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## RESEARCH ARTICLE

## Modulations of serum hepcidin, IL<sub>6</sub> and iron status by different exercise regimens in ovariectomized rats

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### Abstract

**Background:** Hepcidin is a liver-derived regulatory protein playing a crucial role in iron metabolism. It is established that gender differences exist regarding iron storage. However, the effects of sex steroid hormones on iron homeostasis are not completely understood.

**Objective:** Assessing changes in iron status, serum hepcidin & IL<sub>6</sub> in an experimental model of induced menopause, and evaluating modulations induced by different exercise intensities.

**Design:** Forty adult female Wistar albino rats were utilized. They were divided into four equal groups. **Group 1:** non ovariectomized sham operated sedentary group (SHAM/sG). **Group 2:** ovariectomized sedentary group (OVX/sG). **Group 3:** ovariectomized moderately trained (adaptive) exercise group (OVX/ MEG). **Group 4:** ovariectomized strenuously trained exercise group (OVX/ SEG). Serum hepcidin, IL<sub>6</sub> & different parameters assaying iron status were assessed.

**Results:** The present study demonstrated that hepcidin and IL<sub>6</sub> were significantly elevated in OVX/sG versus SHAM/sG. These elevations were associated with significant decreases in the parameters assaying iron status. Following moderate training for 10 weeks, both hepcidin and IL<sub>6</sub> were significantly decreased compared to OVX/sG with significant increases in the parameters assaying iron status. However, following strenuous training for 10 weeks a more significant increase in serum hepcidin and IL<sub>6</sub> compared to their levels in OVX/sG with worsening in the parameters assaying iron status were detected.

**Conclusion:** Experimentally induced menopause was associated with elevated serum hepcidin and IL<sub>6</sub> and deteriorated iron status, which were nearly reversed back to pre-ovariectomized values after moderately adaptive but not strenuous exercise

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## INTRODUCTION

Hepcidin is a 25-amino acid peptide hormone synthesized by hepatocytes. It is involved in iron homeostasis through binding with the iron exporter ferroportin and induction of its internalization and degradation (Nemeth et al., 2004). Ferroportin is expressed on the basolateral membrane of enterocytes, in hepatocytes, and in macrophages. Hepcidin-mediated degradation of ferroportin prevents iron uptake from the intestine as well as release of stored iron from macrophages, thus decreasing the available iron (Nemeth and Ganz, 2009). It may also reduce intestinal iron absorption through ubiquitin dependent proteasome degradation of the divalent metal transporter 1 (DMT1) on the luminal enterocytes membrane (Brasse-Lagnel et al., 2011). Hepcidin is primarily expressed in the liver and its

transcription can be up-regulated by iron overload, inflammation, infection and elevated IL-6, IL-1 $\alpha$ , & IL-1 $\beta$  (Wessling-Resnick, 2010). On the other hand, it is down-regulated by anemia & hypoxia (Krijtet al., 2010). Iron regulates hepcidin expression primarily through the bone morphogenic protein/hemojuvelin (BMP/HJV) pathway, while infection and pro-inflammatory cytokines such as interleukin-6 (IL-6) increase hepcidin transcription mainly through the Janus kinase/ signal transducers and activators of transcription (JAK/STAT) pathway (Zhang and Rovin, 2010).

Also, many recent studies hypothesized that both hepcidin transcription and concentration are altered in response to physical activity and exercise, which in turn affects iron metabolism. Different exercise regimens could induce variable changes in hepcidin levels and subsequently modify iron homeostasis (Sim et al., 2013).

Regarding the regulation of hepatic hepcidin expression by sex steroid hormones; Harrison-Findik (2010) reported the presence of gender variations in the body iron content. Also, Bachman (2010) stated that testosterone could be participating in iron metabolism via decreasing hepatic hepcidin transcription as administration of testosterone was found to decrease serum hepcidin concentrations in men. Moreover the study conducted by Luque-Ramirez (2011) demonstrated that women with polycystic ovary syndrome showed increased testosterone levels & reduced serum hepcidin levels.

