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mRNA levels of low-density lipoprotein receptors are overexpressed in the foci of deep bowel endometriosis

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STUDY QUESTION: Is mRNA expression of LDL receptors altered in deep bowel endometriotic foci?

SUMMARY ANSWER: mRNA expression of LDL receptors is up-regulated in deep bowel endometriotic foci of patients with endometriosis.

WHAT IS KNOWN ALREADY: Several studies have demonstrated the overexpression of low-density lipoprotein receptors in various tumour cell lines and endometriosis has similar aspects to cancer, mainly concerning the pathogenesis of both diseases. This is the first study we know of to investigate lipoprotein receptors expression in deep endometriosis with bowel involvement.

STUDY DESIGN, SIZE, DURATION: During 2014–2015, an exploratory case-control study was conducted with 39 patients, including 20 women with a histological diagnosis of deep endometriosis compromising the bowel and 19 women without endometriosis who underwent laparoscopic tubal ligation.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Peripheral blood samples were collected on the day of surgery for lipid profile analysis, and samples of endometrial tissue and of bowel endometriotic lesions were also collected. The tissue samples were sent for histopathological analysis and for LDL-R and LRP-1 gene expression screening using quantitative real-time PCR.

MAIN RESULTS AND THE ROLE OF CHANCE: Patients with deep endometriosis had lower LDL-cholesterol than patients without the disease (119 ± 23 versus 156 ± 35; $P = 0.001$). Gene expression analysis of LDL receptors revealed that LDL-R was more highly expressed in endometriotic lesions when compared to the endometrium of the same patient but not more than in the endometrium of women without endometriosis (0.027 ± 0.022 versus 0.012 ± 0.009 versus 0.019 ± 0.01, respectively; $P < 0.001$). LRP-1 was more highly expressed in endometriotic lesions, both when compared with the endometrium of the same patient and when compared with the endometrium of patients without the disease (0.307 ± 0.207 versus 0.089 ± 0.076 and versus 0.126 ± 0.072, respectively; $P < 0.001$). The study also showed that LDL-R gene expression in the endometrium of women with endometriosis was higher during the secretory phase of the menstrual cycle ($P = 0.001$). LRP-1 gene expression was increased during the secretory phase in the endometrium of women without the disease ($P = 0.008$).

LIMITATIONS, REASONS FOR CAUTION: In the endometriotic lesions, the presence of fibrosis is substantial, restricting access to the stromal and glandular components of the lesion. Despite that, we found that LDL receptor mRNA was overexpressed. Future studies may perform laser microdissection to isolate the area of interest in the target tissue, excluding fibrosis contamination.

WIDER IMPLICATIONS OF THE FINDINGS: This study supports the feasibility of LDL-R targeted therapy in the treatment of deep endometriosis.

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Key words: endometriosis / receptors, LDL / Low Density Lipoprotein Receptor-Related Protein 1 / cholesterol, LDL / nanoparticles

Introduction

Deep endometriosis is a major cause of pelvic pain and infertility in women. The disease has proliferative characteristics and infiltrates pelvic tissues (Cornillie *et al.*, 1990). It often causes adhesions, retractions and deformities in organs adjacent to the uterus, and it has a strong resemblance to neoplastic disease because of the presence of growth factors and cytokines associated with the regulation of cell proliferation and angiogenesis (Podgaec *et al.*, 2007; Pupo-Nogueira *et al.*, 2007; Podgaec *et al.*, 2012; Bellelis *et al.*, 2013). In addition, analogous to cancer, endometriotic lesions may be single or present in distant foci, mimicking metastases (Thomas and Campbell, 2000; Abrao *et al.*, 2006).

The above data suggest the possibility of a common pathophysiological basis between malignancies and deep endometriosis, allowing us to postulate that the biochemical and metabolic alterations present in cancer patients might also be observed in patients with endometriosis.

