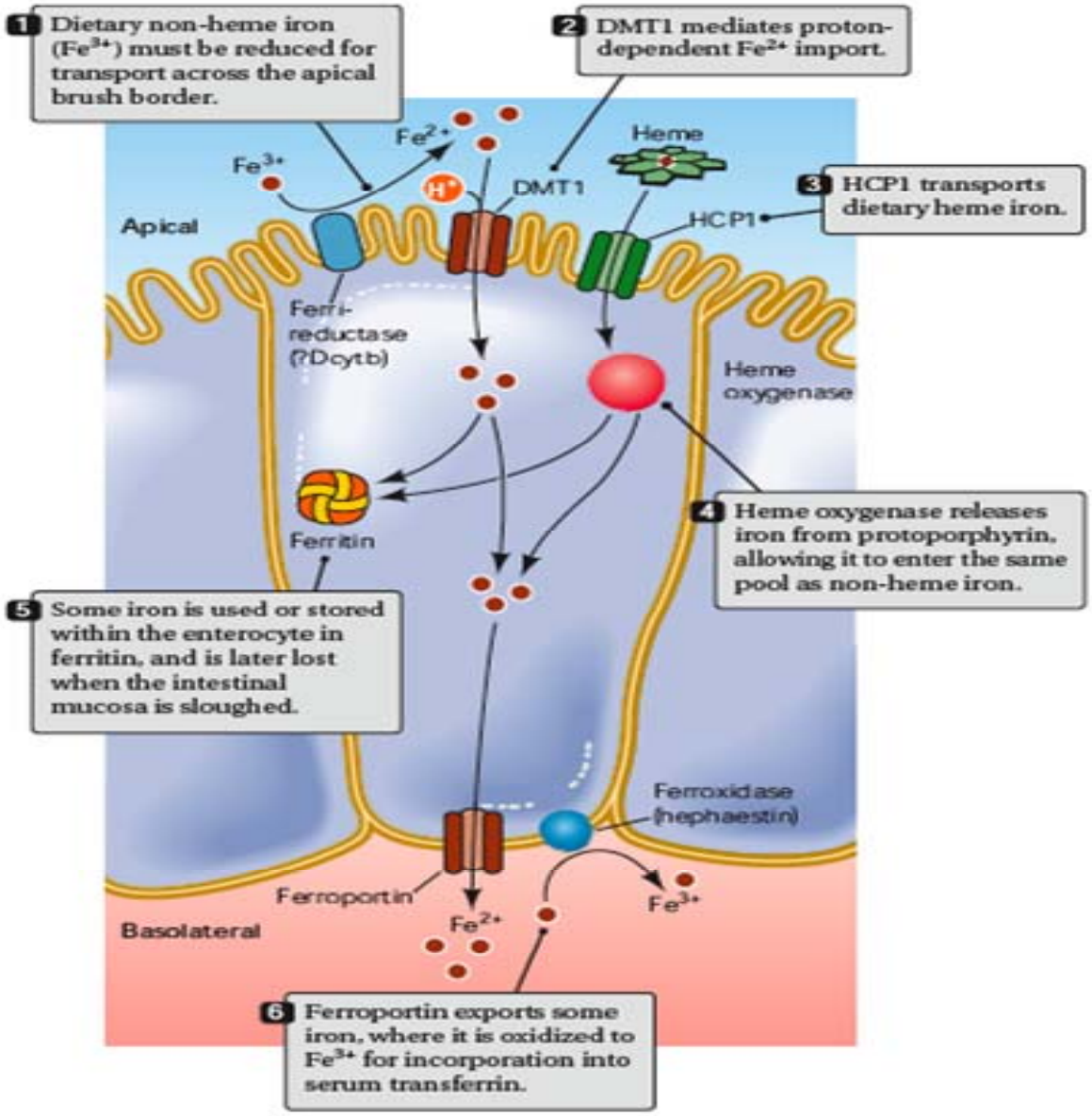
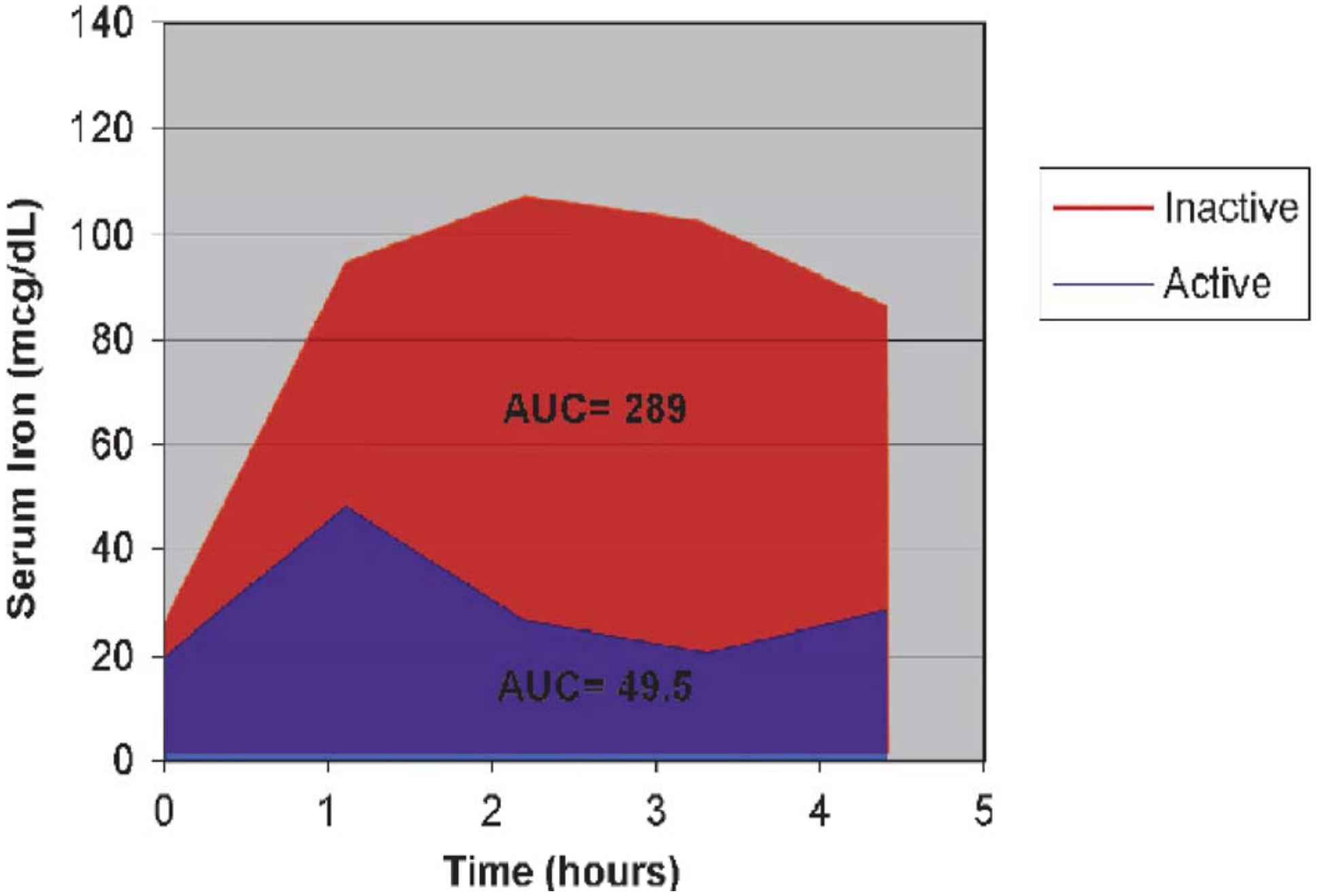
The anti-inflammatory effect of β-carotene reverse iron dependent inta-cellular abnormalities in inflamed Caco-2 cell-line

