Cell PRESS

Special Focus: Fertility and pregnancy

Iron metabolism and the polycystic ovary syndrome

Héctor F. Escobar-Morreale

Diabetes, Obesity and Human Reproduction Research Group, University of Alcalá and Hospital Universitario Ramón y Cajal and Centro de Investigación Biomédica en Red Diabetes y Enfermedades Metabólicas Asociadas CIBERDEM & Instituto Ramón y Cajal de Investigación Sanitaria IRYCIS, Carretera de Colmenar km 9'1, E-28034 Madrid, Spain

The polycystic ovary syndrome (PCOS) is associated with insulin resistance and abnormal glucose tolerance. Iron overload may lead also to insulin resistance and diabetes. Serum ferritin levels are increased in PCOS, especially when glucose tolerance is abnormal, suggesting mild iron overload. Factors contributing to potential iron overload in PCOS include the iron sparing effect of chronic menstrual dysfunction, insulin resistance, and a decrease in hepcidin leading to increased iron absorption. Enhancement of erythropoiesis by androgen excess is unlikely, because soluble transferrin receptor levels are not increased in PCOS. Future venues of research should address the long-term effects of PCOS treatment on iron overload and, conversely, the possible effects of iron lowering strategies on the glucose tolerance of patients with PCOS.

The polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is a frequent androgen excess disorder that, aside from cosmetic and reproductive consequences, is associated with obesity, visceral adiposity, insulin resistance, and disorders of glucose tolerance [1–4]. These associations may be explained by the existence of a vicious circle in PCOS patients, whereby androgen excess favoring the abdominal deposition of fat, further facilitates androgen secretion by their ovaries and adrenals [5].

The association of hemochromatosis or iron overload (see Glossary) with insulin resistance, abnormal glucose tolerance, and diabetes has been known for decades [6]. Yet, milder grades of iron excess may influence glucose tolerance in the absence of severe grades of iron overload as those present in hemochromatosis [7]. This article puts forth an opinion based on recent evidence that in some patients mild iron overload might be associated with PCOS, and discusses the possible clinical and therapeutic consequences of this association.

Iron metabolism in humans

Iron is essential for human life: functions of iron and development of iron sparing mechanisms during evolution as survival advantage.

Iron is a trace mineral oligoelement, essential for human life [8]. Iron is a part of heme, and heme proteins have diverse biological functions including the transportation of diatomic gases, chemical catalysis, diatomic gas detection, and electron transfer [8]. Therefore, iron is essential for many physiological processes in humans, including electron transport in the Krebs cycle and oxygen transport to tissues [8]. In humans, the iron content of the body is tightly regulated by modulating intestinal absorption, and very little iron is lost from the body in the absence of menstruation or blood loss from other sources, pregnancy, or lactation [8].

Iron deficiency is a common cause of infant and maternal mortality and is a risk factor for low birth weight, preterm delivery, and infant developmental delays [9]. Hence, common genetic variants that favor iron accumulation within the body and might provide a survival advantage during times of environmental stress and famine, may induce iron overload when these stressors are no longer present [10,11].

Interestingly, PCOS and associated metabolic conditions such as insulin resistance and obesity may also

Glossary

Ferritin: cellular storage protein for iron, is also an acute phase protein and, hence, serves as a clinical marker of body iron content after significant inflammation has been excluded.
Haptoglobin: forms soluble complexes with hemoglobin after processes of intravascular hemolysis, ameliorating the generation of oxygen reactive species and oxidative stress that might follow the liberation of free iron into the circulation.
Hemochromatosis: also called iron overload, is a disorder that results in too much iron being absorbed from the gastrointestinal tract. Patients with hemochromatosis have hyperferritinemia.
Hepcidin: hormone that acts as the main negative regulator of iron metabolism by inducing the internalization and degradation of the iron transporter ferroportin, thereby reducing intestinal absorption of iron and inhibiting the release of iron from macrophages.
Human hemochromatosis protein (HFE): protein that forms complexes with transferrin receptor and reduces the release of iron from TfR–diferric transferrin complexes within the endosome. Mutations in *HFE* gene are responsible for most cases of hereditary hemochromatosis.
Hyperferritinemia: indicates high serum ferritin levels found in genetic and acquired conditions, that may or may not be associated with iron overload. Patients with hyperferritinemia do not necessarily have hemochromatosis.
Iron: trace element essential for human life, participates in many reactions because of its ability to cycle from ferrous to ferric forms. Free iron is highly toxic because of the potential for generating free radicals by Fenton reaction.
Transferrin: iron transporter in the circulation, may bind one or two ferric ions.
Transferrin receptor (TfR): binds diferric transferrin in a complex that enters the cell by endocytosis. A soluble form (sTfR) is released into the circulation when the receptor is not bound to diferric transferrin, and serves as a marker of the rate of erythropoiesis.
Transferrin saturation: ratio of plasma iron to transferrin (usually between 20% and 50%), increases as a function of iron burden.

