Effects of hypoxia-inducible factor-1α on endometrial receptivity of women with polycystic ovary syndrome

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Abstract. Embryo implantation is associated with an hypoxic endometrial microenvironment. Hypoxia-inducible factor-1α (HIF-1α) is activated under hypoxic conditions. In the present study, the expression pattern of HIF-1α in endometrial tissue was investigated and its effects on endometrial receptivity in patients with polycystic ovary syndrome (PCOS) were examined. A total of 81 patients were enrolled for *in vitro* fertilization and embryo transfer. They were divided into PCOS (n=40) and Control groups (n=41); both groups were further divided based on body weight (overweight and normal weight subgroups). The expressions of HIF-1α, vascular endothelial growth factor (VEGF) and glucose transporter protein (GLUT)-1 and GLUT4 were determined by reverse transcription-quantitative polymerase chain reaction and immunohistochemistry. The results demonstrated that mRNA and protein expression levels of HIF-1α and VEGF in the PCOS group were significantly lower compared with expression levels in the Control group. However, there were no statistically significant differences in the expression levels of GLUT1 and GLUT4 between groups. In patients with PCOS, GLUT1 and GLUT4 were mainly localized in the nuclei and cytoplasm, but not in the cell membrane. Overweight patients had the lowest expression levels of HIF-1α, VEGF and GLUT1 expression compared with normal weight patients. In conclusion, HIF-1α may be involved in the molecular mechanisms of endometrial dysfunction in women with PCOS, particularly in those who are overweight. HIF-1α might therefore be a novel target for improving the endometrial receptivity and successful embryo implantation in PCOS women.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects 5-10% of 20-37 years old women (1). PCOS is characterized by anovulation, hyperandrogenemia and polycystic ovaries. Up to 60% of all patients with PCOS are overweight or obese (2); recent studies have demonstrated the negative effects of increased body weight on reproductive outcomes (3,4). In the past few decades, advances in assisted reproductive techniques allowed for the selection of high quality embryos, but the implantation rates for patients with PCOS remained low in clinical settings (5,6). Poor reproductive outcomes in patients with PCOS are probably associated with decreased endometrial receptivity (7). Therefore, it is important to improve the endometrial receptivity of women with PCOS.

During the window for embryo implantation, a rich vascular network is necessary to supply nutrients and oxygen for cell proliferation and implantation of the blastocyst (8). At this time, the endometrium microenvironment is considered to be hypoxic (9). Hypoxic conditions activate hypoxia-inducible factor (HIF)-1, which is an oxygen-sensitive transcription factor. HIF-1 is a heterodimer, which comprises α and β subunits (10). The HIF1-β subunit is stably expressed, whereas the expression of HIF-1α is regulated by oxygen concentration (11,12). HIF-1α is rapidly degraded through the ubiquitin-proteasome pathway under physiological conditions. Under hypoxic conditions, HIF-1α is stabilized and is involved in the regulation of cellular processes, including angiogenesis, glucose metabolism and cell differentiation (13,14). Previous studies have reported that HIF-1α expression in the endometrium occurs exclusively during the secretory and menstrual phase, which may be associated with the physiological changes associated with menstruation (15).

Vascular endothelial growth factor (VEGF) is an important mediator of angiogenesis (16). Glucose transporter protein (GLUT) is responsible for glucose uptake and storage in the endometrium (17-19). VEGF and GLUT are targets of HIF-1α and their expression is necessary for endometrial receptivity (20). The implantation window is in the mid-secretory phase of human endometrium, that is, 7-10 days following

luteinizing hormone (LH) surge (21). The role of HIF-1α in the regulation of endometrial receptivity is yet to be confirmed. In the present study, the expressions of HIF-1α and its target genes VEGF, GLUT1 and GLUT4 were investigated in the endometrium of women with and without PCOS during the implantation window, and the effects of body weight on their expression levels were also examined.

