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Elevated Levels of Estrogen Suppress Hepcidin Synthesis and Enhance Serum Iron Availability in Premenopausal Women

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Key words
Estrogen, hepcidin, serum iron, premenopausal women

received 09.10.2017
first decision 26.11.2017
accepted 29.11.2017

Bibliography
DOI https://doi.org/10.1055/s-0043-124077
Published online: 2018
Exp Clin Endocrinol Diabetes

ISSN 0947-7349

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ABSTRACT

Clinical and experimental observations have long suggested that elevated levels of estrogen associate with increased serum iron availability. Additionally, recent work has shown that estrogen can downregulate hepcidin synthesis in vitro. This study aims at assessing whether the ability of estrogen to downregulate hepcidin synthesis translates into changes in serum iron status. Hepcidin synthesis was evaluated in MCF-7, Hep-G2 and SKOV-3 cells treated with increasing concentrations of estrogen and cultured for up to 24 h post treatment. The correlation between levels of serum estrogen, hepcidin and iron was assessed using serum samples collected from 153 premenopausal women at random and samples collected from 6 women at days 1, 5, 10, 16, 21 and 28 of the monthly cycle. Estrogen-treated MCF-7 cells showed a significant reduction in hepcidin synthesis, especially at 20 nM/24 h E2 treatment. Hepcidin synthesis was also significantly reduced in Hep-G2 and SKOV-3 cells at 20 nM/24 h E2 treatment. In serum samples collected at random, estrogen ($P = 0.022$; $R = -0.213$) and iron ($P = 0.028$; $R = -0.316$) correlated negatively with hepcidin and positively with each other ($P = 0.033$; $R = 0.319$). An overall similar pattern was also observed in monthly cycle-timed samples. These findings suggest that elevated levels of estrogen reduce hepcidin synthesis as means of enhancing serum iron content in menstruating women.

Introduction

Owing to its significant redox potential and its ability to readily alternate between oxidized and reduced states, iron plays an essential role in the survival and growth of almost all types of cells and organisms. For example, iron serves as a co-factor in numerous proteins involved in cellular energetics, DNA replication, and metabolism. However, excess iron under aerobic conditions leads to the generation and propagation of free radicals through Fenton chemistry. Mammals therefore have evolved intricate mechanisms to tightly regulate iron absorption and release. Ferroportin (FPN) on duodenal enterocytes and reticuloendothelial macrophages efflux iron into the circulation, where it combines with transferrin to then be delivered to target cells though transferrin receptor 1 (TfR1). Iron efflux through FPN is negatively regulated by hepcidin which triggers the internalization, phosphorylation and degradation of FPN [1–3]. Increased demand for iron downregulates hepcidin synthesis via hypoxia inducible factor 1α (HIF-1α) [4] and growth differentiation factor 15 (GDF15) [5] among other potential regulatory proteins. In contrast, decreased demand for iron and inflammation (e. g. IL-6), upregulate hepcidin synthesis [6].

Besides variations in demand and inflammation, there is evidence to suggest that iron homeostasis is influenced by sex hormones; estrogen (E2, 17-β estradiol) in particular [7]. While ovariectomy in rodents leads to decreased serum iron concentration, E2 treatment in ovariectomized mice [8] and oral contraceptive use in humans [9, 10] enhance serum iron availability. Elevated levels of E2 have been shown to associate with upregulated expression of several genes (lactotransferrin, ferroxidase ceruloplasmin, lipocalin 2 and ferroportin) involved in iron metabolism [11]. The exact mechanism underlying the role of E2 in iron homeostasis is not fully clear. Previous studies have suggested that E2 upregulates the expression of HIF-1α in cultured ovarian cells (ES-2 and SKOV-3) [4, 12]; an effect that may negatively impact hepcidin gene expression. This is further supported by the observation that hepcidin mRNA levels are significantly reduced in a mouse model of ovariectomy-related E2 deficiency [13, 14]. Therefore, it is possible that the positive correlation between elevated levels of E2 and serum iron concentration could be explained by the ability of E2 to downregulate hepcidin synthesis in hepatocytes and other cell types. To test this possibility, the capacity of E2 to alter hepcidin synthesis was evaluated in vitro using MCF-7 (breast adenocarcinoma), Hep-G2 (hepatocellular carcinoma) and SKOV-3 (ovarian adenocarcinoma) cell lines. The correlation between E2, hepcidin, and serum iron status was assessed using serum samples collected from premenopausal women at random and at specific time points during the monthly cycle.

