

Original Article

Effects of insulin resistance on myometrial growth

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Abstract: To observe the effects of insulin resistance on gonadal steroid hormone stimulation and the myometrial growth of female rats in order to elucidate the relationship between insulin resistance and the development of uterine leiomyomas. We divided 180 nonpregnant female Wistar rats into three groups as follows: group A, as the control group; group B, as the “model by exogenous sex hormone” group; and group C, as the “model by exogenous sex hormone plus insulin-resistance” group. All the animals were raised for 16 weeks. Uterine coefficient and homeostasis model assessment of insulin resistance (HOMA-IR) index were calculated. Myometrial depth and expression levels of the oestrogen receptor (ER), progesterone receptor (PR), and proliferating cell nuclear antigen (PCNA) were measured. HOMA-IR index, serum oestrogen level, uterine coefficient, and myometrial depth were lower in group B than in group C ($P < 0.05$). The expression levels of ER, PR, and PCNA were higher in group C than in group B ($P < 0.05$). An auxo-action of insulin resistance in myometrial growth was observed when exogenous oestrogen and progesterone were administered to the female rats in this study. Thus, we suspected that insulin resistance may affect the development of uterine leiomyomas.

Keywords: Myometrium, insulin resistance, gonadal steroid hormones, laboratory rats, uterine leiomyomas

Introduction

Leiomyoma, also known as fibroid tumour, is the most commonly reported benign tumour in female reproductive organs, as it originates from the myometrium. The mechanism of the development of leiomyoma remains unclear. Insulin resistance (IR), a novel research field that involves multiple subjects, represents a common condition in human diseases and may play a critical role in various pathologies. Our group intended to investigate the effect of IR on the growth of the myometria of female Wistar rats stimulated by sexual hormones and reveal the potential action of IR in the development of leiomyoma.

Materials and methods

Experimental animals

We purchased 180 nonpregnant female Wistar rats with similar weights from the Laboratory Animal Centre, the Military Medical Science Academy of the People's Liberation Army (permission license No. SCXK-[Jun] 2009-004).

Animal feeding and hormone stimulation

All the rats were subjected to adaptive feeding for 1 week and then randomised into three groups. Group A, the control group, was fed with standard laboratory mouse stocks and injected with 0.2 mL of water via the vastus lateralis every other day. Group B, model by “exogenous sex hormone” group, was fed with standard laboratory mouse stocks and injected with 0.1 mg (in 0.1 mL) of oestradiol benzoate and 1 mg (in 0.1 mL) of progesterone via the vastus lateralis every other day. Group C is the “model by exogenous sex hormone plus insulin-resistance” group, which was fed the sugar- and fat-rich mouse stocks and injected with 0.1 mg (in 0.1 mL) of oestradiol benzoate and 1 mg (in 0.1 mL) of progesterone via the vastus lateralis every other day. Rats in each group were kept in separate cages at ambient temperature and humidity, with free access to food and water, and their body weights were measured and recorded once a week. They were fed and injected with reagents for 16 weeks. At the end of 16 weeks, data were collected 24 hours after discontinuation of feeding and injection, and 12 hours after water deprivation.

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Table 1. Results of the comparisons of FPG level, FINS level, and HOMA-IR index between the groups ($\bar{x} \pm s$)

Group	n	FPG (mmol/L)	FINS (μ U/mL)	HOMA-IR
A	60	9.54 \pm 1.73	12.07 \pm 1.98	4.58 \pm 1.16
B	60	12.36 \pm 2.19	11.54 \pm 2.63	5.87 \pm 1.29
C	60	14.18 \pm 1.87	12.12 \pm 2.09	6.63 \pm 1.56

Table 2. Results of the comparisons of serum oestrogen (E_2) and progesterone levels between the groups ($\bar{x} \pm s$)