Corresponding author: Escobar-Morreale, H.F. (hescobarm.hrc@salud.madrid.org)
Keywords: iron; ferritin; hepcidin; transferrin; haptoglobin; transferrin receptor; HFE; androgen excess; polycystic ovary syndrome; insulin resistance; glucose tolerance; diabetes.

 http://dx.doi.org/10.1016/j.tem.2012.04.003

provide selective advantage during evolution [12]. The increase in assertive behavior resulting from increased androgen secretion might be advantageous during times of environmental stress [12]. The subfertility of women with PCOS could increase the interval between pregnancies, decreasing the birth rate and favoring maternal and infant survival [13]. Furthermore, insulin resistance played a central role in the combination of thrifty genotypes and phenotypes that favored survival during times of environmental stress or food shortage [14]. Insulin resistance increases glucose availability for brain metabolism. It also increases salt and water retention, sympathic tone and blood pressure, increases coagulability, and decreases fibrinolysis. These are defensive mechanisms against trauma and bleeding. But more importantly, insulin resistance favors obesity, protecting against starvation, and obesity contributes to a proinflammatory state through the secretion of several cytokines, contributing to the defense against infection, and possibly to the development of functional hyperandrogenism and PCOS [12]. From this evolutionary perspective, it would be no surprise if PCOS or insulin resistance were associated with genetic or acquired iron sparing mechanisms.

Iron metabolism and its molecular genetics

Iron metabolism is summarized in Figure 1. Absorption of iron in the duodenum is the critical step in the regulation of iron metabolism, because the body has no effective means of excreting iron [8]. Dietary iron comes in heme (fish, meat and poultry) and non-heme (vegetable) forms, the former showing higher bioavailability than the latter [15]. Dietary sources contain mostly ferric iron (Fe^{3+}) that is converted to ferrous iron (Fe^{2+}) during absorption. Non-heme iron is transported across the intestinal epithelium by the divalent metal transporter 1 (DMT1), whereas the mechanisms involved in the absorption of heme iron are less clear [15].

Ferrous iron is then transported across the basolateral membrane by ferroportin, oxidized to ferric iron by hephastin, and loaded into transferrin [15]. Mutations in the ferroportin gene cause rare forms of autosomal dominant hereditary hemochromatosis [15]. Iron absorption increases when iron stores are reduced, in the presence of hypoxia or in situations of increased erythropoiesis [15].

Hepcidin is a circulating protein secreted by the liver in response to increased body iron levels, inflammation, and infection. Hepcidin acts as the main negative regulator of iron absorption in villous enterocytes and also inhibits the