Materials and Methods

Cell culture and treatment

Cell lines MCF-7, Hep-G2 and SKOV-3 (ATCC, Manassas, VA, USA) were used in this study; all three cells lines are E2 receptor (ER)-α^+ and hence responsive to E2 treatment. MCF-7 and Hep-G2 cells were maintained in Dulbecco's modification of Eagle's medium (DMEM) (Sigma Aldrich Aldrich) supplemented with 2 µg/ml insulin, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 4 mM glutamine, 10 % fetal calf serum, and antibiotics (penicillin / streptomycin) at 37 °C and 5 % CO2. SKOV-3 cells were maintained in McCoy′s 5 A medium (Sigma Aldrich Aldrich) supplemented with 2 mM Glutamine, 1 mM sodium pyruvate, 15 % FBS, 1 % antibiotics (penicillin / streptomycin) at 37 °C and 5 % CO2. For E2 treatment, cells were seeded at 5×10^4 cells/ml in 25 cm flasks, at ~70 % confluency, cells were treated with E2 (Oestradiol benzoate [Folone]; Misr Co, Egypt) at 5, 10 or 20 nM and cultured for 6 or 24 h prior to harvesting and assessment. Control cells were left untreated or were treated with equal volumes of the vehicle (ethanol) [15].

Western blotting

Western Blotting was carried out as described previously [16]. Briefly, cells were lysed with ice-cold RIPA buffer containing protease cocktail inhibitor tablets (Catalog No. S8830; Sigma Aldrich). Protein concentration in whole cell lysates was quantified using the standard Braford method (Catalog No. 500-0006; BioRad). Lysate aliquots containing 30 µg protein were separated by 12 % SDS-PAGE gel electrophoresis and transferred onto a Polyvinylidene difluoride (PVDF) membrane (Catalog No. 162- 0177; Biorad). The membrane was blocked with 7 % skimmed milk powder for 1 h at room temperature, washed with T-TBST and reacted with the primary anti-hepcidin IgG1 antibody (Catalog No. 57611; Abcam) at 1:1000 dilution overnight at 4 °C. The secondary anti-IgG antibody (Catalog No. 97040; Abcam) was reacted with the membrane at 1:5000 dilution for 1 h at room temperature. Chemiluminescence was detected using the ECL kit (Catalog No. 32106; Thermo Scientific Pierce). Hepcidin quantification was carried out using the Bio-Rad Bio-Rad Image Lab software (ChemiDoc™ Touch Gel and Western Blot Imaging System, Bio-Rad, Hercules, CA) and the online Image J software, National Institute of Health (NIH), USA (http://rsb.info.nih.gov/ij/index.html). β-actin was used as a normalization control and values of control (untreated) samples were defined as 1.00; values of experimental samples were quantified as relative to that of control.

Immunofluorescence

Staining and analysis of cells for immunofluorescence was carried out as described previously [16]. Briefly, cells were seeded at 10^4/ml on sterile polylysine-coated glass coverslips in 6-well culture plates, 48 h later, cells were starved for 12 h prior to treatment with E2. Cells on slides were then washed with PBS and fixed with 4 % paraformaldehyde for 15 min at room temperature and treated with 0.1 % Triton X-100 for 10 min. Fixed and permeabilized cells were blocked with BSA at 3 % for 1 h, rinsed with 1X PBS and incubated with the primary anti-hepcidin IgG1 antibody (Catalog No. 57611; Abcam) at 5 µg/ml overnight at 4 °C. Samples were subsequently washed with 1X PBS and incubated with the Alexafluor®$_{680}$-labeled secondary antibody (Abcam) for 1 h at 37 °C; excess reagent was rinsed with 1X PBS. Genomic DNA was stained with 4′, 6′-diamidino-2-phenylindole (DAPI) (Catalog No. D1306; Invitrogen) according to manufacturer's instructions. Slides were visualized by fluorescence microscopy using an Olympus BX51 fluorescent microscope (Olympus Corporation, Tokyo, Japan).

RT-PCR

RNA was isolated using TRIzol Tri reagent (Catalog No. 93289; Sigma Aldrich) according to manufacturer's instructions. 1 µg of total RNA was reverse transcribed in a 20 µl reaction volume containing random primers and Go-Script reverse transcription mix. Quantitative RT-PCR was carried out using the GoTaq system (Catalog No. A6010; Promega) and the reaction was run on a Rotor-Gene Q5 RT-PCR cycler (Qiagen corporation, Hilden, Germany). Primers used to test for hepcidin gene expression were (forward: 5′-CTGTTTTCCCACAACAGACG-3′; reverse: 5′-CAGCACATCC-CACACTTTGA-3′) and that for the internal normalizing control GAPDH were (forward: 5′-CCAGGTGGTCTCCTCTGACTTC-3′; reverse: 5′-TCATACCAGGAAATGAGCTTGACA-3′).

