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Original Article

Superimposed effect of ovariectomy on type 2 diabetes mellitus in Wistar rats ☆

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ABSTRACT

Background: Estrogen deprivation in the postmenopausal women plays a critical role in progression of type 2 diabetes mellitus (T2DM).
Aim: The present study investigated the overlaid effect of ovariectomy on T2DM and the possible underlying mechanism.
Materials: Forty female Wistar rats were divided into four groups (10 rats each): sham control, ovariectomized control, sham diabetic and diabetic ovariectomized groups. At the end of experiment, estimation of body weight gain percentage, food intake, fasting blood glucose concentration, and insulin tolerance test were done. Then, rats were euthanized and blood samples were taken for measurement of serum concentration of insulin, HOMA-IR, lipid parameters, tumor necrosis factor-α, interleukin 1 beta, interleukin 4, interleukin 10, malondialdehyde and total thiol. Also, histopathological and immunohistochemical examination of the pancreas were done.
Results: The present study revealed that ovariectomy aggravated the diabetic induced glucose metabolic disturbance as implied by impaired insulin tolerance test, increased insulin resistance alongside lipid dyshomeostasis. These metabolic disturbances might claim to exacerbation of oxidative stress and inflammatory response along with apparent histopathological and immunohistochemical changes on the pancreas.
Conclusion: We concluded that metabolic disturbances induced by diabetes might be getting worse after ovariectomy via intensification of oxidative stress and inflammatory state.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a progressive chronic metabolic disease characterized by elevated blood glucose concentration and insulin resistance.[1]

Estrogen deficiency after menopause correlates with metabolic disorders as T2DM, and cardiovascular disease;[2] this designates its role in the pathogenesis of these conditions.[3] Beside, the role of estrogens in female physiology and reproduction, now they are considering important regulators of multiple physiological and pathological processes.[4] Consequential to menopause, the relation between estrogen concentrations and the risk of developing diabetes is complex. Margolis et al.[5] showed lower risk of diabetes in women taking hormone replacement therapy. Meanwhile, Ding et al.[6] reported insulin resistance and diabetes in women with elevated endogenous estrogen levels after menopause.

Low-grade inflammation plays a critical role in the pathophysiology of various chronic age related conditions such as T2DM,[7] alongside increased level of pro-inflammatory cytokines observed in T2DM.[8] Cytokines are small soluble proteins or membrane-bound proteins. Almost all nucleated cells generate these proteins and respond to them. Cytokines include "interleukins, growth factors, chemokines, hematopoietins and colony stimulating factors".[9]

Estrogen deficiency after menopause is coupled with oxidative stress,[10] a state occurs when the production of reactive oxygen species (ROS) exceed the body's anti-oxidant defenses.[11] ROS are oxidative physiological processes end products[12] that bind with protein, lipid, carbohydrates and DNA within the cells, result in cellular damage include cell membranes and genetic materials.[11] Given that the amplified levels of ROS contribute to the pathogen-

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esis of T2DM,[13] this may suggest an increased risk of development of T2DM in postmenopausal females.

The present study was planned to examine the superimposed impact of surgically induced menopause (ovariectomy) on body weight gain percentage and food intake, glucose and lipid metabolism, insulin tolerance, and insulin resistance in a model of T2DM induced by high fat diet and low dose streptozotocin and to elucidate the potential mechanisms of this effect.

2. Materials and methods

2.1. Animals

Forty Wistar female rats, initially weighing from 150 to 200 grams, aged 3 months obtained from Animal house of Faculty of Medicine, Assiut University. Rats housed in clean stainless steel cages, the cage size was 65 cm × 25 cm × 15 cm, and each cage contained 5 rats. Rats maintained on natural light/dark cycle in an aerated room under controlled hygienic conditions and provided with free access to water. All procedures conducted in accordance to the *Guide of the Care and Use of Laboratory Animals*[14] and approved by the Ethical Committee for Scientific Research at the Faculty of Medicine.

