

Effects of hypercaloric diet-induced hyperinsulinemia and hyperlipidemia on the ovarian follicular development in mice

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Abstract. Long-term hypercaloric diets may adversely affect the development of ovarian follicles. We investigated the effects of high sugar (HS), high fat low sugar (HFLS), and high fat normal sugar (HFNS) diets on the ovarian follicle development in mice fed with these diets as compared to those fed with normal diet (control) for 180 days. Body weight, gonadal fat, glucose, lipid, insulin, estrous cycle, sex hormones and ovarian tissues were examined, and metabolism-related protein expression in the ovaries was evaluated by immunoblotting. The mice fed with hypercaloric diets showed hyperinsulinemia and hyperlipidemia, and exhibited heavier body and gonadal fat weights, longer estrous cycles, and fewer preantral and antral follicles than mice fed with normal diet. The sex hormone levels in the blood were similar to those in controls, except for significantly elevated estradiol levels in the HS diet group. The AMPK α phosphorylation was reduced, while AKT phosphorylation and caspase-3 levels were increased in the ovarian tissues of mice in all three hypercaloric diet groups than those in control. Taken together, the results suggest hyperinsulinemia and hyperlipidemia as possible mechanisms that impair the development of ovarian follicles in response to long-term exposure to unhealthy hypercaloric diets.

Key words: Follicular development, Hypercaloric diets, Hyperinsulinemia, Hyperlipidemia

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Fertility disorders constitute an increasingly common public health problem worldwide [1], and currently affect approximately 13–17% of couples in the reproductive age [2]. The absolute number of infertile couples increased from 42.0 million to 48.5 million between 1990 and 2010 [3], and the lifetime prevalence of infertility is approximately 17–28% [4]. Thus, the increasing incidence of fertility-related disorders has led to recognition of infertility as a global health concern [5].

Genetic factors, advanced age, autoimmune conditions, environmental toxins, and exposure to chemotherapy or radiotherapy cause abnormalities in the development of the primordial follicles, leading to early depletion of the ovarian follicular reserve [6]. There is an upward trend in the incidence of idiopathic infertility that may be associated with an unhealthy diet. Moreover, lifestyle may affect the ovarian function, and unhealthy dietary patterns influence the occurrence of anovulatory infertility [7]. Human fertility depends on nutrition; however, both malnourishment and over-nourishment impair fertility. Hypercaloric diets can lead to the development of obesity and metabolic dysfunction in humans and animals [8], while being underweight or overweight can increase the risk of infertility [9]. In addition, obesity, currently recognized as a pandemic by the World Health Organization (WHO), can lead to variety of secondary

pathologies, including female infertility [10].

Metabolic dysfunction is closely related to reproductive abnormalities [11], and dietary habits have been implicated in significant effects on the ovarian development [12]. Circulating blood metabolites, such as glucose and lipids, and hormones, such as insulin, are known to affect the hypothalamic-pituitary-ovarian axis; thus, dysregulation of these metabolites or signaling molecules can induce infertility in women [13]. A study showed that diet-induced obese mice had significantly more apoptotic ovarian follicles, smaller and fewer mature oocytes, smaller fetuses, and smaller pups than normal mice [14]. Another study showed that diet-induced obesity affects the oocyte meiotic maturation, ovulation, and fertilization [15]. Moreover, such diets can reduce oocyte quality and blastocyst survival rates, cause abnormal embryonic cellular differentiation, and increase frequency of early embryo loss and fetal growth retardation [16].

Liver kinase B1 (LKB1), also known as serine/threonine kinase 11 (STK11), is a serine/threonine protein kinase that has been implicated in several key cellular processes, including regulation of cell proliferation, cell polarity, and energy metabolism [17, 18]. A study by Shaw *et al.* identified LKB1 as a major upstream kinase responsible for the activation of AMP-activated protein kinase (AMPK) that acts as a metabolic rheostat to maintain energy homeostasis in response to a decline in the cellular ATP/AMP ratio [19]. Some studies have also linked LKB1 to phosphorylation-mediated regulation of AMPK [20, 21] and microtubule dynamics [22].

A major downstream signaling pathway controlled by LKB1/AMPK axis is the mammalian target of rapamycin (mTOR) that regulates protein synthesis and cell growth, and confers protection against apoptosis during cellular stress [23]. AKT (also known as protein kinase B) is activated by serine and threonine phosphorylation, and it

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functions as a key mediator of insulin signaling [6]. AKT is involved in many cellular processes, including cell growth, survival, proliferation, and metabolism [24]. The dysregulation of AKT is associated with several human diseases, including infertility and ovarian cancer [25].