Materials and methods

Subjects. The present study recruited 40 women with PCOS and 41 women with tubal blockage who served as controls. Women with PCOS were divided into overweight (OW)-PCOS (n=22) and normal-weight (NW)-PCOS (n=18) subgroups. Women in the control group were also divided into OW-Control (n=21) and NW-Control (n=20) subgroups. All participants were referred to the Reproductive Medicine Center, Yantai Yuhuangding Hospital (Yantai, China) for *in vitro* fertilization pre-embryo transfer. The patients were 20-37 years old with normal basal serum follicle stimulating hormone levels and antral follicle counts (AFC). None of the patients had taken oral contraceptives or other medicines in the preceding 3 months. Hysteroscopic examination was performed to exclude endometrial diseases in all participants. Patients with endometriosis, intrauterine adhesions, endometrial polyps, recurrent history of miscarriage and those with a recurrent implantation failure were excluded from the present study. PCOS was diagnosed according to the 2003 Rotterdam Consensus (22). Overweight and/or obesity were defined according to the 2000 World Health Organization and The International Obesity Task Force diagnostic criteria for the Asia-Pacific population (23). Clinical data, including age, duration of infertility, body weight, height, waist-to-hip ratio (WHR), basal serum follicle-stimulating hormone, LH, total testosterone (TT), fasting glucose (mmol/l), fasting insulin (mIU/l), estradiol (E2) and progesterone (P) levels following human chorionic gonadotropin (HCG) administration during controlled ovarian hyperstimulation (COH), number of oocytes, endometrial thickness and high-quality embryo rate were recorded. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2) and the homeostatic model assessment-insulin resistance (HOMA-IR) was calculated as [(fasting basal blood glucose)x(fasting basal insulin)]/22.5 (24). This study was approved by The Ethics Committee of Yantai Yuhuangding Hospital (Yantai, China), and written informed consent was received from each patient prior to enrolment in the study.

Ovarian stimulation. The gonadotropin-releasing hormone antagonist (GnRH-ant) protocol was used for COH (25). On day 3 of menstruation, 125-225 IU/day of recombinant FSH (Puregon; Merck Sharp & Dohme-Hoddesdon, UK) was used for ovarian stimulation, according to the patient's age, BMI, anti-Müllerian hormone, infertility duration and AFC. A 0.25 mg dose of GnRH antagonist (Orgalutran; Merck Sharp & Dohme-Hoddesdon) was administered daily when the follicle diameter reached 12 mm until the day of HCG (Beijing Saisheng Pharmaceutical Co, Ltd., Beijing, China) administration. Highly purified human menopausal gonadotropin (Menopur; Ferring Pharmaceuticals, Saint-Prex, Switzerland) was administered at the late follicle phase. HCG (6,000 IU) was administered for final oocyte maturation when at least two leading follicles were ≥18 mm in size, and oocytes were retrieved following 34-36 h. All patients received 600 mg/day progestogen (Utrogestan; Besins Manufacturing Belgium, France) for luteal phase support.

Specimens. Endometrial tissues were collected from patients with a curette on day 5 following the oocyte pick-up, certain tissues were fixed in 4% formaldehyde for 12 h at room temperature for paraffin embedding; the other tissues were washed in phosphate-buffered saline and stored immediately at -70˚C for RNA isolation.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from tissues using an RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA), as described previously (26). A BioWaveII spectrophotometer (Biochrom, Ltd., Cambridge, UK) was used to evaluate the concentration and purity of the RNA (A260:A280 ratio). cDNA was produced by reverse transcription using a PrimeScript RT Reagent kit (Takara Bio, Inc., Otsu, Japan), according to the manufacturer's protocol. qPCR was performed in 20 μl reactions using 1 μl of cDNA, 10 μM of each primer and 2X Platinum SYBR Green qPCR Supermix-UDG (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), following the manufacturer's protocol. The following gene-specific primers were purchased from Invitrogen (Thermo Fisher Scientific, Inc.) (27): HIF-1α, forward 5'-CATCAGCTATTT GCGTGTGAGGA-3', reverse 5'-AGCAATTCATCTGTG CTTTCATGTC-3'; VEGF, forward 5'-CCTGGTGGACAT CTTCCAGGAGTACC-3', reverse 5'-GAAGC-TCATCT CTCCTATGTGCTGGC-3'; GLUT1, forward 5'-CCAGCT GCCATTGCCGTT-3', reverse 5'-GACGTAGGGACCACA CAGTTGC-3'; GLUT4, forward 5'-CTGGGCCTCACAGTG CTAC-3', reverse, 5'-GTCAGGCGCTTCAGACTCTT-3'; GAPDH, forward 5'-GGGAAACTGTGGCGTGAT-3', reverse 5'-GAGTGGGTGTCGCTGTTGA-3'. PCR thermocycling was performed at 95˚C for 30 sec, followed by 45 cycles at 95˚C for 5 sec and at 60˚C for 60 sec.

