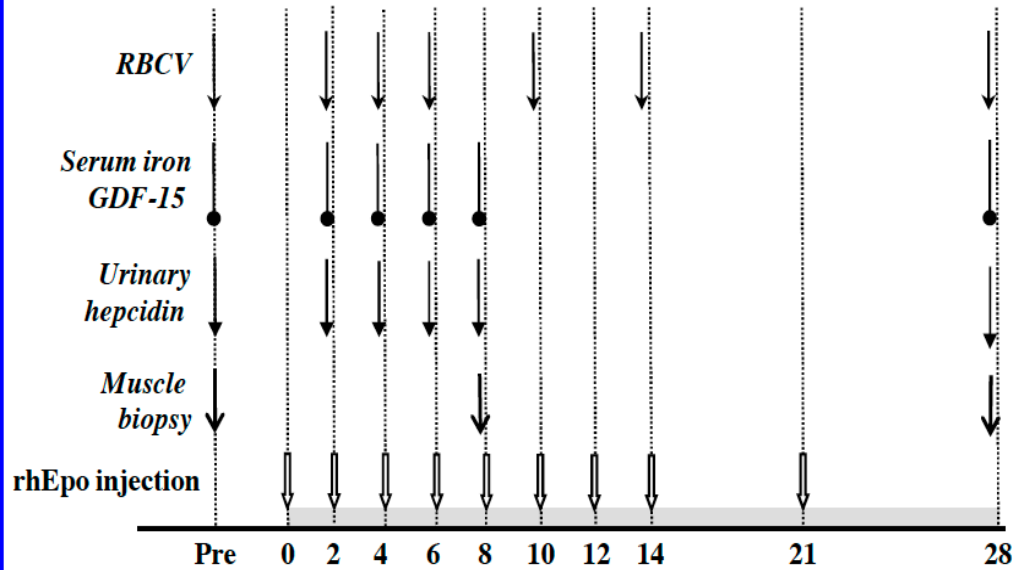


Changes on muscles and iron metabolism following EPO treatment

Prof.Cecilia Gelfi
University of Milano

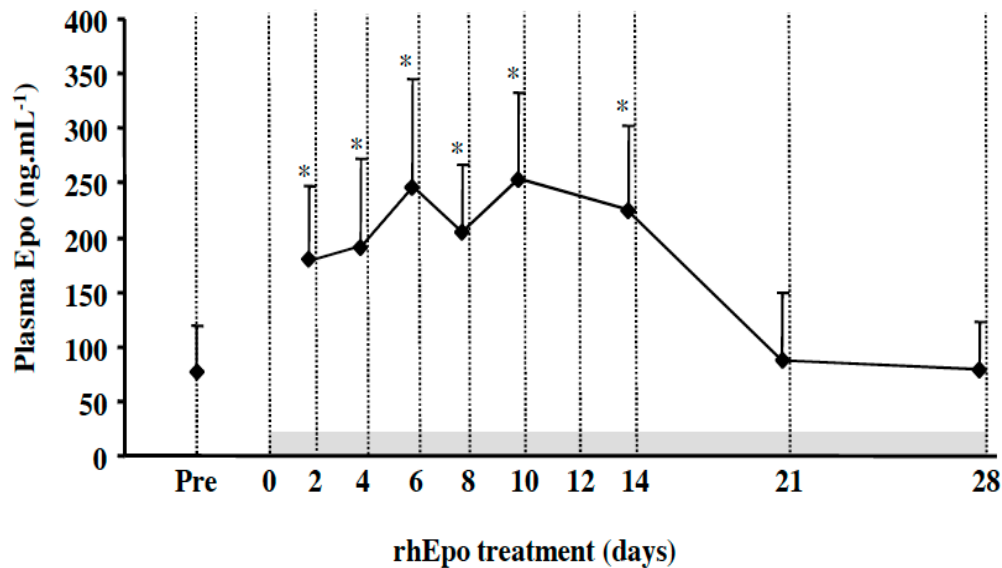
***WADA/JADA Symposium
Tokyo, 7-8 November 2009***

