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Research Article

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The Effect of Obesity on the Expression of *FOXM1* and *PPARγ* Expression in the Placenta of Female Rats

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Abstract

Background and aim: The incidence of obesity is accompanied by the risk of many disorders such as pregnancy complications that may lead to the fetus injury. On the other hand, obesity affects the gene expression in the placenta which is the vital organ for growth and development of fetus. Therefore, in this study, the expression of two important genes, *FOXM1* and *PPARγ*, which are expressed in the placenta and their expression is affected by obesity, was investigated.
Material and Methods: In this study we fed one groups of female rats with normal food and 4 groups with high fat diet food for eight weeks, in order to have normal (control) and obese rats. Then all groups were mated by male rats. 18 days after the pregnancy, we remove the placenta of each rat. Then total RNA of placenta was extracted and cDNA was prepared. Then amplification of DNA was performed by TaqMan real-time PCR according to the mentioned protocol. We compare the expression of *FOXM1* and *PPARγ* compared with *GAPDH* transcription.
Results: The expression of both gene *FOXM1* and *PPARγ* was significantly induced in the placenta of obese rats related to nonobese rats ($p<0.05$).
Conclusion: Obesity makes an impression on the transcription of some genes like *FOXM1* and *PPARγ* and in this way affects the growth and development of the fetus and even maybe lead to some disorders. So understanding this pathway might be the target for new treatment strategies for prevention and treatment of fetal disorders.

Keywords: *Obesity, FOXM1, PPARγ, Gene expression, Placenta*

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Introduction

Obesity in pregnancy is associated to disadvantages both in the mother and the fetus. The incidence of gestational diabetes and preeclampsia is higher in obese women, increasing maternal and infant morbidity and mortality (Beneventi *et al.,* 2023). Maternal obesity often leads to increased levels of inflammatory state in adipose tissue that could disturb placental function and could mediate the opposing consequences (Ehlers *et al.,* 2021).

The placenta is a unique organ for the proper implantation, having serious functions on developing the fetus. Changes in placental gene expression are physiologic reactions to fetal growth and developmental condition. Many other factors also contribute to gene expression in pregnancy pathologies like preeclampsia, gestational diabetes or maternal obesity (Sõber *et al.,* 2015; Gale *et al.,* 2015).

Forkhead box protein M1 (*FOXM1*) is a transcription factor that regulate the expression of numerous cell cycle genes and is expressed in the cytotrophoblast during early pregnancy, and may have some effect on placental formation. *FOXM1* also serves an important role in the development of preeclampsia (Cui *et al.,* 2018). It has been proved that obesity increases the risk of preeclampsia for several populations and even weight loss reduces preeclampsia risk (Roberts *et al.,* 2011). Moreover another article showed that the expression of *FOXM1* is increased by nondiabetic obesity and causes the beta-cell proliferation in adult humans, in order to compensate for insulin resistance. It confirms that the expression of FOXM in human islets is positively correlated with body mass index (Davis *et al.,* 2010) So we hypothesized that maybe the expression of *FOXM1* in the placenta of rat may also be altered in obesity.

Peroxisome proliferator-activated receptors (PPARs) are highly expressed in the placenta and they are essential for development of placenta (Wang *et al.,* 2002). *PPARγ* is a transcription factor which is also abundantly expressed in adipose tissue. Some evidence show that obesity causes a decrease in expression of *PPARγ* that is associated with the pathogenesis of obesity (Diradourian *et al.,* 2005) whereas other studies have shown increased expression of *PPARγ* in adipose tissue of obese persons (Berhouma *et al.,* 2013). Thus according to the controversies, we investigate the relation between obesity and *PPARγ* expression in rat placenta.

Material and Methods

Five groups of adult Wistar female rats were maintained under 12 h light, 12 h dark condition and 22°C temperature. All animal experiments were conducted in accordance with the ethical standards of the Animal Care and Use Committee of Shiraz University of Medical Sciences, Shiraz, Iran.

One group of rat had free access to pelleted food and tap water as control group and 4 groups of animals received high fat diet food for 8 weeks to become fat, based on the Lee obesity index. Obese group weighing 300-350 g and control group was 200-250 g. Then 2 male rats added to each group for mating. On the 18th days of gestation, rats were anesthetized by Ketamine (100 mg/kg ip) and after dissection, the placental tissue were taken. The placenta of each rat was grinding. Then total RNA was extracted from the placenta by RNA kit II (Invitek, Germany) according to the manufacturer's instructions. The extracted RNA was quantitated by $OD_{260/280}$ measurement. Then, the total RNA (10 µg) of tissue extracts was reverse transcribed in a 20-µl volume using random hexamer primers with enzyme and buffers supplied in the cDNA First Strand Synthesis kit (Fermentas, Life Science, EU Germany).