Serum sample collection

Blood samples were collected from 153 young healthy women aging 19–43 years (average 29 + 7.36 years) and from 6 young women aging 22–35 year at days 1, 5, 10, 16, 21 and 28 of their monthly cycles. Pregnant women, women on E2-based contraceptives and those on corticosteroid or hormonal replacement therapy were excluded from the study. The study was approved by the University of Sharjah research ethics committee. Participants (na-

tionals and residents of the United Arab Emirates) were informed of the goals of the study and asked to sign a consent form on the understanding that their personal information will be kept confidential. Serum was collected from fresh blood samples and stored frozen until further use.

Measurement of serum levels of E2, hepcidin, ferritin, total iron binding capacity (TIBC), and iron

Commercially available ELISA kits were used to measure the concentration of E2, hepcidin, and ferritin (DRG diagnostics, Frauenbergstr, Marburg, Germany) using a Hospitex Diagnostics ELISA plate reader (Hospitex Diagnostics V.S.4e, Sesto Fiorentino, Italy). Serum iron concentration and total iron binding capacity were assessed using commercially available colorimetric assay kits (BioMèreux, Marcy-ÍEtoile, France). Serum ferritin and TIBC were only tested in timed monthly-cycle samples; all tests were done according to manufacturer's instructions.

Statistical analysis

For western blot and RT-PCR data, independent t test was used to generate P values for comparisons between different groups in each data set (Graph Prism Pad 5, GraphPad Software Inc., La Jolla, CA); $P < 0.05$ was considered as significant. For serum samples, data analysis was carried out using the SPSS statistical software package (version 17.0; SPSS Inc, Chicago, IL). The presence of significant differences in the mean values of E2, ferritin, hepcidin, iron and TIBC among the various E2-based groups was evaluated by independent sample tests (Levene's test for equality of variance and t-test for equality of means), $P < 0.05$ was considered as significant. As the data set derived from serum samples taken from premenopausal women was not normally distributed and as sample size for timed samples was small, mean values and levels of correlation (statistical dependence) between various paired combinations of variables was assessed using the nonparametric Spearman's correlation (ρ) test; correlation was considered as significant at 0.05 level (2-tailed).