Experimental design

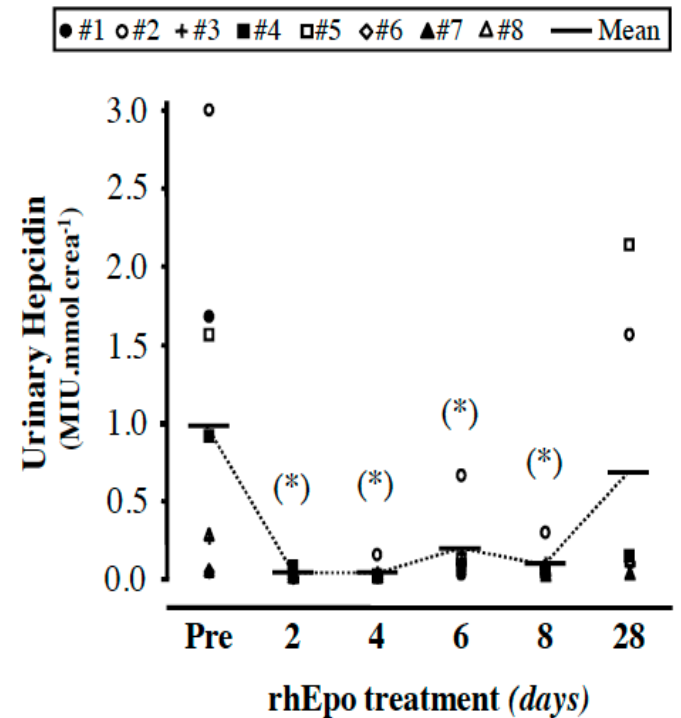


Alteration of iron metabolism

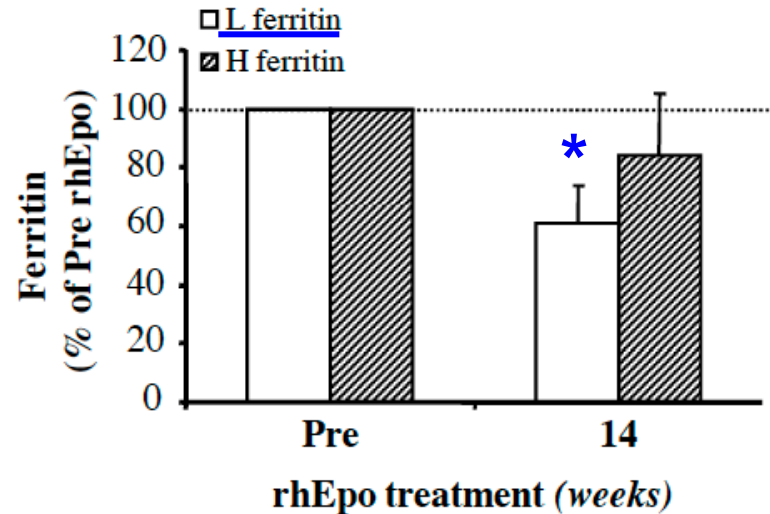
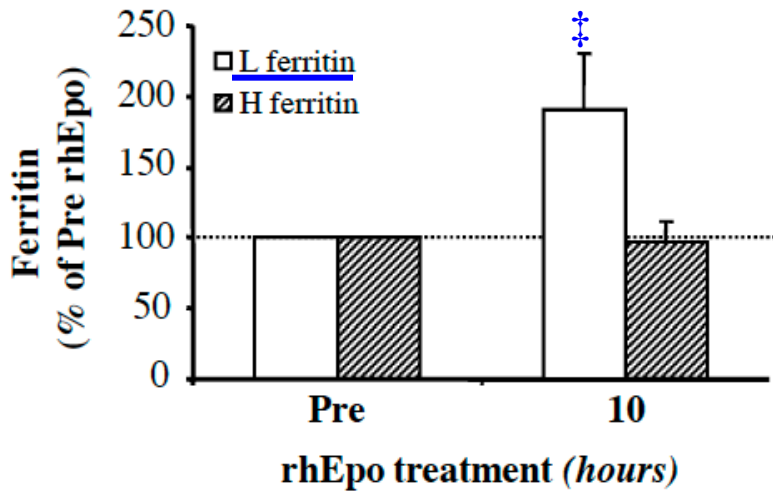
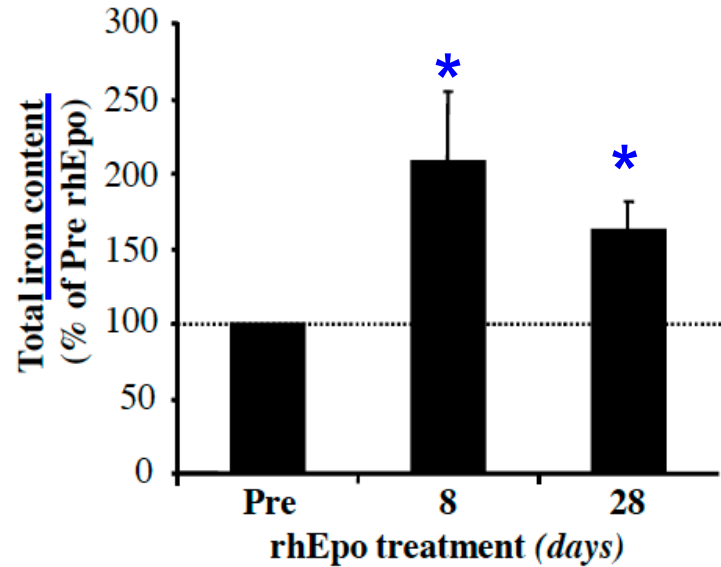
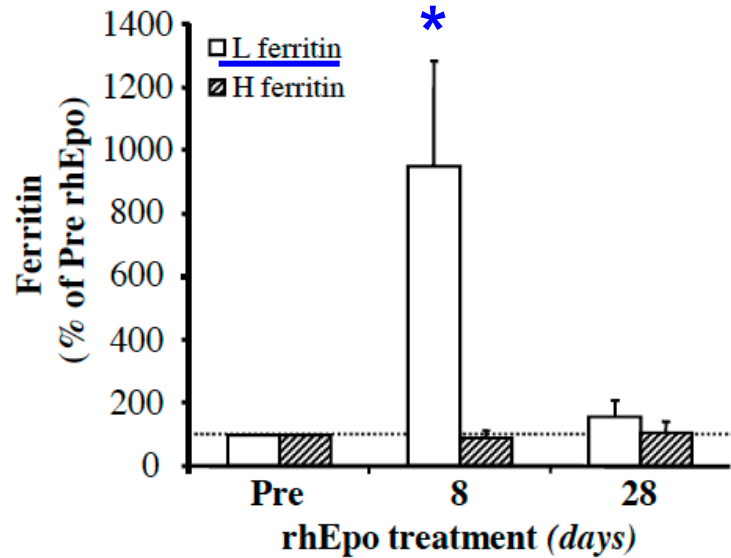
Plasma Epo levels



