Iron storage is significantly increased in peritoneal macrophages of endometriosis patients and correlates with iron overload in peritoneal fluid

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Objective: To further investigate peritoneal iron disruption in endometriosis by studying iron storage in peritoneal macrophages of patients with endometriosis compared with controls.

Design: Cross-sectional study.

Setting: Academic gynecology research unit in a university hospital.

Patient(s): Fifty patients undergoing laparoscopy.

Intervention(s): Collection of peritoneal fluid samples (N = 50) from patients with (n = 27) and without (n = 23) endometriosis undergoing laparoscopy.

Main Outcome Measure(s): Quantification of peritoneal macrophage ferritin by immunocytochemical staining and immunodensitometry and measurement of peritoneal iron, transferrin, ferritin, and prohepcidin concentrations.

Result(s): The optical density of peritoneal macrophage ferritin staining was statistically significantly higher in endometriosis patients than in controls. Higher iron concentrations, transferrin saturations, and ferritin concentrations were also detected in case of endometriosis. A statistically significant positive correlation was found between the optical density of macrophage ferritin staining and peritoneal iron concentrations in endometriosis and control patients.

Conclusion(s): Iron storage is statistically significantly increased in peritoneal macrophages of patients with endometriosis and correlates with iron overload in peritoneal fluid. The potential implications of iron accumulation in peritoneal macrophages in case of endometriosis are discussed. (Fertil Steril® 2009;91:1668–75. ©2009 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, macrophages, peritoneal fluid, iron, transferrin, ferritin, oxidative stress

Endometriosis is a gynecologic disorder affecting 10% to 15% of women in their reproductive years. This pathology is characterized by the presence of endometrial glands and stroma outside the uterine cavity. Despite an increasing number of studies on its physiopathology, the etiology of endometriosis remains unclear, in part, to its multifactorial characteristics. Indeed, a growing body of evidence indicates that a combination of genetic, hormonal, environmental, immunologic, and anatomic factors may play a role in the pathogenesis of this disorder (1).

In most menstruating women, menstrual effluent containing endometrial cells and blood is transported into the abdominal cavity through the fallopian tubes (2, 3). This phenomenon, known as retrograde menstruation, was first described in 1927 by Sampson (4), who suggested that it may be the origin of pelvic endometriosis. Several theories have been proposed to explain why ectopic endometrial tissue implants in approximately 10% to 15% of patients while, in the majority of women, endometrial cells are resorbed by the peritoneal environment. It has been postulated that peritoneal protective mechanisms may be defective or overwhelmed by high amounts of retrograde menstruation in some women, permitting implantation and growth of endometrial cells, resulting in endometriosis (5, 6).

Peritoneal macrophage number and concentrations are enhanced in the peritoneal fluid of endometriosis patients, and these cells are also more strongly activated in the peritoneal cavity (7, 8). As in most tissues, pelvic macrophages have two important functions, first, in the regulation of the inflammatory response, and second, in iron homeostasis. Iron in macrophages originates from phagocytosis of red blood cells or endocytosis of the hemoglobin–haptoglobin complex (9). Metabolism of hemoglobin and heme releases iron,
which is then incorporated into ferritin in macrophages or returned to the iron transporter transferrin in peritoneal fluid. Hepcidin is a recently discovered peptide that appears to be the homeostatic regulator of iron metabolism by macrophages, acting by inhibiting iron efflux through ferroportin, thereby inducing cellular iron sequestration (10). Hepcidin synthesis is up-regulated by inflammation and iron overload, and occurs predominantly in the liver. However, inflammatory cells, in particular macrophages and neutrophils, can also directly express this peptide (11, 12).

A number of studies have demonstrated the presence of iron overload in the peritoneal cavity of endometriosis patients. Higher levels of iron were detected in the peritoneal fluid of women with endometriosis (13), and iron concentrations were related to the severity of the disease (14). Van Langendonck et al. (15) reported increased iron and ferritin concentrations in the peritoneal fluid of patients developing endometriosis, and showed higher rates of ferritin and hemosiderin deposits in peritoneal tissue adjacent to lesions. Iron transport also appears to be enhanced in pelvic endometriosis, as expression of transferrin receptors by peritoneal macrophages was found to be higher (16), and transferrin concentrations in peritoneal fluid greater (17), than in control patients.