Results

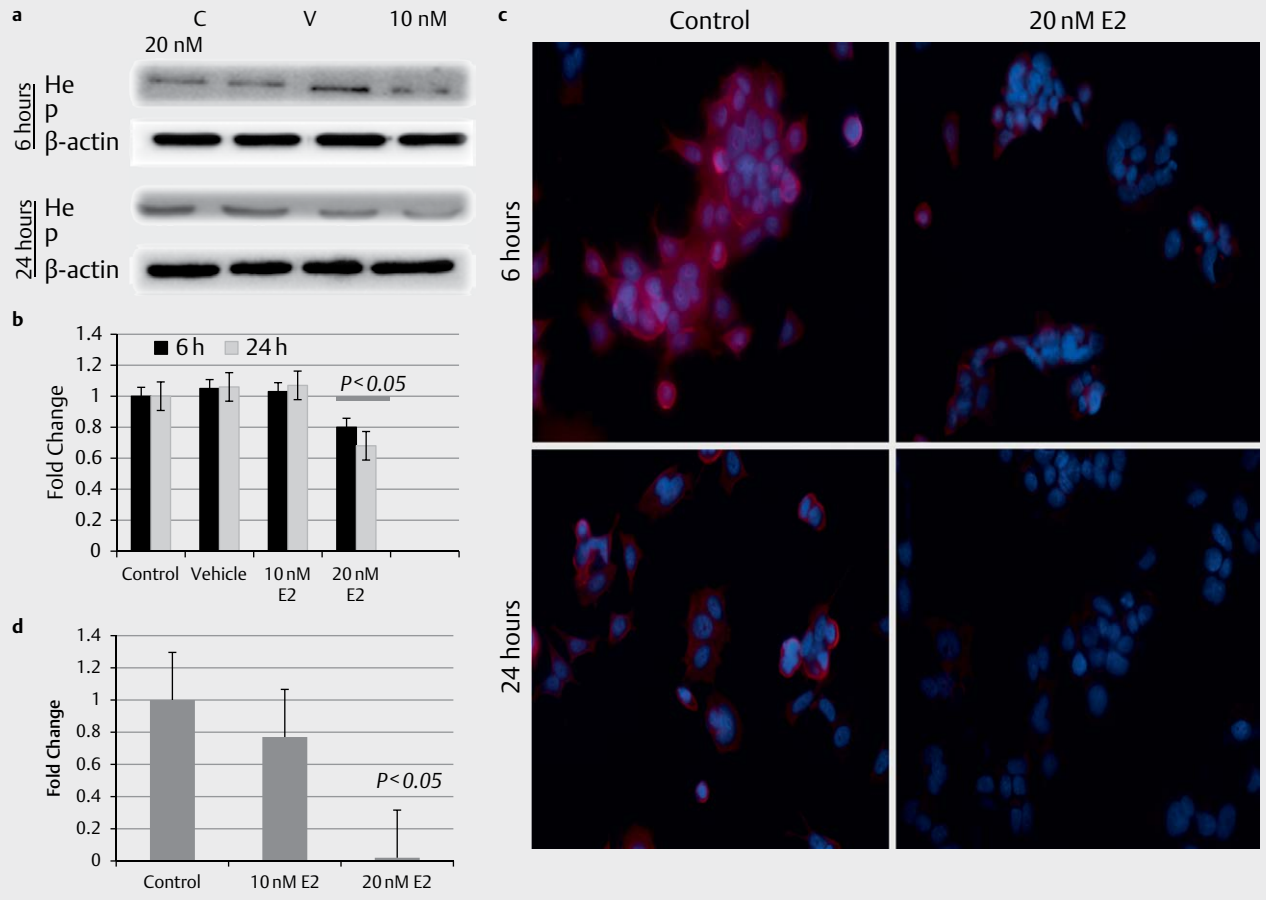
To investigate the effect of E2 on hepcidin synthesis, MCF-7, Hep-G2 and SKOV-3 cells were treated with increasing concentrations of E2 and evaluated for hepcidin synthesis at different time points post treatment. As shown in ▶ **Fig. 1**, cells treated with 10 nM E2 expressed hepcidin at levels slightly higher than that in control cultures. In contrast, cells treated with 20 nM E2 showed reduced levels of hepcidin protein synthesis as compared with that in controls especially at 24 h post treatment (▶ **Fig. 1a-c**). The level of hepcidin mRNA also dropped significantly especially at 20 nM E2 treatment (▶ **Fig. 1d**). To test whether the ability of E2 to downregulate hepcidin synthesis can be replicated in target cells other than MCF-7, Hep-G2 (liver) and SKOV-3 (ovarian) cells were treated with increasing concentrations of E2 and assessed for the expression of hepcidin at 24 h post treatment (▶ **Fig. 2**). E2 treatment resulted in a significant reduction in hepcidin synthesis in both cell types, especially at 10 nM E2 for Hep-G2 and 10 and 20 nM for SKOV-3.

To address the question of whether E2-induced downregulation of hepcidin synthesis at the cellular level translates into detectable changes in serum hepcidin concentration and hence serum iron status, serum samples collected from premenopausal women at random were assessed for E2, hepcidin and serum iron levels. The correlation between E2, hepcidin and serum iron was evaluated by nonparametric Spearman's best fit correlation analysis. As shown in ▶ **Fig. 3**, E2 negatively correlated with hepcidin at a significant level ($P = 0.022$; $R = -0.213$). Furthermore, a significant negative correlation was noted between iron and hepcidin ($P = 0.028$; $R = -0.316$) and a significant positive correlation was noted between E2 and iron ($P = 0.033$; $R = 0.319$) (▶ **Fig. 3**).

To assess whether such an "E2-iron" axis is sensitive to monthly cycle-related changes in E2 concentration, blood samples collected from 6 premenopausal women at days 1, 5, 10, 16, 21 and 28 of the monthly cycle were tested for E2, hepcidin, ferritin, TIBC, and serum iron. ▶ **Fig. 4a** shows the relationship between these 5 parameters based on mean values of 6 samples/time point. Overall and with the exception of iron and TIBC, the concentration of E2, hepcidin or ferritin did significantly change over the various phases of the cycle. Nonparametric best fit correlation however yielded a pattern of correlation between the various parameters (▶ **Fig. 4b**) similar to that seen in serum samples collected from premenopausal women at random (▶ **Fig. 3**). In that, E2 correlated negatively with hepcidin ($P = 0.064$; $R = -0.322$) and positively, but weakly, with iron ($P = 0.488$; $R = 0.143$). As expected, a negative correlation was noted between hepcidin and ferritin ($P = 0.001$; R -0.540); the correlation between hepcidin and iron, though negative, was week ($P = 0.358$; R -0.163) and that between hepcidin and TIBC, though positive, was also weak ($P = 0.273$; R 0.194). TIBC negatively, but weakly, correlated with both iron ($P = 0.794$; R -0.047) and ferritin ($P = 0.314$; R -0.184).

