

# Effects of an Iron Supplementation Combined to either a Zinc Deficiency or a Zinc Supplementation in Pregnant Rat

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**Abstract:** ***Objectives:** This article reports the effects of an Iron overload on oxidative stress, and the protective role of Zinc. Pregnancy was chosen because it enhances the interactions and the effects of both metals on physiological processes and on oxidative stress. **Design and methods:** Four groups of pregnant rats were compared. The first 16-rats were included in the control group (Group I), a second 16-rats group (Group II) was supplemented with Iron, in the third group (Group III) 16 rats were supplemented both with Iron and Zinc and in the last group (Group IV), the 16 rats were supplemented with Iron but fed a Zincdeficient diet. **Results:** In the groups II and IV, plasma malondialdehyde concentrations were significantly higher than in the groups I and III while plasma SOD was lower in the groups II and IV than in the two others. Plasma  $\alpha$ -tocopherol concentrations were lower in the groups IV,II and III as compared to the control group. **Conclusion:** This experiment shows the deleterious effects of Iron and the protective effect of Zinc. Indeed, oxidative stress was increased in the Zinc deficient group.*

**Keyword:** Iron; Zinc; supplement; rat; hemochromatosis; active species; pregnant; malondialdehyde; superoxide dismutase; protection

## 1. Introduction

Zinc and Iron are respectively implicated in the protection or the generation of free radicals. Zinc is an essential trace element that takes part in the active site of more than 600 enzymes, these enzymes acting in all parts of metabolism. As Zinc has only one possible ionization degree it can also protect against the deleterious effects of Iron simply by displacing it from its protein combinations [1, 2, 3, 4] Indeed, contrary to Zinc, Iron has multiple possible oxidation numbers and can promote the Fenton reaction and the Haber-Weiss cycle that initiate free radical production [5, 6, 7, 8, 9]. Hence Iron needs to be carefully handled inside and out of the cells mainly by means of tightly chelating proteins [10, 11]. But despite the defenses developed by tissues to protect against the free forms of this metal, Iron overload have been described to favour or promote various diseases [12] like sepsis [13], ageing [14] and Alzheimer dementia [15,16], carcinogenesis [17], atherosclerosis, liver failure [18] and cardiac infarction [19].

Zinc has been shown to decrease Iron-induced atherosclerosis in rabbits and lipid peroxidation [20]. Reactive oxygen species (ROS) include Superoxide, hydroxyle radicals and singlet-oxygen. They can alter biological molecules and cell structures by triggering lipid peroxidation, protein and enzyme oxidation and DNA alterations. Such data have been long known but to date no experimental work could study the interactions between these trace elements in vivo. In this study, we investigated the respective roles of an Iron supplementation and of either a Zinc supplementation or an experimental Zinc deficiency in pregnant rats.

In pregnant rats, the metabolism of Iron is modified to meet the increased needs due to fetus growth [21]. Iron is then stored in the placenta where it is sequestered by transferrin and released to the fetus when it needs [22 23. 24]. In humans, Iron absorption is maximum at the end of gestation, when the fetus size is maximum. In the rat, maximal absorption occurs between day 16 and the term at day 21

This study aimed at studying the effects of an Iron overload on stress and biochemical indexes, and the role of Zinc on overload. As Iron absorption has been known to increase during pregnancy, the choice of pregnant rat seemed interesting because it could enhance the deleterious effects of this metal in animals

## 2. Material and Methods

### 2.1. Animals

A total of 64 female pregnant Wistar albinos rats 7 to 8 weeks old, and weighing between 180 to 200 g. were randomly included into 4 groups of 16 rats. It was the first gestation for all the rats that were included as soon as they became pregnant. The first group (Group I) consisted in control rats, in the second group (Group II), rats were supplemented with one intraperitoneal dose of iron (10 mg/ Kg body weight), in the third group (Group III), animals were supplemented with Iron and Zinc simultaneously (6.5 mg/ Kg), and in the last group (Group IV), rats were simultaneously Zinc deficient and supplemented with Iron (10 mg/ Kg). On the 21<sup>st</sup> day of pregnancy, after an overnight fasting, rats were anesthezied and blood was collected from the animal's eye. Blood was then

immediately centrifuged for 10 minutes at +4°C and at 3000 g.