Young women of reproductive age and even prepubescent girls often consume hypercaloric diets with excessive sugar and/or fat that contributes to occurrence of hyperinsulinemia and hyperlipidemia. The cellular mechanisms that function in the energy balance required for reproduction remain poorly understood, and the effects of these unhealthy diets on female fecundity have not been thoroughly studied. Here, we investigated the possible effects of different unhealthy diets on the ovarian follicular development in prepubescent C57BL/6J female mice to evaluate the hypothesis that long-term consumption of hypercaloric diets may lead to abnormalities in glucose and lipid metabolism. We also examined whether these effects could eventually impair the ovarian follicular development in female mice. We fed mice with three different hypercaloric diets, including high sugar (HS), high fat low sugar (HFLS), and high fat normal sugar (HFNS). Further, we studied the molecular mechanisms by investigating the signaling pathways altered by these hypercaloric diets; the analyses suggested that dysfunction of pathways that influence cell proliferation, growth, survival, energy metabolism, and so on may deleteriously affect the ovarian follicular development.

Materials and Methods

Animal experiments

C57BL/6J female mice (weighing 13–16 g, 28-day-old) were obtained from the Laboratory Animal Center of the Xi'an Jiaotong University (Xi'an, Shaanxi, China). Approximately five mice were housed per cage in a temperature-controlled room with a 12/12 h light/dark cycle. The mice were fed with one of the diets detailed in Table 1 (HS, HFLS, HFNS, or standard Purina rodent chow) for approximately 180 consecutive days. The mice were weighed every two weeks. There were four groups with 30 mice in each group. Mouse chow was purchased from Beijing Keaoxieli Feed Co., Ltd., Beijing, China. All animals were provided food and water *ad libitum*. Animal experiments were approved by the Committee on the Use and Care of Animals of the Xi'an Jiaotong University (Xi'an, Shaanxi, China), and all experimental procedures were performed in accordance with the approved animal protocols.

Sample collection

One week before ending the 180-day diet, venous blood samples were collected from the tails of each mouse using glass capillaries. These samples were used for monitoring blood lipids (after the mice were fasted for 12 h). At the end of the experiment (day 180), all

mice were weighed, fasting blood glucose (FBG) was measured, and they were anesthetized with intraperitoneal injection of pentobarbital (150 mg/kg; Shanghai Zhixin Chemical Co., Ltd., Shanghai, China). The blood samples were collected by cardiac puncture from the mice after fasting for 12 h and placed in tapered plastic centrifuge tubes. The blood samples were centrifuged (ST8, Thermo Fisher Scientific Inc., Waltham, USA) at $1,500 \times g$ for 15 min, and the serum was isolated and stored at -80°C for assays of sex hormones and insulin. Tissues, including the ovaries and gonadal fat, were collected. The freshly dissected ovaries were flash-frozen in liquid nitrogen for further analysis of protein lysates or were immediately fixed in 4% paraformaldehyde (Xi'an Qiyue Biotechnology Co., Ltd., Xi'an, China) for histopathology. The right and left ovaries were used for histological and molecular studies, respectively.

Blood sample assays

The levels of blood lipids, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were analyzed using commercial kits (Biosino, Beijing, China). On the day of sacrifice (day 180), FBG levels were measured using a glucometer with glucose strips (Roche, Mannheim, Germany). Estradiol (E_2), fasting blood insulin (FBI), and testosterone (T) levels were measured by ELISA kits (ALPCO, Keewaydin, USA). The homeostasis model assessment index of insulin resistance (HOMA-IR) value was calculated using the formula: blood glucose (mmol/l) \times serum insulin (mIU/l)/22.5.

Estrous cycle

To study the effects of hypercaloric diets on the estrous cycle, vaginal smears were obtained using a simple miniature cotton swab, and cytologically evaluated microscopically using hematoxylin and eosin (H&E), after feeding mice with various diets for 180 days. The vaginal smears were obtained for 15 consecutive days, starting 15 days before the end of the experiment (on day 166–180).

Ovarian section

The ovaries were fixed in 4% paraformaldehyde for 24 h, dehydrated in increasing concentrations of ethanol, followed by immersion in xylene, and embedded in paraffin wax. The ovary samples were serially sectioned at 5 μm thickness using Leica microtome (RM 2235, Leica Microsystems Ltd., Heidelberg, Germany). The paraffin sections were deparaffinized in xylene twice and rehydrated with decreasing concentrations of ethanol. Further, the ovarian sections were stained with H&E and analyzed under conventional light microscope (CX23, Olympus Corporation, Tokyo, Japan). The sections used for follicle counting were randomly selected by taking every fifth section from the serial sections of the ovary samples.

Table 1. The composition of chow nutrients for the normal (Control), HS, HFLS, and HFNS diets

Nutrients	Control diet		HS diet		HFLS diet		HFNS diet	
	%	Kcal %	%	Kcal %	%	Kcal %	%	Kcal %
Protein	20.0	24.4	16.5	18.5	18.9	15.1	13.2	12.3
Fat	4.5	12.3	3.8	9.5	33.5	59.9	19.1	39.8
Carbohydrate	52.0	63.3	64.3	72.0	31.5	25.0	52.0	47.9
Total calories (Kcal/g)	–	3.3	–	3.6	–	5.0	–	4.3

The left column of each treatment shows the percentage of total proteins, fats, and carbohydrates; the remaining percentages comprising vitamin, mineral, and salt components are excluded. The right column shows the percentage of calories consisting of protein, fat, or carbohydrate in each dietary treatment (total calorie = 100%). HS, high sugar; HFLS, high fat low sugar; HFNS, high fat normal sugar.