On the other hand, the interaction of estrogen with iron at systemic level has been long suspected (Jian et al., 2009). As the most common diseases affecting women's health in the post-menopausal life, e.g. osteoporosis and breast cancer are closely related to alternations of endogenous estrogen and variations in serum iron levels (Huang, 2008; Eckard et al., 2010; Gabriel and Domchek, 2010; Yanget al., 2010).

The direct drive linking estrogen with iron homeostasis is limited. As, there are a conflicting data regarding the direct effects of the female sex hormones on hepcidin level and iron metabolism

Ikeda et al (2012) found that estrogen- deficient conditions after ovariectomy resulted in augmented iron absorption in the duodenum because of the down regulation of hepcidin in the liver which contributed to increased body iron storage.

While, on contrary other studies suggested that elevated levels of estrogen manipulate iron homeostasis. For instance, ovariectomy results in decreased serum iron, iron binding capacity, and iron response protein-1 binding activity (Mattace-Raso et al., 2009). In addition, the use of oral contraceptives in humans (Campesiet al., 2012), and estrogen treatment in ovariectomized mice (Ulas and Cay, 2011) were both associated with increased levels of serum iron and total iron-binding capacity.

The intensity and duration differences in physical exercise result in various reactions of hematologic parameters and iron status. Most researchers investigated the acute effect of a period of physical activity on the iron status while few researchers investigated the effects of a prolonged training period (adaptation to training) especially on menopausal females (Shabkhiz et al., 2009).

In face of this controversy, the role of female sex hormones in the regulation of iron homeostasis and the involvement of this disturbed iron homeostasis in the pathogenesis of the most prevalent post-menopausal diseases. The present work was carried out to assess serum hepcidin, IL-6 levels and their influence on iron status in an experimental model of induced menopause; Moreover, evaluating the modulatory effect of different intensities of training exercise programs on serum hepcidin, IL-6 levels and iron status aiming to minimize /or attenuate the possible existing post- menopausal iron imbalance was performed.

## Materials and methods

### Animals

Forty adult female Wistar albino rats weighing 200-240g and aged 14 weeks old, supplied from faculty of Veterinary Medicine, Zagazig University, were enrolled in the present study. Rats were housed in stainless steel rodent cages under environmentally controlled conditions and were allowed one week for acclimatization at room temperature (23  $\pm$  2°C), with a 12 hour dark / light cycle before beginning the experimental work. During the acclimatization period and throughout the study period, rats were kept in the animal unit of the physiology department, faculty of Medicine, Zagazig University. They were fed the standard commercial rodent chow and had free access to water. All surgical procedures and protocols used were approved by the Zagazig University Ethical Committee and were conducted in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

### Experimental design:

After one week of acclimatization, rats were randomly divided into: four equal groups (n = 10, for each). **Group1:** non ovariectomized Sham-operated sedentary group (SHAM/sG). **Group2:** ovariectomized sedentary group (OVX/sG). **Group3:** Ovariectomized moderately trained (adaptive) exercise group (OVX/ MEG). **Group4:** Ovariectomized strenuously trained exercise group (OVX/SEG).

***Oophorectomy procedure:***

At the onset of the study, OVX rats were bilaterally ovariectomized under anesthesia (ketamine, 100 mg/kg; xylazine, 5 mg/kg i.p., purchased from Sigma Chemicals) and, L-shaped incision was made on either side of the abdominal wall. The ovaries were dissected and removed. The surgical wound was sutured and waterproof plaster was applied. A single dose of Penicillin (100,000 units) was given to these rats to prevent infection, and allowed to recover from surgery. The SHAM animals were subjected to the same general surgical procedure as OVX groups; except that the ovaries were not excised (**Ganaraja et al., 2004**). The exercise was initiated one week after recovery from ovariectomy (**Souza et al., 2007**).

***Exercise regimen:***

Swimming was selected as our exercise regimen as it belongs to the natural behavior of rodents. In addition swimming is less stressful and can avoid foot injury, which may generate an unexpected iron reaction to exercise (**Souza et al., 2007**).