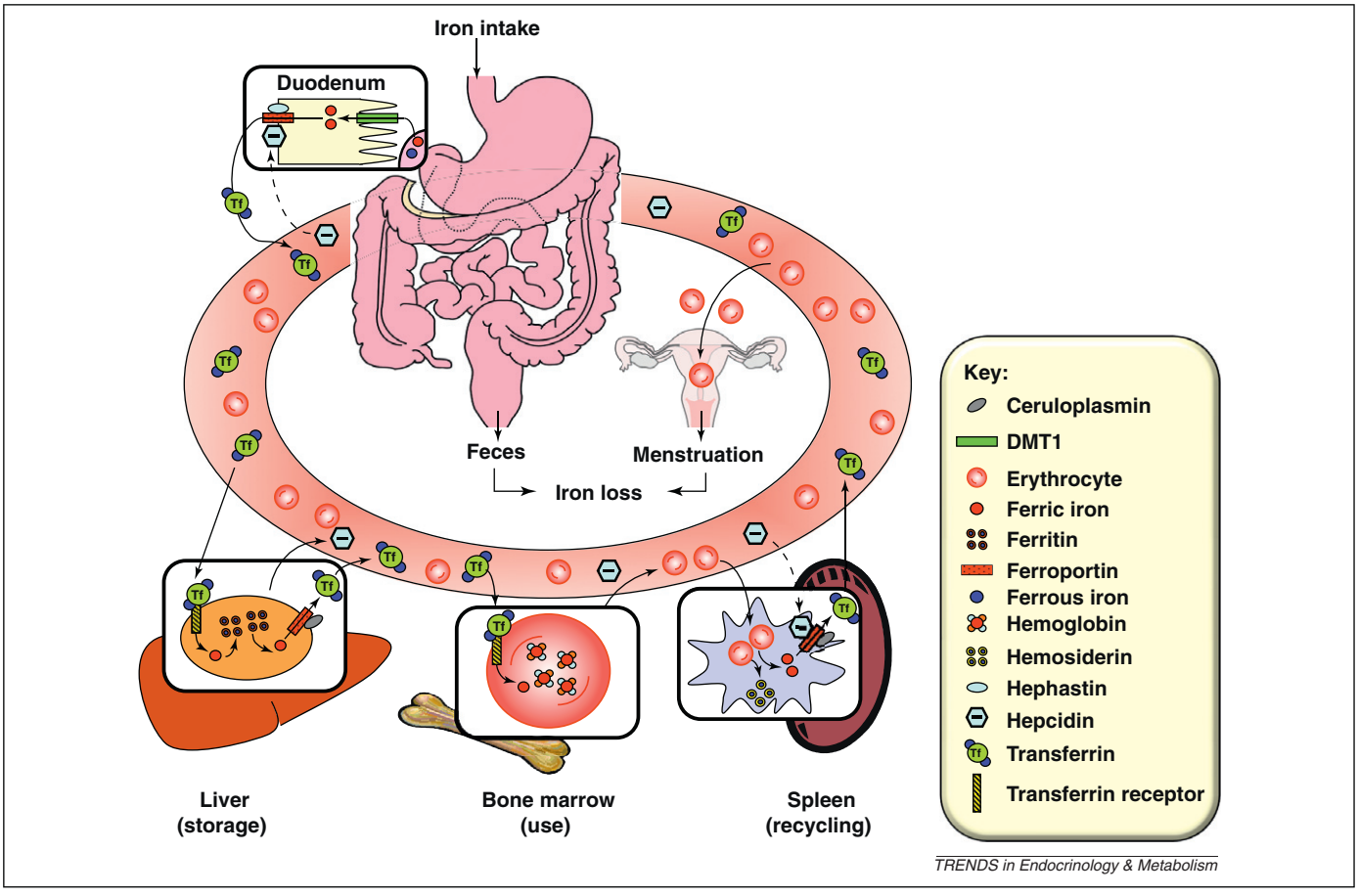

Figure 1. Schematic representation of iron metabolism. Iron is absorbed in the duodenum. Non-heme iron enters using the divalent metal transporter 1 (DMT1), whereas the mechanisms used by heme iron in humans are unclear. Ferrous iron is then transformed in ferric ion by hephastin and exported by ferroportin into the circulation bound to transferrin (Tf). Diferric Tf is imported into sites of storage and used by a specific receptor (TfR). In the liver, iron is stored as ferric ion into ferritin, and is released by ferroportin and ceruloplasmin bound to Tf, as needed. In the bone marrow, iron is used by erythroid precursors to synthesize hemoglobin. Iron is recycled by macrophages, especially in the spleen. In macrophages, iron may accumulate as hemosiderin or released back into the circulation by ferroportin and ceruloplasmin. Hepcidin, a hormone synthesized by the liver, acts as the main negative regulator of iron absorption in the duodenum and of iron release by macrophages. Iron is lost in very small quantities in feces, but menstruation, pregnancy, and lactation may cause important iron loss in women.

release of iron from macrophages. The effects of hepcidin are mediated by the internalization of the iron transporter ferroportin, that is then degraded by lysosomes [15]. Constitutive overexpression of hepcidin results in severe iron deficiency anemia at birth and, conversely, inactivating mutations in hepcidin results in a rare form of juvenile hemochromatosis [15]. Mutations in hemojuvelin, a regulator of hepcidin secretion, are responsible for the common form of juvenile hemochromatosis [15].

Transferrin is the major transporter of iron in the circulation, and may bind one or two ferric ions. The saturation of transferrin increases in situations when iron supply exceeds iron demands, and decreases in iron deficiency [15]. Iron enters cells in sites of use and storage by endocytosis, in a process that requires the binding of transferrin to the transferrin receptor (TfR). A soluble form (sTfR) is released into the circulation when the receptor is not bound to diferric transferrin. sTfR levels correlate directly with the rate of erythropoiesis [15].

The protein that stores iron in cells is ferritin, and its circulating levels serve as a marker for body iron stores [15]. The synthesis of TfR and ferritin is regulated reciprocally at the post-transcriptional level and depends on the cellular iron status [15]. Accordingly, in iron overload, ferritin increases to permit adequate iron storage and TfR decreases to minimize iron uptake. The opposite is observed in iron deficiency [15].

Finally, TfR is associated in a complex with the human hemochromatosis (HFE) protein, encoded by the *HFE* gene [15]. The *C282Y* and *H63D* mutations in the *HFE* gene are responsible for most cases of hereditary hemochromatosis [11]. The HFE–TfR complex competes for diferric transferrin and reduces the release of iron from TfR–diferric transferrin complexes within the endosome [11]. The *HFE C282Y* mutation prevents HFE to appear on the cell membrane, and the *H63D* mutation reduces the affinity of HFE for TfR [11]. Although how *HFE* mutations increase the intestinal absorption of iron is unclear [11], the HFE–TfR complex may influence hepcidin, as hemochromatosis patients showing *HFE* mutations have decreased hepcidin levels that do not respond to an oral iron challenge [16]. Also, in epithelial cells of the crypts of the duodenum, the HFE–TfR complex modulates the iron-absorbing function that the cell will have after migration to the tip of the villus [8]. Furthermore, HFE also binds TfR2, which is believed to sense transferrin saturation and, when mutated, causes type 3 hereditary hemochromatosis (a non-HFE form of hemochromatosis) [15].

HFE mutations might have provided survival advantage during evolution [10,11]. Carriers of the *C282Y* mutation might have a moderate increase in intestinal iron absorption protecting them from iron deficiency anemia, although this has not been fully demonstrated [17]. Furthermore *HFE* mutations also result into low concentrations of iron in macrophages, and this provides an advantage against infective microorganisms such as *Salmonella typhi* and *Mycobacterium tuberculosis* that require iron-rich macrophages for their virulence and replication [17]. These selective advantages may explain the high frequencies of carriers of *HFE* mutations among Caucasians [11].