Follicle count

The numbers of primordial, primary, preantral, and antral follicles were counted in each of the selected ovarian sections. The counting was performed by a single, blinded person to avoid interpersonal variations, and only the follicles containing an oocyte with a visible nucleolus were counted. The number of follicles in the selected sections was then multiplied by 5 to estimate the total number of follicles in each ovary [26–30]. Follicle classification and statistical analysis were performed as per previously published methods [31, 32]. Briefly, primordial follicles were identified as having a compact oocyte surrounded by a single layer of flattened (fusiform) granulosa cells (GCs). The primary follicles were identified based on presence of an enlarged oocyte surrounded by a single layer of cuboidal GCs. The preantral follicles were defined by an enlarged oocyte surrounded by at least a partial or complete second layer, but no more than four layers of cuboidal GCs. The antral follicles were identified as having more layers of cuboidal GCs and with formation of a follicle cavity [31, 32].

Western blotting

The ovarian protein samples were mixed with sodium dodecyl sulfate buffer and boiled for 8 min. Equal amounts of proteins were loaded onto a sodium dodecyl sulfate-polyacrylamide gel (Neovander Science & Technology Co., Ltd., Beijing, China), electrophoresed, and subsequently transferred onto polyvinylidene fluoride membranes. The membranes were blocked in 5% nonfat milk (Heilongjiang Feihe Dairy Industrial Co., Ltd., Heilongjiang, China) for 2 h at 20°C, incubated with primary antibodies at an appropriate dilution for 12 h at 4°C, and incubated for 60 min at 20°C with secondary antibodies at 1:3,000 dilution (goat anti-rabbit GGHL-15p and goat anti-mouse GGHL-90p, Beijing Zhongbei Linge Technology Development Co., Ltd., Beijing, China). Chemiluminescence reagent (OH189776, Thermo Fisher Scientific Inc., Waltham, USA) was used to visualize the bands. Densitometric analysis of protein levels in the ovaries from the experimental and control mice was performed. The western blotting analyses were repeated at least three independent times. The antibody information is provided in Supplementary Table 1.

Statistical analysis

All data are presented as mean \pm standard error (SE). Statistical analyses were performed using IBM SPSS Statistics for Windows, version 19.0. All data were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test. A $P < 0.05$ was considered to be statistically significant.

Results

Changes in the body and gonadal fat weights in mice fed with hypercaloric diets

The body weights of the mice failed to differ between the control and experimental groups during the pretreatment phase ($P > 0.05$). The weights fluctuated, but at day 180, the body weights of all mice fed with hypercaloric diets were significantly higher than those fed with normal diet ($P < 0.05$); and, mice fed with HFNS diet exhibited the most significant increase in body weight (Fig. 1A). The weights of the gonadal fat pads and ratio of gonadal fat pad to body weight in all mice fed with hypercaloric diets were significantly higher than those fed with normal diet ($P < 0.05$, $P < 0.01$, or $P < 0.001$) (Figs. 1B and C).

Lipid and glucose metabolic abnormalities in mice fed with hypercaloric diets

After 180 days, we observed significant increase in the levels of TG, TC, and HDL-C in all mice fed with hypercaloric diets than in control mice ($P < 0.05$, $P < 0.01$, or $P < 0.001$). However, the LDL-C levels failed to significantly differ ($P > 0.05$) between mice fed with the HFNS diet and control mice (Fig. 1D). The FBG levels were also similar ($P > 0.05$) between mice fed with the hypercaloric diet and normal diet. The FBI levels were significantly increased in all mice fed with hypercaloric diets than in control mice ($P < 0.05$), and the HOMA-IR values were significantly elevated in all mice fed with hypercaloric diets than in control mice ($P < 0.05$, $P < 0.01$), with mice fed with the HS diet exhibiting the highest HOMA-IR values (Fig. 1E).

Sex hormone levels and estrous cycle in mice fed with hypercaloric diets

There were no significant changes ($P > 0.05$) in the serum concentrations of T in mice fed with HS, HFNS, or HFNS diets for 180 days. However, E_2 concentrations were significantly increased in mice fed with the HS diet than in those fed with normal diet ($P < 0.05$; Fig. 2A).

The mice fed with the HS, HFNS, and HFNS diets had extended estrous cycles, and prolonged proestrus, metestrus, and diestrus phases than control mice ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively), but the estrus phase was not significantly different ($P > 0.05$) between the treatment groups (Fig. 2B).

The ovarian histology and follicle count in mice fed with hypercaloric diets

Macroscopic observation of the ovaries revealed that the control mice exhibited morphologically normal follicles and normal corpora lutea, whereas the number of follicles was significantly decreased in the ovaries of mice fed with each of the three hypercaloric diets (Fig. 3). Moreover, the numbers of the preantral and antral follicles were significantly decreased in all experimental mice than in control mice ($P < 0.05$; Figs. 3A–D).