The expression of two genes was evaluated by TaqMan Real Time PCR. For Quantitative real time PCR, 5 µg cDNA was added to Taq man master mix (TaKaRa, Takara Shuzo, Otsu, Japan). The final volume of the PCR was 20 µl: 10 µl Master Mix, 0.6 µl of each primer, 0.6 µl prob, 0.4 µl reference dye, and 2.8 µl dH2O. Amplification of DNA was performed under the following conditions: 10 min at 95ºC, 10s at 95ºC, and 30s at 60º for 40 cycles. Primers and probe used for real-time PCR were selected using rat genomic sequences as templates and NCBI (http://www.ncbi.nlm.nih.gov/pubmed) and Allele ID programs. *GAPDH* was selected as endogenous control and the transcription of *PPARγ*, and *FOXM1* was checked in relation to *GAPDH*. Each experiment was repeated 3 times (Table 1).

Gene	Forward primer	Reverse primer	Probe
GAPDH	GGCTCTCTGCTCCTCCCTGTTC	CGGCCAAATCCGTTCACACCGA	GCCGCATCTTCTTGTGCAGTGCCAGCC
PPARγ	CAGAGGGACAAGGATTCATGACC	TTCACAGCAAACTCAAACTTAGGC	AGTCACCAAAGGGCTTCCGCAGGC
FOXM1	CAAGGTAAAAGCCACGTCTAAGC	GGAGCAGCAGGTGACTAATGG	TGGGCATTTCCTGGTCCTCACGGC

Table 1. The sequence of primers and probes.

Statistical analysis

The data are shown as mean ± SE. For evaluation of each gene transcription, 6 test groups were compared to the controls and each experiment was repeated 3 times. Different groups were compared through one-way ANOVA. $p<0.05$ was considered as statistically significant. The statistical analyses were performed using SPSS 27 and design of the graphs by Microsoft Excel.

Results

In order to evaluate the effect of obesity on gene expression, we assess the transcription of *PPARγ*, and *FOXM1* in the placenta of normal and obese rats.

1- The result shows that *PPARγ* expression significantly increased in all test groups (obese) versus control (nonobese) ($P<0.05$) (Figure 1).

2- The result demonstrate significant increase in *FOXM1* expression in all test groups (obese) versus control one (nonobese) ($P<0.05$) (Figure 2).

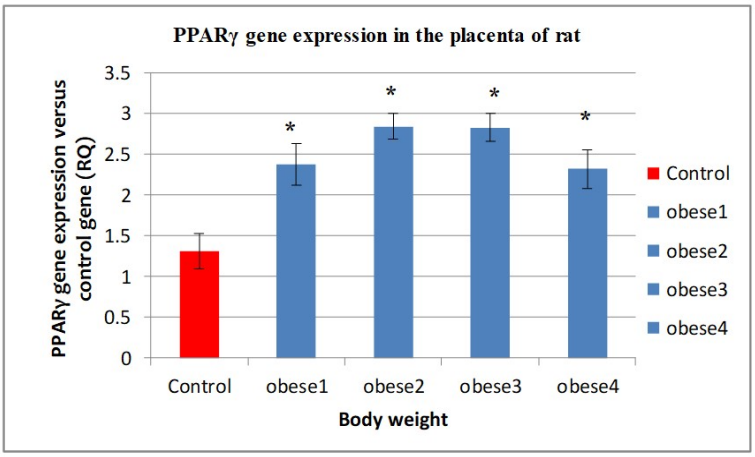

Figure 1. The effect of obesity on *PPARγ* gene expression in rat. Obese groups weighing 300-350 g and control group was 200-250 g. The data are shown as mean ± SD. * $p<0.05$ for each group versus control; analysis by one-way ANOVA followed by Tukey's test, (n=5).

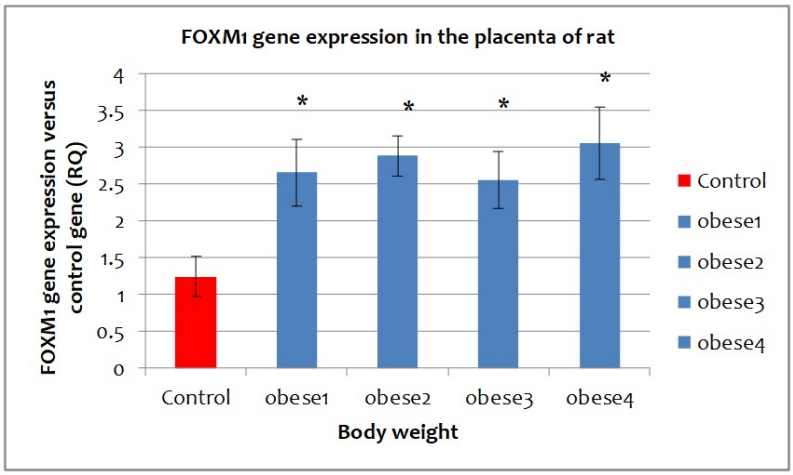

Figure 2. The effect of obesity on *FOXM1* gene expression. Obese groups weighing 300-350 g and control group was 200-250 g. The data are shown as mean ± SD. * $p<0.05$ for each group versus control; analysis by one-way ANOVA followed by Tukey's test, (n=5).