## 2.2. Measurements

Cholesterol, triglycerides, ferritin and plasma Iron were measured using a Roche-Cobas Integra (Roche, Meylan France) in the Blood Analysis Laboratory Ibn Sina (Constantine, Algeria). Zinc measurements were performed by the National Research Centre in Cairo (Egypt) using flame atomic absorption. Erythrocyte Cu-Zn superoxide dismutase (SOD) activity was measured after hemoglobin precipitation by monitoring the autooxidation of pyrogallol. One unit of SOD is defined as the amount of the enzyme required to inhibit the rate of pyrogallol autoxidation by 50%. Fat-soluble vitamins and carotenoids were separated with a 3 µ Adsorbosphere HS C18 150 x 4.6 ID column from Alltech (Alltech Associates, Inc. Deerfield IL, USA), vitamin C was analyzed with a 5 µ Nucleosil 100 AB 150 x 4.6 mm ID column from Macherey Nagel (Macherey Nagel Sarl, Hoerdt, France). Malondialdehyde (MDA) was measured by HPLC with Visible detection at 532 nm after derivation with thiobarbituric acid [25]. Separations were performed with a Alltech Adsorbosphere C18 5µm column (250 x 4.6 mm ID) and a Shimadzu Prominence series 20 HPLC system. Methanol, acetonitrile, dichloromethane, hexane, tetrahydrofuran, ethanol were purchased from Riedel-de Haën (Sigma-Aldrich Corp, St Louis, MI, USA). Retinol, α-tocopherol, β-carotene were from Sigma.

## 2.3. Diet and Supplements

Rat food was manufactured by UAR (Usine d'Alimentation Rationnelle, Villemoisson, France) and provided the following nutrients (g/Kg rat weight): egg albumin 200, glucose 300, maize starch 300, maize oil 60 and the mineral mixture UAR 205B 70 g/Kg, the vitamin mixture UAR 200 10 g/Kg. The mineral UAR 205B mixture provided the following amounts (g/kg food): Ca 8; K 4.8; Na 3.2; Mg 0.2; Fe 0.24; Mn 0.064; Cu 0.01; Zn 0.036 (except in the groups IV); Co 0.00008; I 0.00008. The UAR 200 vitamin mixture provided the following amounts (mg/kg food): retinol 6; cholecalciferol 0.06; thiamin 20; riboflavin 15; pantothenic acid 70; pyridoxine 10; inositol 150; cyanocobalamin 0.05; ascorbic acid 0.8; dl -tocopherol 170; menadion 40; nicotinic acid 100; para amino benzoic acid 50; folic acid 5; biotin 0.3; choline 1.36. Starch was washed with a 1% EDTA solution in the Group IV and then washed with distilled water. Supplementations were made with Iron gluconate and Zinc sulphate, 7H<sub>2</sub>O (Sigma, L'Isle d'Abeau, France).

## 2.4. Statistical Analysis

Statistical analysis was performed with the SPSS Software (SPSS Inc. Chicago IL.) using the General Linear Model (GLM) analysis [26]. When significant, the GLM was followed by a Student-Newmann Keuls post-hoc test to detect specific inter-group significances. Differences were considered as significant when  $p < 0.05$ .

## 3. Results

Results are summarized in figure 1 to 8.

Plasma malondialdehyde concentrations and plasma triglycerides were significantly higher in Groups II and IV than in the Groups I and III. plasma cholesterol was significantly higher in Group II than in all other Groups. As expected, plasma Zinc concentrations were significantly different in every group, they were higher in the Group III, and lower respectively in the Groups I, II and IV. The concentrations of plasma iron were significantly increased in rats treated groups compared to the control group, while his groups were homogeneous between them. Plasma ferritin concentrations were significantly higher in Group IV and II than the Group I. Erythrocyte SOD was lower in Groups II and IV than in Groups I and III. Plasma α-tocopherol concentrations were significantly reduced in rats treated groups as compared to the control group.

## 4. Discussion

Plasma ferritin concentrations show that Iron supplementation of rats with 10mg/Kg an increase Iron stores in the liver and induces a hemochromatosis. At such levels the metal, which is normally bound to proteins, binds to low molecular weight compounds and may become toxic by producing the highly toxic hydroxyle ions [1, 2].