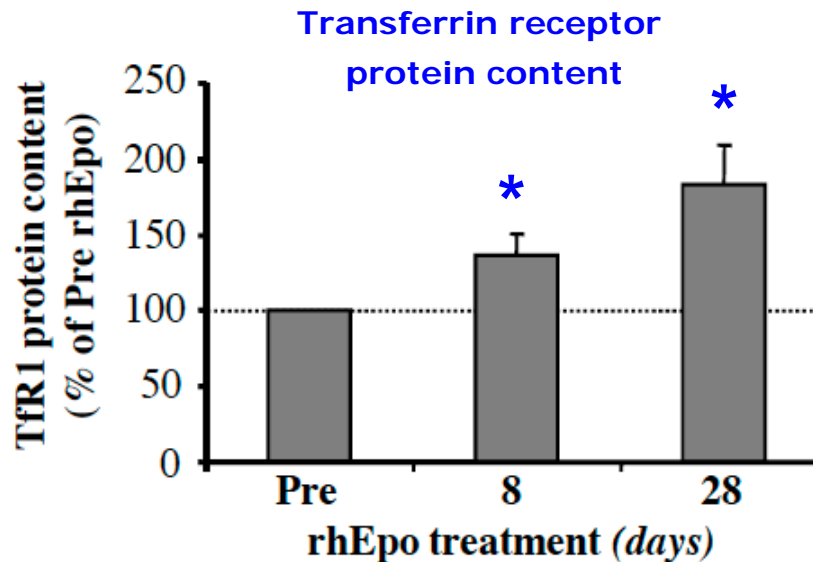
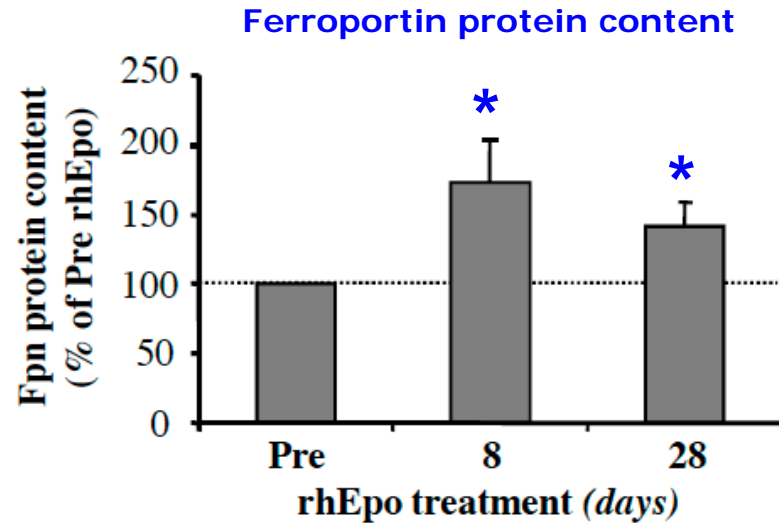
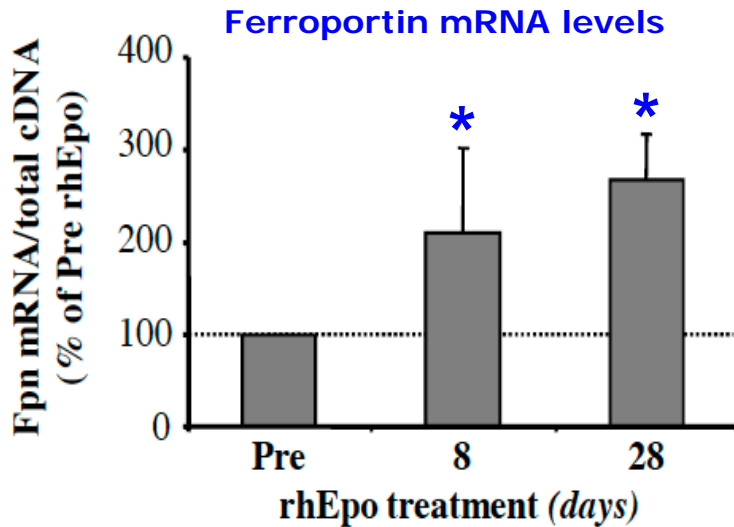
Urinary hepcidin levels



