Effects of testosterone and metformin on glucose metabolism in endometrium

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Objective: To investigate the effects of testosterone and metformin on glucose metabolism in endometrium.
Design: In vitro experiment.
Setting: Reproductive and Genetic Center, Obstetrics and Gynecology, The First Hospital of Peking University, Beijing, China.
Patient(s): Premenopausal women undergoing total abdominal hysterectomy for uterine myoma or adenomyosis.
Intervention(s): Endometrial glandular epithelial cell culture.
Main Outcome Measure(s): Insulin receptor substrate-1 (IRS-1) and glucose transporter 4 (GLUT-4) expression in endometrial glandular epithelial cell.
Result(s): After the action of testosterone solution, the protein expressions of IRS-1mRNA, IRS-1, and GLUT-4 in endometrial glandular epithelial cells all decreased significantly. Metformin may reverse this effect.
Conclusion(s): The high androgen levels in patients with polycystic ovary syndrome could induce insulin resistance in endometrial glandular epithelial cell, whereas metformin has an antagonizing effect. (Fertil Steril® 2010;93:2295–8. ©2010 by American Society for Reproductive Medicine.)

Key Words: PCOS, metformin, endometrium, IRS-1, GLUT-4

As a common syndrome among child-bearing women, polycystic ovary syndrome (PCOS) is caused by the disturbed balance of hypothalamic-pituitary-ovary axis. The main clinical manifestations include hyperandrogenemia, oligomenorrhea or amenorrhea, infertility, polycystic ovary–like changes, hirsutism, insulin resistance (IR) and resulting hyperinsulinemia, etc. The incidence of PCOS among childbearing women is 5%–10%. In recent years, the relationship between hyperinsulinemia and hyperandrogenemia in PCOS has gradually become the hot research spot. The studies of how the high androgen influences the local glucose metabolism in endometrium remain scant. The present study chose the ex vivo cultured endometrial glandular epithelial cells as the study subject to explore the effects of the high androgen and metformin levels on glucose metabolism in endometrial glandular epithelial cell. This study obtained institutional review board approval in the First Hospital of Peking University in Beijing, China.

MATERIALS AND METHODS

Main Reagents and Instruments

The cell culture medium was DMEM/F-12 culture medium (GIBCO, Grand Island, NY) with a pH value of approximately 7.2. The cell digestion solution was 0.1% collagenase IV (Sigma Chemical Co., St. Louis, MO). monoclonal mouse-anti-human insulin receptor substrate-1 (IRS-1) antibody, monoclonal sheep-anti-human glucose transporter 4 (GLUT-4) antibody (Santa Cruz Biotechnology Inc., St. Lukes, Bolivia); testosterone (10^{-3} M; Sigma Chemical Co.); 7300 real-time quantitative PCR apparatus (Applied Biosystems, Foster City, CA); Alpha ImagerTM2200 image analysis and processing system (Nature Gene Corporation, Medford, NJ).

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Experimental Methods

Isolation and culture methods of endometrial glandular epithelial cells Fifty-two patients undergoing total abdominal hysterectomy for uterine myoma or adenomyosis at our hospital during September 2006 to March 2007 were selected. These patients were 32–45 years old and with a body mass index of 19.3–25.5. The late proliferative phase (menstrual days 12–14) endometrial tissues were collected. All of the specimens were obtained from the nonmenopausal patients who had normal menses and no abnormal bleeding before the surgery. All of the postoperative pathological analysis was of normal proliferative phase endometrium. In the operating room, the sterile and fresh endometrial tissues were harvested, washed thoroughly, and scissored into small blocks (1mm × 1mm) with ophthalmologic dissecting scissors in sterile culture dishes. The specimens were then transferred into sterile centrifuge tubes; 0.1% collagenase IV was added. After incubating in water bath tank, the solutions were filtered through 100-mesh and 400-mesh screens. A large quantity of D-Hank's solution was used to rinse the screens repeatedly to wash away the glandular cell mass for inoculation. The large tissue pieces on the surface of the 100-mesh screen were transferred into centrifuge tubes. Collagenase was added continuously for the second digestion. The steps of digestion, filtration, and inoculation were repeated.