**Sedentary groups:** remained sedentary in a pool filled with water to a depth of 5cm, when the exercise rat groups swam in swimming pool (130cm×90cm×70cm) filled with water to a depth of 50cm (**Brasse-Lagnelet et al., 2011**), the water temperature was maintained at 32±1c.

**-Moderately trained exercise group:** The rats were initially acclimatized to the exercise of swimming for longer duration from about 15 minutes on the first day to 2 hours by the third week. Then, they swam 2 hours/day for the remainder of the 10 weeks (**Elhaimour et al., 2003**).

**-Strenuously trained exercise group:** The rats followed the same above exercise program, but they were trained to swim with different loads. The loads were increased by 1% from the 2<sup>nd</sup> week to the 10<sup>th</sup> week. Lead pieces (different from 2 to 10% of rats' body weight) were attached to the tails as loads, so their leg movements were not limited (**Dawson, and Horvath 1970; Elhaimour. et al., 2003**)

***Blood samples***

Blood was collected following 10 weeks of swimming. The rats were fasted for 16 h before blood sampling. The rats were anesthetized with 0.4% pentobarbital sodium (1ml/100 g body weight), and were sacrificed by decapitation 36 h after the last exercise (**Liu et al., 2011**). Blood samples were drawn into three eppendorf tubes, one with ethylenediamine tetra acetic acid (EDTA, K<sub>2</sub>) as an anticoagulant, while the other two with serum separator.

***Assessment of serum iron status***

-The blood samples with an anticoagulant were taken for hemoglobin concentration (Hb), hematocrit value (PCV), and mean corpuscular hemoglobin (MCH), using an automatic blood analyzer (Coulter LH 750 Hematology Analyzer).

-The other two tubes with serum separator were centrifuged within 2 hours for 15 min at 5000 rpm/min at 4 °C and the supernatant was collected and frozen at -70 °C in iron free tubes (**Liu et al., 2006**). Serum samples were assayed later for:

- Serum Iron (SI), total iron binding capacity (TIBC), and transferrin saturation % (TS). All were determined by using commercially available kits, and according to the method described by **Burits and Ashwood, 1999**.

- Serum ferritin (SF) and serum soluble transferrin receptor (sTfR) were assayed using commercial rat enzyme linked immunosorbent assay (ELISA) kits (R&D systems, Minneapolis, MN, USA), with an intra-assay coefficient of variation of 4% and intersay coefficient of variation of 10% (**Berenshtein, et al., 2002, Davis et al., 2008**).

***Assessment of serum hepcidin and interleukine 6:***

Serum levels of hepcidin and IL<sub>6</sub> were quantified by applying; rat serum hepcidin ELISA kit (USCN life Co., Houston, TX, USA) & rat serum IL<sub>6</sub> ELISA kit (R&D Systems, Inc., Minneapolis, MN) and according to manufacturer's instruction.

All pervious parameters were measured by using commercial kits

***Statistical methods***

Statistical methods used in this study for analysis of data were, according to the statistical analysis, SPSS released 10.0 program for Windows (SPSS Inc. Chicago, IL, USA). All data were expressed as mean (SD (Standard Deviation)). Analysis of variance (one way ANOVA of F test): Used for comparison of means of more than two groups. The correlation between serum hepcidin with IL<sub>6</sub> as well as parameter assaying iron status were tested with spearman rank correlation. All data were considered statistically significant if P<0.05

**Results*****-Hepcidin, iron status and IL<sub>6</sub> in the OVX groups:-***

The conducted results revealed significant elevation in serum Hepcidin and IL<sub>6</sub> in the OVX sedentary group compared to their levels in the non OVX rats (p<0.05 & p<0.01 respectively). The elevations in Hepcidin and IL<sub>6</sub>

were associated with changes in the iron status, which was represented by significant decreases in Si ( $p<0.05$ ), Hb ( $p<0.05$ ), PCV ( $p<0.05$ ), MCH ( $p<0.05$ ), Tsat (%) ( $p<0.05$ ) & SF saturation ( $p<0.01$ ), as well as significant increases in TIBC and sTfR in ( $p<0.05$  &  $p<0.01$  respectively) (Tables 1 & 2).