Specificity of the PCR reactions was assessed by melting curve analysis. Correct melting temperatures were obtained for all products. The relative gene expression levels in each sample were normalized to the expression levels of GAPDH, which was used as the housekeeping gene, and were analyzed using the $2^{-\Delta\Delta Cq}$ method (28,29). This normalization could account for the inherent variability in the efficiency of the reverse transcription reactions. Each set of RT-qPCR reactions was repeated three times.

Hematoxylin and eosin (H&E) and immunohistochemical staining of endometrial sections. H&E staining was used for examination of the morphological characteristics of the endometrium and for demonstration of tissue integrity (30). Briefly, the tissues were hydrated in H_2O for 30 sec. Then the slides were immersed in hematoxylin (Beyotime Institute of Biotechnology, Shanghai, China; cat. no. C0105-1) for 30 sec and rinsed in H_2O for 1 min. Subsequently, the slides were stained with 1% eosin Y solution (Beyotime Institute of Biotechnology; cat. no. C0105-2) for 30 sec and dehydrated in 95 and 100% alcohol.

Table I. Clinical characteristics and laboratory parameters after controlled ovarian hyperstimulation in PCOS and controls.

Variable	PCOS (n=40)	Controls (n=41)
Age (years)	31.10±2.62	31.12±2.43
Infertility duration (years)	3.96±2.69	3.74±2.76
BMI (kg/m^2)	26.12±3.63	25.05±3.67
WHR	0.87±0.05	0.85±0.03
Follicle-stimulating hormone (mIU/ml)	5.78±2.15	6.48±1.51
LH (mIU/ml)	9.48±5.54	**5.67±2.16**[a]
TT (ng/ml)	0.43±0.20	**0.30±0.18**[a]
Fasting glucose (mg/dl)	5.2±0.19	**4.65±0.68**[a]
Fasting insulin (mIU/ml)	14.45±6.24	**7.93±2.33**[a]
HOMA-IR	3.38±1.54	**1.66±0.54**[a]
E2 levels on the day of HCG (pg/ml)	3,468.64±1,935.33	3,124.83±1,862.69
P levels on the day of HCG administration (ng/ml)	0.93±0.38	0.88±0.34
Endometrial thickness on HCG day (mm)	10.43±2.09	10.59±1.64
Number of oocytes	11.00±5.64	9. 95±4.28
High-quality embryo rate (%)	65.37±25.96	70.68±21.76

[a]P<0.05. BMI, body mass index; COH, controlled ovarian hyperstimulation; E2, estradiol; HCG, human chorionic gonadotropin; HOMA-IR, homeostasis model assessment-insulin resistance; LH, luteinizing hormone; P, progesterone; POCS, polycystic ovary syndrome; TT, total testosterone; WHR, waist-to-hip ratio.