In line with this reasoning, several studies have demonstrated the overexpression of low-density lipoprotein receptors (LDL-R and LRP-1) in various tumour cell lines (Ho *et al.*, 1978; Rudling *et al.*, 1990; Maranhão *et al.*, 1994; Guo *et al.*, 2011; Liu *et al.*, 2013). These receptors are glycoproteins that are synthesized in the endoplasmic reticulum and are expressed on the surface of most cells, especially in the liver. Cells can obtain cholesterol and other lipids in the plasma through the uptake of these lipoproteins by LDL receptors (Brown and Goldstein, 1986; Goldstein and Brown, 2009).

Overexpression of LDL receptors occurs as a result of the increased need for cholesterol for synthesis of cell membranes consequent to the rapid proliferation of malignant tumour cells, a fact that explains the crucial role of cholesterol in tumourigenesis (Webb, 1901; Gabitova *et al.*, 2014). Furthermore, because of the amount of cholesterol required by tumour cells, other studies have shown a reduction in serum total cholesterol (TC) and LDL concentrations resulting from the accelerated removal of this lipoprotein from the bloodstream (Ho *et al.*, 1978; Hungria *et al.*, 2004; Pinheiro *et al.*, 2006; Kok *et al.*, 2011; Simko and Ginter, 2014; Sun *et al.*, 2016).

Endometriosis is known to be an oestrogen-dependent hormonal disease, and the presence of oestrogen is essential for inducing the growth of endometriotic foci in a manner similar to its action on the endometrium (Giudice, 2010). Endometriosis-related changes in cholesterol levels occur not only through systemic oestrogenic stimulation but also via local production in the focus of the disease, where cholesterol is converted into steroid hormones promoted by specific enzymes (Huhtinen *et al.*, 2012).

Considering the relationship of cholesterol with fundamental cellular mechanisms in tumourigenesis and its crucial role as a precursor of steroid hormones, we suggest that it may be involved in the pathophysiology of deep endometriosis because of this disease's similarities to cancer and its oestrogen dependence.

The aim of this study was to investigate whether LDL receptors (LDL-R and LRP-1) are overexpressed in deep bowel endometriotic foci compared with the eutopic endometrium and to determine the concentration of LDL-cholesterol in women with the disease.

Materials and Methods

Ethical approval

The study was approved by the Research Ethics Committee (CAPPesq #0417/11) of the Hospital das Clínicas, School of Medicine, University of São Paulo, in the years from 2014 to 2015. All patients were informed in advance about the study objectives, and samples were collected only after the participants signed a consent form.

Patients

This was a prospective, exploratory case-control study with 39 patients, of whom 20 had a histological diagnosis of deep endometriosis with bowel involvement; 19 did not have endometriosis. The study was conducted at the Gynaecological Clinic of the Hospital das Clínicas and in the Lipid Metabolism Laboratory of the Heart Institute, both of which are in the School of Medicine, University of São Paulo, SP, Brazil.

The following inclusion criteria were adopted for patients in both groups: were of reproductive age, had eumenorrheic cycles, were not suffering from autoimmune or neoplastic diseases and had not used antidyslipidaemic or hormonal therapy in the 3 months prior to surgery.

Women in the endometriosis group presented with cyclic dyschezia that was not responsive to 6–12 months of medical treatment and with sonographically demonstrated deep lesions (>5 mm) on the rectosigmoid where there was progression of symptoms or increase in the size of the lesion at imaging. Women may have also presented with sub-occlusion, defined by symptoms such as nausea and stomach upset during menses, long periods of constipation and early satiety, associated with the presence of deep rectosigmoid lesions observed at transvaginal ultrasound (Riazi *et al.*, 2015). Histologically, these lesions all compromised the inner muscular layer of the bowel.

Women in the comparison group without endometriosis were submitted to pelvic examination by videolaparoscopy, which confirmed the absence of lesions. Women who had incidental findings of superficial or deep endometriosis at the time of tubal ligation were excluded from the study.

The day of the menstrual cycle was recorded at the time of the surgery, and the patients were divided according to whether they were in the proliferative phase, i.e. between the first and 14th day of the cycle, or the secretory phase, i.e. between the 14th and the last day of the menstrual cycle (Ecochard and Gougeon, 2000).