Dosing methods and grouping When the endometrial glandular epithelial cells grew fully at the bottom of the culture

flask and became close to 80%–85%, 3%ct-FBS in de-phenol red DMEM/F-12 culture medium was changed to continue incubation for 24 hours, to synchronize the cellular status and simultaneously remove the interference of endogenous estrogen and growth factors in serum. Three milliliters 3%ct-FBS in de-phenol red DMEM/F-12 culture medium and each dosing group were subsequently added into the culture flasks. After 48 hours of incubation, the total cellular protein was collected and the Western blot method was used to measure the protein expression amounts of GLUT-4 and IRS-1. The total cellular mRNA was collected and the real-time PCR method used to measure the expression amount of IRS-1 mRNA. The groups were divided according to the below method. Each group was conducted at least in triplicates:

1. Blank group: no dosing
2. 10^{-6} M testosterone group: 10^{-6} M testosterone solution added and acted for 48 hours
3. Metformin group: 10^{-4} M metformin added and acted for 48 hours
4. Metformin plus testosterone group: 10^{-4} M metformin and 10^{-6} M testosterone solutions added simultaneously and dosed for 48 hours

Insulin receptor substrate-1 primer Insulin receptor substrate-1 primer was designed with the help of Teacher Pu Dingfang at the Central Laboratory of our institute who searched the gene sequence of IRS-1 from Genebank and selected the following primer:

IRS-1: IRS1-F: 5'-TAAGCGCCTATGCCAGCAT

IRS1-R: 5'-AGGATTTGCTGAGGTCATTTAGGT

IRS1-TaqMan: 5'-TTTCCAGAAGCAGCCAGAGGACC GTC

Target fragment length: 105 bp

Internal reference β-Actin was chosen as the internal reference in Western blot and GAPDH as the internal reference in real-time PCR.

Statistic Processing

The statistic software SPSS10.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. All of the results agreed with the normal distribution. The data were described with mean ± SD and statistically analyzed with one way ANOVA. The statistic difference was considered significant when $P < 0.05$.

RESULTS

Effect of Testosterone and Metformin Solutions on the Expression of GLUT-4 Protein in Endometrium (Table 1)

Compared with blank group, metformin caused no significant change ($P > 0.05$) in the expressions of IRS-1 and GLUT-4. Compared with blank group, testosterone caused the expressions of IRS-1 and GLUT-4 to decrease significantly ($P < 0.05$). Compared with testosterone, group metformin plus testosterone caused the expressions of IRS-1 and GLUT-4 to be markedly higher than those in the testosterone group ($P < 0.05$).

TABLE 1

Protein expression amounts of IRS-1 and GLUT-4 in endometrial glandular epithelial cell after the actions of testosterone and metformin.

Group	IRS-1 (mean ± SEM)	GLUT-4 (mean ± SEM)
Blank	0.834 ± 0.067	0.643 ± 0.068
M(10^{-4})	0.840 ± 0.084[a]	0.662 ± 0.071[a]
T (10^{-6})	0.613 ± 0.042[b]	0.481 ± 0.025[b]
M (10^{-4}) + T (10^{-6})	0.788 ± 0.068[a]	0.609 ± 0.041[a]

[a] Compared with testosterone group, $P < 0.05$.
[b] Compared with blank group, $P < 0.05$.

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Effects of Testosterone and Metformin on Relative Expression Amounts of IRS-1mRNA in Endometrial Glandular Epithelial Cell (Table 2)

Compared with blank group, all of the IRS-1 expressions in the metformin group had no significant change ($P > 0.05$). Compared with blank group, testosterone caused IRS-1 expression to decrease significantly ($P < 0.05$). Compared with testosterone group, metformin plus testosterone caused IRS-1 expression to be up-regulated significantly ($P < 0.05$).

DISCUSSION

Insulin receptor substrate-1 and glucose transporter 4 are the key mediators of insulin actions. The lower expressions of two can indicate the existence of IR. For this present study,

TABLE 2

Effects of metformin plus testosterone on relative expression amounts of IRS-1mRNA in endometrial glandular epithelial cell.

Group	IRS-1/ GAPDH ($\times 10^{-5}$) (mean ± SEM)
Blank	0.904 ± 0.032
M (10^{-4} M)	0.923 ± 0.110[a]
T (10^{-6} M)	0.654 ± 0.095[b]
M (10^{-4} M) + T (10^{-6} M)	0.846 ± 0.069[a]

[a] Compared with testosterone group, $P < 0.05$.
[b] Compared with blank group, $P < 0.05$.

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