Immunohistochemical staining of 4 μm thick sections of the formaldehyde-fixed and paraffin-embedded endometrial tissue was performed, as described previously (31). Briefly, the tissue sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Endogenous peroxidase activity was blocked by incubation of sections in 3% hydrogen peroxide in methanol for 10 min at room temperature. Following three washes in PBS, the sections were placed in a blocking solution sheep heat-inactivated serum (Beyotime Institute of Biotechnology; cat. no. C0265) for 30 min at oom temperature to block nonspecific binding sites. Subsequently, sections were incubated with the following primary antibodies: Rabbit polyclonal anti-HIF-1α (1:700; Abcam, Cambridge, UK, cat. no. ab85886), rabbit polyclonal anti-VEGF (1:200; Abcam; cat. no. ab46154), rabbit monoclonal anti-GLUT1 (1:150; Abcam; cat. no. ab150299) and rabbit polyclonal anti-GLUT4 (1:400; Abcam; cat. no. ab654), overnight at 4˚C. The sections were washed three times in PBS and were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (1:50, Beyotime Institute of Biotechnology; cat. no. A0208) at 37˚C for 1 h. 3,3-diaminobenzidin was used as the chromogen and counterstaining was done with hematoxylin.

Sections were examined with an Olympus CX31-LV320 light microscope (Olympus Corporation, Tokyo, Japan) at x20 magnification. Reactions demonstrating the specificity of primary antibodies were also carried out by omission of the antibodies in the incubating medium; no immunoreactivity was observed in these sections (data not shown). Experiments were repeated at least three times. The immunostaining density of HIF-1α, VEGF, GLUT1 and GLUT4 was evaluated using a semi-quantitative method, as previously described (32). The Image-Pro Plus 6.0 software (Media Cybernetics company, USA) was used to calculate the integrated optical density (IOD) and areas, three fields were examined in per slide. Data are presented as mean density (IOD/area) to indicate the relative expression (33).

Statistical analysis. Data analysis was performed using SPSS 19.0 (IBM Corp., Armonk, NY, USA). Data were expressed as the mean ± standard deviation (or standard error of the mean for the RT-qPCR experiments). Inter-group differences with respect to normally distributed variables were assessed with Student's t-test; differences pertaining to non-normally distributed variables were assessed with the Mann-Whitney U test. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical and COH characteristics of patients. Serum levels of LH, TT, fasting insulin, fasting glucose and HOMA-IR in patients with PCOS were significantly higher than those in the controls (P<0.05; Table I). No significant inter-group differences were observed with respect to E2, P level on HCG day and high-quality embryo rate. OW-PCOS patients exhibited higher BMI, WHR, fasting insulin and HOMAR-IR compared with NW-PCOS patients. In the control group, only BMI and WHR were significantly different between the OW-Control and NW-Control groups (P<0.05; Table II).

Endometrial mRNA expression levels of HIF-1α, VEGF, GLUT1 and GLUT4 during the implantation window. In both the PCOS and the Control groups, mRNA expression levels of *HIF-1α* were notably higher compared with the mRNA expression levels of of *VEGF*, *GLUT1* and *GLUT4*. mRNA expression

Table II. Clinical characteristics of overweight and normal-weight subgroups of the PCOS and controls.

Variable	NW-PCOS (n=18)	OW-PCOS (n=22)	NW-Control (n=20)	OW-Control (n=21)
Age (years)	30.94±2.65	31.18±2.75	30.65±2.94	31.57±1.77
Infertility duration (year)	3.25±1.68	4.48±3.17	4.60±3.22	2.93±2.01
BMI (kg/m^2)	22.64±1.73	**28.66±2.27**[a]	22.0±1.97	**27.94±2.27**[b]
WHR	0.82±0.01	**0.93±0.04**[a]	0.83±0.01	**0.89±0.02**[b]
FSH (mIU/ml)	5.47±1.68	6.00±2.45	6.67±1.33	6.31±1.69
LH (mIU/ml)	10.89±6.64	8.46±4.48	5.85±1.78	5.49±2.51
TT (ng/ml)	0.44±0.25	0.43±0.18	0.29±0.20	0.30±0.15
Fasting glucose (mg/dl)	5.11±0.43	5.11±0.21	4.75±0.77	4.70±0.67
Fasting insulin (mIU/ml)	9.72±3.99	**16.78±5.91**[a]	7.73±2.83	8.25±2.12
HOMA-IR	2.22±0.95	**3.84±1.41**[a]	1.63±0.64	1.73±0.52
Endometrium thickness on the day of HCG administration (mm)	10.31±2.30	10.52±1.99	10.60±1.53	10.57±1.77
Number of oocytes	11.00±5.52	11.00±5.85	11.00±4.07	8.95±4.34
High-quality embryo rate (%)	70.5±24.4	61.4±20.42	73.62±20.70	68.0±22.85