Collection, processing and storage of biological samples

On the morning of surgery, prior to administration of anaesthesia, 20 ml of peripheral blood was collected from each patient after a 12-h fast. The blood samples were sent immediately to the Lipid Metabolism Laboratory,

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where they were centrifuged at 1500*g* for 10 minutes to separate the serum and plasma.

In both groups, eutopic endometrial samples were obtained using a Pipelle® curette immediately after the induction of anaesthesia and the positioning of the patient in a dorsal recumbent position. Deep endometriotic foci were completely resected according to the surgical treatment proposed to eradicate the disease. The tissue fragments collected during surgery were immediately stored in RNA*Later*® solution (Ambion by Life Technologies, USA) and sent to the Lipid Metabolism Laboratory.

Serum, plasma and tissue samples were stored at −80°C for further analysis.

Fragments of endometriotic lesions were sent to the Pathological Anatomy Laboratory of the Hospital das Clínicas, School of Medicine, University of São Paulo, for histological confirmation of the disease.

Lipid profile

Serum TC and triglycerides levels were determined through enzymatic methods (Labtest, Brazil). HDL-cholesterol was measured with the same method used for TC after lipoprotein precipitation with magnesium phosphotungstate. LDL-cholesterol (LDL-c) values were calculated according to the Friedewald formula (Friedewald *et al.*, 1972). Serum concentrations of Apo AI and Apo B-100 were measured using immunoturbidimetry (Roche, Germany).

Gene expression analysis of LDL receptors by quantitative real-time PCR

Total RNA was isolated from deep endometriosis and eutopic endometrium tissues. Tissue processing of all the samples was handled in the same way: 100 mg of tissue fragment was homogenized in 1 ml of TRIzol® reagent (Invitrogen by Life Technologies, USA) using a polytron homogenizer Power Gen 125™ instrument (Thermo Fisher Scientific, USA). Subsequently, RNA isolation was performed according to the manufacturer's instructions. RNA purification and quantification were evaluated by measuring absorbance at 260 and 280 nm with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc., USA). Measurements of the 260/280 ratio that ranged from 1.8 to 2.0 were considered to indicate satisfactory purity. The quality of the samples was assessed by performing denaturing agarose gel electrophoresis. The first strand of cDNA was synthesized from 250 ng of total RNA using the Superscript® VILO™ Master Mix 5X (Invitrogen by Life Technologies, USA) according to the manufacturer's specifications.

The gene expression levels of LDL receptors were determined by quantitative real-time PCR (qRT-PCR) using the TaqMan detection system. Reactions were performed in duplicate and run on a StepOnePlus Real-Time PCR System (Applied Biosystems by Life Technologies, USA). Quantitative data were normalized relative to the glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH) as an internal housekeeping control gene. TaqMan gene expression assays were used for LDL-R (Hs00181192_m1) and LRP-1 (Hs00233856_m1), and pre-developed TaqMan assay reagents were used for human GAPDH (Applied Biosystems by Life Technologies, USA). The $2^{-\Delta Ct}$ method was applied to calculate the mRNA expression levels of LDL-R and LRP-1 (Livak and Schmittgen, 2001; Schmittgen and Livak, 2008).

Statistical analysis

Statistical analysis was conducted using SPSS 20.0 statistical software (SPSS® Advanced Statistics, IBM Corporation, Chicago, IL, USA). A power calculation indicated that with $n = 16$ in each group, we would have 80% power (95% CI) to detect a difference in gene expression values at least 1 SD among the study groups.

Patient characteristics and lipid profile were compared using an unpaired Student's *t*-test. Fisher's exact test was used to analyse percentages. The data were analysed according to the phase of the menstrual cycle, and their associations with study groups were determined using the chi-square test (χ^2). Gene expression analysis was assessed using the Mann–Whitney test or generalized estimation equations with Gamma distribution and identity link function. The Bonferroni multiple comparison correction was used to compare mRNA expression levels between groups or sites. Correlations among age, BMI and gene expression were assessed using Spearman's rho correlation test. Lipid profile and gene expression analyses were adjusted by BMI and the phase of the menstrual cycle. In all analyses, parameters were considered significantly different when $P < 0.05$. Data were expressed as the mean ± SD.

