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Obesity accelerates ovarian follicle development and follicle loss in rats

Na Wang[a], Li-Li Luo[b], Jin-Jie Xu[a], Ming-Yan Xu[a], Xing-Mei Zhang[b], Xiao-Ling Zhou[b], Wei-Juan Liu[b], Yu-Cai Fu[a,*]

[a] Laboratory of Cell Senescence, Shantou University Medical College, Xinling Road 22, Shantou 515041, P.R. China
[b] Department of Gynaecology and Obstetrics of the First Affiliated Hospital, Shantou University Medical College, Shantou, P.R. China

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ABSTRACT

Objective. Studies have shown that excess body fat negatively affects reproductive functions in females. However, whether obesity affects the ovarian follicle development and ovarian lifespan and the underlying mechanism has not been well elucidated. The aim of the present study was to investigate the association between obesity and ovarian follicle development.

Methods. Adult female Sprague–Dawley rats (n = 36) were randomly divided into three groups: the normal control (NC) group, the caloric restriction (CR) group (fed 70% food of the NC group) and the high-fat diet (HF) group. They were maintained on these regimens for 18 weeks.

Results. The body weight, ovary weight and visceral fat in the HF group were significantly higher than those in the NC group and the CR group at the end of treatment. Histological analysis showed that the HF rats had significantly less number and percentage of primordial follicles, but greater number and percentage of developing and atretic follicles than the NC rats and CR rats. Western blot analysis demonstrated that the level of mTORC1 and p-S6K1 proteins significantly increased in the ovaries of HF rats, whereas that of SIRT1, SIRT6, FOXO3a and NRF-1 decreased compared to the NC rats. In contrast, the expression of mTORC1 and p-S6K1 dramatically declined, while that of SIRT1, SIRT6, FOXO3a and NRF1 increased in the ovaries of CR rats.

Conclusions. Our study suggests that the HF diet induced obesity may accelerate the ovarian follicle development and rate of follicle loss through activating mTOR and suppressing SIRT1 signaling, thus leading to POF, and that CR may inhibit the activation of primordial follicles, follicular development and loss, thus extending the ovarian lifespan through suppressing mTOR and activating SIRT1 signaling.

Abbreviations: AB, apoptotic body; CLC, corpora lutea cell; CO, cumulus oophorus; CR, calorie restriction; DAB, 3, 3′-diaminobenzidine; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; FOXO3a, forkhead box group O; GC, granular cell; GE, gross energy; GTP, guanosine triphosphate; H&E, hematoxylin and eosin; HF, high fat; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; NC, normal control; NRF-1, nuclear respiratory factor 1; ON, oocyte nucleus; PAGE, polyacryl amide gel electrophoresis; PBS, phosphate buffer solution; PG, pre-granular cell; POF, premature ovarian failure; S6K1, p70 ribosomal protein S6 kinases; SDS, sodium dodecyl sulfate; S.E.M, standard error of mean; SIRT, silent information regulator; SPSS, Statistical Package for the Social Sciences; TC, thecal cell; TSC, the tuberous sclerosis complex.

* *Corresponding author.* Tel.: +86 754 88900431; fax: +86 754 88557562.
E-mail address: ycfu@stu.edu.cn (Y.-C. Fu).

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1. Introduction

The prevalence of obesity has increased dramatically around the world, and studies have shown that excess intake of high-calorie food can raise the risk factors for severe health problems including cardiovascular disease, diabetes, osteoarthritis, cancer and metabolic disorders [1], as well as disruptions in reproduction [2]. It was recently reported that oocytes from obese women undergoing assisted reproduction and female mice with diet-induced obesity gave rise to blastocysts of poorer quality [3,4]. Additionally, the increased childhood weight gain and fat acquisition have also been demonstrated to be positively associated with earlier pubertal maturation in several studies [5–7]. Generally, children who develop puberty earlier are more likely to be overweight [8]. Moreover, epidemic investigations clearly show that fat in excess of the normal can lead to menstrual abnormality, anovulation, sub-fecundity and infertility[9–11], further shedding light on the negative effects of obesity on the reproductive functions in females. However, whether obesity affects the ovarian follicle development, the reserve of follicle pool or the ovarian lifespan, and the underlying mechanisms remain unknown.