Iron overload in the peritoneal cavity may induce oxidative stress, involving peritoneal macrophages in particular (18). Indeed, iron accumulation in macrophages may severely compromise their function as a result of excessively increased production of reactive oxygen species (ROS) and enhanced activation of the proinflammatory transcriptional factor nuclear factor-kappa B (NF-kB) (19). In a recent study, we demonstrated greater NF-kB activation in peritoneal macrophages of endometriosis patients than controls (20). However, iron storage has never been investigated in peritoneal macrophages of patients with endometriosis compared with controls.

To further assess peritoneal iron disruption in endometriosis, and particularly in peritoneal macrophages, which play a major role in the development of endometriosis, our study evaluated and compared iron accumulation by these cells in patients with and without endometriosis. Peritoneal macrophage ferritin was quantified by immunocytochemical staining and correlated with peritoneal ferric iron concentrations, transferrin saturations, and ferritin and prohepcidin concentrations.

### MATERIALS AND METHODS

#### Patients and Sample Collection

Peritoneal fluid samples were collected from 50 patients with (n = 27) and without (n = 23) endometriosis undergoing laparoscopy during the proliferative (endometriosis, n = 12; control, n = 10) and secretory (endometriosis, n = 15; control, n = 13) phases of the menstrual cycle. All patients had regular cycles and had received no hormonal treatment for at least 3 months before surgery. Clinical information on endometriosis and control patients is summarized in Table 1. Control patients (n = 23; mean age, 35.9 ± 7.1 years) undergoing laparoscopy for tubal ligation (n = 12) or before intrauterine insemination for male factor infertility (n = 11) showed no endometriotic lesions or any other disease at laparoscopy. Endometriosis patients (n = 27; mean age, 34.4 ± 5.8 years) had moderate (n = 8) or severe (n = 19) endometriosis according to the revised American Society for Reproductive Medicine (ASRM) classification (21) and were undergoing surgery for ovarian endometriosis (n = 9), deep endometriosis (n = 15), or associated ovarian and deep endometriosis (n = 3). All of them also presented with peritoneal endometriotic lesions at laparoscopy that were later confirmed histologically. All patients included in the study (endometriosis and control) had patent fallopian tubes.

Collection of peritoneal fluid samples was approved by the ethics review board of the Catholic University of Louvain. Peritoneal fluid was collected during laparoscopy after insertion of the suction probe through the first counterincision, before any manipulation. Care was taken to avoid contamination of the sample with blood from the abdominal wall. The fluid was aspirated from the cul-de-sac into a sterile syringe and immediately transported on ice to the laboratory.

#### Peritoneal Macrophage Isolation

A Percoll discontinuous density gradient was used to isolate peritoneal macrophages from other cells, as previously described elsewhere (20). Briefly, peritoneal fluid was centrifuged at 400 × g for 5 minutes to separate cells and peritoneal fluid supernatant. Peritoneal fluid supernatant was frozen at −20°C until analysis, while peritoneal cells were immediately resuspended in 2 mL of 45% Percoll solution (Amersham Pharmacia Biotech AB, Upplands, Sweden). Successive density layers were subsequently added to a 15-mL conical tube: 2 mL of 55% Percoll solution, 2 mL of 50% Percoll solution, 2 mL of 45% Percoll solution containing peritoneal cells, 2 mL of 30% Percoll solution, and, finally, 2 mL of culture medium on top: DMEM-F12 containing 10% decomplemented fetal bovine serum (FBS) (GIBCO BRL, Paisley, Renfrewshire, Scotland, United Kingdom), 100 IU/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B (GIBCO BRL). The gradient tube was centrifuged at 300 × g for 20 minutes. The top two cell fractions, which contained the greatest proportion of macrophages, were fixed in formal and projected onto SuperFrost Plus Slides (Menzel-Gläser, Braunschweig, Germany) by centrifugation (Cytospin Thermo Electron Corporation, Marietta, GA) at 55 × g for 5 minutes to perform ferritin immunostaining and assessment of macrophage isolation.