Discussion

Findings presented here suggest that there is a positive correlation between elevated levels of E2 and serum iron concentration and that such a correlation can be explained by the ability of E2 to downregulate hepcidin gene expression. This is based on the observation that serum E2 levels correlated negatively with those of hepcidin and positively with those of iron and that levels of serum hepcidin correlated negatively with those of iron (▶ **Fig. 3** and **4**). It is also based on the finding that in vitro treatment with E2 resulted in a significant downregulation of hepcidin mRNA and protein synthesis (▶ **Fig. 1** and **2**). The latter observation is consistent with previously published reports, which have suggested that E2 treatment suppresses hepcidin mRNA expression [4, 7, 11–14, 17]. It is also consistent with the observation that treatment at 20 nM E2 for 24 h reduces hepcidin protein synthesis and enhances FPN expression [16] and that E2 downregulates FPN gene expression [18]. Previous studies have also shown that E2 treatment enhances the expression HIF-1α, which could in turn downregulate hepcidin synthesis [12]. Inconsistent with our findings however is the observation that E2 treatment upregulates hepcidin gene expression in a GPR30-bone morphogenetic protein 6 (BMP6)-dependent manner [19]. It is possible that the outcome of E2 treatment on intracellular iron metabolism varies depending on the receptor it engages, a possibility has yet to be addressed. It is worth noting that the effects of E2 on hepcidin synthesis and iron metabolism seem

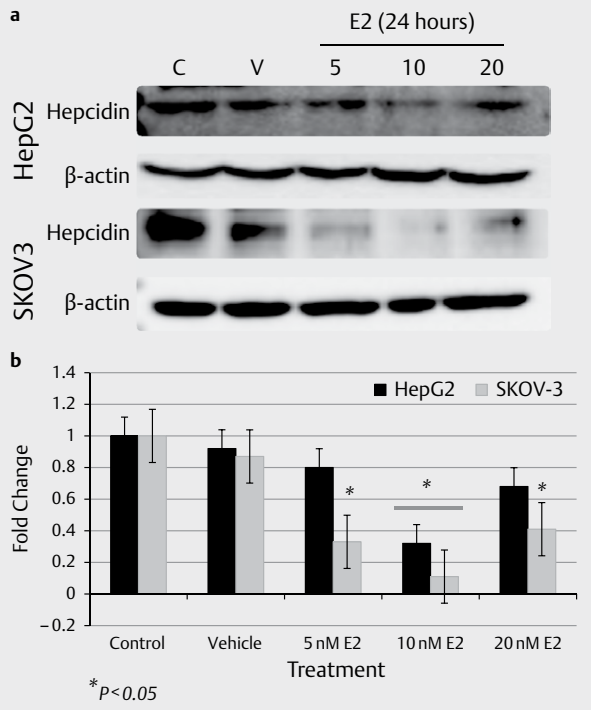


▶ **Fig. 1** The effect of E2 treatment on hepcidin synthesis in MCF-7 cells. **a** Hepcidin protein expression in MCF-7 cells treated with E2 at 10 or 20 nM for 6 or 24 h. **b** Average fold change ± SEM in hepcidin synthesis based on three separate experiments as explained in A. **c** Hepcidin immunofluorescence staining pattern in MCF-7 cells treated with E2 at 20 nM for 6 and 24 h; data shown is representative of three separate experiments each. **d** Levels of hepcidin mRNA isolated from cells treated with 10 or 20 nM E2 for 6 h as well as untreated cells as detected by RT-PCR, GAPDH was used for normalization; data presented as mean ± SEM of three separate experiments where each was run as a triplicate. Independent t test was used to test for the presence of a significant difference in hepcidin protein concertation or mRNA expression as compared with control; $P < 0.05$.