Group	n	E_2 (pg/mL)	Progesterone (ng/mL)
A	60	33.23 \pm 11.16	0.86 \pm 0.29
B	60	38.65 \pm 9.87	0.76 \pm 0.45
C	60	48.35 \pm 12.54	0.84 \pm 0.37

Rat treatment procedure

All the rats were treated according to procedure as follows: (1) measuring the body weights; (2) fixing rats on an operating table after anaesthesia, harvesting 5 mL of blood sample through euthyphoria open-heart surgery and taking one drop from the sample for blood sugar assay with remains stored in tubes; (3) opening the abdominal cavity of the rat, separating the uterus, peeling off the surface adipose tissue of the uterus and fixing the uterus in 10% solution of formaldehyde after measurement of its wet weight; (4) killing the rats; and (5) collecting 1 mL of serum after centrifuging the blood samples and storing the serum sample at -80°C for examination.

Biochemical assays

Biochemical assays of the following were performed: fasting plasma glucose (FPG), fasting serum insulin (FINS), serum oestrogen (E_2), and serum progesterone. The homeostasis model assessment of insulin resistance index was calculated ($\text{HOMA-IR} = [\text{FPG} \times \text{FINS}] / 22.5$) to estimate IR, where a higher value indicates a more severe insulin resistance.

Pathological assays

The organ index of the rats was calculated as follows: organ index for the uterus = wet weight of the uterus (in milligrams)/body weight (in gram) \times 100%. The uterine tissues of the rats were pathologically examined. The uterine tis-

sue samples were fixed in 10% formaldehyde solution for 48 hours, embedded with paraffin, stained with hematoxylin-eosin as 5- μm sections, and examined under light microscopy, with the smooth muscle thickness calculated. The smooth muscle thickness was measured using the imaging software Image-Pro Plus 6.0; the real value represented 1/200 of the measurement readout, and three fields of vision were selected randomly and averaged for each sample. Immunohistochemical assays were performed to determine the expression levels of oestrogen receptor (ER), progesterone receptor (PR), and proliferating cell nuclear antigen (PCNA) in the uterus; a positive reaction was identified as particle aggregation, brown to dark brown in colour. Each section was subjected to random selection of a positively stained high-density area using low-power lens, examined within five fields using high-power lens (all smooth muscle layers) and then analysed using the software Image-Pro Plus 6.0, thereby the mean integrated optical intensity was computed statistically, and the positive expression ratios of ER and PR, as well as the proliferative index of PCNA, were calculated.

Statistical analysis

The statistical software package SPSS (version 19.0) was used for the statistical analysis, and all the data were presented as mean \pm standard deviation ($\bar{x} \pm s$). The results obtained were tested for normality and homogeneity of variance. The groups were compared using one-way analysis of variance, and paired groups with differences were further compared using the q test, with the test level $\alpha = 0.05$. Immunohistochemical results were analysed using Image-Pro Plus 6.0.

Results

Results of the comparisons of the FPG level, FINS level, and HOMA-IR index between the groups are shown in **Table 1**. The FPG level in group A was lower than that in groups B and C ($P < 0.05$), and that in group B was lower than that in group C ($P < 0.05$). No significant difference in FINS level was observed between the three groups ($P > 0.05$). The HOMA-IR index was significantly lower in group A than in groups B and C ($P < 0.001$), and that in group B was lower than that in group C ($P < 0.05$). These results imply that the sugar- and fat-rich mouse diets induced IR in the rats.