The purity of macrophage isolation was evaluated immunohistochemically using mouse monoclonal antibody to human CD163 (clone RM3/1; Sanbio, Uden, the Netherlands) (1:900 dilution), which is a specific marker for monocye-macrophage lineage.
Staining and Quantification of Iron Storage in Peritoneal Macrophages

Ferritin immunocytochemical staining was performed on peritoneal macrophages fixed in formol and projected onto slides (Fig. 1). An immunoperoxidase method using rabbit antibody against human ferritin (Dakocytomation, Glostrup, Denmark) was applied. Briefly, endogenous peroxidase activity quenching, heat epitope retrieval, and blocking of nonspecific staining were performed. The cells were then incubated in a 1:1600 dilution of the primary antibody overnight at 4°C, followed by incubation with a secondary antibody conjugated to peroxidase (EnVision+; Dakocytomation). The presence of peroxidase was revealed using 3,3′-diaminobenzidine (Dakocytomation).

Immunodensitometry has been used to quantify ferritin immunocytochemical staining, as described by Rahier et al. (22). Images of projected cells were obtained at 160× magnification and measurement fields (90,000 μm²/752 × 560 pixels) were digitized through a Zeiss microscope using a KS-400 system (Zeiss, Munich, Germany) coupled to a CCD72 camera (Dage-MTI, Michigan City, IN) at a wavelength of 450 nm. Immunocytochemical staining was determined by gray level threshold to separate the cellular signal from the extracellular background. For each pixel from the cellular area, the intensity of labeling was evaluated by the computer, which attributed a gray level from 0 (black) to 250 (white). The mean gray level is the sum of the gray level of each pixel divided by the number of pixels of the reference area. Transmittance (T) is linearly related to the gray level and optical density (OD) is obtained by OD = −log T.

Measurement of Iron, Transferrin, and Ferritin Concentrations in Peritoneal Fluid

Iron, transferrin, and ferritin concentrations were measured in peritoneal fluid supernatant. Iron was dissociated from transferrin at an acidic pH and measured using a colorimetric method (Unicel DxC 800 Synchron; Beckman Coulter).

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control patients (n = 23)</th>
<th>Endometriosis patients (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at time of surgery</td>
<td>35.9 ± 7.1</td>
<td>34.4 ± 5.8</td>
</tr>
<tr>
<td>Age at first diagnosis of endometriosis</td>
<td>—</td>
<td>32.63 ± 6.33</td>
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<tr>
<td>Disease recurrence</td>
<td>—</td>
<td>11/27 (40.74%)</td>
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<tr>
<td>Menstrual cycle phase</td>
<td></td>
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<tr>
<td>Proliferative</td>
<td>10/23 (43.48%)</td>
<td>12/27 (44.44%)</td>
</tr>
<tr>
<td>Secretory</td>
<td>13/23 (56.52%)</td>
<td>15/27 (55.56%)</td>
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<tr>
<td>Surgical indications</td>
<td></td>
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<tr>
<td>Tubal ligation:</td>
<td>12/23 (52.17%)</td>
<td>Peritoneal endometriosis:</td>
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<td></td>
<td></td>
<td>27/27 (100%)</td>
</tr>
<tr>
<td>Preoperative</td>
<td>10/23 (43.48%)</td>
<td>Associated ovarian endometriosis:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9/27 (33.33%)</td>
</tr>
<tr>
<td>Secretory</td>
<td>13/23 (56.52%)</td>
<td>Associated deep endometriosis:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15/27 (55.56%)</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td>Associated ovarian and deep endometriosis:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/27 (11.11%)</td>
</tr>
<tr>
<td>Revised ASRM classification</td>
<td>—</td>
<td>Stage III (moderate: 16–40): 8/27 (29.63%)</td>
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<td></td>
<td></td>
<td>Stage IV (severe: &gt;40): 19/27 (70.37%)</td>
</tr>
<tr>
<td>Patent fallopian tubes</td>
<td>23/23 (100%)</td>
<td>27/27 (100%)</td>
</tr>
</tbody>
</table>

Note: Age at time of surgery and at first diagnosis are expressed as mean (± standard deviation).


FIGURE 1

Ferritin immunocytochemical staining on isolated peritoneal macrophages fixed in formol and projected onto slides. Original magnification ×200.
Fullerton, CA). Ferric iron was reduced to ferrous iron and formed a colored complex with ferrozine. Absorbance was read at 560 nm and was proportional to the total iron concentration.