Metabolism-related proteins in mice fed with hypercaloric diets

There were no significant differences ($P > 0.05$) in the levels of p-LKB1 and p-mTOR, whereas those of p-AMPK α were significantly decreased ($P < 0.05$) in the ovaries of mice fed with HS, HFNS, and HFNS diets than in control mice (Fig. 4). We failed to observe any significant differences ($P > 0.05$) in the levels of p85 and p110 between the treatment groups. However, levels of p-AKT and caspase-3 were significantly increased ($P < 0.05$) in the ovaries of mice fed with HS, HFNS, and HFNS diets than in control mice (Fig. 5).

Discussion

We studied the effects of different hypercaloric diets on the ovarian follicular development at both the tissue and molecular levels using a mouse model. We found that mice fed with hypercaloric diets for 180 days developed hyperinsulinemia and hyperlipidemia, with significantly increased body and gonadal fat weights. The hypercaloric diets reduced the number of preantral and antral follicles, and prolonged estrous cycle. We also observed disequilibrium in energy metabolism in the ovarian tissues of mice fed with hypercaloric diets, as indicated by significantly decreased AMPK activation, but increased AKT activation in the ovaries. Taken together, the data suggest possible mechanisms that govern the known negative

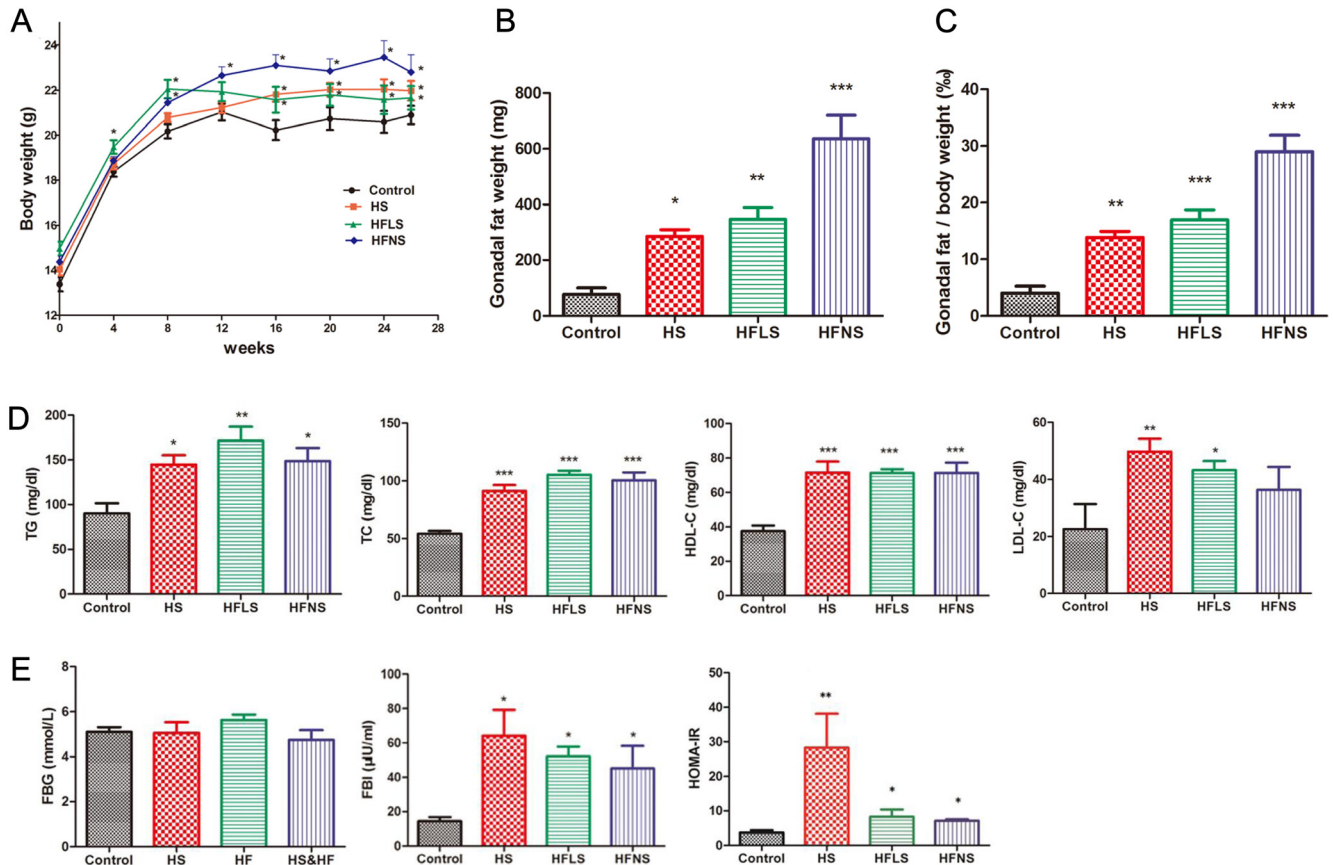


Fig. 1. Body weight change curves and the weight of gonadal fat pads in mice fed with normal (control) or hypercaloric diets. (A) Body weight change curves in mice fed with normal (control), HS, HFLS, or HFNS diets; (B) The weight of gonadal fat pads; (C) The ratio of gonadal fat pad weight to body weight. Data are represented as mean \pm SE from 30 different mice per experimental group. C57BL/6J female mice were 28-day-old, weighing 13–16 g at the start of the diet treatments. * $P < 0.05$ vs. Control. Summary of lipid and glucose metabolic parameters in mice fed with normal (Control) or hypercaloric diets for 180 days. (D) The levels of serum TG, TC, HDL-C, and LDL-C; (E) The levels of FBG, FBI, and HOMA-IR. Data are presented as mean \pm SE from 20 different mice per experimental group. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. Control. HS, high sugar; HFLS, high fat low sugar; HFNS, high fat normal sugar; SE, standard errors; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; FBI, fasting blood insulin; HOMA-IR, homeostasis model assessment index of insulin resistance.

effects of long-term consumption of hypercaloric diets on female fecundity. Moreover, the results indicate that hyperinsulinemia and hyperlipidemia may play a role in the abnormal development of ovarian follicles and possibly in the pathogenesis of disorders related to folliculogenesis.