Results

Initially, the present study enrolled 20 women with endometriosis and 23 women without the disease. Four patients were excluded because they presented incidental findings of superficial or deep endometriosis at the time of tubal ligation. In total, 19 women without endometriosis were included.

Weight, height, BMI and serum LDL-c are decreased in endometriosis

Women with endometriosis had a lower mean weight ($P = 0.001$), height ($P = 0.046$) and BMI ($P = 0.006$) than those without the disease, as shown in Table I.

Serum LDL-c is decreased in endometriosis ($P = 0.001$) (Table II).

LDL receptors gene expression is increased in endometriosis

In the analysis of the mRNA expression levels of LDL receptors, determined by real-time PCR, we compared the eutopic endometrium of women without endometriosis (control group) versus women with endometriosis (endometrium group) and versus endometriosis lesions (endometriosis group). Gene expression was calculated based on the $2^{-\Delta Ct}$ method (Figs 1 and 2).

Table I Physical characteristics and phase of the menstrual cycle in control group and in women with endometriosis. Data are expressed as the mean ± SD.

	Control group ($n = 19$)	Group with endometriosis ($n = 20$)	*P*-value
Physical characteristics			
Age (years)	33.6 ± 5.7	35.7 ± 6.3	0.288
Weight (kg)	73.7 ± 8.4	62.6 ± 10.5	***0.001***
Height (m)	1.65 ± 0.06	1.61 ± 0.07	***0.046***
BMI (kg/m^2)	26.9 ± 2.9	24.1 ± 3.2	***0.006***
Phase of the menstrual cycle (%)			
Proliferative	10 (52.6)	11 (55.0)	0.882*
Secretory	9 (47.4)	9 (45.0)	

*P-values were calculated by Student's *t*-test or Chi-square test.

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Table II Lipid profile of control group and women with endometriosis. Data are expressed as the mean ± SD.

	Control group ($n = 19$)	Group with endometriosis ($n = 20$)	*P-value
Cholesterol (mg/dl)			
Total	194 ± 37	187 ± 27	0.604
High density lipoprotein	43 ± 14	42 ± 9	0.639
Low density lipoprotein	156 ± 35	119 ± 23	***0.001***
Triglycerides (mg/dl)	119 ± 55	130 ± 40	0.274
Apolipoproteins (mg/dl)			
AI	136.5 ± 19.9	128.1 ± 33.3	0.419
B-100	91.7 ± 30.8	76 ± 20.9	0.280

*Student's *t*-test adjusted for BMI.

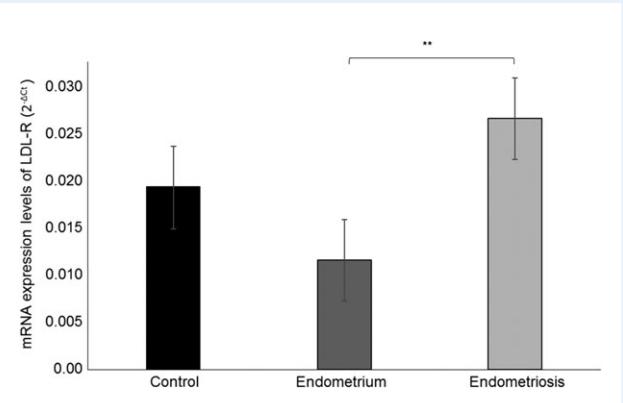

Figure 1 Increased low-density lipoprotein receptor (LDL-R) gene expression in endometriosis. mRNA expression levels of LDL-R were determined in endometrium of 19 women without endometriosis (control), compared to 20 women with endometriosis in endometrium (endometrium) and endometriotic lesions (endometriosis). Total RNA was reverse-transcribed into cDNA and analysed by quantitative real-time PCR using the TaqMan detection method. Quantitative data were normalized relative to the glyceraldehyde 3-phosphate dehydrogenase gene as an internal housekeeping control gene. The differences in expression levels among the groups were determined using the Mann–Whitney test. The Bonferroni multiple comparison correction was used to compare gene expression levels between groups or sites. Data were adjusted for BMI. Results are shown as the mean ± SD of each group. Endometriosis tissue presented increased LDL-R gene expression levels when compared to endometrium of women with the disease (**$P < 0.001$).