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Table 3. Results of the comparison of organ index for the uterus between the groups ($\bar{x} \pm s$)

Group	n	Body weight (g)	Uterus weight (mg)	Organ index for the uterus (mg/g \times 100%)
A	60	296.35 \pm 14.45	161.76 \pm 22.80	54.45 \pm 10.51
B	60	256.08 \pm 10.73	221.34 \pm 18.36	86.43 \pm 9.75
C	60	243.62 \pm 16.89	229.45 \pm 27.53	94.18 \pm 10.97

Table 4. Results of the comparison of myometrial thickness between the groups ($\bar{x} \pm s$, mm)

Group	n	Myometrial thickness
A	60	28.37 \pm 3.69
B	60	44.15 \pm 8.36
C	60	49.82 \pm 6.51

Results of the comparisons of serum oestrogen (E_2) and progesterone levels between the groups are shown in **Table 2**. The E_2 level in group A was lower than that in groups B and C ($P < 0.001$), and that in group B was lower than that in group C ($P < 0.05$). No significant difference in serum progesterone level was observed between all the groups ($P > 0.05$).

Results of the comparison of the organ index for the uterus between the groups are shown in **Table 3**. The rats in group A were significantly heavier than those in groups B and C ($P < 0.001$), but no significant difference was observed between groups B and C ($P > 0.05$). The wet weight of the uterus was significantly lesser in group A than in groups B and C ($P < 0.001$), but no significant difference was observed between groups B and C ($P > 0.05$). The organ index for the uterus was significantly lower in group A than in groups B and C ($P < 0.001$), and the organ index for the uterus was lower in group B than in group C ($P < 0.05$).

Results of the comparison of myometrial thickness between the groups are shown in **Table 4**. The myometrial thickness in group A was significantly less than that in groups B and C ($P < 0.001$), and that in group B was less than that in group C ($P < 0.05$).

Results of the comparisons of the expression levels of ER, PR, and PCNA in the smooth muscles of the uterus between the groups are shown in **Table 5**. The expression levels of ER

(**Figure 1**), PR (**Figure 2**), and PCNA (**Figure 3**) were higher in groups B and C than in group A ($P < 0.05$), and group C had higher expression levels of ER, PR, and PCNA than group B ($P < 0.05$).

Discussion

Uterine leiomyoma is a kind of benign tumour formed by proliferative myometrium and connective tissues, usually occurring in women between 35 and 35 years old and representing the most common reason for surgical removal of the uterus. It is a monoclonal tumour derived from a single smooth muscle cell in the uterus and classified as an ovarian hormone-dependent tumour, in which the levels of the oestrogen and progesterone hormones in circulation play a critical role in the onset of uterine leiomyoma. There are oestrogen and progesterone receptors in the myometrium of the uterus, and the effect of the hormones in the target tissues depends on the content of their receptors, where a higher content of the receptors means a more significant effect. PCNA content affects the proliferative activity of cells and serves as an accurate indicator of proliferative activity of tumour cells, making it useful in prognostic evaluation, where an increase in PCNA content in the smooth muscle layer of the uterus indicates an elevated proliferative activity of the smooth muscle cell in the uterus, as well as a hyper-proliferative state of the smooth muscle tissue of the uterus.

IR refers to a pathological condition characterised by decreased sensitivity of surrounding tissues to circulatory insulin, insensitivity of tissue to insulin, and resistance of peripheral tissue against the promoting effect of insulin on glucose uptake, which is the pathological basis for diabetes. In recent years, IR has attracted extensive attention from research in gynaecology and obstetrics, which is focused on the mechanism of IR in pregnancy, the effect of IR on physiology and pathology in pregnancy, the correlation between hypertensive disorders in pregnancy and IR, and the correlation between polycystic ovarian syndrome and IR. Tan *et al.* [1] reported a significant increase in HOMA parameters, and triglyceride, low-density lipoprotein, apolipoprotein B levels, and a significant decrease in high-density lipoprotein

Table 5. Results of the comparisons of the expression levels of ER, PR, and PCNA in the myometrium between the groups ($\bar{x} \pm s$, %)