Transferrin concentrations were assayed using an immunoturbidimetric method (Unicel DxC 800 Synchron; Beckman Coulter) (23), with a reagent consisting of sheep antihuman transferrin antibodies. In the presence of transferrin, complexes are agglutinated in a dose-dependent manner, causing increased turbidity. The increase in turbidity was detected at 340 nm. Transferrin saturation (percentage of transferrin with iron bound to them, TS) was obtained by TS (%) = [(iron (μg/dL) × 100)/(transferrin (mg/dL) × 1.43)].

Ferritin was measured by a paramagnetic chemiluminescent immunoassay (UniCel Dxi 800; Beckman Coulter) (24).

Detection of Hepcidin Propeptide in Peritoneal Fluid by Competitive Binding Assay
Peritoneal prohepcidin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (DRG Diagnostics, Marburg, Germany). The ELISA tests were performed in duplicate according to the manufacturer’s instructions, and prohepcidin concentrations were expressed in ng/mL.

Statistical Analysis
Statistical analysis was first applied to compare samples according to the presence or absence of endometriosis. To examine whether the phase of the ovulatory cycle could influence these data, patient samples were then divided into four groups according to the phase of the cycle (proliferative or secretory) as well as the presence or absence of endometriosis. Statistical analysis was also performed to evaluate the potential influence of a number of variables in endometriosis patients such as disease severity, endometriotic lesion subtype, and age at first diagnosis. Data are expressed as medians and percentiles (P25 and P75). Because they were not normally distributed, the nonparametric two-tailed Mann-Whitney U test was used. Correlations between the optical density of macrophage ferritin staining and peritoneal iron, transferrin, ferritin, and hepcidin concentrations were analyzed using Spearman’s rank correlation coefficient. P < .05 was considered statistically significant. Statistical analysis was performed using SPSS 14.0 software (SPSS Inc., Chicago, IL).

RESULTS
Peritoneal Macrophage Isolation
A Percoll discontinuous density gradient was used to isolate peritoneal macrophages from other peritoneal cells. Using a mouse monoclonal antibody to human CD163, which is a specific immunohistochemical marker for monocyte-macrophage lineage, we assessed macrophage enrichment of at least 90% for each sample (data not shown).

Iron Storage in Peritoneal Macrophages
Ferritin immunocytochemical staining was analyzed in isolated peritoneal macrophages from 27 endometriosis patients and 23 control patients. The optical density of peritoneal macrophage ferritin staining was found to be statistically significantly higher in women with endometriosis than in controls (P = .008) (Fig. 2A).

To examine whether the phase of the ovulatory cycle could influence this variable, the following groups were then evaluated: control patients during the proliferative phase (n = 10), endometriosis patients during the proliferative phase (n = 12), control patients during the secretory phase (n = 13), and endometriosis patients during the secretory phase (n = 15). The Mann-Whitney statistical test revealed that the optical density of ferritin staining was statistically significantly higher during the secretory phase of the menstrual cycle in women with endometriosis than in controls (P = .029) (Table 2). However, optical density did not differ during the proliferative phase (see Table 2) nor between the two different phases in patients with and without endometriosis (data not shown).

In endometriosis patients, we found no statistically significant difference according to the revised ASRM stage of endometriosis (moderate versus severe), the subtype of endometriotic lesions (ovarian versus deep), or the age at first diagnosis (≤ 30 or > 30 years) (data not shown).

Iron, Transferrin, and Ferritin Concentrations in Peritoneal Fluid
Peritoneal fluid of patients with endometriosis showed statistically significantly higher peritoneal iron concentrations (P = .048), transferrin saturations (P = .015), and ferritin concentrations (P = .000) than that of controls (Fig. 2B–D). Peritoneal transferrin concentrations were not found to differ between women with and without endometriosis (median: 140.0 mg/dL, P25 and P75: 115.0 and 175.0 mg/dL; median: 161.0 mg/dL, P25 and P75: 127.0 and 190.0 mg/dL, respectively).

Statistical analysis by menstrual cycle phase revealed a statistically significant increase in peritoneal iron concentrations (P = .006) and transferrin saturations (P = .022) during the secretory phase in patients with endometriosis compared with controls, and ferritin concentrations were found to be statistically significantly increased during both the proliferative (P = .014) and secretory phases (P = .029) (see Table 2). Peritoneal transferrin concentrations did not differ according to menstrual phase in women with and without endometriosis. Peritoneal iron and transferrin concentrations, transferrin saturations, and ferritin concentrations did not differ between the cycle phases in patients with and without endometriosis (data not shown).