Recently, mammalian target of rapamycin (mTOR), a serine/threonine kinase that regulates cell growth and proliferation in response to stress, nutrients and growth factors, has emerged as a fundamental cellular regulator in many obesity-induced diseases [12]. mTOR is part of two distinct multiprotein complexes: rapamycin sensitive mTOR complex 1 (mTORC1) and rapamycin insensitive mTORC2 [13,14]. The upstream of mTORC1, the TSC1/TSC2 protein complex, suppresses the activation of mTORC1 through a GTPase activating protein domain located in TSC2 [15]. The major downstream effectors of mTOR known so far are 4E-BP1 and p70S6K, both regulators of protein translation [16]. The TOR signaling has been implicated in longevity in lower organisms and mice [17,18]. Recent studies demonstrated that the deletion of Tsc1 or Tsc2 in mouse oocytes led to the over-activation of primordial follicles and premature ovarian failure (POF) [19], suggesting that the activation of mTOR signaling may promote the follicle development and loss.

However, CR extends lifespan of many species of animals [20], and also extends the lifespan of mammalian ovary [21,22]. Sir2 or sirtuins are the key genes mediating the life-extending effects of CR. The mammalian genome encodes seven sirtuins (SIRT1-7) [23]. Mice with deletion of Sirt1 gene can not survive after birth, and both male and female Sirt1$^{-/-}$ mice are infertile [24,25], whereas SIRT1 transgenic mice displayed prolonged lifespan, inhibited ovarian follicular development and delayed sexual maturity [26]. FOXO3a is an important substrate of SIRT1, and Foxo3a$^{-/-}$ mice showed over-activation of primordial follicles and POF [27]. In addition, SIRT6-deficient mice are small and develop abnormalities usually associated with aging by 2–3 weeks of age and overexpression of SIRT6 protects against the damage caused by high-fat diet-induced obesity [28]. But whether SIRT6 affects the ovarian function has not been reported. Our previous study demonstrated that CR extended ovarian lifespan by increasing the follicle reserve, and the level of SIRT1 and SIRT6 expression in the ovaries was also increased [29], suggesting the involvement of SIRT1 and SIRT6 in CR-mediated regulation of ovarian follicular development.

In fact, both TOR and sirtuin signalings are components of the same longevity pathway that extends lifespan in lower organisms and mice. CR has been shown to involve the TOR signaling for lifespan extension in yeast [17]. Inhibition of TOR has been shown to extend lifespan in yeast by increasing Sir2p activity [30]. Resveratrol, a known activator of SIRT1, has been demonstrated to inhibit mTOR activity and cellular senescence [31]. In a recent extensive study, rapamycin, the inhibitor of mTOR, was shown to extend the median and maximal lifespan of mice [18]. Furthermore, we recently demonstrated that inhibition of mTOR by rapamycin resulted in suppressed ovarian follicle development and augmented the follicle pool reserve, and meanwhile, upregulated the expression of SIRT1 and SIRT6 proteins in adult rat ovaries, suggesting that both mTOR and sirtuin signalings may be involved in regulating ovarian follicle development [32].

Based on these findings, we hypothesized that obesity may cause POF by activating the activity of mTOR signaling, and CR, on the contrary, may suppress the ovarian follicle development and rate of loss, thus extending ovarian lifespan by inhibiting mTOR signaling. However, the mechanisms need to be elucidated. The present study examined effects of high-fat diet induced obesity and CR on the follicle development and reserve, as well as the expression of genes in mTOR and sirtuin signalings such as mTORC1, p-S6K1, SIRT1, SIRT6, FOXO3a and NRF1, and discussed the possible mechanisms of regulation.

2. Materials and methods

2.1. Animals and diets

Thirty-six female Sprague–Dawley rats (8-weeks old, 180–200 g) were supplied by the Vital River Laboratories of Beijing. After a four-week adaptation period, rats were randomly divided into three diet groups: the normal control (NC) group fed *ad libitum* standard rodent food (4.84% fat, 7.34% fiber, 20.11% protein, plus all necessary vitamins and minerals, GE = 3.92 kcal/g); the high-fat (HF) group fed *ad libitum* a high-fat diet prepared by adding 20% lard to the standard chow(GE = 5.80 kcal/g) [33]; and the CR group fed 70% of the food intake from the NC group. We recorded daily food intake of NC rats, and the food supply of the CR group was adjusted accordingly. All animals had access to the three feeding conditions for 18 weeks.

All of the rats were housed individually in steel cages and had free access to tap water. The rats were maintained under a 12/12 h dark/light cycle at 21–24 °C. Each rat was weighed weekly. The study protocol was approved by the Animal Care and Use Committee of Shantou University Medical College.

2.2. Estrous cycle analysis

Vaginal cytology was assessed daily between 8:00 and 9:00 AM before and until the end of treatment to determine the estrous cycle of each rat. Vaginal cells were collected via a sterile cotton swab saturated with normal saline (NaCl, 0.9%), and