Oksana Katz, M.Sc.1 *, Ram Reifen M.D.1* Aaron Lerner M.D., M.H.A2

1-Faculty of Agriculture, Food, and Environment, Institute of Biochemistry, Food Science and Nutrition, School of Nutritional Sciences, The Hebrew University of Jerusalem, Rehovot

2- Pediatric Gastroenterology Unit, Carmel Medical Center,
B. Rappaprot School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.


1 Dietary non-heme iron (Fe^{3+}) must be reduced for transport across the apical brush border.
2 DMT1 mediates proton-dependent Fe^{2+} import.
3 HCP1 transports dietary heme iron.
4 Heme oxygenase releases iron from protoporphyrin, allowing it to enter the same pool as non-heme iron.
5 Some iron is used or stored within the enterocyte in ferritin, and is later lost when the intestinal mucosa is sloughed.
6 Ferroportin exports some iron, where it is oxidized to Fe^{3+} for incorporation into serum transferrin.
Fe^{3+}
Fe^{2+}
H^+
DMT1
Heme
HCP1
Apical
Ferri-reductase (?Dcytb)
Heme oxygenase
Ferritin
Ferroxidase (hephaestin)
Ferroportin
Fe^{2+}
Fe^{3+}
Basolateral


140
120
100
80
60
40
20
0
Serum Iron (mcg/dL)
AUC= 289
AUC= 49.5
0
1
2
3
4
5
Time (hours)
Inactive
Active

Introduction

Anemia of chronic disease, also called anemia of inflammation can lead to the development of anemia due to iron sequestration in enterocytes resulting in a decrease in body iron availability.

The hypoferremia associated with chronic inflammation is mediated by the acute-phase protein hepcidin, largely induced by pro-inflammatory cytokines. Pro-inflammatory cytokines and chemokines are produced in the intestine and play an important role in the pathogenesis of intestinal injury in critical illness.

The mechanisms involved in controlling gut-based inflammatory response and their effect of local iron metabolism despite being critical, were insufficiently studied.

The aims

1. To explore the influence of iron on intra-cellular iron-dependent proteins (ferritin H+L, FPT, TfR) either in presence or absence of inflammatory environment.

2. To examine whether this can be reveres by carotenoids, by turning-off the pro-inflammatory cascade.

Material and methods

1.**Caco2** Cells were placed in serum-free media and treated with **FeSO4**x7H2O in different concentration (0µmol/L, 10µmol/L 100µmol/L, 200µmol/L, 250µmol/L and 350mol/L) at 4, 12 and 24 hour. **Dose and time response**

2. After treatment, medium was collected for **IL8** measurement, cells were harvested.

3.Inflammatory condition was induced by **IL1β** (10ng/ml) or **IL1β** (10 ng/ml) together with **FeSO4**x7H2O (250µmol/L).

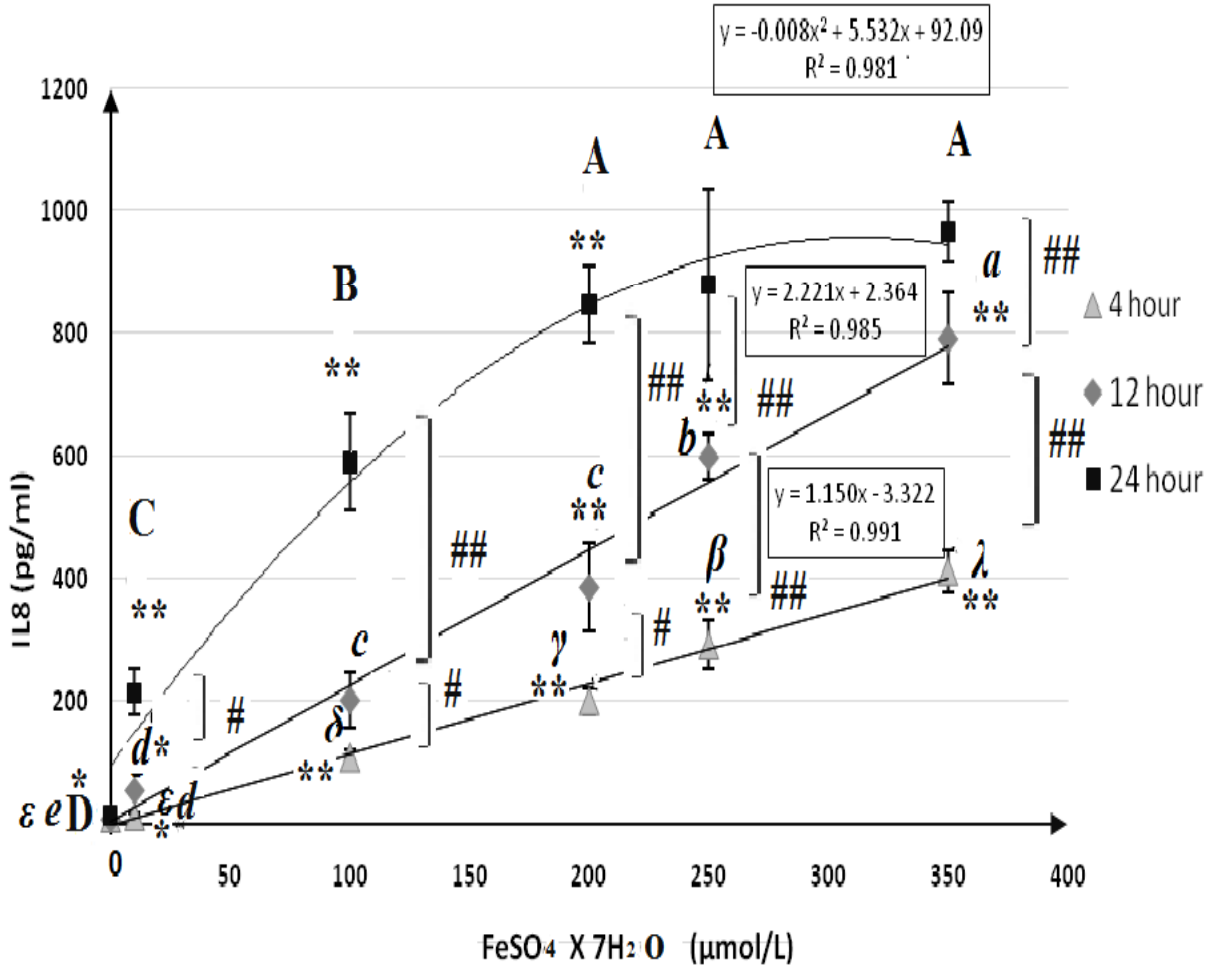
4. In a separate experiment cells were treated with **IL1β** (10ng/ml) or **IL1β** (10ng/ml) together with **FeSO4**x7H2O (250µmol/L) or with FeSO4x7H2O (250µmol/L) alone and **β-carotene** at final medium concentration of 3.4µmol/L or **vitamin A** at a final concentration in the medium of 3.3µmol/L, added subsequently.