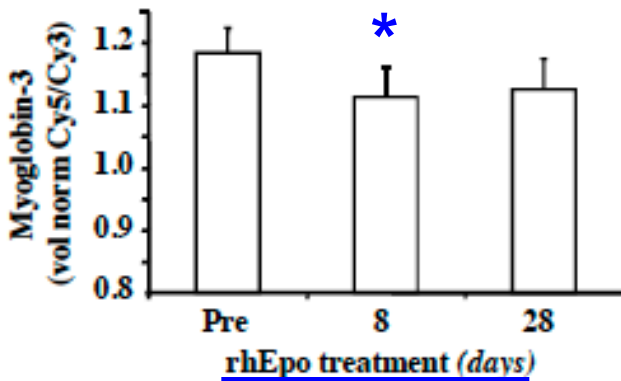
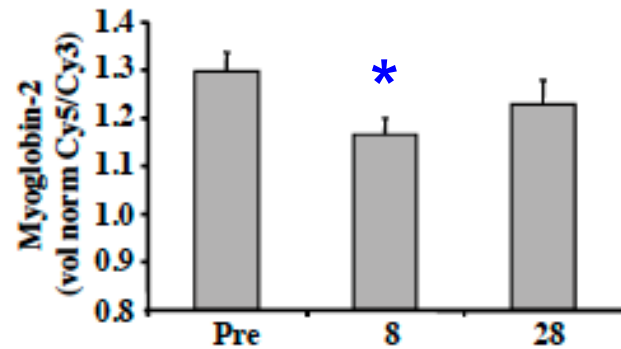
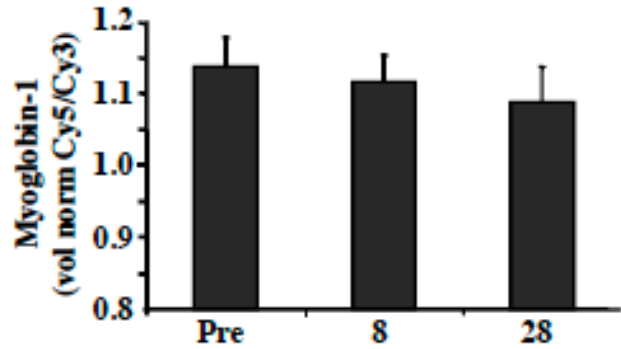
Muscle iron parameters