Plasma MDA monitors effects of ROS on cell membranes [27]. Superoxide dismutase (SOD) is a Copper-Zinc metalloenzyme which fights Superoxide radicals by converting them into hydrogen peroxide and water [28]. These results show that Iron increases ROS activity and that this change is either enhanced by Zinc deficiency or decreased by Zinc supplements [29]. Iron has been known to produce Superoxide and Hydroxyle radicals [30] through the Fenton and the Haber-Weiss cycle [31]. These radicals - particularly •OH - are very unstable and toxic. As expected, the Groups II and IV show the highest levels of malondialdehyde. This work also show that Zinc protects from the deleterious effects of Iron as in Group III the MDA concentrations are not significantly different than in the control group.

Iron supplements increases plasma cholesterol probably by altering liver functions. A similar mechanism is seen in alcohol toxicity where patients exhibit high cholesterol level at least at the first steps of the intoxication. The hypercholesterolemia induced by Iron supplements is corrected by a Zinc supplementation, and - as Zinc deficiency hampers its synthesis - plasma cholesterol is decreased in the Zinc-deficient group. Plasma triglyceride concentrations showed similar changes, but contrary to cholesterol, they are higher in the Zinc deficient group than in all the others. Indeed, most part of the triglycerides is synthesized out of the liver.

Plasma Zinc concentrations are much higher in the Zinc supplemented group and drop in the rats fed a Zinc deficient diet. But Zinc is also lower in the Iron supplemented group, indeed Iron competes with the intestinal absorption of Zinc. Similar results were found for plasma ferritin levels. This

data shows a competition between Zinc and Iron absorptions as confirmed by ferritine concentrations that are lower in the Iron + Zinc supplemented group than in the Iron alone supplemented. Ferritine levels are highest in the Iron supplemented /Zinc deficient group. These results show the interactions that exist in the intestinal absorption of these two metals. They also show that Zinc protects against Iron effects primarily by decreasing its intestinal absorption. But Zinc is also an indirect antioxidant [32, 33], because it is a component of SOD, because it competes with transition metals in the Fenton reaction, because it protects protein-thiol groups from oxidation and because it stabilizes proteins and cell membranes. Zinc induces the synthesis of the cysteine-rich metallothioneins that fight directly free radicals. In this study, decreased erythrocyte superoxide dismutase concentrations in Groups II and IV can be explained by a relative Zinc deficiency that can be caused either by a high Iron intake alone or by the same intake associated with a Zinc deficient diet. The influence of Zinc on SOD is confirmed by its higher levels in the Group III.

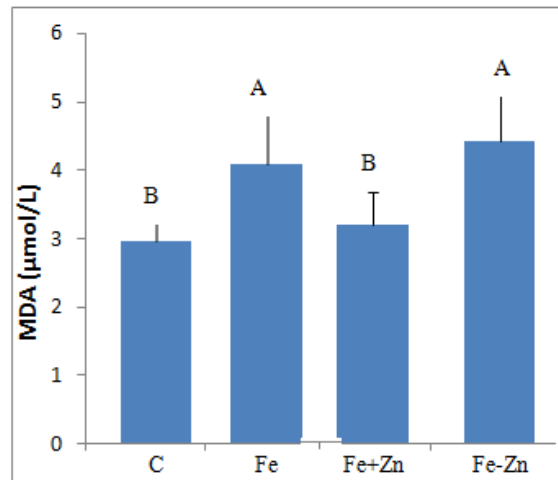
In this study, the reduction in the concentration of  $\alpha$ -tocopherol, can be interpreted by increasing its use in the fight against the oxidative stress caused by iron overload. The  $\alpha$ -tocopherol may be oxidized by peroxide radicals resulting from the oxidation of unsaturated fatty acids. The  $\alpha$ -tocopherol is known as one of the most important antioxidants, it provides protection against attack by free radicals, in particular peroxide. The  $\alpha$ -tocopherol inhibits the third phase of lipid peroxidation and prevents the spread of oxidation reactions by interacting with peroxy radicals which transforms them into peroxides [38].