5 Cell's culture was incubated additional 4 hours, medium was collected for **IL8** measurement, and cells were harvested for **Western blotting.**

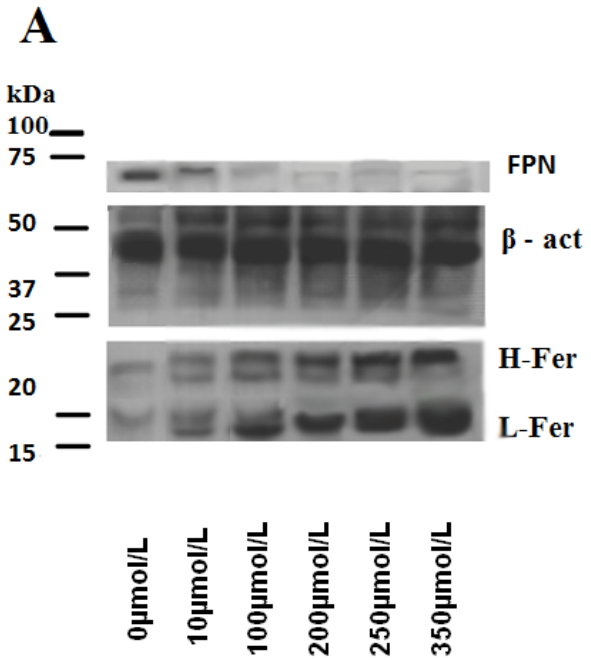
6. **Intracelular Fe** was measured by inductively coupled plasma mass spectometry