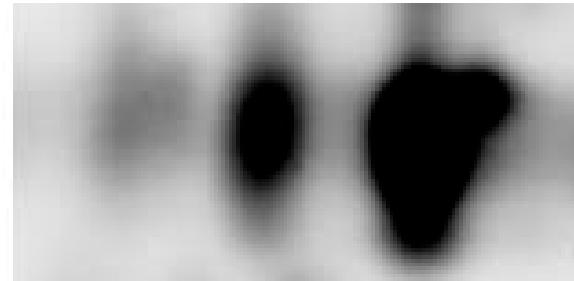
Muscle iron parameters



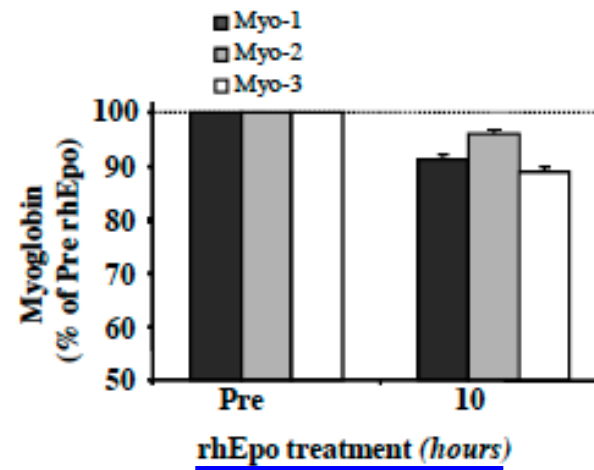
Myoglobin levels



Myo-3 Myo-2 Myo-1

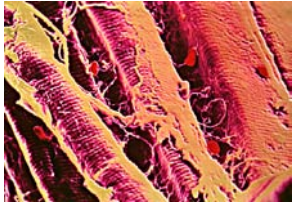


Myoglobin isoforms



Experimental procedure

Muscle protein extraction



Protein CyDye labelling



Each sample with
Cy5 Fluor Dye

The internal standard with
Cy3 Fluor Dye

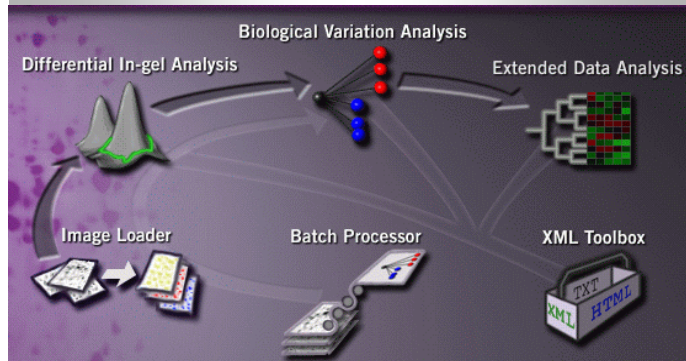
Two dimensional electrophoresis



First dimension
IPGstrip pH gradient 3-10 NL

Second dimension
12% acrylamide-bisacrylamide gel

DeCyder Software image analysis

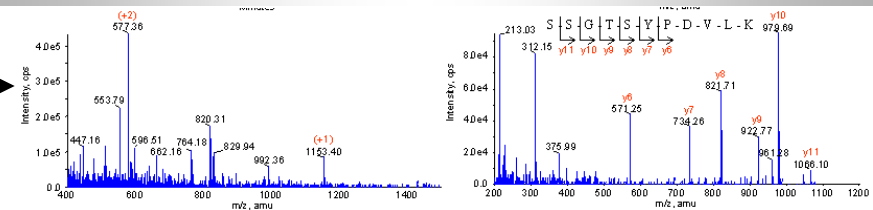


Gel image acquisition



Mass spectrometry (ESI MS/MS) identification

Statistical analysis
(Paired Student's T-test)