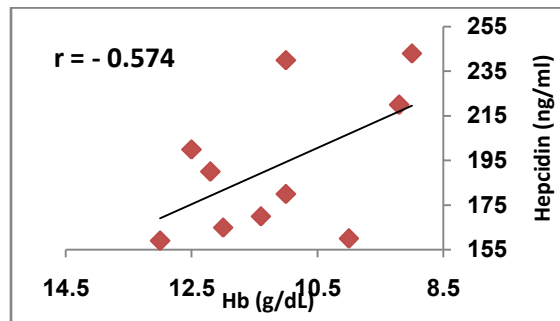
**Table (1): iron status in sham operated sedentary group (SHAM/sG), ovariectomized sedentary group (OVX/sG), ovariectomized moderately trained exercise group (OVX/ MEG), and ovariectomized strenuously trained exercise group (OVX/ SEG) (n=10/ group).**

Parameters	SHAM/sG	OVX/sG	OVX/MEG	OVX/SEG
Hb (g/d)	14.02±2.14	11.74 ± 2.38 <sup>a</sup>	13.91 ± 3.62 <sup>b</sup>	9.57 ± 2.14 <sup>aab</sup>
PCV (%)	43.8 ± 9.12	36.3 ± 10.17 <sup>a</sup>	42.63 ± 7.19 <sup>b</sup>	29.8 ± 6.39 <sup>aab</sup>
MCH (pg)	18.53 ± 4.74	14.74 ± 3.93 <sup>a</sup>	18.136 ± 4.86 <sup>b</sup>	11.50 ± 3.27 <sup>aab</sup>
SI (µg/dl)	190.43 ± 45.16	154.46 ± 41.49 <sup>a</sup>	188.35 ± 49.57 <sup>b</sup>	121.54 ± 33.90 <sup>aab</sup>
TIBC (µg/dl)	601.9 ± 94.09	681.20 ± 103.03 <sup>a</sup>	602.8 ± 59.27 <sup>b</sup>	750.6 ± 90.38 <sup>aab</sup>
Tsat (%)	29.52 ± 7.72	21.02 ± 6.7 <sup>a</sup>	28.56 ± 9.28 <sup>b</sup>	5.54 ± 1.44 <sup>aaabbb</sup>
SF (ng/ml)	40.62 ± 10.21	28.0 ± 8.8 <sup>aa</sup>	39.22 ± 9.44 <sup>b</sup>	52.83 ± 10.62 <sup>aaabbb</sup>
sTfR (ng/ml)	4.34 ± 1.22	6.91 ± 1.55 <sup>aa</sup>	5.02 ± 1.33 <sup>b</sup>	8.44 ± 2.75 <sup>aaabbb</sup>

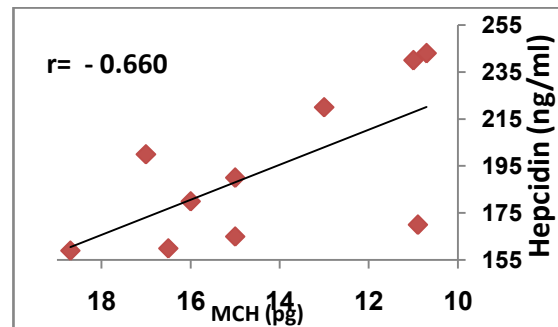
Values are means ± standard deviation ( $\bar{x} \pm SD$ ). Least significant difference (LSD) among values was analyzed by one way ANOVA, when the Interaction was significant ( $P<0.05$ ). <sup>a</sup> $P<0.05$ , <sup>aa</sup>  $P<0.01$  & <sup>aaa</sup>  $P<0.001$  versus SHAM/sG group and). <sup>b</sup> $P<0.05$ , <sup>bbb</sup> $P<0.001$  versus OVX/sG

#### **-Hepcidin, iron status and IL<sub>6</sub> in the OVX exercised groups:-**

As regards, the OVX rats that followed moderately trained exercise for 10 weeks, both serum levels of hepcidin, and IL<sub>6</sub> were nearly improved back to the pre-ovariectomized values with non-significant differences between their mean values after exercise and mean values in the SHAM/sG ( $P>0.05$  for both).