Extensive previous work has established that female fertility is affected by obesity at all stages of reproduction, including the oocyte development [13, 16, 33–35]. The present study focused on the impact of unhealthy diets on the ovarian follicular development. We fed female mice with hypercaloric diets with different ratios of sugar and fat (and very less differences in protein levels) to mimic several unhealthy human diets. The total body weight of all mice fed with hypercaloric diets was significantly higher than that of control mice after 180 days.

Examination of specific tissues after sacrificing the mice revealed that the gonadal fat weights of the mice fed with hypercaloric diet were also significantly higher than those of control mice. There was a significant trend for weight gain in the fat deposits near the reproductive tissues, and the hypercaloric diets with a relatively higher fat content led to more substantial weight gain. Fat deposits have been reported to induce lipotoxic effects on female fertility [36]. Analyses of blood samples in the present study revealed that

each of the hypercaloric diets significantly increased blood lipid levels—TG, TC, and HDL-C—than normal diet. Moreover, it is known that an overabundance of lipids and insulin circulating in the blood leads to an unfavorable reproductive environment. In addition, hyperinsulinemia causes an early response to luteinizing hormones by the GCs of small follicles, leading to anovulation due to premature differentiation of these cells [37]. Interestingly, we also observed hyperinsulinemia in all mice fed with the hypercaloric diets.

Further, the analyses suggested that mice fed with hypercaloric diets had significantly longer estrous cycles, with prolonged proestrus, metestrus, and diestrus phases than control mice, suggesting the potential effects on reproductive capacity. The metabolic disturbances identified in the study were the primary reasons for delayed estrous cycles and hindered oocyte and GC development; these findings are consistent with those of Minge *et al.* [16].

The ovaries are target organs for insulin activity, and insulin receptors are present on both the stromal and follicular compartments. Furthermore, insulin stimulates steroidogenesis in the theca cells and GCs, *in vitro*, and participates in the follicular development and GC proliferation [38]. GCs provide nutritional support to the oocyte, regulate oocyte growth, and maintain meiotic arrest; while hyperinsulinemia can affect follicle development and ovulation