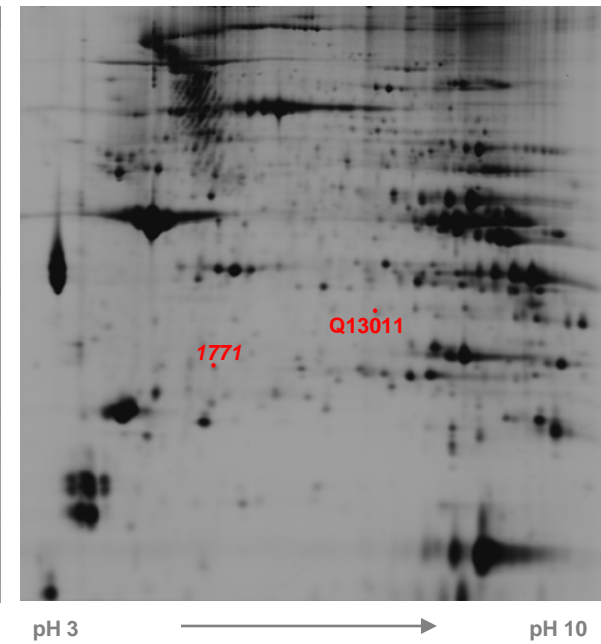
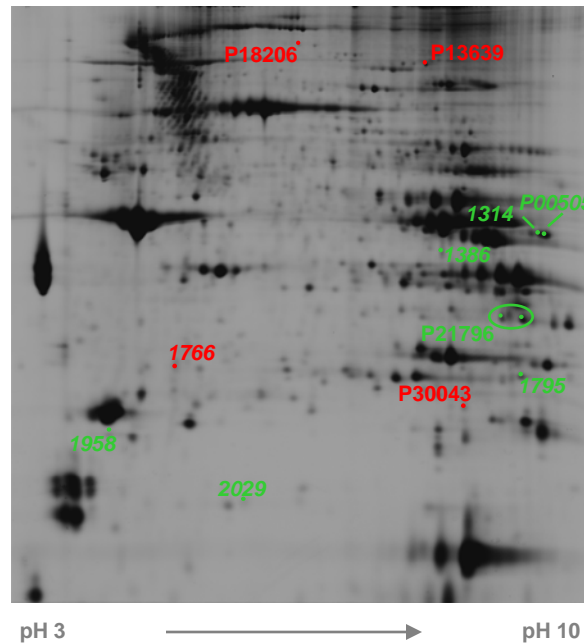
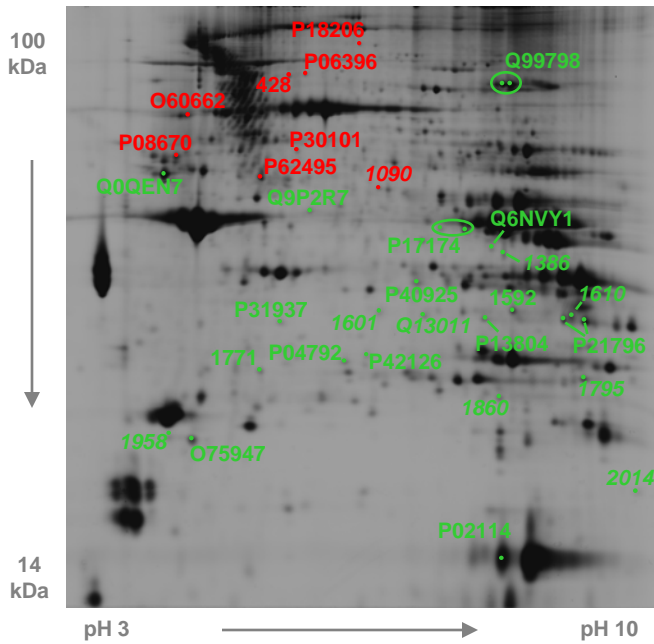


Muscle proteome signature

Changed spots (34) after 8 days

Changed spots (12) after 28 days

Changed spots (2) from 8 to 28 days



■ underexpressed spots

■ overexpressed spots

Unidentified spots

Summarizing

- Epo treatment induces in muscles:
- An **increase** in:
 - Ferroportin
 - Transferrin Receptor
 - Ferritin
 - Total iron cellular levels
 - Cytoskeletal proteins
- A **decrease** in :
 - Epicidin levels
 - Myoglobin
 - Oxydative metablolism

Conclusions

After EPO treatment, muscle activates a system to protect itself from the negative effects of increased iron by storing it on **ferritin, reducing metabolism**, which generates free radicals, increasing radical scavenger protecting membrane from oxydation.

Aknowledgements

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d'Alpinism, Chamonix***

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