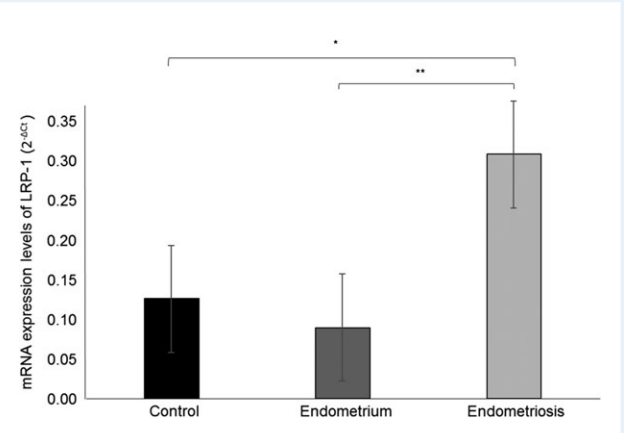

Figure 2 Increased low density lipoprotein receptor-related protein 1 (LRP-1) gene expression in endometriosis. mRNA expression levels of LRP-1 were determined in endometrium of 19 women without endometriosis (control), compared to 20 women with endometriosis in endometrium (endometrium) and endometriotic lesions (endometriosis). Total RNA was reverse-transcribed into cDNA and analysed by quantitative real-time PCR using the TaqMan detection method. Quantitative data were normalized relative to the glyceraldehyde 3-phosphate dehydrogenase gene as an internal housekeeping control gene. The differences in expression levels among the groups were determined using the Mann–Whitney test. The Bonferroni multiple comparison correction was used to compare gene expression levels between groups or sites. Data were adjusted for BMI. Results are shown as the mean ± SD of each group. Endometriosis tissue presented increased LRP-1 gene expression levels when compared to endometrium of women with the disease (**$P < 0.001$) and when compared to endometrium of women without the disease (*$P = 0.004$).

As shown in Fig. 1, an increase in the mRNA expression levels of LDL-R was clearly observed in the endometriotic tissue samples from women with endometriosis when compared with endometrium samples from the same patients (1.9-fold difference). In patients with endometriosis, 85% of the endometriotic tissue samples (17 cases out of 20 patients) presented elevated LDL-R gene expression compared with endometrium samples from the same patients ($P < 0.001$).

Figure 2 shows that the mRNA expression levels of LRP-1 were higher in endometriotic lesions than in the endometrium of women with (5-fold difference) and without endometriosis (2.4-fold difference). In patients with endometriosis, 95% of the endometriotic tissue samples (19 cases out of 20 patients) presented an elevated LRP-1 gene expression compared with the endometrium samples from the same patients ($P < 0.001$).

Compared to the control group, 90% of the endometriotic tissue samples (18 cases out of 20 patients) presented elevated LRP-1 gene expression ($P = 0.004$). LDL-R gene expression in patients with endometriosis was not different from that in the control group.

LDL receptors gene expression was correlated with BMI and the phase of the menstrual cycle

As shown in Table III and Fig. 3, a positive correlation between BMI and mRNA expression levels of LRP-1 in endometriotic lesions was found ($r = 0.501$ and $P = 0.024$).

However, mRNA expression levels of LDL-R did not correlate with BMI or age, as shown in Table III.

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Table III Spearman's correlations of age and BMI with mRNA expression levels of low-density lipoprotein receptor (LDL-R) and low density lipoprotein receptor-related protein 1 (LRP-1) between groups with or without endometriosis and in the different sites (eutopic endometrium and endometriosis lesion).