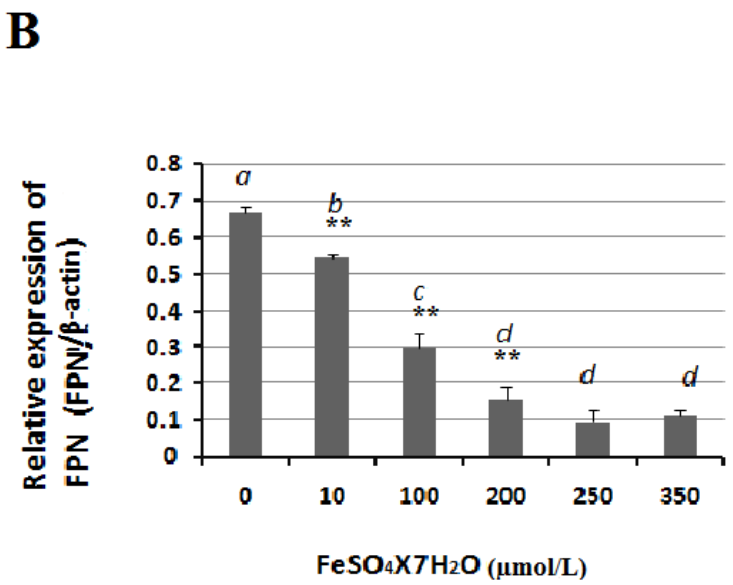
RESULTS

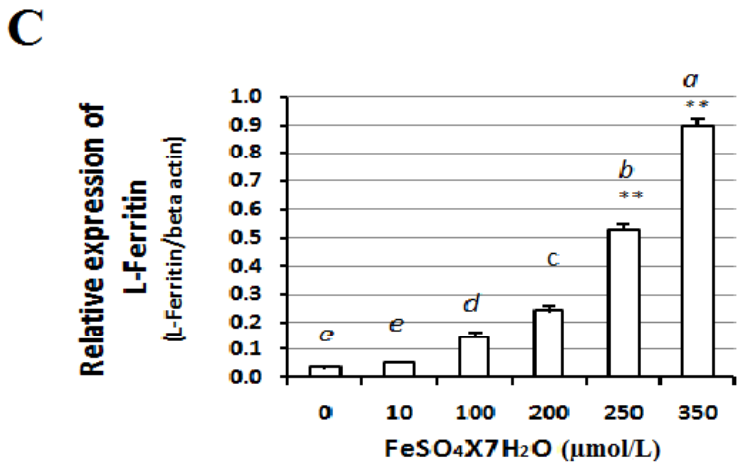
IL8 induction by iron

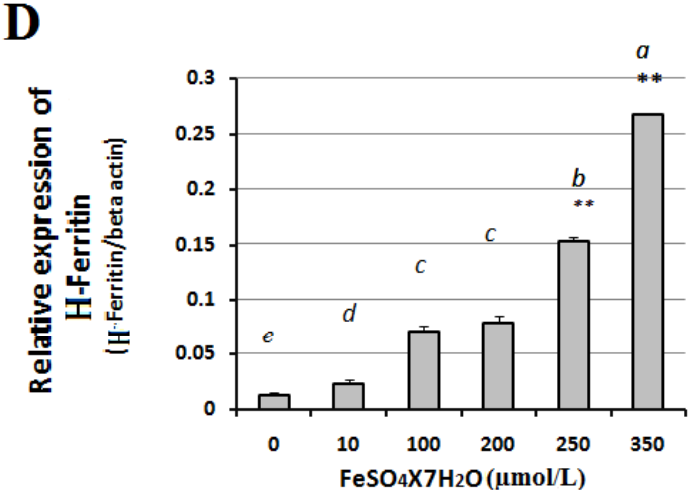


Crosstalk between iron, iron-related proteins and IL8 in Caco-2 cell's culture