[a]P<0.05 vs. NW-PCOS. [b]P<0.05 vs. NW-Control. BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; HCG, human chorionic gonadotropin; HOMA-IR, homeostasis model assessment-insulin resistance; NW, normal weight; OW, overweight; TT, total testosterone; WHR, waist-to-hip ratio.

levels of *HIF-1α* and *VEGF* in patients with PCOS were significantly lower compared with the respective expression levels in the Control patients (P<0.05; Fig. 1A). *GLUT4* mRNA expression levels were very low in both groups and there was no statistically significant inter-group differences with respect to *GLUT1* and *GLUT4* expression levels (P>0.05; Fig. 1A).

Association between HIF-1a, VEGF, GLUT1 and GLUT4 mRNA expression levels with body weight. mRNA expression *levels* of *HIF-1α* and *VEGF* in OW patients were significantly lower than in the NW patients in both the PCOS and Control groups (P<0.05; Fig. 1B and C, respectively). *GLUT1* mRNA expression levels were significantly lower in OW-Control patients compared with expression in the NW-Controls (Fig. 1C; P<0.05); no statistically significant differences in *GLUT4* expression levels were identified between OW and NW patients with our without PCOS (P>0.05; Fig. 1B and C, respectively).

Endometrial histology and protein expression levels of HIF-1α, VEGF, GLUT1 and GLUT4 during implantation window. The histological stage of endometrium in PCOS and control groups was in secretory phase according to the Noyes standard (Fig. 2) (34). In the secretory endometrium, strong brown immunostaining for HIF-1α and VEGF were observed in the nuclei of epithelial and stroma cells in the PCOS and control groups. Positive cytoplasmic and nuclear immunostaining for GLUT1 and GLUT4 was detected in the epithelial cells of OW-PCOS and NW-PCOS patients. However, these were localized mainly in the cell membrane of cells in the OW-control and NW-control group (Fig. 2).

Semi-quantitative IOD protein expression levels of HIF-1α and VEGF in PCOS samples were significantly lower compared with expression levels in the controls (P<0.05; Fig. 3A). No

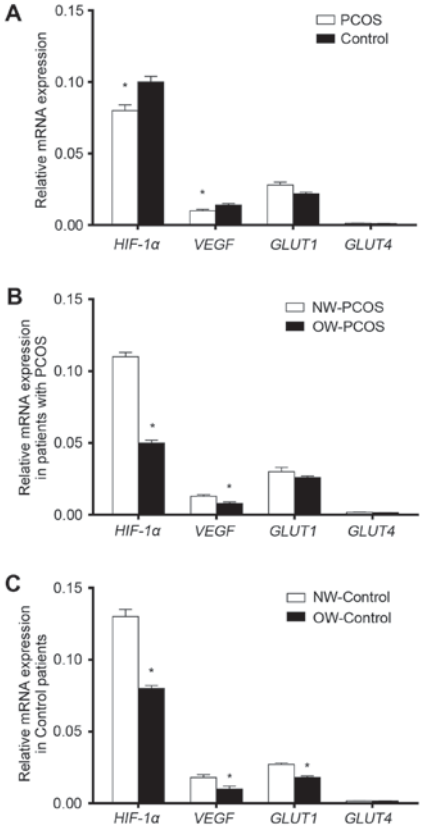

Figure 1. mRNA expression levels of *HIF-1α*, *VEGF*, *GLUT1* and *GLUT4*. Reverse transcription-quantitative polymerase chain reaction was used to examine mRNA expression level in the endometrium of (A) POCS and non-POCS Control groups, as well as in (B) NW-PCOS vs. OW-PCOS subgroups and in (C) NW-Control vs. OW-Control subgroups. Data are presented as the mean ± standard error of the mean; *P<0.05. GLUT, glucose transporter protein; HIF-1α, hypoxia-inducible factor-1α; NW, normal weight; OW, overweight; POCS, polycystic ovary syndrome; VEGF, vascular endothelial growth factor.