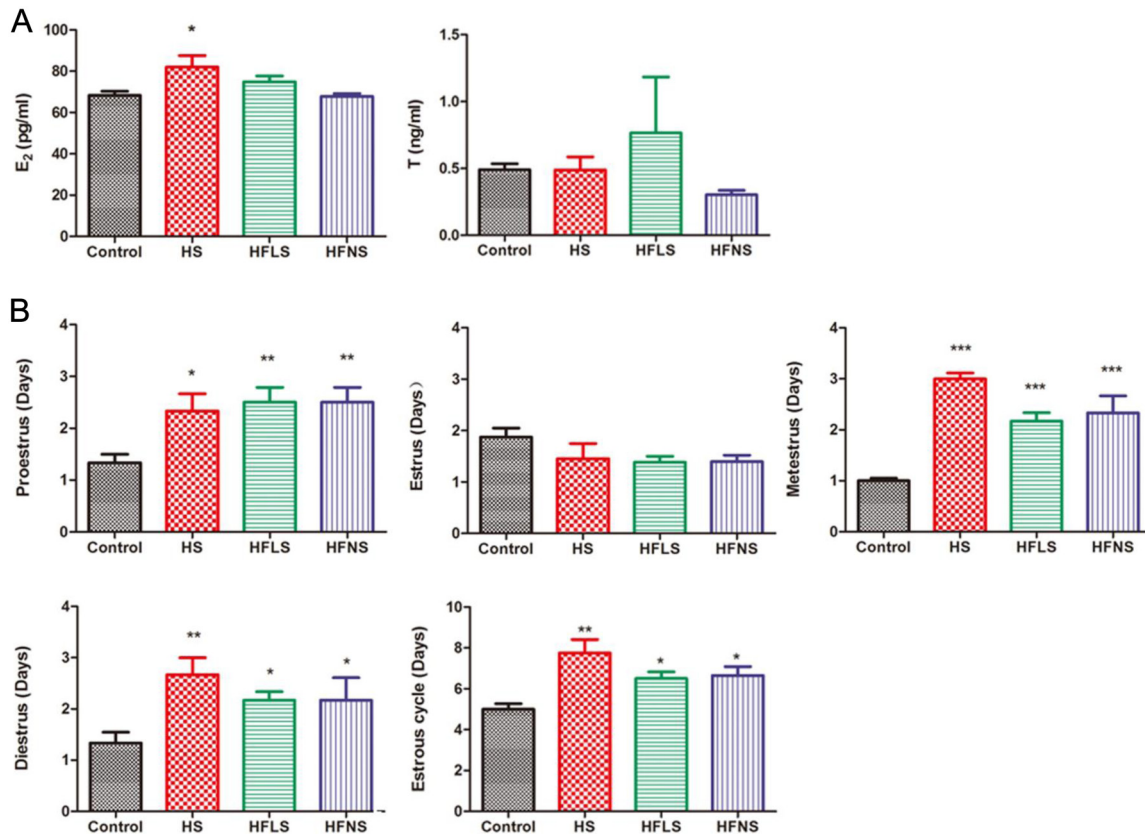


Fig. 2. The concentrations of serum sex hormones in mice fed with normal (Control) or hypercaloric diets. (A) The average serum concentrations of estradiol (E₂) and testosterone (T) from 20 different mice per experimental group; (B) The proestrus, estrus, metestrus and diestrus phases, and estrous cycle in 10 different mice per experimental group. Data are presented as mean \pm SE. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs. Control. HS, high sugar; HFLS, high fat low sugar; HFNS, high fat normal sugar.

[39]. A study by Wu *et al.* identified significant increase in the cell cycle arrest and apoptosis in the ovarian GCs of high-fat diet mice than in control mice, that led to aberrant follicle development [40]. The results of the present study indicate that exposure to long-term hypercaloric diet can interfere with the follicular growth, specifically by reducing the number of preantral and antral follicles and by inhibiting the growth of primary follicles. Further, previous studies investigating the reproductive disorders in mice showed that high-fat hypercaloric diets negatively affect folliculogenesis, fertilization, and oocyte quality [41, 42]. Hypercaloric diets have also been shown to cause mitochondrial abnormalities and meiotic defects [43]. In addition to inducing polycystic ovaries and irregular estrous cycling, high-fat and high-sugar diets induce metabolic dysfunction, including elevated FBI, FBG, and HOMA-IR in rats; and RNA-seq analyses of the ovarian tissues further revealed dysregulation of steroid biosynthesis and folliculogenesis-related genes in rats [44]. These findings indicate that hypercaloric diets can potentially affect female fecundity.

Female fertility is highly dependent on the regulation of energy metabolism. AMPK is an important cellular energy sensor. The activation of AMPK by energetic stresses activates catabolic pathways that generate ATP, while it inactivates biosynthetic pathways that consume ATP [45, 46]. AMPK has been reported to be involved in reproduction, especially in the development of ovarian follicles [12]. The present study found that hyperinsulinemia and hyperlipidemia in mice fed with hypercaloric diets may reduce p-AMPK α in the ovarian tissue. The results were similar to those of p-AMPK levels in the

livers of high-fat-diet/streptozocin-induced type 2 diabetes mellitus mice [47]. The inhibition of AMPK phosphorylation, along with activation of caspase family proteins, including caspase-3, promotes apoptosis in the hepatocytes in mice [48]. Another study showed that the number of GCs in the high-fat diet mice was reduced than that in control mice [40]. Abnormal lipid metabolism in the ovaries of ob/ob mice increases oxidative stress and caspase-3 levels [49, 50] that impairs the growth of follicles [51]. Moreover, peripheral administration of insulin temporarily increases AKT phosphorylation [52]. Therefore, we hypothesized that hyperinsulinemia in mice fed with hypercaloric diets promotes insulin-driven AKT phosphorylation in the ovaries. The analyses indicated reduction in p-AMPK α levels, while increase in p-AKT and caspase-3 levels in the ovaries of female mice fed with the three hypercaloric diets. These results suggest that the ovaries of mice fed with hypercaloric diets exhibit increased apoptosis. We believe that these initial insights into the association between apoptosis and excessive energy intake should be assessed using spatially resolved sampling from mice or by manipulating the micronutrient sources in culture media *in vitro*.