**Figure (1): Spearman, s correlation between serum Hepcidin level (ng/ml) and Hb concentration (g/dL) ( $r=-0.574$ ,  $P<0.05$ ) in the ovariectomized sedentary group (OVX/sG) n=10.**



**Figure (2): Spearman, s correlation between serum Hepcidin level (ng/ml) and MCH (g/dL) ( $r=-0.660$ ,  $P<0.01$ ) in the ovariectomized sedentary group (OVX/sG) n=10.**

Also, these decreases in hepcidin and IL<sub>6</sub> were accompanied by a correction in the disturbed iron status. As there were non-significant changes between almost all parameters assaying iron status after 10 weeks of moderate exercise versus non-ovariectomized sedentary group ( $P>0.05$  for all) (Table 1 & 2).

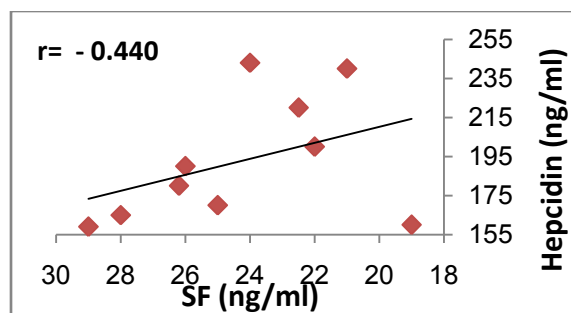


Figure (3): Spearman, s correlation between serum Hepcidin level (ng/ml) and serum ferritin (SF) g/dL ( $r=-0.440, P<0.05$ ) in the ovariectomized sedentary group (OVX/sG)  $n=10$ .

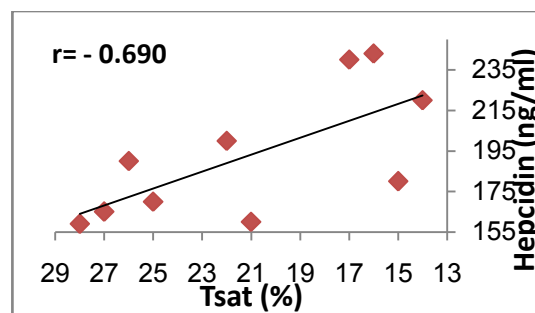


Figure (4): Spearman, s correlation between serum Hepcidin level (ng/ml) and transferrin saturation % (Tsat%) ( $r=-0.690, P<0.01$ ) in the ovariectomized sedentary group (OVX/sG)  $n=10$ .

However, in the ovariectomized rats that followed strenuously trained exercise for the same period, both hepcidin and  $IL_6$  were significantly higher than their estimated levels in OVX/sG ( $p<0.05$  &  $p<0.01$  respectively), and the iron status had further significant deterioration than its values in the OVX/sG. As, Serum iron, Hb, MCH, PCV, and Tsat (%) were significantly decreased when compared to their values in OVX /sG ( $p<0.05$  for all &  $p<0.001$  for Tsat). In addition, these decreases in the previous parameters were paralleled with significant increases in TIBS and sTfR ( $p<0.001$  for both) (Table 1&2)...

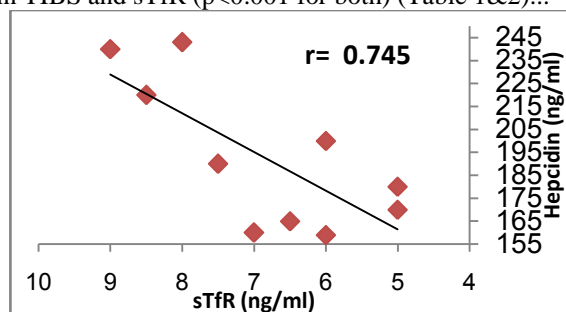


Figure (5): Spearman, s correlation between serum Hepcidin level (ng/ml) and serum soluble transferrin receptor (sTfR) concentration ng/ml ( $r=0.745, P<0.001$ ) in the ovariectomized sedentary group (OVX/sG)  $n=10$ .