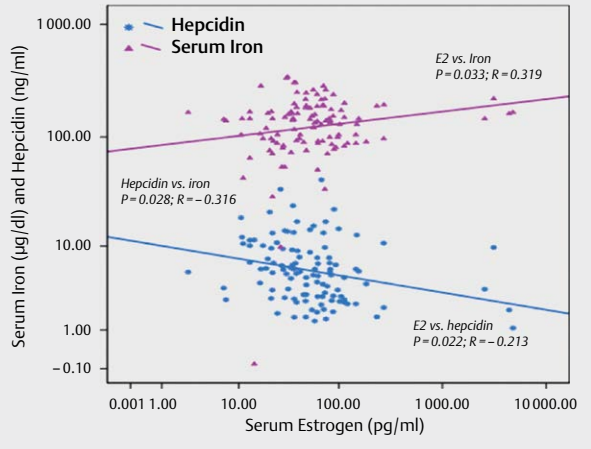
to extend to other sex hormones as elevated levels of testosterone have been reported to enhance hematopoiesis through its ability to suppress hepcidin synthesis and enhance serum iron availability [20, 21].

Given the non-normal distribution of the data set obtained from serum samples collected at random (▶ **Fig. 3**) or at specific time points of the monthly cycle (▶ **Fig. 4a**), it was no surprise that no statistically significant differences in serum hepcidin concentration in relation to E2 concentration were discernable. Nonparametric (spearman's test) statistical analysis however revealed that serum E2 correlates positively with serum iron and negatively with serum hepcidin (▶ **Figs. 3** and ▶ **4**b). This is consistent with previous reports which have suggested that elevated levels of serum E2 associate with increased serum iron availability [7–11, 13]–[14]. Furthermore, the patterns noted in ▶ **Fig. 4b** regarding serum ferritin and serum TIBC in relation to iron are consistent with the well-established understanding of how this set of serum iron indicators relate to serum iron [22, 23]. Taken together, these findings suggest that E2-induced suppression of hepcidin synthesis at the cellular level translates into detectable changes in serum hepcidin levels and serum iron status.

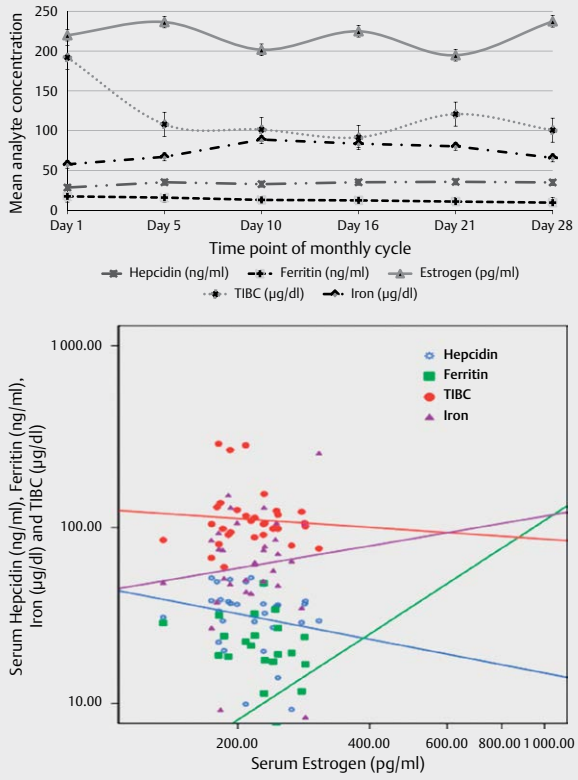
The ability of E2 to act as a regulator of iron homeostasis may relate to the need to compensate for E2-triggered iron loss through menstruation and E2-dependent pregnancy in premenopausal women [11]. As iron deficiency could result from monthly cycle-related changes in E2 and the consequent menstruation and iron loss in premenopausal women, a compensatory mechanism that offsets iron loss is likely to ensue. The ability of E2 to suppress hepcidin synthesis may serve to orchestrate responses mediated by HIF-1α [4] and GDF15 [5] or may serve as a redundant and possibly independent mechanism. In that, while some studies have shown that E2 enhances the expression HIF-1α as means of down-regulating hepcidin synthesis [4, 12], others have suggested that it directly targets E2 response elements within the hepcidin gene [13, 14]. Either way, the involvement of E2 in iron homeostasis seems logical given that its levels are related to blood loss through menstruation and pregnancy and hence partially reflective of systemic iron status.



▶ **Fig. 2** The effect of E2 treatment on hepcidin synthesis in Hep-G2 and SKOV-3 cells. **a** Hepcidin protein expression in Hep-G2 and SKOV-3 cells treated with E2 at 5, 10 or 20 nM for 24 h. **b** Average fold change ± SEM in hepcidin synthesis based on three separate experiments as explained in A. Independent t test was used to test for the presence of a significant difference in hepcidin protein expression as compared with control; P <0.05.