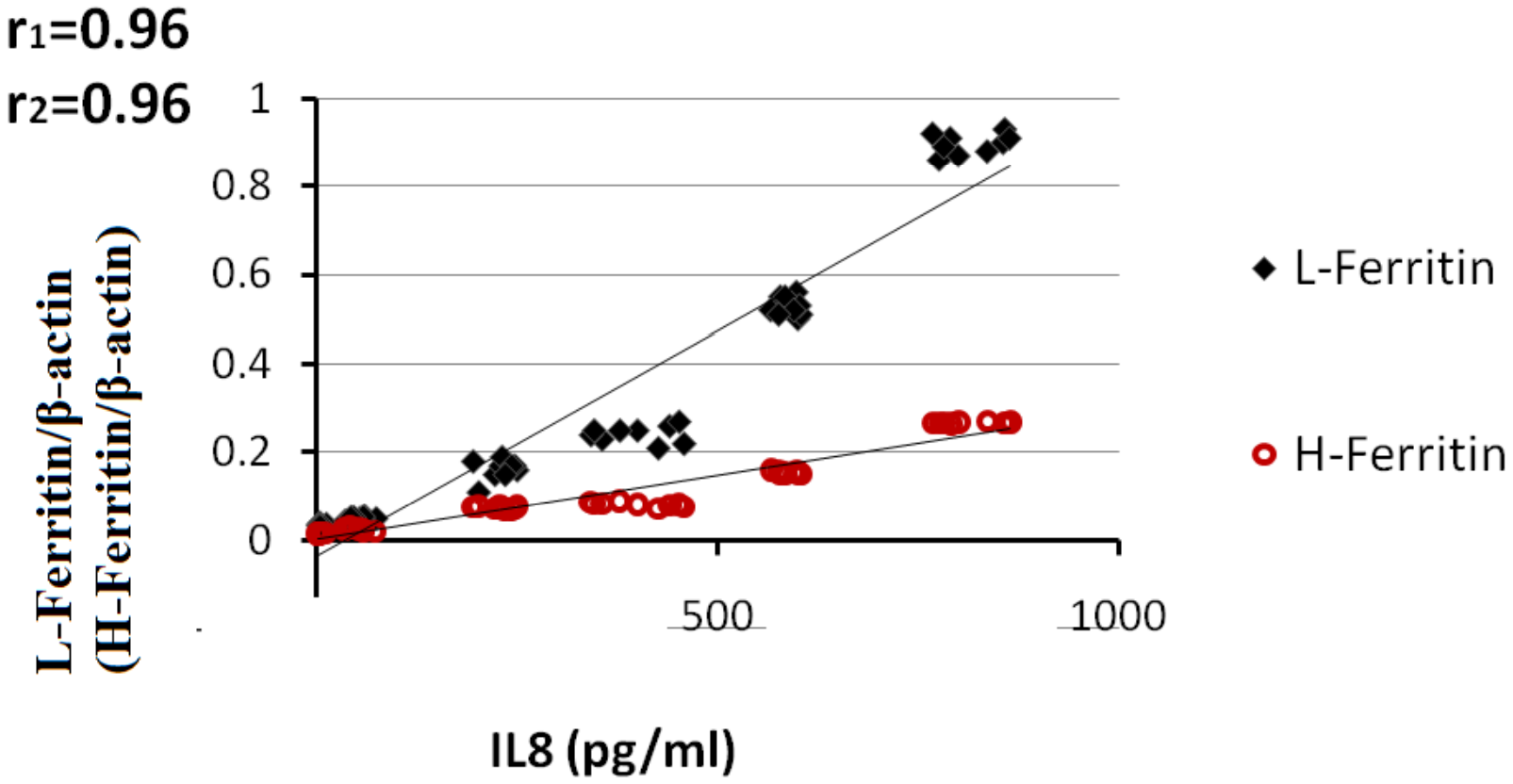




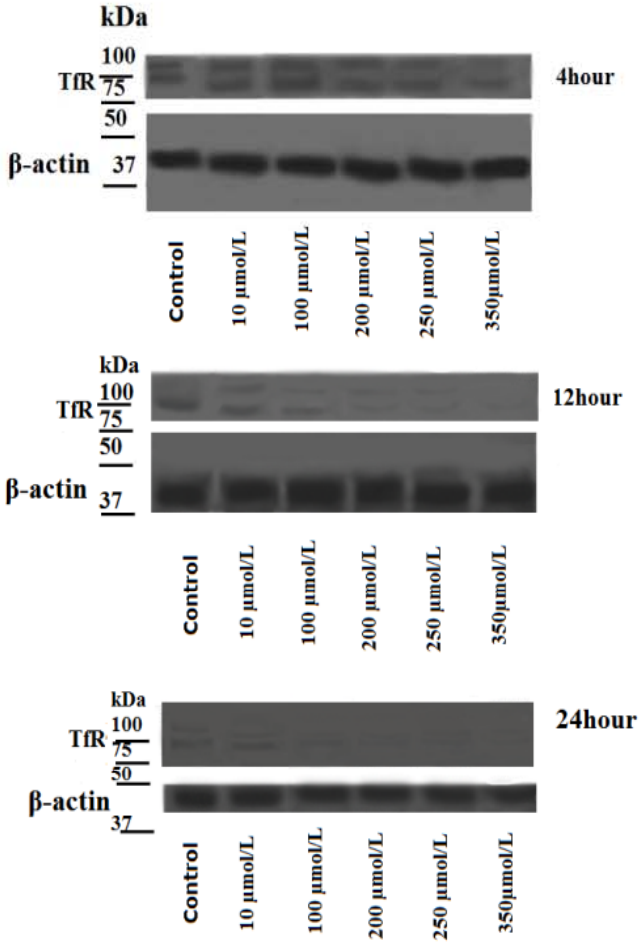


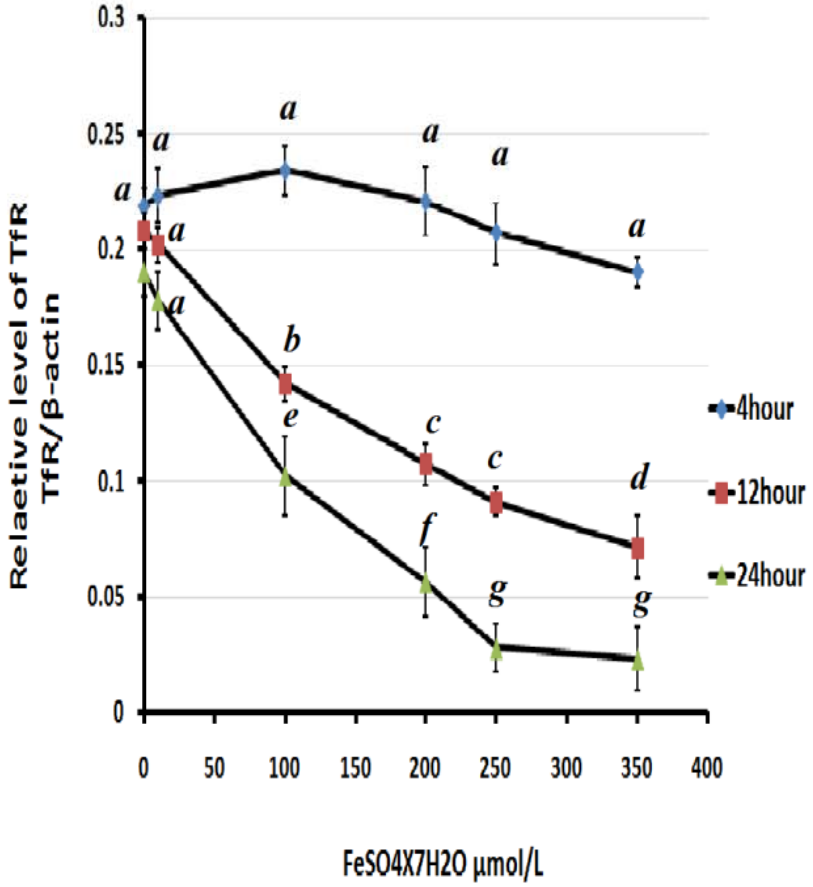


increased concentration of iron induced IL-8 production

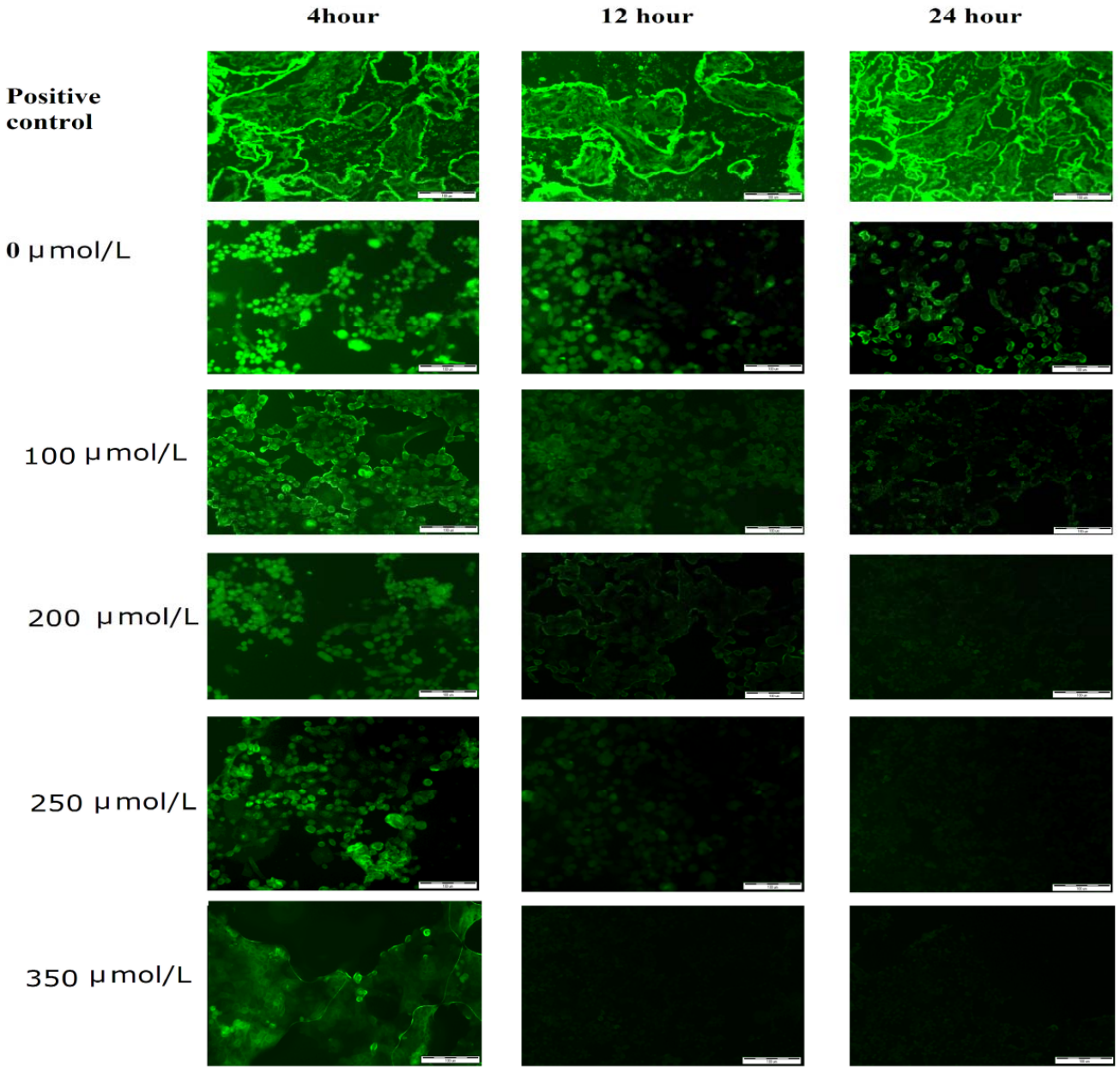


Influence of iron loading on TfR protein