Spearman's correlation	Control group		Group with endometriosis			
	Eutopic endometrium		Eutopic endometrium		Endometriosis lesion	
	Age (years)	BMI (kg/m^2)	Age (years)	BMI (kg/m^2)	Age (years)	BMI (kg/m^2)
mRNA levels						
LDL-R						
r	−0.003	0.204	−0.070	−0.076	0.039	0.372
P-value	0.991	0.401	0.769	0.750	0.870	0.107
LRP-1						
r	0.098	0.107	−0.136	−0.284	0.078	0.501
P-value	0.690	0.663	0.566	0.224	0.743	***0.024***

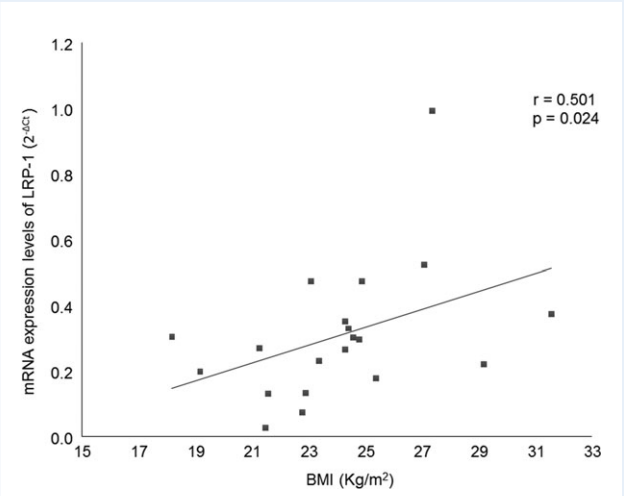

Figure 3 Positive correlation between BMI and low density lipoprotein receptor-related protein 1 (LRP-1) gene expression. The coefficient of correlation (*r*) shows difference between BMI and mRNA expression levels of LRP-1 in endometriotic lesions ($r = 0.501$ and $P = 0.024$, Spearman's correlation test).

Analysis of the correlation between the gene expression data and the phase of the menstrual cycle demonstrated that mRNA expression levels of LDL-R in the endometrium of women with endometriosis were higher during the secretory phase ($P = 0.001$). In the same way, the mRNA expression levels of LRP-1 were higher during the secretory phase in the endometrium of women without the disease ($P = 0.008$), as shown in Table IV.

Discussion

Studies showing the role of cholesterol in tumourigenesis have been of clinical importance in demonstrating that tumour cells have greater LDL uptake than normal cells (Ho *et al.*, 1978; Pinheiro *et al.*, 2006; Kok *et al.*, 2011; Gabitova *et al.*, 2014; Simko and Ginter, 2014). LDL is the major cholesterol transporter in the body, accounting for ~70% of total plasma cholesterol (Vitols *et al.*, 1992). Some solid tumours, mainly those with intense proliferation, have an increased uptake of cholesterol, as cell division increases the need for cholesterol to synthesize membranes (Rudling *et al.*, 1990; Shrivastava *et al.*, 2014; Vasseur and Guillaumond, 2015). Accumulation of cholesterol in mitochondria has emerged as a molecular element that regulates some of the metabolic alterations in cancer cells by impairing mitochondrial function. As a consequence, mitochondrial cholesterol loading in cancer cells may contribute, in part, to the stimulation of aerobic glycolysis to meet the energetic demands of proliferating cells while protecting cancer cells against mitochondrial apoptosis due to changes in mitochondrial membrane dynamics (Ribas *et al.*, 2016).

Consequently, our attention was strongly drawn to the results of the lipid profile evaluation, particularly LDL-c concentration. Patients with deep bowel endometriosis had lower LDL-c levels than the control group, which was similar to the results observed in patients with multiple myeloma (Hungria *et al.*, 2004), Hodgkin's and non-Hodgkin's lymphoma (Pinheiro *et al.*, 2006), prostate cancer (Kok *et al.*, 2011), ovarian and endometrial cancer (Sun *et al.*, 2016).