Clinical disorders resulting from iron overload

Whether resulting from hereditary hemochromatosis or from other causes such as chronic ineffective erythropoiesis or hematologic disorders requiring repeated blood transfusions, severe iron overload, if left untreated, may lead over the course of years to the insidious development of fatigue, liver fibrosis and cirrhosis, diabetes, skin pigmentation, hypogonadism and hypothyroidism, arthropathy, cardiomyopathy, and cardiac arrythmias [11]. Although a detailed review of the management of hemochromatosis and other iron overload disorders is beyond the scope of this article, it must be highlighted that nowadays screening strategies allow a diagnosis of hemochromatosis at early stages, and that treatment by repeated phlebotomies or iron-chelating agents may prevent the development of irreversible organ damage [11].

However, less pronounced grades of iron overload have been proposed to play a role in the pathophysiology of other chronic disorders such as diabetes, heart disease, and cancer [18]. I will discuss the evidence supporting a participation of mild iron overload in the pathophysiology of insulin resistance, diabetes, and PCOS in the next sections.

Laboratory markers of iron metabolism

Laboratory markers of iron metabolism include serum iron and transferrin concentrations, transferrin saturation, ferritin and sTfR levels, and the sTfR/ferritin ratio. In the absence of confounding factors – inflammation, vitamin C deficiency, oxidative stress, hepatocyte dysfunction, and increased cell death – serum ferritin is found in proportion to the size of cellular iron stores [19], whereas sTfR levels are directly proportional to the erythropoietic rate. Hence, the sTfR/ferritin ratio serves as an indicator of the appropriateness of cellular iron demands for the total body iron contents [15].

Definite demonstration of iron overload requires direct measurement of increased iron concentrations in storage organs such as the liver [18]. Indirect techniques using quantitative magnetic resonance imaging or biomagnetic liver susceptometry have been developed [19]. However, the invasive nature of the former and the technical complexity of the latter preclude their broad application. Furthermore, the sensitivity of indirect techniques of iron assessment in measuring lesser grades of iron excess than those present in genetic or acquired iron overload syndromes remains to be established [19].

Iron overload may result in increased serum iron and transferrin levels, increased transferrin saturation, increased serum ferritin concentrations, and decreased sTfR concentrations [11,18,20,21]. Serum ferritin remains essential in assessing iron overload as these assays are available worldwide, relatively well standardized, and inexpensive [19]. The clinical utility of circulating hepcidin as a marker of intestinal iron absorption still needs to be established [21].

Associations between iron metabolism, intermediate metabolism, and diabetes

Severe iron overload may result in glucose intolerance and diabetes [11]. Although the risk for diabetes in subjects with hemochromatosis is related predominantly to family

history of diabetes [22], beta cell (β cell) dysfunction secondary to iron accumulation in the pancreas [22] and insulin resistance [6,23] are involved in the development of diabetes in these patients. However, iron influences glucose metabolism, even in the absence of severe iron overload.

Mounting epidemiological evidence suggest that, in the general population, body iron stores and iron intake are positively associated with the development of disorders of glucose tolerance, including diabetes [24]. As elegantly proposed by Fernández-Real *et al.* [7], iron and glucose metabolism maintain a crosstalk based on two-directional influences: iron influences insulin secretion and sensitivity, and insulin influences iron metabolism.

On the one hand, iron interferes with insulin's ability to inhibit hepatic glucose production, and both the extraction and metabolism of insulin in the liver are reduced with increasing hepatic iron stores, leading to systemic hyperinsulinemia [7]. On the other hand, insulin facilitates iron uptake and ferritin synthesis in several animal and human cell lines, in parallel with the effects of insulin on glucose transport [7].

In addition, both iron and glucose metabolism may be influenced by third parties such as chronic inflammation and oxidative stress, which are involved in the pathogenesis of many complex metabolic disorders including PCOS [12,25,26]. Free iron is highly toxic and participates, through the Fenton reaction, in the formation of free radicals including hydroxide and superoxide anions, thereby contributing to oxidative stress [7]. Moreover, inflammatory cytokines increase transferrin receptors on the cell surface favoring iron deposition in tissues, and are as well intimately involved in the development of obesity-associated insulin resistance [25].

The causal role of iron for the development of insulin resistance and diabetes is suggested by the observation that reduction of iron stores exerts beneficial effects on insulin resistance and glucose control, because repeated phlebotomies in diabetic patients with hemochromatosis may improve metabolic control [22,27].

The association of iron stores with disorders of glucose tolerance is not restricted to patients with severe iron overload or hemochromatosis: (i) the risk for diabetes is increased in parallel with an increase in the dietary intake of heme-iron [28] and insulin sensitivity increases in relation to low iron status in lacto-ovo vegetarians, compared with meat eaters [29]; (ii) frequent blood donations improve insulin sensitivity and are protective against the development of diabetes [30]; and (iii) in patients with type 2 diabetes, repeated phlebotomies result in an improvement in insulin resistance, β cell function, and vascular reactivity [31,32]. Taken together, these findings suggest that iron depletion might prove useful for the prevention and treatment of type 2 diabetes and conditions in which insulin resistance plays a pathophysiological role [7].