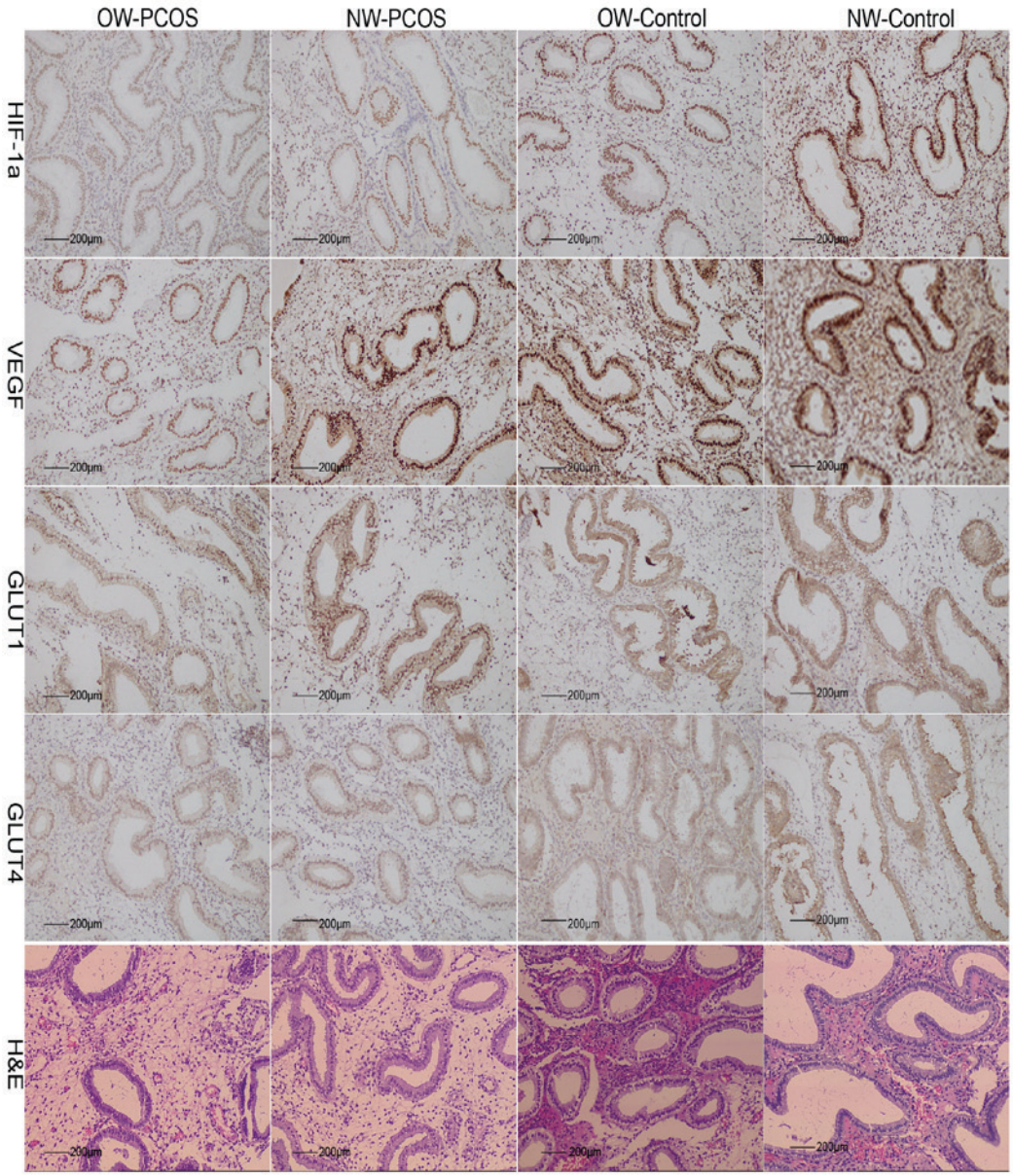

Figure 2. Immunohistochemical and H&E staining of endometrial tissues. Immunohistochemical detection of HIF-1α, VEGF, GLUT1 and GLUT4 proteins in the endometrium of OW-PCOS, NW-PCOS, OW-Control and NW-Control patients. Brown positive immunostaining was detected in epithelial and stroma cells of endometria for all antigens. H&E, hematoxylin and eosin. HIF-1α, hypoxia-inducible factor-1α; GLUT, glucose transporter protein; NW, normal weight; OW, overweight; POCS, polycystic ovary syndrome; VEGF, vascular endothelial growth factor.