The study identified increase in phosphorylation of AKT in mice fed for long-term with hypercaloric diets and increase in accumulation of caspase-3. In the future, we will determine the stage at which follicles undergo this change. The effects of high sugar and high fat diets on cellular energy metabolism and programmed cell death in the ovaries remains to be clarified. Nevertheless, the present study provides preliminary evidence supporting hypercaloric diet-induced hyperinsulinemia and hyperlipidemia in the dysregulation

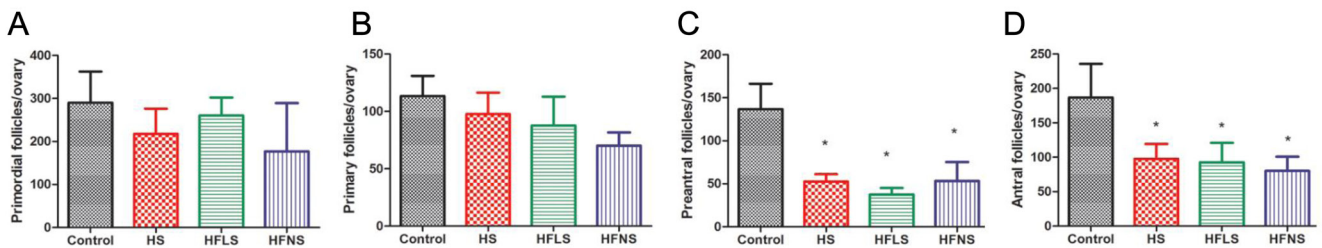
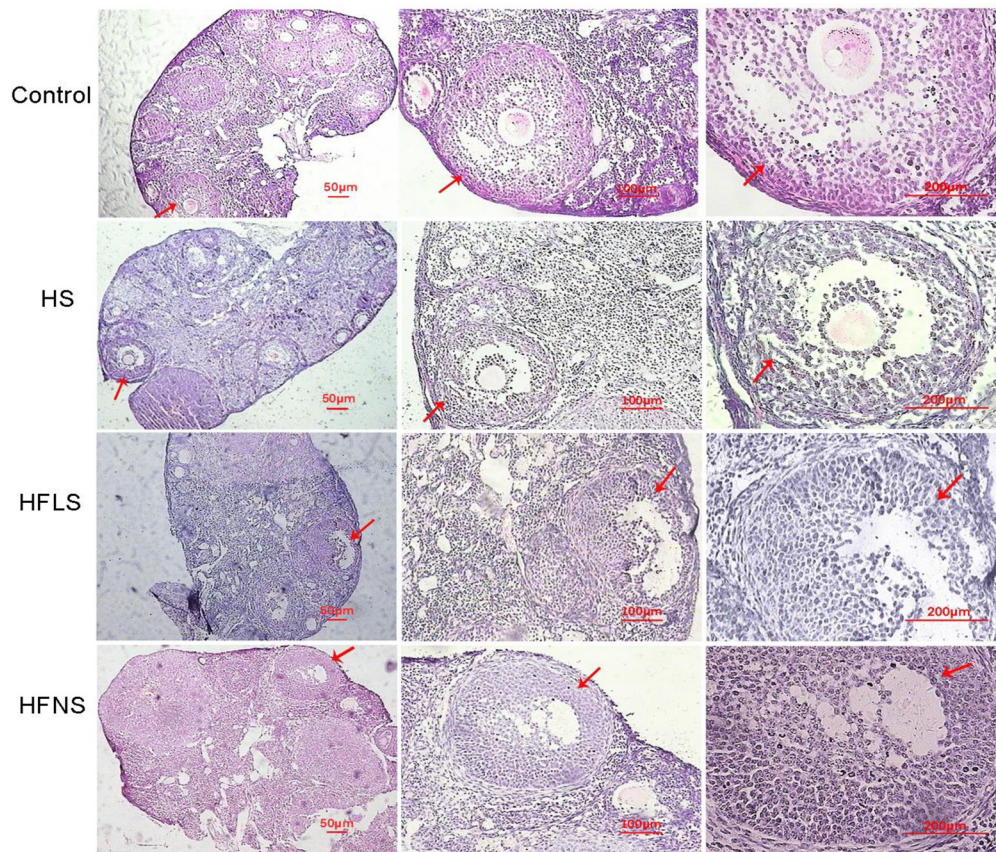


Fig. 3. Hematoxylin and eosin histopathological assessment of morphological changes in the ovaries of mice fed with normal (Control), HS, HFLS, or HFNS diet for 180 days. The representative ovary from the normal mouse shows follicles at different stages, including primordial follicles, primary follicles, preantral follicles and antral follicles, and corpora lutea in the cortical ovarian zone. The morphological abnormalities and decreased follicle number in mice fed with the HS, HFLS, or HFNS diets are visible. Arrows indicate the same follicle in each group. The number of follicles at different stages in mice fed with normal (Control) or hypercaloric diets. (A) The number of primordial follicles per ovary; (B) The number of primary follicles per ovary; (C) The number of preantral follicles per ovary; (D) The number of antral follicles per ovary. Data are presented as mean \pm SE from six different mice per experimental group. * $P < 0.05$ vs. Control. HS, high sugar; HFLS, high fat low sugar; HFNS, high fat normal sugar.