Is there a link between iron overload and PCOS?

Evidence supporting the existence of mild iron overload in PCOS

Considering that abdominal adiposity and insulin resistance play a major role in the pathogenesis of PCOS [5], the possibility that mild iron overload might also be associated with PCOS has been recently explored. The presence of hyperferritinemia in PCOS patients, in the absence of confounding factors such as inflammation or infection, suggests increased body iron stores in these women. However, definite proof of iron overload is lacking because direct assessment of liver iron concentrations has not been conducted in these women owing to ethical constraints.

Serum ferritin levels were found to be increased in overweight and obese patients with PCOS, compared with their control counterparts, in a study with 121 premenopausal women subjects, including 78 patients with PCOS and 43 controls (matched for body mass index and grade of obesity). This increase was independent of changes in serum high sensitivity C-reactive protein concentrations, indicating that chronic inflammation is not decisive for the increase observed in ferritin levels. Moreover, these women were young and did not have neoplastic, inflammatory, or infectious disorders or other confounding factors that might increase their ferritin concentrations. Of note, serum ferritin levels were markedly increased in both patients and controls presenting with abnormalities of glucose tolerance.

A subsequent, extended study of 149 women with PCOS and 108 controls confirmed these findings [33]. Patients with PCOS showed increased ferritin concentrations compared to controls, as did premenopausal women with abnormal glucose tolerance, compared to those presenting with normal glucose tolerance [33]. These increases in ferritin levels occurred both in non-obese and obese women, and obesity did not influence serum ferritin concentrations after controlling for both PCOS and glucose tolerance (Figure S1) [33].

Several molecules involved in iron metabolism have been identified as potential biomarkers for PCOS. Using two different proteomic techniques, Matharoo-Ball *et al.* [34] identified haptoglobin α and β chains, among four other potential biomarkers, as circulating a biomarker of PCOS. Haptoglobin is a circulating acute phase response protein that forms soluble complexes with hemoglobin, during intravascular hemolysis, ameliorating the generation of oxygen reactive species and oxidative stress that might follow the liberation of free iron into the circulation from the rupture of red blood cells [35].

Two separate studies have confirmed the involvement of haptoglobin in the pathogenesis of PCOS. First, a common polymorphism in the gene encoding the α chain of haptoglobin results in the existence of two different alleles, Hp^1 and Hp^2 [36]. The Hp^2/Hp^2 genotype reduces plasma haptoglobin levels, decreases its hemoglobin-binding capacity and antioxidant properties and the secretion of anti-inflammatory cytokines, while increasing immunological reactivity [37]. PCOS is associated with Hp^2 suggesting that the antioxidant and anti-inflammatory properties of haptoglobin may be reduced in these patients [38]. Second, a nontargeted proteomic approach that examined the plasma of patients with PCOS and controls found that haptoglobin β-chain and α2-macroglobulin proteins were decreased only in the plasma of patients with PCOS, whereas transferrin was overabundant in these women [39]. A decrease in haptoglobin may contribute to the

oxidative stress seen in PCOS patients [40], whereas an increase in transferrin would be the expected response, if iron overload is present in patients with PCOS. Moreover, it must be highlighted that α2-macroglobulin is the hepcidin high-affinity transporter in the circulation, and, because α2-macroglobulin rapidly targets ligands to cells via receptor-mediated endocytosis, the binding of hepcidin to α2-macroglobulin may influence its functions [41]. Therefore, both targeted and nontargeted experimental approaches suggest that abnormalities in iron metabolism might somehow be linked to PCOS.

Potential contributors to iron overload in PCOS

Several, non-mutually exclusive mechanisms exist that might participate in the mild iron overload present in some patients with PCOS.

Stimulation of erythropoiesis by androgen excess Androgens stimulate erythropoiesis [42], and increased erythropoiesis in a major stimulus for the intestinal absorption of iron overcoming any inhibitory influence [8]. Although some patients with PCOS present with increased ferritin concentrations, their sTfR levels are not increased and their sTfR/ferritin ratios are adequately decreased. These findings make it unlikely that androgen-dependent enhancement of erythropoiesis participated in the mild iron overload of these hyperandrogenic women [43].

Iron sparing effects of chronic oligomenorrhea The oligo-ovulation of PCOS frequently results in chronic oligomenorrhea or even in periods of amenorrhea. Regular menstrual losses are one of the few mechanisms by which the female body losses iron in significant quantities. Hence, the iron sparing effects of chronic oligomenorrhea might contribute to the increased iron stores found in some individuals with PCOS [44]. In the studies discussed above, serum ferritin levels were shown to increase in a fashion parallel to the severity of menstrual dysfunction [33,43,44], and menstrual dysfunction is among the major determinants of serum ferritin concentrations in premenopausal women (Figure S2) [33].

Association with the C282Y and H63D variants in HFE The possibility that variants related to iron metabolism and genetic variants involved in the pathogenesis of PCOS could have cosegregated in pedigrees surviving during periods of environmental stress, also exists. PCOS is common in women homozygous for *HFE C282Y* mutations, and these women may develop earlier age-of-onset hemochromatosis phenotypes compared to women homozygous for *C282Y* but without androgen excess [45]. However, in the general population no association among PCOS and the *HFE C282Y* and *H63D* variants [46] has been found to date, yet the *HFE H63D* variant was among the major determinants of serum ferritin concentrations in premenopausal women, irrespective of PCOS status (Figure S2) [33].