▶ **Fig. 3** The relationship between serum E2, hepcidin and iron in premenopausal women: Best fit correlation between hepcidin and iron in relation to E2 in premenopausal women. Individual values of hepcidin and iron were plotted against E2 concentration (X-axis) on the assumption that E2 is the dependent variable; P and R values were calculated using nonparametric Spearman's test statistic.



▶ **Fig. 4** The relationship between E2, hepcidin and serum iron indicators during various phases of the monthly cycle: **a** monthly cycle-related changes in the concentration of E2 (pg/ml), hepcidin (ng/ml), ferritin (ng/ml), TIBC (µg/dl) and serum iron (µg/dl) were calculated as the mean value/parameter/time point based on samples collected from 6 females at days 1, 5, 10, 16, 21, 28 of the monthly cycle. **b** Serum samples collected the same group of females at the same time points (36 samples in total) were tested for the concentration of E2, hepcidin, ferritin, TIBC and serum iron. Best fit correlation was discerned by plotting individual values of the various parameters against E2 as the dependent variable.

Besides its role in iron homeostasis in premenopausal women, the E2-iron axis may provide further insight into the immunosuppressive [24–31] and anti-carcinogenic effects of E2 [16, 32–35]. The relationship between E2 and iron homeostasis is also worth considering when assessing the risks and benefits of E2-based hormonal replacement therapy [36].

Funding

This work was supported by research grant 15010501005-P/VCGSR (MH), University of Sharjah, UAE and Al-Jalila Foundation research grant AJF201664 (MH), Al-Jalila Foundation Research Center, Dubai, UAE.

Authors' contributions

M. Hamad was responsible for the conception of the idea, literature review, hypothesis formulation, statistical analysis, data interpretation and manuscript preparation. H. Allam and M. Madkour worked on blood sample collection and testing. K. Bajbouj, J. Shafarin, and D Sandeep performed the in vitro studies, western blotting and immunofluorescence assays with the help of A. El-Serafy. S. Awadallah participated in the conception of the idea, statistical analysis, data interpretation and manuscript preparation.

Acknowledgments

We wish to thank the generous in-house support of the Sharjah Institute for Medical Research (SIMR).

Conflict of Interest

All authors declare that they have no conflict of interest regarding any material included in this manuscript.

References

[1] Nemeth E, Tuttle MS, Powelson J et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306 2004; 306: 2090–2093

[2] De Domenico I, Lo E, Ward DM et al. Hepcidin-induced internalization of ferroportin requires binding and cooperative interaction with Jak2. Proc Natl Acad Sci 2009; 106: 3800–3805

[3] De Domenico I, Ward DM, Langelier C et al. The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Mol Biol Cell 2007; 8: 2569–2578

[4] Peyssonnaux C, Zinkernagel AS, Schuepbach RA et al. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). J Clin Inves 2007; 117: 1926–1932

[5] Tanno T, Bhanu NV, Oneal PA et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med 2007; 13: 1096–1011

[6] Nemeth E, Rivera S, Gabayan V et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004; 113: 1271–1276

[7] Harrison-Findik DD. Gender-related variations in iron metabolism and liver diseases. World J Hepatol 2010; 2: 302–310

[8] Mattace Raso G, Irace C, Esposito E et al. Ovariectomy and estrogen treatment modulate iron metabolism in rat adipose tissue. Biochem Pharmacol 2009; 78: 1001–1007

[9] Campesi I, Sanna M, Zinellu A et al. Oral contraceptives modify DNA methylation and monocyte-derived macrophage function. Biol Sex Differ 2012; 3: 4

[10] Ulas M, Cay M. Effects of 17β-estradiol and vitamin E treatments on blood trace element and antioxidant enzyme levels in ovariectomized rats. Biol Trace Elem Res 2011; 139: 347–355

[11] Stuckey R, Aldridge T, Lim FL et al. Induction of iron homeostasis genes during estrogen-induced uterine growth and differentiation. Mol Cell Endocrinol 2006; 253: 22–29

[12] Hua K, Din J, Cao Q et al. Estrogen and progestin regulate HIF-1 alpha expression in ovarian cancer cell lines via the activation of Akt signaling transduction pathway. Oncol Rep 2009; 21: 893–898

[13] Yang Q, Jian J, Katz S et al. 17β-estradiol inhibits iron hormone hepcidin though an estrogen responsive element half-site. Endocrinology 2012; 153: 3170–3178

