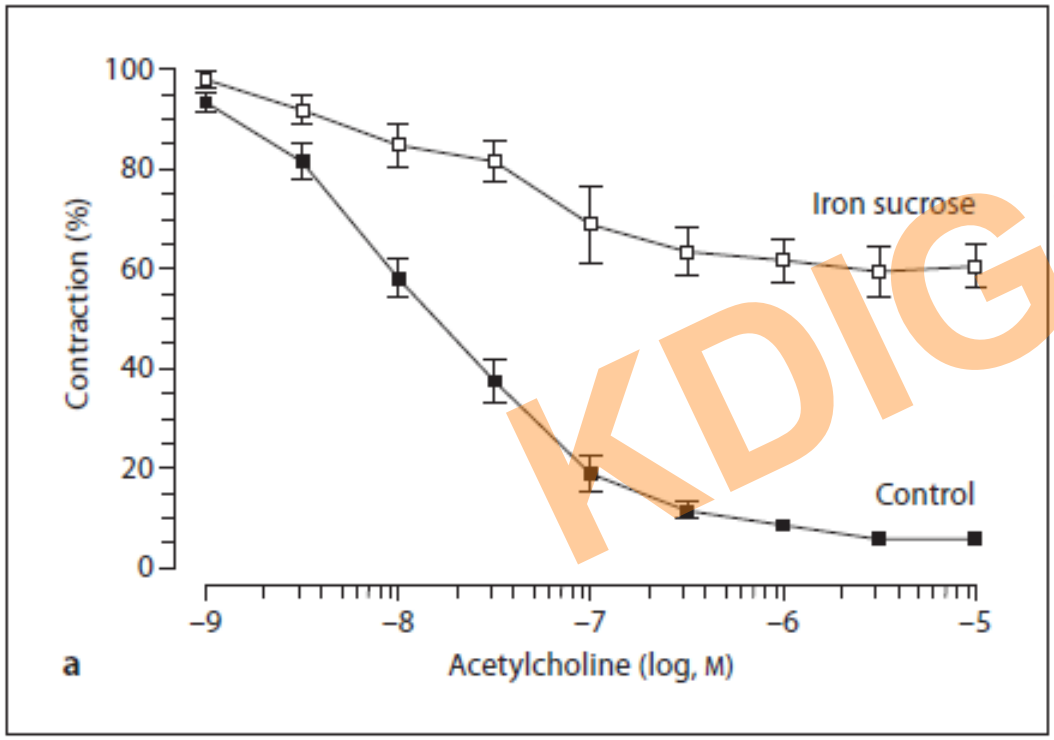


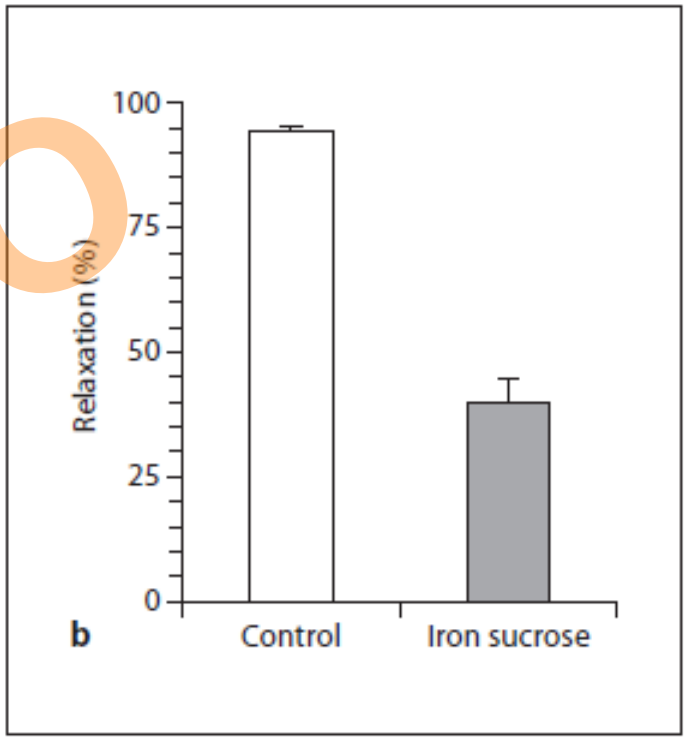
Figure 1



Effect of iron sucrose on monocyte adhesion to endothelial cells. HAEC were treated with iron sucrose (10-100 mg/ml) for 4 h. Fluorescently labeled monocytic THP-1 cells were added to the HAEC. After 1 h, monocyte adhesion to HAEC were assessed by measuring fluoresce intensity







a- Relaxation response curve to acetylcholine (ACh) in phenylephrine-precontracte aortic rings incubated for 4 hrs in media without and with iron sucrose (200 mg/l) for 4 hrs

b-Maximal relaxation response to ACh in in aortic rings pre-incubation in media with and without iron sucrose (200 mg/l)

Potential role of catalytically active iron in micro-capillary occlusion and dialysis amyloidosis

Abnormal interactions in plasma and tissue proteins are prevented by having the hydrophobic groups facing the interior of their tridimensional structures which is sustained by disulfide bonds.

Disruption of disulfide bonds results in unfolding of the polypeptide chains and exposure of the hydrophobic domains. This allows formation of intermolecular bonds that leads to generation of large pro-inflammatory aggregates which are resistant to proteolytic enzymes (exemplified by human prion proteins)

Generation of .OH by catalytically active iron in blood converts circulating fibrinogen to a plasmin-resistant insoluble fibrin-like complex (parafibrin). This process can lead to micro-vascular occlusion and capillary rarefation in the heart, brain, and other organs causing cardiomyopathy, neurodegenerative disorders among others.

Likewise unfolding of proteins at the tissue level can contribute to formation of amyloid like deposits as commonly seen in dialysis patients

Role of iron overload in vascular calcification

- By promoting oxidative stress which is known to trigger transformation of VSMC from contractile to osteogenic phenotype, iron overload can potentially facilitate vascular calcification

- In addition iron plays a critical role in the development of calcific arteriolopathy (calciphylaxis) which is a catastrophic complications in ESRD population

Conclusions

There is irrefutable evidence supporting the role of iron overload in the pathogenesis of diabetes and CVD in patients with hereditary disorders of iron metabolism and in patients with transfusion related iron overload in the absence of advanced kidney disease.

There is no reason to assume that presence of CKD/ESRD mitigates the effects of iron overload on pancreatic endocrine system or cardiovascular system. On the contrary CKD and underlying diabetes tend to amplify the vulnerability to the damaging effects of iron overload.

Given the well-documented occurrence of iron overload with the currently accepted anemia treatment guidelines and the high risk of iron overload in ESRD population, reconsideration of the current guidelines is urgently needed

Thank you