It has been shown that a consequence of Zinc deficiency is a marked increase in membrane and cellular Iron concentration [34, 35]. Moreover, Zinc reduces the Iron- and Copper-induced damage to the DNA and this ability of Zinc is greater against Iron than against Copper [36]. This study confirms the deleterious effect of an experimental Iron overload and that Iron effects can be modulated by Zinc intakes. Zinc deficiency enhances the effects of Iron overload while Zinc supplements can decrease its effect [37]. These facts may be used in patients who exhibit an Iron overload and particularly those who suffer from thalassemia or haemoglobin abnormalities.

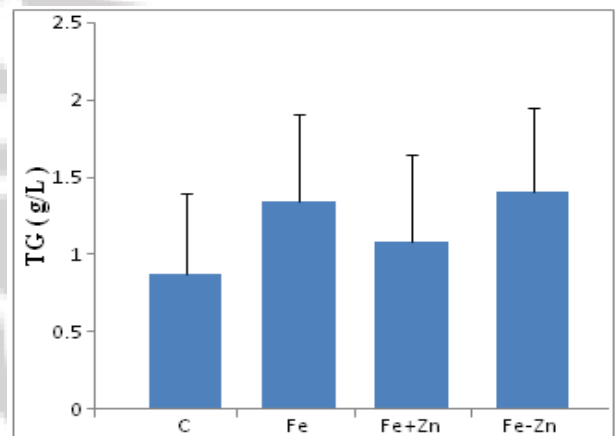
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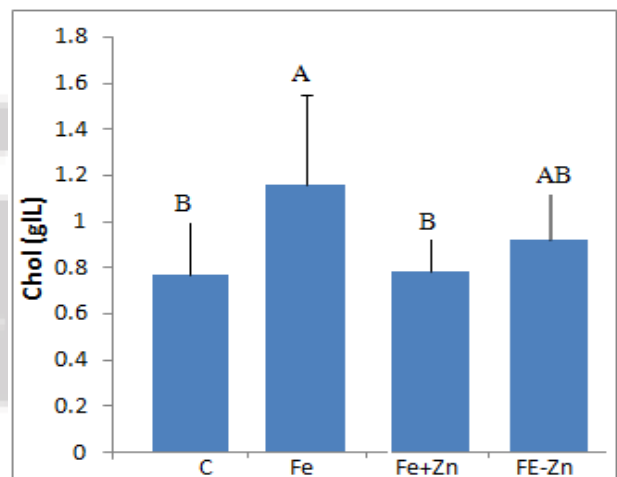
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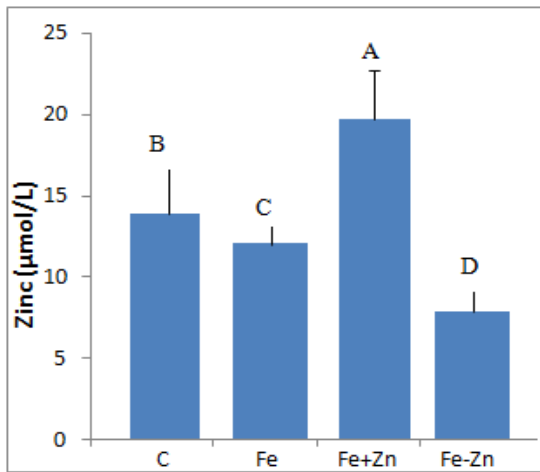
**Figure 1:** Plasma malondialdehyde concentrations in the four groups of pregnant rat's. Different letters mean that differences are significant. Between-group significance  $P < 0.001$



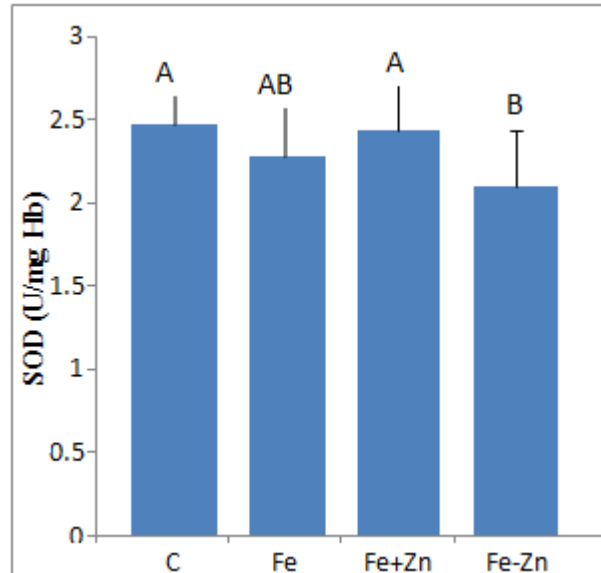
**Figure 2:** Plasma triglyceride concentrations in the four groups of pregnant rats. Different letters mean that differences are significant. Between-group significance  $p < .026$