Group	n	ER-positive ratio (%)	PR-positive ratio (%)	PCNA proliferative index (%)
A	60	25.67 ± 6.59	30.57 ± 6.91	20.65 ± 2.73
B	60	41.83 ± 8.92	44.96 ± 9.01	33.67 ± 8.43
C	60	54.26 ± 10.41	59.88 ± 11.72	48.91 ± 10.15

and apolipoprotein A levels in patients with hypertensive disorders in pregnancy compared with that in normal pregnant women. They concluded that IR and abnormal lipid metabolism are present in hypertensive disorders in pregnancy and proposed a correlation among them. Wang *et al.* [2] showed that the IR in polycystic ovarian syndrome decreases the expression level of insulin receptor substrate 2 (IRS-2) and involves the onset of disruption of ovarian reproductive function by increasing the expression of insulin receptor substrate 1 (IRS-1) in ovarian granulosa cells. Meanwhile, the relationship between of IR and tumourigenesis is attracting attention, as various tumours can be accompanied by IR while it facilitates the onset and progress of some tumours. Most related research focused on colorectal, breast, and endometrial cancers. Flood *et al.* [3] revealed that the risk of colon cancer is 1.5-fold higher in patients with IR than in the healthy population and is especially higher in female patients diagnosed with IR for more than 11-15 years, indicating a critical role of chronic IR in the development of colon cancer. Adiponectin secreted by adipocytes can increase the sensitivity of tissues to insulin and serves as an alternative marker of IR owing to its negative correlation with IR. Mantzoros *et al.* [4] investigated serum adiponectin levels in 74 patients with breast cancer and 169 control samples, and demonstrated a significant negative correlation between postmenopausal adiponectin level and breast cancer risk. In addition, the negative correlation was independent of known risk factors such as insulin-like growth factor, and obesity, indicating an independent correlation of IR to the development of breast cancer. Soliman *et al.* [5] conducted a comparison study by determining serum adiponectin levels in 117 samples of endometrial cancer tissue and 238 tissue samples from patients without history of tumour and showed that IR is clearly

related to endometrial carcinoma and also play an important role in the development of endometrial cancer in women without obesity. Accordingly, the authors proposed that IR is independently related to endometrial cancer.

Based on existing literatures [6, 7] and our previous experiments, the present study stimulated the growth of uterine smooth muscle by simultaneously administering oestrogen and progesterone hormones, induced IR by providing rats with sugar- and fat-rich diets, and explored the allosteric effect of IR and sexual hormone stimulation on the growth of rat uterine smooth muscles. The present study showed that IR can enhance the effect of stimulation of sexual hormones on serum E₂ level in rats, without significant effect on serum progesterone level, implying that insulin shares a similar signalling pathway with oestrogen hormones. Moreover, IR can facilitate sexual hormones to stimulate an increase in the organ index for the uterus, myometrial thickness, and ER, PR, and PCNA expression levels in rats. It can enhance the effect of the stimulation of sexual hormones on the growth of rat uterine smooth muscles, implying a possible effect of IR in the development of uterine leiomyoma. Regarding the relationship between IR and uterine leiomyoma, various reports have differing points. Some studies showed [8] that insulin facilitates the production of hormones and decreases the association between sexual hormones and globulins, resulting in increased levels of oestrogen hormones and other tumour-promoting factors. Meanwhile, insulin can vary the expression of receptors of tumour cells by modifying the signalling pathway of receptor tyrosine kinase and promotes tumourigenesis. A patient-control comparison study by Takeda *et al.* [9] showed a correlation of metabolic syndromes characterised by obesity, hypertension, and hyperglycaemia to uterine leiomyoma, and proposed that uterine leiomyoma is a potential pathological feature of the progression of metabolic syndromes. By contrast, a study by Sadlonova *et al.* [10] that compared between 56 patients with uterine leiomyoma and 20 healthy control subjects demonstrated no significant differences in the IR parameters between the experimental and control groups by determining levels of fast