Insulin resistance Insulin resistance is common in PCOS patients [5], and compensatory hyperinsulinemia may facilitate iron uptake by different tissues [7]. Recent data support this hypothesis and show that insulin sensitivity is inversely related to ferritin concentrations in premenopausal women, and that women with abnormal glucose tolerance have increased ferritin levels [33,43,44].

Increased intestinal absorption Although direct assessment of iron absorption has not been performed in women with PCOS, the decreased hepcidin levels of patients with PCOS suggest a reduced inhibitory effect on ferroportin that might result in increase intestinal absorption of iron and a decreased iron release from macrophages in these women [47]. Moreover, their ferritin/hepcidin ratio is increased inappropriately [47]. These abnormalities increase as a function of the severity of menstrual dysfunction, in parallel to a decrease in insulin sensitivity and an increase in serum androgen levels [47]. In conceptual agreement, testosterone administration to men suppresses hepcidin [48]. As a whole, these results may suggest that decreased hepcidin may be the mechanism by which insulin resistance and androgen excess associate with iron overload in PCOS patients.

In summary, the iron sparing effects of reduced menstrual losses and a decrease in serum hepcidin concentrations (influenced by insulin resistance and androgen excess) that may increase iron absorption and decrease iron release from macrophages, might lead to mild iron overload in some women with PCOS (Figure 2).

Clinical implications

Mild iron overload as a risk factor for the development of disorders of glucose tolerance in premenopausal women In the data discussed above, controls and patients with PCOS presenting with abnormalities of glucose tolerance, showed, as a group, increased serum ferritin levels [33,43,44]. Having a serum ferritin level above the median of the population carries a 2.4 odds ratio of having abnormal glucose tolerance [33]. Separate analyses indicate that serum ferritin levels are associated with an imbalance

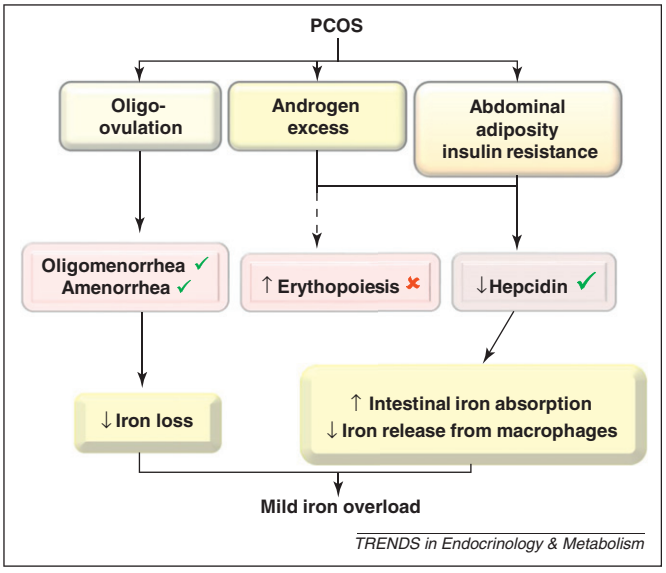

Figure 2. Potential contributors to mild iron overload in polycystic ovary syndrome (PCOS). PCOS is characterized by oligo-ovulation and androgen excess, and associates abdominal adiposity and/or insulin resistance frequently. Oligo-ovulation may result in oligomenorrhea and/or amenorrhea reducing iron loss. Androgen excess may enhance erythropoiesis and, together with insulin resistance, may decrease hepcidin secretion thereby increasing iron absorption. Of these potential mechanisms, data in humans support the association of reduced menstrual losses and decreased hepcidin concentrations with the mild iron overload of PCOS, whereas erythropoiesis does not appear to be enhanced by androgen excess.

between insulin resistance and secretion, only in women without androgen excess [49]. These findings suggest a causal role of iron overload in the development of abnormalities in glucose tolerance; however, this association could also be spurious, because women with increased ferritin were those with the worse insulin resistance and the higher androgen levels [33,43,44].

Effects of short-term therapeutic intervention on iron overload in patients with PCOS A randomized clinical trial comparing the insulin sensitizer metformin with an antiandrogenic contraceptive pill for 6 months showed that it was insulin sensitization, and not the resultant menstrual regularity, the short-term therapeutic strategy that decreased serum ferritin levels in these women [50]. These treatments showed no statistically significant changes in hepcidin levels, yet the increased ferritin/hepcidin ratio normalized after 6 months on contraceptive pills suggesting that amelioration of androgen excess might have beneficial effects on iron metabolism [47].

Future venues for research Further intervention studies are needed before implying causality to the association of increased ferritin levels and abnormal glucose tolerance in women (Box 1). Perhaps, the most needed step is evaluating if iron-lowering strategies improve indexes of insulin sensitivity and ameliorate disorders of glucose tolerance in premenopausal women with mildly increased iron stores (including those with PCOS). However, the long-term effects on iron stores of strategies directed toward amelioration of insulin resistance, menstrual dysfunction, and androgen excess in patients with PCOS have also not been established to date, and these data are definitely needed for a better understanding of the mechanism underlying the relationship between iron overload, insulin resistance, and androgen excess.