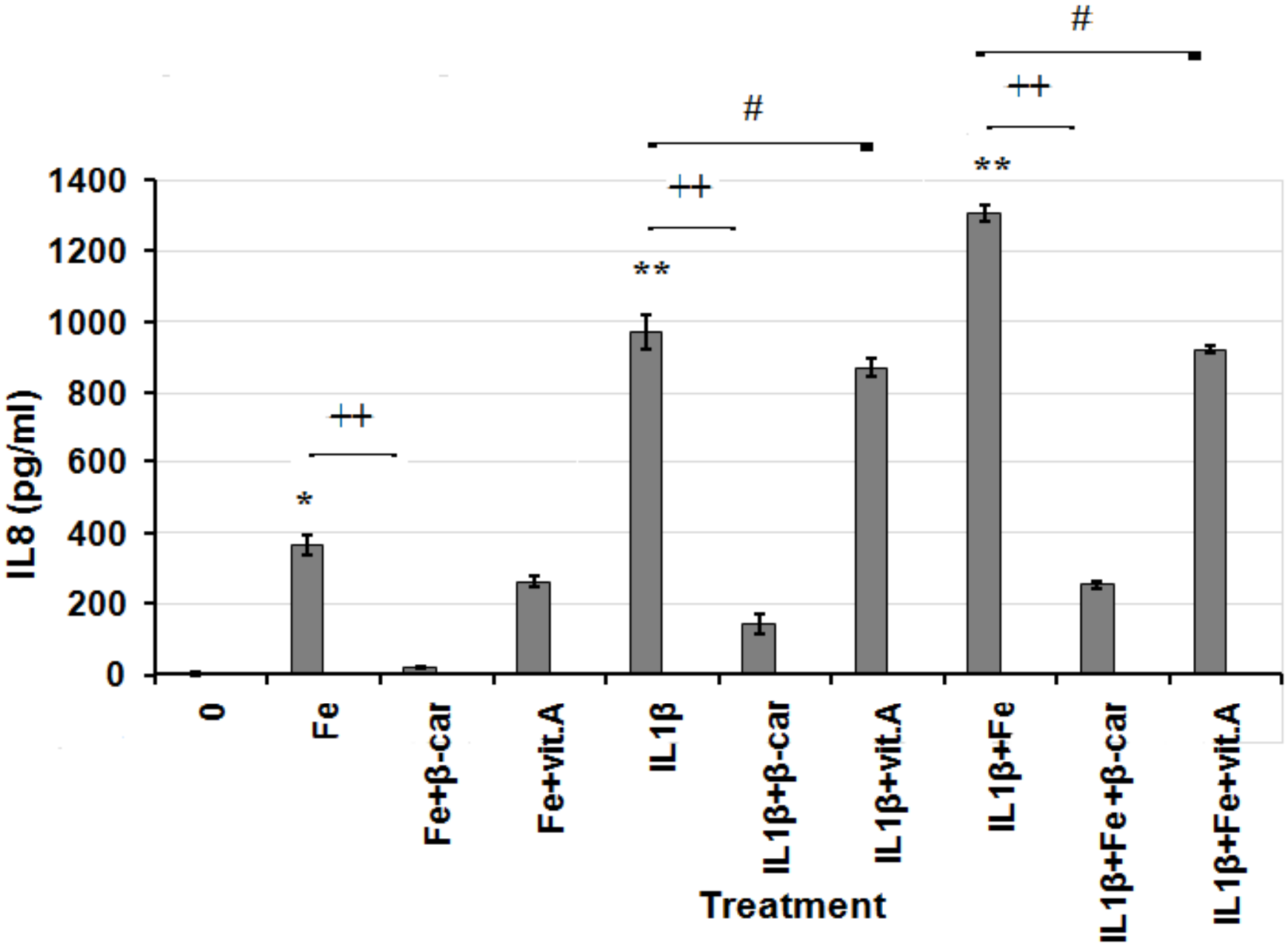




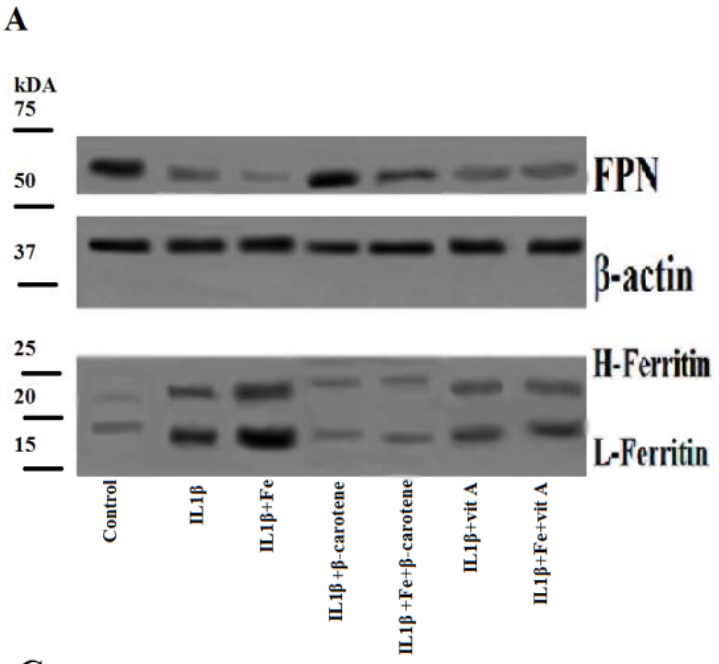
immune fluorescence microscopy was performed

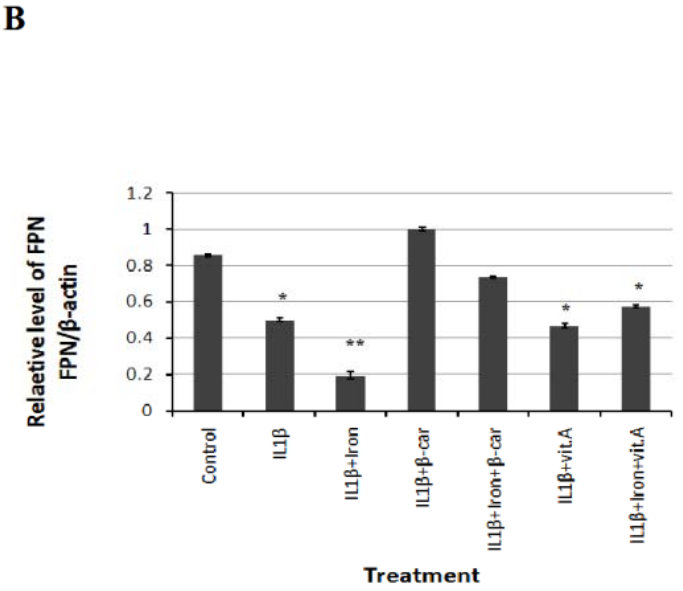


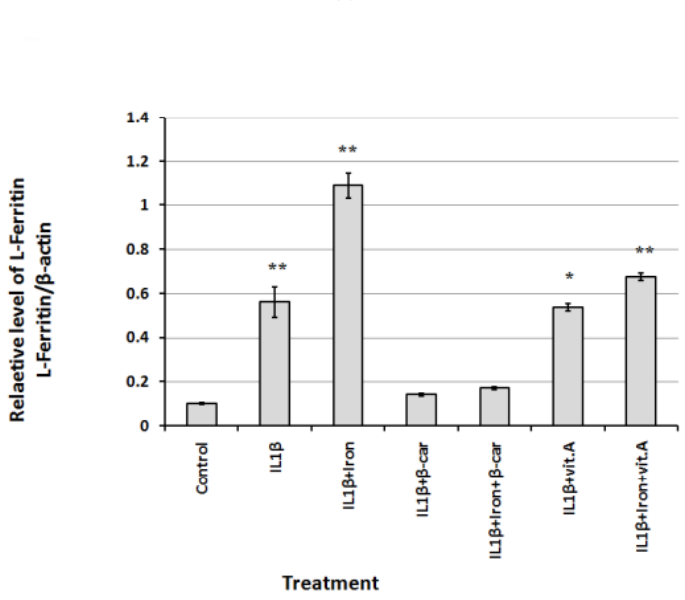
Influence of carotenoids on IL-8 production