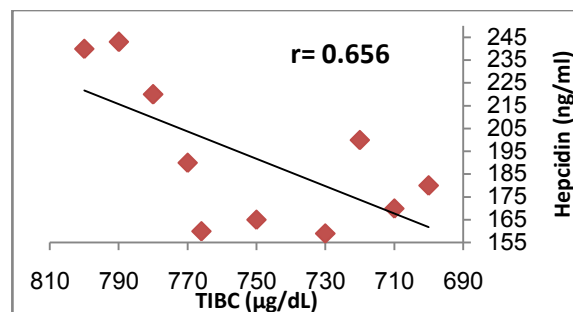


Figure (6): Spearman, s correlation between serum Hepcidin level (ng/ml) and Total iron binding capacity (TIBC)  $\mu\text{g/dL}$  ( $r=0.656, P<0.01$ ) in the ovariectomized sedentary group (OVX/sG)  $n=10$ .

The significant increase in SF value of OVX/SEG versus SF of both SHAM/sG & OVX/sG may be explained by the presence of the other factor affecting SF level rather than the change in the serum iron level e.g. acute phase reactant associated with this type of exercise.

Table (2): Serum hepcidin and  $IL_6$  levels in in sham operated sedentary group (SHAM/sG), ovariectomized sedentary group (OVX/sG), ovariectomized moderately trained exercise group (OVX/ MEG), and ovariectomized strenuously trained exercise group (OVX/ SEG) ( $n=10$ / group).

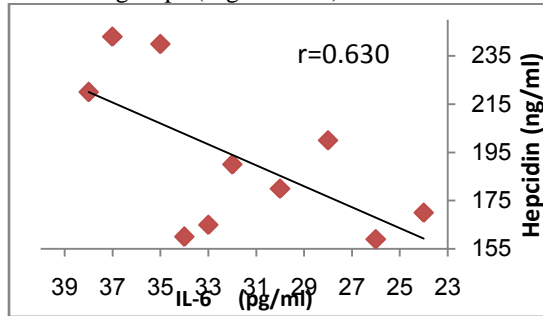
Parameters	SHAM/sG	OVX/sG	OVX/MEG	OVX/SEG
Hepcidin (ng/ml)	161.28 $\pm$ 42.07	203.30 $\pm$ 39.80 <sup>a</sup>	166.61 $\pm$ 53.65 <sup>b</sup>	236.24 $\pm$ 45.69 <sup>aab</sup>
$IL_6$ (pg/ml)	21.18 $\pm$ 4.95	31.08 $\pm$ 6.99 <sup>aa</sup>	22.09 $\pm$ 3.22	41.51 $\pm$ 9.52 <sup>aaabb</sup>

Values are means  $\pm$  slandered deviation ( $\bar{x} \pm SD$ ). Least significant difference (LSD) among values was analyzed by one way ANOVA, when the Interaction was significant ( $P<0.05$ ).<sup>a</sup> $P<0.05$ , <sup>aa</sup> $P<0.01$  & <sup>aaa</sup> $P<0.001$  versus SHAM/sG group and). <sup>b</sup> $P<0.05$ , <sup>bb</sup> $P<0.01$  versus OVX/sG

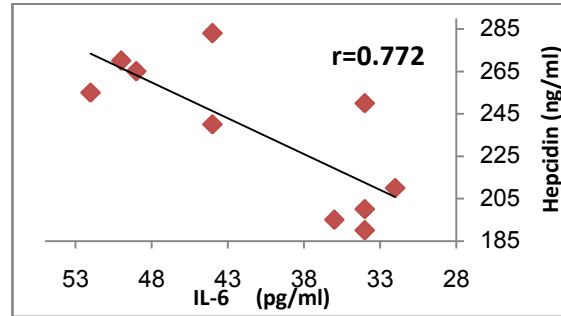
### -The correlation between hepcidin and iron status parameters and IL<sub>6</sub>.