statistically significant inter-group differences were identified with respect to GLUT1 and GLUT4 immunostaining (P>0.05; Fig. 3A).

Association between body weight and protein expression levels of HIF-1α, VEGF, GLUT1 and GLUT4. Semi-quantitative protein expression levels of HIF-1α and VEGF in OW patients were significantly lower compared with expression levels in the NW patients in both groups (P<0.05; Fig. 3B and C). Protein expression levels of GLUT1 were significantly lower in OW-Control patients compared with expression levels in the NW-Control group (Fig. 3C; P<0.05). No statistically significant differences in GLUT4 protein expression levels were identified between the two subgroups of patients with or without PCOS (P>0.05; Fig. 3B and C, respectively).

Discussion

Human embryogenesis takes place in a hypoxic environment. At the time of embryo implantation, cell proliferation and implantation of the blastocyst requires an increasing supply of nutrients and oxygen, which promotes the establishment of the vascular network at the implantation site. Hypoxia-induced synthesis of HIF-1α modifies the endometrial microenvironment and contributes to an improvement in uterine receptivity (35,36). In the present study, quantitative mRNA and semi-quantitative protein expression levels of HIF-1α were determined in the endometrium during the implantation window. To the best of our knowledge, this is the first study to investigate the role of HIF-1α in determining endometrial receptivity for embryo implantation.