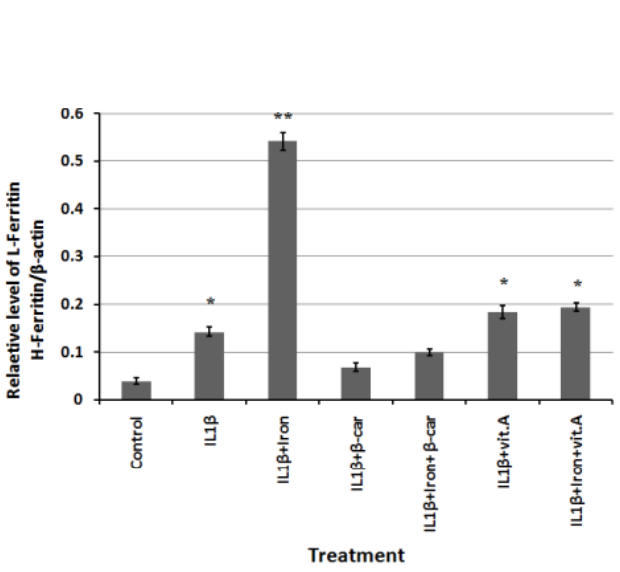


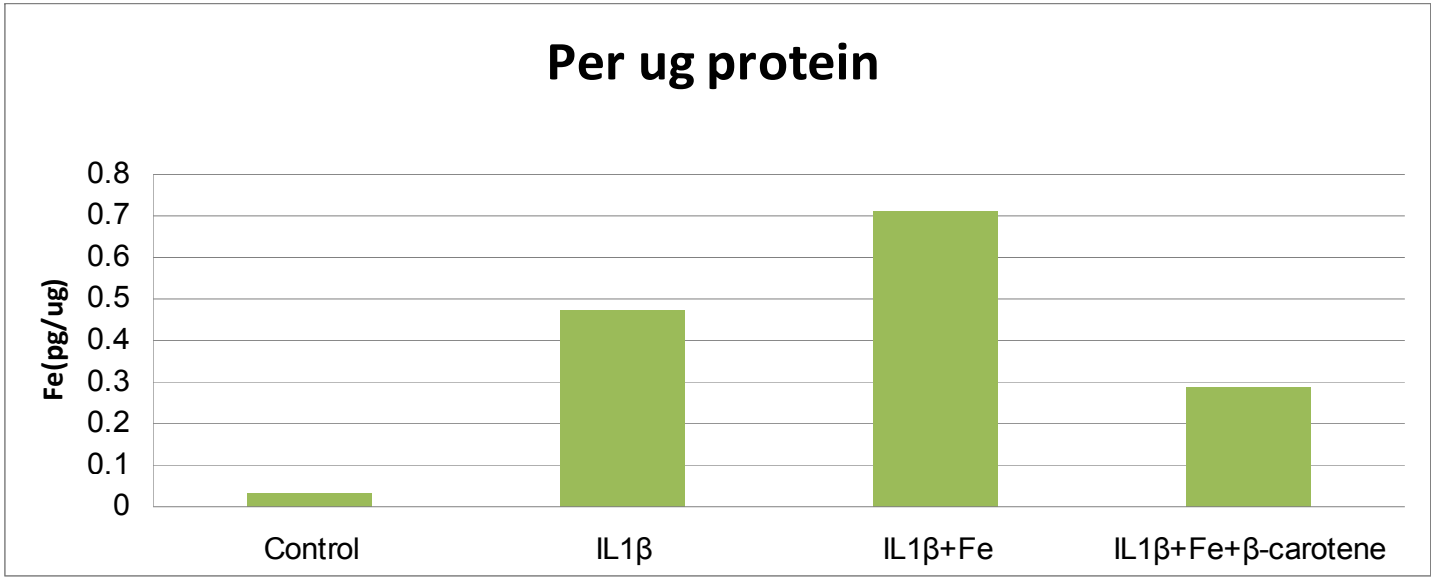
L-, H-ferritin and FPN under treatment of IL1β ± iron