Spearman's correlation was used to evaluate any correlations between hepcidin, and IL<sub>6</sub> as well as indicators of iron status in the studied groups. There were negative correlations between serum hepcidin level and Hb; MCH; SF and Tsat% in the OVX sedentary group. Moreover, there were positive correlation between serum hepcidin level and TIBC&TfR in the same group (Figures 1-6).

Also, hepcidin levels were positively correlated with serum IL6 levels in both OVX sedentary and OVX strenuously exercised groups (Figures 7&8).



**Figure (7): Spearman, s correlation between serum Hepcidin level (ng/ml) and serum IL-6 level (pg/ml) ( $r=0.630$ ,  $P<0.01$ ) in the ovariectomized sedentary group (OVX/sG)  $n=10$ .**



**Figure (8): Spearman, s correlation between serum Hepcidin level (ng/ml) and serum IL-6 level (pg/ml) ( $r= 0.772$ ,  $P<0.001$ ) in ovariectomized strenuously trained exercise group (OVX/ SEG) $n=10$ .**

## Discussion

Iron is an essential element for humans, being involved in oxygen transport, energy metabolism and DNA synthesis. Iron homeostasis is tightly governed by the hepcidin–ferroportin axis, of which hepcidin is the master regulator (Nemeth and Ganz, 2009). Excess iron is associated with various diseases, including cancer breast and osteoporosis, which are closely related to alternation in the endogenous estrogen (Huang, 2008; Jian et al., 2009). The regulation of hepcidin by female sex hormones has not yet been decided. So, the conducted work established a model of female sex hormone deficiency induced by ovariectomy to verify the biological effect of female sex hormones on iron metabolism.

Unlike animals, the serum iron may be elevated after ovariectomy or in the early stage of the menopausal period in humans, due to the cessation of menstrual bleeding. This is usually followed by reducing serum iron induced by increased iron demand in the subsequent periods of the menopause as a result of decreased physical activity. So, there are different factors influencing iron levels in humans, and masking the proper effect of female sex hormones on iron metabolism.

However, the female rats do not experience menstrual bleeding. So, the only regulatory mechanism affecting iron metabolism after ovariectomy is expected to be through a deficiency in female sex hormones governing iron-hepcidin axis (Borràs, 1998).

In the present study, serum hepcidin and IL<sub>6</sub> were significantly elevated in ovariectomized sedentary rats compared to the control sham operated group. These elevations were accompanied by a significant reduction in different iron parameters.

In concordance with the present findings, the previous clinical studies conducted by Casabellata et al., (2007) detected time-dependent increase of iron stores in oral contraceptive female users compared to non-users, and Galeslout et al., (2011) & Itkonen et al., (2011) who demonstrated that serum hepcidin levels were lower in women than in men. Moreover, the pre-menopausal women had lower serum hepcidin concentrations than postmenopausal women.

On the other hand, the experimental studies reported that hepatic hepcidin mRNA is inhibited in mice by nanomolar concentrations of E<sub>2</sub>, which is physiologically or pharmacologically relevant to young women at the preovulatory phase or those taking contraceptives (Schiavon et al., 1988). Furthermore, Qing Yan et al., (2012) discovered an estrogen response element (ERE) in the promoter region of the hepcidin gene of murine models. They postulated that estrogen greatly contributes to iron homeostasis by regulating hepatic hepcidin expression directly through a functional ERE in the hepcidin gene promoter. Whereas, binding of estrogen to this site in the murine hepcidin gene was reported to down-regulate hepcidin transcription and increased iron release. This effect can be reversed by the addition of estrogen antagonists (ICI 182780).

Also, it was proved that the elevation in estrogen levels was associated with suppressed production of IL-6 and other inflammatory cytokines that induce hepcidin synthesis (**Hamad M1.and Awadallah, 2013**).

But in controversy to the conducted results, the study carried by **Ikeda et al., (2012)** reported that 17 $\beta$ -estradiol (E2) increased the expression of hepcidin mRNA in mice hepatic G (HepG2) cells in a concentration-dependent manner. In vitro. E2-induced hepatic hepcidin up-regulation which was not inhibited by ICI 182720, an inhibitor of the estrogen receptor, instead, hepcidin expression was increased by ICI 182720. The discrepancy between the results of our study and that of the previous study could be explained by species difference and differences in the experimental models (in vivo versus in vitro).