In contrast to our findings, one study showed an unfavourable lipid profile, with an increase of 38% in LDL-c levels in patients with endometriosis (Melo *et al.*, 2010). However, in that study, patients considered to not have endometriosis did not have laparoscopic confirmation of the absence of the disease; instead, the absence of endometriosis was surmised from the fact that they did not exhibit infertility or pelvic pain. This may represent a selection bias because the literature notes incidental findings of endometriosis in up to 22% of patients undergoing tubal ligation (Cramer and Missmer, 2002; Murphy, 2002). Moreover, that study did not differentiate between different forms of endometriosis, i.e. whether it was the superficial, ovarian or deep form. In our study, the objective was to evaluate only the deep bowel form of the disease because of its similarity to expansive tumour lesions, regardless of the presence of adhesions or other findings, such as ovarian endometrioma.

The main objective of this study was to evaluate the gene expression of LDL receptors (LDL-R and LRP-1) to understand the cell behaviour of deep bowel endometriosis with respect to cholesterol uptake, specifically, whether the nodule is avid for this metabolite.

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Table IV Low-density lipoprotein receptor (LDL-R) and low density lipoprotein receptor-related protein 1 (LRP-1) gene expression in women with and without endometriosis during the proliferative and secretory phases of the menstrual cycle. Data are expressed as the mean ± SD.

Group	Site	Gene expression	Phase of the menstrual cycle		*P-value
			Proliferative	Secretory	
Control	Eutopic endometrium	LDL-R	0.015 ± 0.008	0.024 ± 0.011	0.065
		LRP-1	0.093 ± 0.038	0.162 ± 0.085	***0.008***
Endometriosis	Eutopic endometrium	LDL-R	0.007 ± 0.003	0.017 ± 0.01	***0.001***
		LRP-1	0.108 ± 0.097	0.067 ± 0.03	0.710
Endometriosis	Endometriosis lesion	LDL-R	0.025 ± 0.023	0.029 ± 0.02	0.261
		LRP-1	0.291 ± 0.159	0.327 ± 0.264	0.941

*Mann–Whitney test.

Our results indicated that the LDL-R mRNA expression levels were higher in endometriotic lesions than in the endometrium of women with endometriosis and that the LRP-1 mRNA expression levels were higher in endometriotic lesions than in the endometrium of women with and without endometriosis.

We therefore consider that LDL receptors overexpression in deep endometriotic foci may be highly important in terms of the pathophysiology of the development and progression of this disease. In addition to acting as a substrate for cell division and membrane component synthesis, cholesterol can act as a precursor of steroid hormones, and this function may represent a major role in the growth of deep endometriotic lesions.

It is known that oestrogenic activity plays a key pathophysiological role in endometriosis, explaining both lesion activity and the pain mechanism (Brown and Farquhar, 2014). Steroidogenic acute regulatory protein is responsible for the initial stage of oestrogen formation, i.e. cholesterol uptake into the mitochondria (Miller, 2013). Thereafter, five proteins catalyse the enzymatic steps that convert cholesterol into biologically active oestradiol. The main stage, conversion of C19 steroids into oestrogen, is catalysed by aromatase, the inhibition of which effectively ends the entire oestrogen production process (Bulun *et al.*, 2005).

Oestrogen production in women with endometriosis occurs via three distinct pathways: oestradiol secreted by the ovary; the conversion of androstenedione into oestrone and then to oestradiol, a phenomenon catalysed by aromatase in adipose and skin tissue; and cholesterol, which is converted into oestradiol locally in endometriotic tissue because it expresses a complete set of steroidogenic genes, including the aromatase gene (Bulun *et al.*, 2005).

High expression of aromatase in endometriosis foci has been shown previously (Noble *et al.*, 1996; Kitawaki *et al.*, 1997; Bulun *et al.*, 2002, 2005), as has the low expression or even absence of this enzyme in the eutopic endometrium of women without endometriosis. We therefore hypothesize that the overexpression of LDL receptors in endometriotic foci may be related to cholesterol uptake and could contribute to autochthonous oestrogen production and, consequently, affect the progression of the disease.