[14] Hou Y, Zhang S, Wang L et al. Estrogen regulates iron homeostasis though governing hepatic hepcidin expression via an estrogen response element. Gene 2012; 511: 398–403

[15] Kantner I, Nubbemeyer R, Schkoldow J et al. Estradiol release kinetics determine tissue response in ovariectomized rats. Endocrinol 2012; 153: 1725–1733

[16] Shafarin J, Bajbouj K, El-Serafy A et al. Estrogen-Dependent Downregulation of Hepcidin Synthesis Induces Intracellular Iron Efflux in Cancer Cells In Vitro. Biol Med (Aligarh) 2016; 8: 7 doi:10.4172/0974-8369.1000356

[17] Lehtihet M, Bonde Y, Beckman L et al. Circulating Hepcidin-25 Is Reduced by Endogenous Estrogen in Humans. PLoS ONE 2016; 11: e0148802

[18] Qian Y, Yin C, Chen Y et al. Estrogen contributes to regulating iron metabolism though governing ferroportin signaling via an estrogen response element. Cell Signal 2015; 27: 934–942

[19] Ikeda Y, Tajima S, Izawa-Ishizawa Y et al. Estrogen Regulates Hepcidin Expression via GPR30-BMP6-Dependent Signaling in Hepatocytes. PLoS ONE 2012; 7: e40465 doi:10.1371/journal.pone.0040465

[20] Guo W, Bachman E, Li M et al. Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells. Aging Cell 2013; 12: 280–291

[21] Latour C, Kautz L, Besson-Fournier C et al. Testosterone perturbs systemic iron balance though activation of epidermal growth factor receptor signaling in the liver and repression of hepcidin. Hepatology 2014; 59: 683–694

[22] Peter F, Wang S. Serum Iron and Total Iron-Binding Capacity Compared with Serum Ferritin in Assessment of Iron Deficiency. Clin Chem 1981; 27: 276–279

[23] Wish JB. Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation. Clin J Am Soc Nephol 2006; 1 (S4/S8): doi:10.2215/CJN.01490506

[24] Tai P, Wang J, Jin H et al. Induction of regulatory T cells by physiologic levels of estrogen. J Cell Physiol 2008; 214: 456–464

[25] Marx J. Iron and infection: competition between host and microbes for a precious element. Best Pract Res Clin Haematol 2002; 15: 411–426

[26] Ganz T. Hepcidin-a peptide hormone at the interface of innate immunity and iron metabolism. Curr Top Microbiol Immunol 2006; 306: 183–198

[27] Mencacci A, Cenci E, Boelaert JR et al. Iron Overload Alters Innate and T Helper Cell Responses to Candida albicans in mice. J Infect Dis 1997; 175: 1467–1476

[28] Krausse-Opatz B, Wittkop U, Gutzki FM.et.al. Free iron ions decrease indoleamine 2,3-dioxygenase expression and reduce IFN gamma-induced inhibition of Chlamydia trachomatis infection. Microb Pathog 2009; 46: 289–297

[29] Hamad M, Awadallah S. Estrogen-dependent changes in serum iron levels as a translator of the adverse effects of estrogen during infection: A conceptual framework. Med Hypotheses 2013; 81: 1130–1134

[30] Ibrahim AS, Spellberg B, Edwards J. Iron acquisition: a novel perspective on mucormycosis pathogenesis and treatment. Curr Opin Infect Dis 2008; 21: 620–625

[31] Hamad M, Bajbouj K. The re-emerging role of iron in infection and immunity. Integrative Mol Med 2016; 3: 807–810

[32] Maximov PY, Lewis-Wambi JS, Jordan VC. The paradox of oestradiol-induced breast cancer cell growth and apoptosis. Curr Signal Transduct Ther 2009; 4: 88–102

[33] Fan P, Griffith OL, Agboke FA et al. c-Src modulates estrogen-induced stress and apoptosis in estrogen-deprived breast cancer cells. Cancer Res 2013; 73: 4510–4520

[34] Pinnix ZK, Miller LD, Wang W et al. Ferroportin and iron regulation in breast cancer progression and prognosis. Sci Transl Med 2010; 2: 43–56

[35] Manz DH, Blanchette NL, Paul BT et al. Iron and cancer: Recent insights. Ann N Y Acad Sci 2016; 1368: 149–161 doi:10.1111/nyas.13008

[36] Jinlong Jian J, Pelle E, Huan X. Iron and Menopause: Does Increased Iron Affect the Health of Postmenopausal Women? Antioxid & Redox Signal 2009; 11: 2939–2943