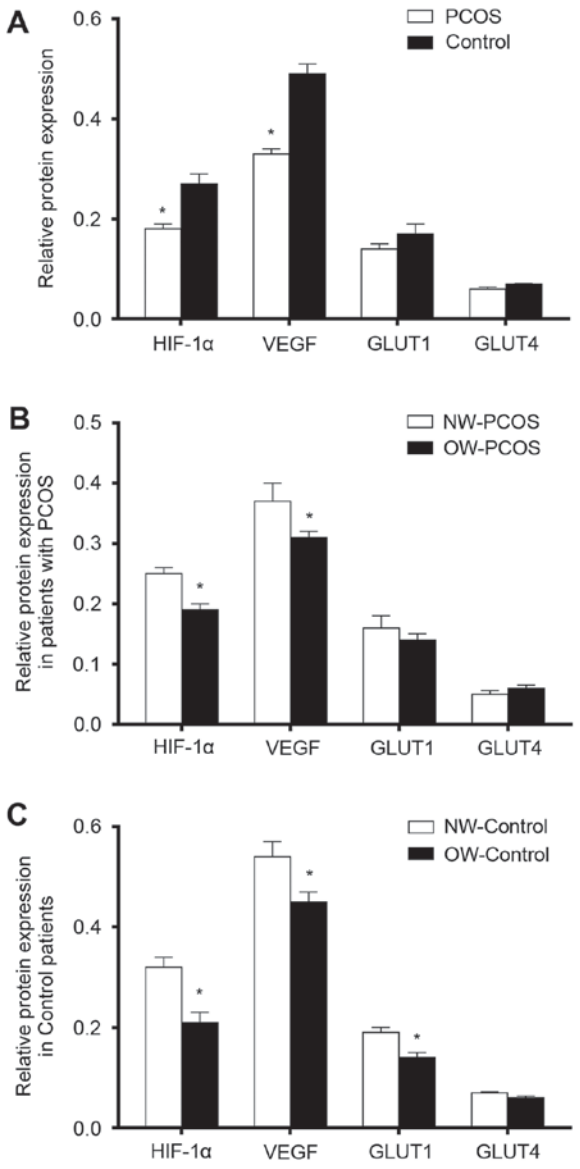

Figure 3. Semi-quantitative integrated optical density analysis of positive immunostaining of endometrial epithelial cells. (A) Relative protein expression levels in POCS and control groups. (B) Relative protein expression levels in NW-PCOS and OW-PCOS groups. (C) Relative protein expression levels in NW-Control and OW-Control groups. Data are presented as the mean ± standard error of the mean; *P<0.05. GLUT, glucose transporter protein; HIF-1α, hypoxia-inducible factor-1α; NW, normal weight; OW, overweight; POCS, polycystic ovary syndrome; VEGF, vascular endothelial growth factor.

Previous reports have demonstrated that HIF-1α expression peaks during the endometrial secretory phase, suggesting its involvement in endometrium repair (15). It was also observed that HIF-1α expression in endometrium peaked at the implantation window at the mRNA but not at the protein levels, suggesting stabilization of HIF-1α in the hypoxic endometrial microenvironment and that HIF-1α may serve an important role in the establishment of uterine receptivity. This may be due to protein turnover rates affecting protein concentrations. In biological systems, there is a high protein turnover in proliferative and dividing cells; however, a steady state may be reached following certain stimuli (37). Therefore, the inconsistency between mRNA and protein expression levels of HIF-1α may reflect rapid protein degradation that occurs with alleviation of hypoxia during the time window for embryo implantation.

It is well known that HIF-1α may participate in the establishment of endometrial receptivity during the time window for embryo implantation (38). However, in the present study, both mRNA and protein expression levels of HIF-1α were significantly decreased in patients with PCOS, which suggested that the hypoxic endometrial microenvironment in patients with PCOS may be impaired. A recent study reported significant changes in uterine receptivity in overweight or obese women (4). The decreased HIF-1α expression in OW-PCOS patients may be a cause of endometrial dysfunction in these patients.

HIF-1α is a co-activator of estrogen-dependent VEGF synthesis (39,40). The expression of VEGF is associated with vascular density. A number of previous studies have suggested that the vascularization of endometrium is a critical factor for successful implantation. VEGF is an important mediator of angiogenesis in female genital organs (41). VEGF expression was significantly reduced in women with unexplained infertility and recurrent spontaneous abortions (42,43), which indicated it was important to the endometrial receptivity and embryo development. VEGF expression levels in the endometrium of patients with PCOS was significantly decreased during the implantation window, which might reduce the vascular density of endometrium and cause implantation failure.

HIF-1α is known to activate the expression of hypoxic-sensitive genes *GLUT1* and *GLUT4*. During the window for embryo implantation, large amounts of energy are required to fulfill the endometrial function and glucose is the main energy source of endometrial cells (38). GLUT1 is a trans-membrane glycoprotein that is responsible for the uptake and storage of glucose in the endometrium, whereas GLUT4 is an insulin-dependent glucose transporter that regulates fast glucose uptake (44). GLUT4 expression levels in the present study were the lowest among those factors. This observation was consistent with a previous study in which GLUT1, but not GLUT4, was demonstrated to be essential for the decidualization of the secretory endometrium (45). There were no statistically significant differences in GLUT1 and GLUT4 expression levels between the POCS and Control groups; however, immunohistochemistry demonstrated differences in their intracellular localization. In Control patients, these proteins were localized in the cell membrane of epithelial cells, while in patients with PCOS, they were observed mainly in the cytoplasm and nucleus. Under basal conditions, GLUT1 and GLUT4 are localized into intracellular vesicles and glucose uptake is completed when the vesicles translocate to the cell surface (46). Reduced translocation of GLUT1 vesicles to the cell membrane of cells in patients with PCOS was observed, which probably impaired glucose uptake by the cells and affect the decidualization of the endometrium. Therefore, the impaired endometrial receptivity in PCOS patients may be associated with impaired GLUT1 functions.

In conclusion, the present study indicated that HIF-1α may be involved in the molecular mechanisms underlying endometrial dysfunction in patients with PCOS, particularly in those who are overweight. Further functional studies need to be conducted in these patients to confirm these findings.

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