Several studies have shown that LRP-1 is expressed in the eutopic endometrium (Moestrup *et al.*, 1992; Zheng *et al.*, 1994; Sayegh *et al.*, 1995; Foca *et al.*, 2000). In the present study, we observed that LRP-1 mRNA levels were higher in the endometrium in the secretory phase when compared to the proliferative phase in women without endometriosis. These findings were consistent with those of another study, which showed that LRP-1 expression in the endometrium was greater during the secretory phase than during the proliferative phase of the menstrual cycle (Foca *et al.*, 2000).

Finally, the results of our study showed that BMI was positively correlated with LRP-1 mRNA expression levels in endometriotic lesions, suggesting that individuals with an unfavourable height/weight ratio can show greater expression of this receptor in endometriotic lesions. However, there is no study in the literature that has evaluated the association of BMI with the expression of LDL receptors in the endometrium or in endometriotic lesions, which creates an opportunity for a new line of research.

These results are encouraging in terms of the direction of new treatment strategies, such as the use of statins, 3-hydroxy 3-methylglutaryl coenzyme A reductase inhibitors used to reduce serum cholesterol levels. *In vitro* and *in vivo* studies started in 2007 have shown the benefits of statins in the treatment of endometriosis due to their antiproliferative, anti-inflammatory, antioxidant, antiangiogenic and metalloproteinase matrix-inhibiting actions (Gibran *et al.*, 2014).

Another promising possibility is the use of lipid nanoparticles that are able to bind antiproliferative drugs in their interior. Lipid nanoparticles carrying chemotherapeutic agents have been used to treat various types of cancer (Rodrigues *et al.* 2002, Valduga *et al.*, 2003; Azevedo *et al.*, 2005; Mendes *et al.*, 2009; Pires *et al.*, 2009), atherosclerosis (Maranhão *et al.*, 2008; Tavares *et al.*, 2011; Bulgarelli *et al.*, 2013; Leite *et al.*, 2015) and rheumatoid arthritis (Mello *et al.*, 2013, 2016). Once injected into the bloodstream, lipid nanoparticles obtain apolipoprotein E via adhesion upon contact with original LDL molecules and are directed to tissues with LDL receptors overexpression; thus, the drug can be delivered to the inside of the lesion while minimizing potential side effects, increasing the half-life of the drug and reducing the required dosage (Dias *et al.*, 2007; Teixeira *et al.*, 2008). The combination of lipid nanoparticles associated with a chemotherapeutic agent is yet to be tested for both safety and efficacy in young women of reproductive age.

This pilot study evaluated the mRNA expression levels of LDL receptors in this demographic, both with and without endometriosis. Although, in the endometriotic lesions the presence of fibrosis is substantial, restricting access to the stromal and glandular components of

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the lesion, it was possible to obtain a favourable amount of RNA to perform the gene expression analysis. Future studies may perform laser microdissection to isolate the area of interest in the target tissue, excluding contamination by fibrosis.

In conclusion, LDL receptors are up-regulated in deep bowel endometriotic tissues of women with endometriosis, similar to malignant tumours. Lower LDL-c levels were also observed in women with deep endometriosis, suggesting an increase in cholesterol uptake by endometriotic tissues in those women. These findings may pave the way for novel treatment strategies for the disease, such as the use of statins or nanoparticles that target LDL receptors for drug delivery to the endometriotic tissues, especially for treatment of women with lesions that are difficult to address surgically.

Authors' roles

L.G. assisted in the design of the study; conceptualized the paper, wrote the first draft and took the lead on subsequent edits; performed the surgical procedures and collected the biological material. R.C.M. assisted in the design of the study and provided substantive edits to the manuscript. E.R.T. performed biochemical and statistical analysis and provided substantive edits to the manuscript. P.O.C. performed gene expression and statistical analysis and provided substantive edits to the manuscript. M.S.A. assisted in conceptualizing the paper and provided substantive edits to the manuscript. S.P. assisted in the design of the study, assisted in conceptualizing the paper, oversaw the statistical analysis and provided substantive edits on the analytic plan and the manuscript.

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Conflict of interest

None declared.

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