Therefore, from the interplay between the present results and the previously mentioned studies, we can suggest that the female sex hormones have the potential to directly reduce hepcidin synthesis through affection of hepcidin gene expression and/ or inhibition of the pro inflammatory cytokine IL<sub>6</sub>.

Hence, these effects appear to increase the serum iron level compensating for its loss during the reproductive period in humans.

As regard the modulatory effect of different exercise intensities on the changes in serum hepcidin level and consequently on iron status after ovariectomy, our results detected that 10 weeks of moderately trained exercise decreased the elevation in serum hepcidin and improved the iron status observed in the ovariectomized sedentary group. However, strenuous exercise for the same duration induced a further significant increase in hepcidin level as well as adding more worsening in the iron status than the ovariectomized sedentary group.

Also, the results revealed a positive correlation between the changes in the serum hepcidin and the changes in the serum IL<sub>6</sub> of that exercised groups.

In agreement with these findings, the results obtained from pervious sport researches performed by **Mettler and Zimmermann, (2010)** demonstrated that excess body iron may be common in male recreational marathon runners. Furthermore, **Shabkhiz et al., (2009)** stated that moderate continuous aerobic training for 12 weeks can increase serum iron and hematological parameters in female rats after menopause

Also, **Liu et al., (2006)** detected that moderate physical exercise could promote a physio-adaptation to exercise. As, they detected that 10 weeks of moderate physical exercise in female rats decreased expression of hepcidine mRNA and increased the expression of divalent metal transporter 1 (DMT1) and iron exporter ferroportin (FPN1). DMT1 in the apical membrane of the enterocyte increased iron absorption, and FPN1 in the basolateral membrane increased iron transfer to the circulation. The net results were increased serum iron level, improvement in iron status and development of adaptation to exercise.

However, in contrary to the present results, **Troadec et al.,(2009)** found that submaximal cycling exercise e.g. low intensity exercise did not significantly change serum iron, and serum or urinary hepcidin levels in healthy untrained individuals. In addition, **Bourquet al.,(1997)** demonstrated that participation in 12 weeks of moderate-intensity endurance exercise training ; walking/running or cycling was not associated with changes in measured iron status in healthy, previously untrained women with normal iron stores

The differences between the conducted results and the last studies may be explained by differences in the species and/or the difference in the applicable exercise programs, whence the type, duration and training.

On the other aspect, in concordance with present results many studies carried on animals and human detected increases in serum hepcidin level and decreased in iron levels after strenuous exercise (**Banzet et al., 2012; Auersperger et al.,2012;Antosiewicz et al., 2013**). Moreover, **Liu et al., (2011)** demonstrated that an increase in hepatic hepcidin expression after strenuous exercise was associated with decreased expression of FPN1 at the enterocytes of exercise group compared with control group which ultimately blocked the release of iron from them.

Whereas, **Helge et al., (2003)** recognized that contracting skeletal muscle may synthesize and release IL<sub>6</sub> into the interstitium as well as into the systemic circulation in response to the bouts of exercise. Also, **Bergfors et al., (2005)** found that the exercise-induced IL<sub>6</sub> response was dependent on the intensity and magnitude of exercise. As, the strenuous exercise which involves several large muscle groups leads to dramatic increases in plasma IL<sub>6</sub>. However, moderate trained exercise reduces plasma IL<sub>6</sub> production that appears as a character of normal training adaptation (**Fischer, 2006**). So, the modulation of IL<sub>6</sub> synthesis by different exercise programs intensities may be involved in the alternations of hepcidin transcription and subsequently its serum levels associated with these physical activities.

## Conclusion

Experimentally induced menopause was associated with elevated serum hepcidin and IL6 and deteriorated iron status, which were nearly reversed back to pre-ovariectomized values after moderately adaptive but not strenuous exercise.

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