In the endometriosis group, no statistically significant difference was found according to the revised ASRM stage, the endometriotic lesion subtype, or the age at first diagnosis (data not shown).
Spearman’s rank correlation coefficient showed a strong positive correlation between peritoneal iron concentrations and transferrin saturations in samples from patients with and without endometriosis (endometriosis: rho = 0.696, P = .000; controls: rho = 0.806, P = .000). Furthermore, we found a positive correlation between peritoneal macrophage ferritin staining and peritoneal iron concentrations in both endometriosis and control patients (endometriosis: rho = 0.388, P = .046; controls: rho = 0.438, P = .037).

Hepcidin Propeptide in Peritoneal Fluid
Prohepcidin concentrations were measured in the peritoneal fluid of patients with and without endometriosis but were not found to differ between the two groups (median: 51.81 ng/mL, P25 and P75: 32.51 and 71.25 ng/mL; median: 50.34 ng/mL, P25 and P75: 31.49 and 62.10 ng/mL, respectively). Analysis by menstrual cycle phase did not reveal any difference between patients with and without endometriosis (see Table 2) or between the two cycle phases in each group. In endometriosis patients, no difference was found according to the revised ASRM stage, the lesion subtype, or the age at first diagnosis (data not shown).

No correlation was found between peritoneal prohepcidin concentrations and macrophage ferritin staining, or peritoneal iron and transferrin concentrations, or transferrin saturations and ferritin concentrations in either group (data not shown).

DISCUSSION
Iron overload has been evidenced in the peritoneal cavity of endometriosis patients (13, 15, 17) but not in peritoneal...
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<tr>
<th></th>
<th>Proliferative phase</th>
<th>Secretory phase</th>
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<tbody>
<tr>
<td></td>
<td>Control (n = 10)</td>
<td>Endometriosis (n = 12)</td>
</tr>
<tr>
<td>Optical density of macrophage ferritin staining</td>
<td>Median (P25, P75) 0.22 (0.20, 0.35)</td>
<td>0.34 (0.27, 0.52)</td>
</tr>
<tr>
<td>Peritoneal fluid iron concentrations (mg/mL)</td>
<td>Median (P25, P75) 51.00 (36.25, 80.50)</td>
<td>55.00 (38.25, 88.75)</td>
</tr>
<tr>
<td>Peritoneal fluid transferrin concentrations (mg/dL)</td>
<td>Median (P25, P75) 163.50 (154.00, 179.50)</td>
<td>148.00 (106.50, 207.00)</td>
</tr>
<tr>
<td>Peritoneal fluid transferrin saturations (%)</td>
<td>Median (P25, P75) 23.59 (15.30, 33.52)</td>
<td>33.49 (14.44, 45.07)</td>
</tr>
<tr>
<td>Peritoneal fluid ferritin concentrations (ng/mL)</td>
<td>Median (P25, P75) 17.50 (9.30, 102.30)</td>
<td>156.20&lt;sup&gt;d&lt;/sup&gt; (103.70, 618.48)</td>
</tr>
<tr>
<td>Peritoneal fluid hepcidin concentrations (ng/mL)</td>
<td>Median (P25, P75) 38.12 (23.51, 53.28)</td>
<td>46.00 (17.49, 73.49)</td>
</tr>
</tbody>
</table>

Note: The nonparametric two-tailed Mann-Whitney U test was used, and values are expressed as median and percentile (P25 and P75).<ref>
<sup>a</sup> P = .029.
<sup>b</sup> P = .006.
<sup>c</sup> P = .022.
<sup>d</sup> P = .014.
<sup>e</sup> P = .029.
</ref>
macrophages. As these cells play an important role in iron homeostasis and the development of endometriosis, our study was designed to evaluate and compare iron accumulation by peritoneal macrophages in patients with and without endometriosis.

Our results showed that iron storage is statistically significantly increased in peritoneal macrophages of endometriosis patients compared with controls. In line with our previous study (15), we found peritoneal iron and ferritin concentrations to be statistically significantly increased in endometriotic peritoneal fluid samples. Furthermore, we demonstrated higher peritoneal transferrin saturation in case of endometriosis.