ER expression

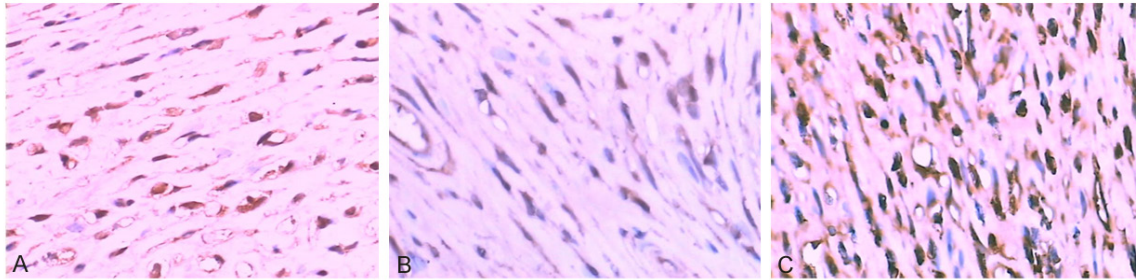


Figure 1. Immunohistochemical staining for ER. Strong expression of ER was observed in (C) compared with (A and B) (a significant correlation ($P < 0.05$) was observed).

PR expression

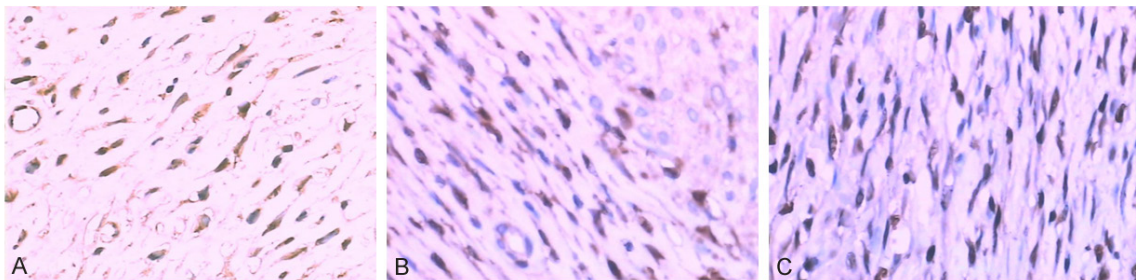


Figure 2. Immunohistochemical staining for PR. Strong expression of PR was observed in (C) compared with (A and B) (a significant correlation ($P < 0.05$) was observed).

PCNA expression

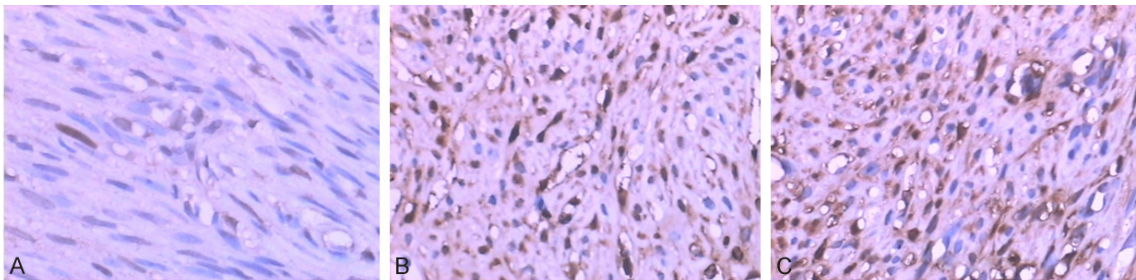


Figure 3. Immunohistochemical staining for PCNA. Strong expression of PCNA was observed in (C) compared with (A and B) (a significant correlation ($P < 0.05$) was observed).

plasma glucose, insulin, C peptides, and sex hormone-binding globulins. Accordingly, they proposed that IR is not a risk factor of uterine leiomyoma.

The present study indicates the allosteric effect of IR and that sexual hormone stimulation can promote the generation of rat uterine smooth muscles at an animal experiment level. However, the underlying mechanism and the relationship between IR and human uterine

leiomyoma are yet to be confirmed by future research.

Disclosure of conflict of interest

None.

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