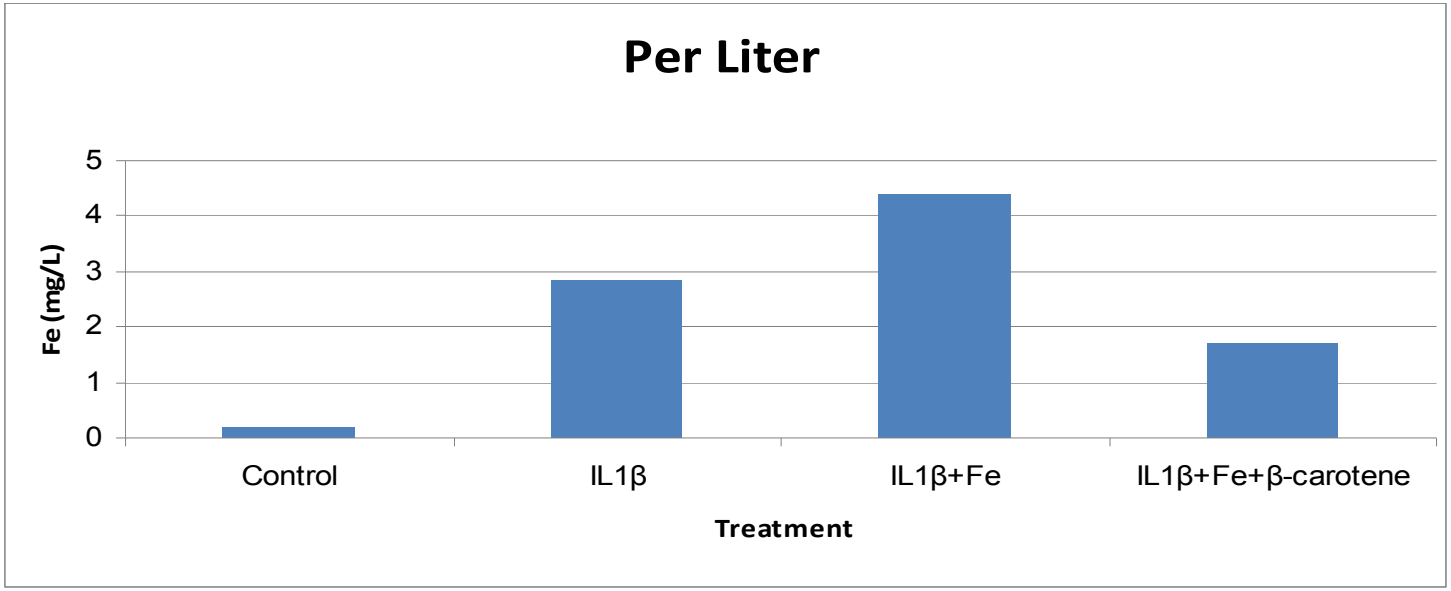


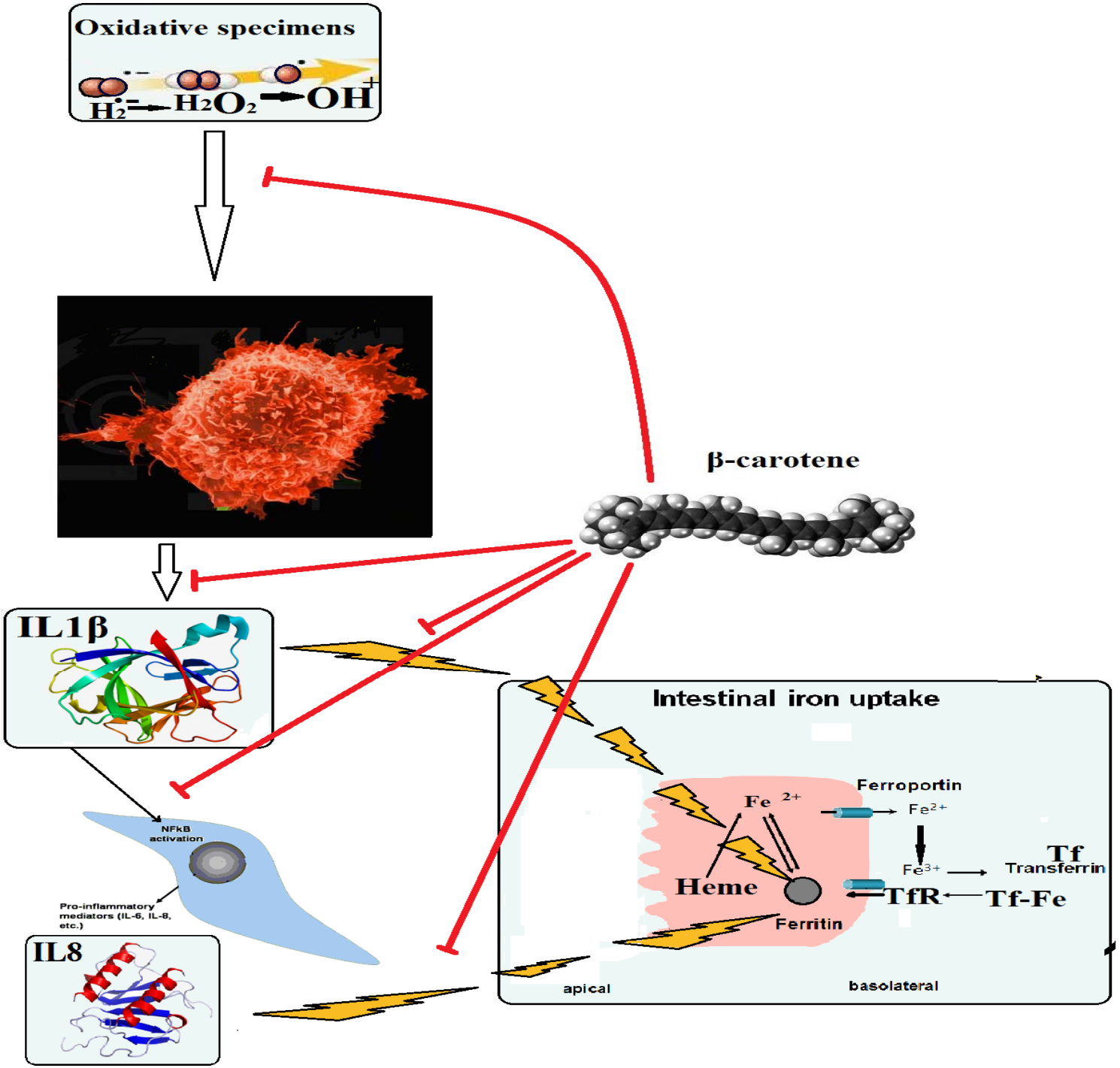







Per ug protein
Fe(pg/ug)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
IL1β
IL1β+Fe
IL1β+Fe+β-carotene


Per Liter
Fe (mg/L)
5
4
3
2
1
0
Control
IL1β
IL1β+Fe
IL1β+Fe+β-carotene
Treatment


Oxidative specimens
$H_2^{\bullet-}$ → H_2O_2 → $OH^{\bullet}$
β-carotene
IL1β
NFkB activation
Pro-inflammatory mediators (IL-6, IL-8, etc.)
IL8
Intestinal iron uptake
Fe 2+
Ferroportin
Fe2+
Fe3+
Tf
Transferrin
Heme
TfR
Tf-Fe
Ferritin
apical
basolateral

Conclusions:

- Application of iron and/or IL1β on Caco2 cells induces inflammation.
- In inflammatory state, enterocyte H-,L-ferritin increases, FPT and TfR decrease.
- β-carotene normalized the main iron related proteins and diminished pro-inflammatory cytokine production.
- Iron application and Inflammation augment while antioxidants decrease inta-cellular iron trapping.
- In iron restricted anemia of Inflammation, iron intra-enterocyte blockage, by closing the iron exit gates, may have deleterious, local and systemic effects.
- One should weight the possibility to encourage IV>PO iron therapy in active Crohn's disease.
- Widening the knowledge of the enterocyte's iron handling during intestinal inflammation is necessary, in the vision of developing new strategies that on one hand induce anti-inflammatory activity and on the other will positively enhance iron bioavailability and delivery.