This peritoneal iron overload may be a consequence of increased influx caused by erythrocyte degradation, resulting either from more abundant menstrual reflux or bleeding lesions, or it may be due to a deficiency in the peritoneal iron metabolism system (18, 25). The hypothesis of increased local iron release is supported by the observation of higher peritoneal concentrations of erythrocytes in women with endometriosis during menstruation (26) and even beyond the perimenstrual period (2). This suggests that bleeding lesions, which are not under hormonal control (27), may contribute to the accumulation of erythrocytes in peritoneal fluid throughout the menstrual cycle.

Iron is an essential metal for many aspects of cellular function because of its involvement in numerous iron-containing enzymes and proteins. However, excess iron exposure can generate oxidative stress, leading to macromolecular oxidative damage, tissue injury, and chronic inflammation (28). For this reason, iron metabolism and distribution in the peritoneal cavity are essential processes to prevent iron-associated generation of oxidative stress.

Our results highlight the involvement of peritoneal macrophages in iron metabolism (9) (Fig. 3). Cellular iron storage within ferritin limits the capacity of iron to generate free radicals and confers an antioxidant effect (29). However, continued delivery of iron to macrophages can overwhelm the capacity of ferritin to store and sequester the metal, inducing oxidative injury to cells. When ferritin staining was evaluated in peritoneal macrophages according to the phase of the menstrual cycle, we found a statistically significant increase only during the secretory phase. This may suggest that the macrophage iron detoxification system could be progressively overwhelmed during the menstrual cycle in endometriosis patients, leading to abnormal macrophage activation. However, more experiments are required to confirm this hypothesis.

To further investigate iron disruption in the peritoneal cavity, peritoneal prohepcidin concentrations were measured in endometriosis and control patients, as hepcidin appears to be the homeostatic regulator of iron metabolism by macrophages (10). No statistically significant difference was found between patients with and without endometriosis, suggesting that iron mobilization/sequestration is regulated by other pathways or that hepatic synthesis, which is the major source of hepcidin, is not affected by local peritoneal iron overload.

This study does have a number of limitations, however. It was designed to better assess peritoneal iron disruption, particularly in peritoneal macrophages of endometriosis patients. Due to the cross-sectional nature of the study, we cannot formally exclude the possibility that aberrations in iron storage are not a consequence but rather a cause of endometriosis.

Santanam et al. (30) proposed a hypothesis on the occurrence of oxidative stress in the peritoneal cavity in case of endometriosis, implicating peritoneal macrophages, increased levels of oxidized lipoproteins, and the presence of inflammatory cytokines and growth factors. Our hypothesis is that iron overload observed in the different compartments of the peritoneal cavity of endometriosis patients, particularly macrophages, may be involved in the pathogenesis of endometriosis by inducing oxidative stress. Indeed, iron accumulation in macrophages leads to excessive production of ROS and enhanced activation of NF-κB (19). This transcriptional factor has been implicated in endometriosis (31–33) and shown to induce expression of multiple genes encoding proinflammatory cytokines, growth and angiogenic factors, adhesion molecules and inducible enzymes (inducible nitric oxide synthase [iNOS], cyclooxygenase-2 [COX-2]) (34), which are all expressed by activated peritoneal macrophages. In line with this hypothesis, increased activation of NF-κB has recently been reported in peritoneal macrophages from endometriosis patients compared with controls (20).

This study does have a number of limitations, however. It was designed to better assess peritoneal iron disruption, particularly in peritoneal macrophages of endometriosis patients. Due to the cross-sectional nature of the study, we cannot formally exclude the possibility that aberrations in iron storage are not a consequence but rather a cause of endometriosis.
Furthermore, because of the multiple variables that could intervene (menstrual cycle phase, revised ASRM stage, endometriosis subtype, and/or age at first diagnosis), further experiments are needed to increase the statistical power of our data.

Identification of the pathological pathways involved in macrophage dysfunction in endometriosis may lead to better targeted therapies in the future, while research into molecules inhibiting oxidative stress and NF-κB pathways in activated macrophages could well prove to be a major field of investigation in the development of medical therapy for endometriosis.

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REFERENCES