Discussion

The numerous functions of PPARs in implantation, trophoblast differentiation and placental function, as well as in embryonic and fetal development, approve their critical role during gestation. So variations made in this signaling pathway may accompanying with the pathogenesis of gestational diseases (Jawerbaum *et al.,* 2011).

Obesity is one of the most probable risk factors of gestational diabets and we investigate maternal obesity on the activation of this pathway. Our results clearly show that *PPARγ* transcription increases in placenta of obese rats. Other researches also proved that maternal disorders, like intrauterine inflammatory environment caused by maternal obesity or diabetes activate *PPARγ* transcription and this activation of different PPARs controls lipid metabolism and anti-inflammatory pathways that could prevent damages in intrauterine injuries induced by maternal diabetes or obesity (Jawerbaum *et al.,* 2011).

FOXM1 has been considered an important transcription factor for development of diabetes and its complications (Zhao *et al.,* 2023). Also other studies associated *FOXM1* to other pregnancy complications such as preeclampsia (Cui *et al.,* 2018).

Moreover another study proved that obesity alters *FOXM1* expression in human islets. They found that the expression of *FOXM1* and its target genes is associated with body mass index and β-cell proliferation to compensate for insulin resistance (Davis *et al.,* 2010).

So we investigated other probable role of *FOXM1* expression during obesity and the results clearly indicate that maternal obesity lead to placental up regulation of this factor and by this way affect the growth and development of the fetus. Of course more researches should be done to clarify the details of these pathways, but our study would be useful in this approach.

Conclusion

Obesity makes an impression on the transcription of some genes like *PPARγ* and *FOXM1* in the placenta and in this way affect the growth and development of the fetus and even might be lead to some serious disorders like preeclampsia, diabetes mellitus and obesity. So understanding this pathway may help us to prevent the disorders or supposing the new managements.

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اثر چاقی بر بیان ژن‌های *FOXM1* و *PPARγ* در جفت موش‌های صحرایی ماده

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چکیده

زمینه و هدف: وقوع چاقی با بسیاری از اختلالات از قبیل عوارض دوران بارداری همراه است که ممکن است به آسیب جنین ختم شود. به عبارت دیگر چاقی بر بیان بعضی ژنها در جفت تاثیر می گذارد که اندام مهم و حیاتی در رشد و تکوین جنین محسوب می شود. بنابراین در این مطالعه به بررسی بیان دو ژن مهم *FOXM1* و *PPARγ* که در جفت بیان می شوند و بیان آنها تحت تاثیر چاقی قرار می گیرد، پرداخته شد.

مواد و روش‌ها: در این مطالعه یک گروه از مش های صحرایی ماده با غذای نرمال (گروه کنترل) و ۴ گروه با غذای پرچرب به مدت ۸ هفته تغذیه شدند تا ۴ گروه رات چاق داشته باشیم. همه گروه ها توسط موش های صحرایی نر جفت گیری شدند. ۱۸ روز پس از بارداری، جفت ها از موش های صحرایی جدا شدند و سپس RNA جفت اسخراج و cDNA از آنها تهیه شد. از روی cDNA کپی سازی به روش Taqman Real Time PCR طبق پروتکل انجام شد. بیان ژن های *FOXM1* و *PPARγ* نسبت به ژن *GAPDH* سنجیده شد.

یافته‌ها: بیان هر دو ژن *FOXM1* و *PPARγ* در جفت موش های صحرایی چاق در مقایسه با جفت موش های صحرایی نرمال بطور معنی داری افزایش یافته بود ($p<۰/۰۵$).

نتیجه گیری: چاقی بر بیان بعضی ژنها مانند *FOXM1* و *PPARγ* تاثیر مهمی دارد و از این طریق بر رشد و تکوین جنین تاثیر می گذارد. بنابراین درک این مسیر ممکن است هدف روش های درمانی جدید برای جلوگیری و درمان اختلالات جنینی باشد.

واژه‌های کلیدی: *چاقی، FOXM1، PPARγ، بیان ژن، جفت*

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