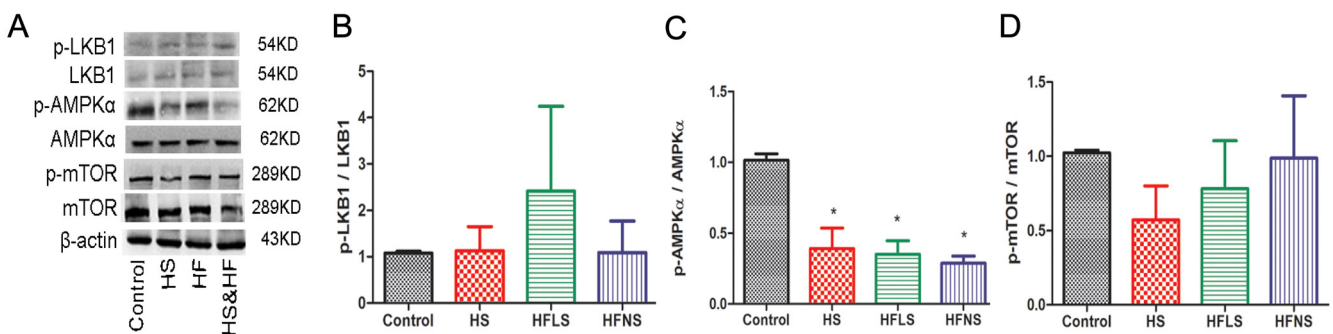


Fig. 4. Effects of hypercaloric diets on levels of AMPK/mTOR signaling pathway in the ovarian tissues in mice fed with normal (Control) or hypercaloric diets. (A) Western blotting analyses of p-LKB1, LKB1, p-AMPK α , AMPK α , p-mTOR, mTOR, and β -actin; Quantitative analyses of: (B) p-LKB1/LKB1; (C) p-AMPK α /AMPK α ; and (D) p-mTOR/mTOR. β -actin is used as an internal loading control. Data are presented as mean \pm SE from six different mice per each experimental group. * $P < 0.05$ vs. Control.

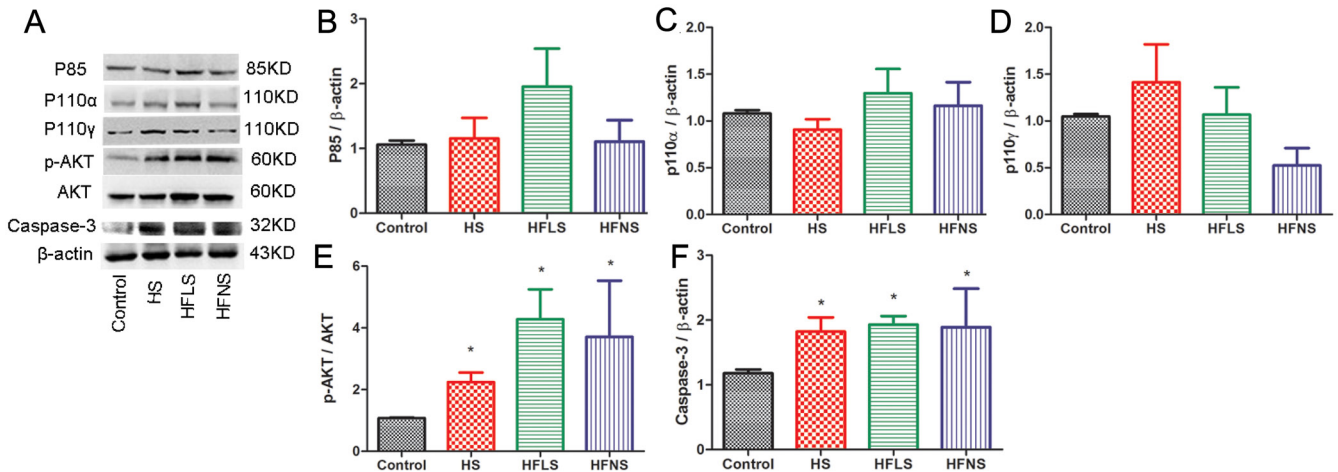


Fig. 5. Effects of hypercaloric diets on levels of PI3K/AKT signaling pathway in the ovarian tissues in mice fed with normal (Control) or hypercaloric diets. (A) Western blotting analyses of p85, p110 α , p110 γ , p-AKT, AKT, caspase-3, and β -actin; Quantitative analyses of: (B) p85/ β -actin; (C) p110 α / β -actin; (D) p110 γ / β -actin; (E) p-AKT/AKT; and (F) Caspase-3/ β -actin. β -actin is used as an internal loading control. Data are presented as mean \pm SE from six different mice for each experimental group. * $P < 0.05$ vs. Control.

of folliculogenesis, and thus, deleterious effects on female fecundity. Therefore, the study may help develop novel preventive measures and interventions in women with unhealthy diets seeking improved reproductive follicular development, a health problem with major clinical significance.

Conflict of interests: The authors declare that they have no competing interests.

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