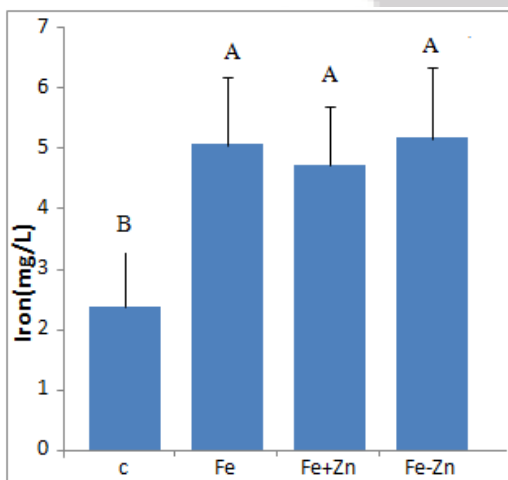
**Figure 3:** Plasma cholesterol concentrations in the four groups of pregnant rats. Different letters indicate mean that differences are significant. Between-group significance  $p < .001$



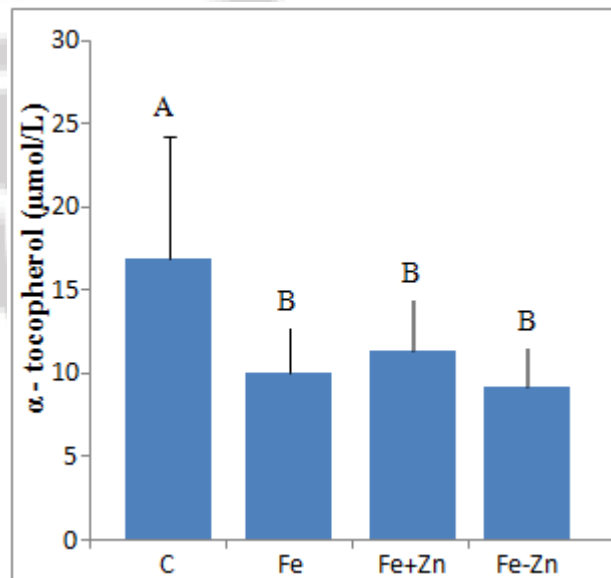
**Figure 4:** Plasma zinc concentrations in the four groups of pregnant rats. Different letters mean that differences are significant. Between-group significance  $p < .001$ .



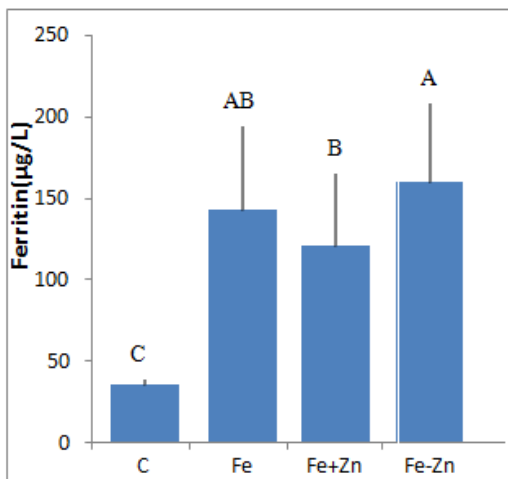
**Figure 7:** Erythrocyte superoxyde dismutase activities in the four groups of pregnant rats. Different letters mean that differences are significant. Between-group significance  $p < .002$ .



**Figure 5:** Plasma iron concentrations in the four groups of pregnant rats. Different letters mean that differences are significant. Between-group significance  $P < .001$ .



**Figure 8:** plasma α- in the four groups of pregnant rats. Different letters mean that difference are significant. Between-group significance  $P < .004$ .



**Figure 6:** Plasma ferritin concentrations in the four groups of pregnant rats. Different letters mean that differences are significant. Between-group significance  $p < .001$ .