Concluding remarks and future perspectives

Several lines of evidence suggest a role of increased body iron stores in the development of insulin resistance and disorders of glucose tolerance in premenopausal women, especially in women with PCOS. Decreased hepcidin concentrations are associated with insulin resistance and possibly androgen excess in patients with PCOS, and therefore increased iron absorption, enhanced by the iron sparing effect of chronic menstrual dysfunction, might play a role in the development of mild iron overload in these women. On the contrary, a putative enhancement of erythropoiesis by androgen excess is an unlikely contributor. Because mild iron overload confers a risk for the development of abnormal glucose tolerance in these women, the possible role of iron-lowering strategies in the prevention and treatment of these metabolic abnormalities warrants further research in this area.

Box 1. Outstanding questions

Chronic oligomenorrhea, insulin resistance, and possibly androgen excess may contribute to mild iron overload in patients with PCOS, and iron excess might contribute to the development of abnormalities of glucose tolerance in these women. However, further research is needed to answer outstanding questions:

- Do therapeutic strategies for PCOS such as lifestyle modification, oral contraceptive pills or insulin sensitizers ameliorate the iron burden of these women on the long term?
- Will iron lowering therapies such as bloodletting improve insulin resistance and glucose metabolism in women with PCOS presenting with increased body iron stores?

Acknowledgments

This work was supported by the Spanish Ministry of Economy and Competitiveness, Instituto de Salud Carlos III, grants FIS PI080944 and PI1100357. CIBERDEM is also an initiative of Instituto de Salud Carlos III.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tem.2012.04.003.

References

1 Asuncion, M. *et al.* (2000) A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J. Clin. Endocrinol. Metab.* 85, 2434–2438
2 Sanchón, R. *et al.* (2012) Prevalence of functional disorders of androgen excess in unselected premenopausal women: a study in blood donors. *Hum. Reprod.* 27, 1209–1216
3 Azziz, R. *et al.* (2006) Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J. Clin. Endocrinol. Metab.* 91, 4237–4245
4 Azziz, R. *et al.* (2009) The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil. Steril.* 91, 456–488
5 Escobar-Morreale, H.F. and San Millan, J.L. (2007) Abdominal adiposity and the polycystic ovary syndrome. *Trends Endocrinol. Metab.* 18, 266–272
6 Eskind, I.B. *et al.* (1953) Insulin-resistant diabetes mellitus associated with hemochromatosis. *Ann. Intern. Med.* 38, 1295–1306
7 Fernández-Real, J.M. *et al.* (2002) Cross-talk between iron metabolism and diabetes. *Diabetes* 51, 2348–2354
8 Fairbanks, V.F. and Beutler, E. (2001) Iron metabolism. In *Williams Hematology* (Beutler, E. *et al.*, eds), pp. 295–304, McGraw-Hill
9 Pazirandeh, S. *et al.* (2012) Overview of dietary trace minerals. In *UpToDate* (Lipman, T.O., ed.), Wolters Kluwer Health
10 Rochette, J. *et al.* (1999) Multicentric origin of hemochromatosis gene (HFE) mutations. *Am. J. Hum. Genet.* 64, 1056–1062
11 Fairbanks, V.F. and Beutler, E. (2001) Disorders of iron storage and transport. In *Williams Hematology* (Beutler, E. *et al.*, eds), pp. 489–502, McGraw-Hill
12 Escobar-Morreale, H.F. *et al.* (2005) The molecular–genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. *Endocr. Rev.* 26, 251–282
13 Witchel, S.F. *et al.* (1997) Evidence for a heterozygote advantage in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J. Clin. Endocrinol. Metab.* 82, 2097–2101
14 Eaton, S.B. and Konner, M. (1985) Paleolithic nutrition. A consideration of its nature and current implications. *N. Engl. J. Med.* 312, 283–289
15 Camaschella, C. and Schrier, S.L. (2012) Regulation of iron balance. In *UpToDate* (Mentzer, W.C., ed.), Wolters Kluwer Health
16 Piperno, A. *et al.* (2007) Blunted hepcidin response to oral iron challenge in HFE-related hemochromatosis. *Blood* 110, 4096–4100
17 Weinberg, E.D. (2008) Survival advantage of the hemochromatosis C282Y mutation. *Perspect. Biol. Med.* 51, 98–102
18 Beutler, E. *et al.* (2003) Iron deficiency and overload. *Hematol. Am. Soc. Hematol. Educ. Program* 40–61
19 Fischer, R. and Harmatz, P.R. (2009) Non-invasive assessment of tissue iron overload. *Hematol. Am. Soc. Hematol. Educ. Program* 215–221
20 Khumalo, H. *et al.* (1998) Serum transferrin receptors are decreased in the presence of iron overload. *Clin. Chem.* 44, 40–44
21 Moyer, T.P. *et al.* (2011) Hereditary hemochromatosis: laboratory evaluation. *Clin. Chim. Acta* 412, 1485–1492

22 Dymock, I.W. *et al.* (1972) Observations on the pathogenesis, complications and treatment of diabetes in 115 cases of haemochromatosis. *Am. J. Med.* 52, 203–210
23 Dandona, P. *et al.* (1983) Insulin resistance and iron overload. *Ann. Clin. Biochem.* 20, 77–79
24 Swaminathan, S. *et al.* (2007) The role of iron in diabetes and its complications. *Diabetes Care* 30, 1926–1933
25 Fernandez-Real, J.M. and Ricart, W. (2003) Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr. Rev.* 24, 278–301
26 Escobar-Morreale, H.F. *et al.* (2011) Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis. *Fertil. Steril.* 95, 1048–1058
27 Niederau, C. *et al.* (1996) Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology* 110, 1107–1119
28 Jiang, R. *et al.* (2004) Dietary iron intake and blood donations in relation to risk of type 2 diabetes in men: a prospective cohort study. *Am. J. Clin. Nutr.* 79, 70–75
29 Hua, N.W. *et al.* (2001) Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians. *Br. J. Nutr.* 86, 515–519
30 Fernández-Real, J.M. *et al.* (2005) Iron stores, blood donation, and insulin sensitivity and secretion. *Clin. Chem.* 51, 1201–1205
31 Fernandez-Real, J.M. *et al.* (2002) Blood letting in high-ferritin type 2 diabetes: effects on vascular reactivity. *Diabetes Care* 25, 2249–2255
32 Fernandez-Real, J.M. *et al.* (2002) Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes* 51, 1000–1004
33 Martinez-Garcia, M.A. *et al.* (2009) Body iron stores and glucose intolerance in premenopausal women: role of hyperandrogenism, insulin resistance and genomic variants related to inflammation, oxidative stress and iron metabolism. *Diabetes Care* 32, 1525–1530
34 Matharoo-Ball, B. *et al.* (2007) Characterization of biomarkers in polycystic ovary syndrome (PCOS) using multiple distinct proteomic platforms. *J. Proteome Res.* 6, 3321–3328
35 Langlois, M.R. and Delanghe, J.R. (1996) Biological and clinical significance of haptoglobin polymorphism in humans. *Clin. Chem.* 42, 1589–1600
36 Bowman, B. (1993) Haptoglobin. In *Hepatic Plasma Proteins* (Bowman, B.H., ed.), pp. 159–167, Academic Press
37 Asleh, R. *et al.* (2005) Haptoglobin genotype- and diabetes-dependent differences in iron-mediated oxidative stress in vitro and in vivo. *Circ. Res.* 96, 435–441
38 Alvarez-Blasco, F. *et al.* (2009) Role of haptoglobin in polycystic ovary syndrome (PCOS), obesity and disorders of glucose tolerance in premenopausal women. *PLoS ONE* 4, e5606
39 Insenser, M. *et al.* (2010) Proteomic analysis of plasma in the polycystic ovary syndrome identifies novel markers involved in iron metabolism, acute-phase response, and inflammation. *J. Clin. Endocrinol. Metab.* 95, 3863–3870
40 Sabuncu, T. *et al.* (2001) Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. *Clin. Biochem.* 34, 407–413
41 Peslova, G. *et al.* (2009) Hepcidin, the hormone of iron metabolism, is bound specifically to α-2-macroglobulin in blood. *Blood* 113, 6225–6236
42 Zitzmann, M. and Nieschlag, E., eds (2004) *Androgens and Erythropoiesis*, Cambridge University Press
43 Escobar-Morreale, H.F. and Luque-Ramirez, M. (2011) Role of androgen-mediated enhancement of erythropoiesis in the increased body iron stores of patients with polycystic ovary syndrome. *Fertil. Steril.* 95, 1730–1735
44 Escobar-Morreale, H.F. *et al.* (2005) Body iron stores are increased in overweight and obese women with polycystic ovary syndrome. *Diabetes Care* 28, 2042–2044
45 Barton, J.C. (2011) Hemochromatosis, HFE C282Y homozygosity, and polycystic ovary syndrome: report of two cases and possible effects of androgens and hepcidin. *Acta Haematol.* 126, 138–140
46 Botella-Carretero, J.I. *et al.* (2006) Mutations in the hereditary hemochromatosis gene are not associated with the increased body iron stores observed in overweight and obese women with polycystic ovary syndrome. *Diabetes Care* 29, 2556
47 Luque-Ramirez, M. *et al.* (2011) Role of decreased circulating hepcidin concentrations in the iron excess of women with the polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 96, 846–852
48 Bachman, E. *et al.* (2010) Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis. *J. Clin. Endocrinol. Metab.* 95, 4743–4747
49 Luque-Ramírez, M. *et al.* (2010) The determinants of insulin sensitivity, β-cell function, and glucose tolerance are different in patients with polycystic ovary syndrome and in non-hyperandrogenic women. *Fertil. Steril.* 94, 2214–2221
50 Luque-Ramirez, M. *et al.* (2007) Increased body iron stores of obese women with polycystic ovary syndrome are a consequence of insulin resistance and hyperinsulinism and are not a result of reduced menstrual losses. *Diabetes Care* 30, 2309–2313