Following acclimation to laboratory circumstances, 24 h food intake and body weight measured 3 times per week for 2 weeks between 8.00 a.m. and 10.00 a.m. These values utilized as baseline values. 24 h food intake estimated by offering a standard amount of rodent food pellets (70 g for each rat) every day. Then, food intake measured by subtracting the leftover food from what added at the previous day.

2.2. Experimental design

The rats randomly divided into four groups (10 rats per group):

1. Group 1 (Sham control group): rats subjected to simulated surgery.
2. Group 2 (Ovariectomized control group): rats subjected to bilateral ovariectomy.
3. Group 3 (Sham diabetic group): T2DM was induced then; rats underwent sham surgical procedure.
4. Group 4 (Diabetic ovariectomized group): diabetic rats were subjected to bilateral ovariectomy.

2.3. Induction of T2DM

T2DM was induced as mentioned by Wang et al.[15] by feeding rats with a high-fat diet (HFD), which prepared by adding 20% sucrose (w/w) and 10% ram tail fat (w/w) into basal diet, for 4 weeks, then rats injected with a single intraperitoneal (i.p.) administration of freshly prepared streptozotocin (STZ) dissolved in ice-cold sterile saline 9% (CAT #18883664; Sigma, Germany) in a dose of 30 mg/kg body weight, the combination of HFD and low-dose STZ surrogates as an alternative animal model resembling the human type 2 diabetes. This STZ low dose exhibited a slight trauma to pancreatic beta cells to simulate the condition of chronic hyperinsulinemic insulin resistance.[16] To prevent the STZ induced hypoglycemia, rats injected with 10% dextrose solution 6 h after STZ administration and for the next 24 h.[17] After that rats continued to feed HFD for another 4 weeks.

2.4. Surgical procedure: ovariectomy

After establishment of diabetes, rats sedated with pentobarbital sodium anesthesia (60 mg/kg, i.p.; Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Bilateral ovariectomy performed according to the method of Khajuria et al.[18] Sham-operated rats anaesthetized, the skin and muscle layers opened; ovaries manipulated but not removed.

Rats allowed to recover in individual cages for one week, during which they received a commercial pelleted diet and water ad libitum, anti-inflammatory drug ketoprofen (2 mg/kg) for three days, and antibiotic ampicillin sodium (30 mg/kg) for five days as described by Tavares et al.[19]

2.5. Blood glucose Determination

Occurrence of diabetes was confirmed by measuring fasting blood glucose concentration (FBG) with strips using glucometer (Accu-Chek Active, Roche Diagnostic Corporation, Mannheim, Germany). Rats with FBG concentrations more than 200 mg/dl considered diabetic.[20]

2.6. Insulin tolerance test

Rats were fasted for 6 hours (from 7 a.m. to 1 p.m.) and each rat received a single intraperitoneal injection of insulin (1 IU/kg body weight) and tail blood samples used for determining concentration of blood glucose at 0, 30, 60, and 120 min.

2.7. Animal euthanize

Fasting rats (18 h) anesthetized and euthanized by lethal dose of pentobarbital (100 mg/kg body weight).[21] 4 ml of blood collected from the retro-orbital venous plexus before euthanize. Serum separated by centrifugation at 3000*g* for 20 min. The separated serum aliquotted and stored frozen at −20 °C until use.

2.8. Determination of homeostasis model of insulin resistance (HOMA-IR)

Serum insulin concentration measured using a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (CAT # RAB0904; Sigma, Germany) according to the manufacturer's instructions. The insulin resistance index (IRI) assessed by homeostasis model assessment estimate of insulin resistance (HOMA-IR):

$$\text{HOMA} - \text{IR} = \text{Fasting insulin}(1\text{IU/ml}) \times \text{Fasting glucose}(\text{mmol/L})/22.5$$

2.9. Determination of serum lipids

Total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-C) determined by enzymatic methods using an automated analyzer (Dimension RxL Max, DADE Behring, Marburg, Germany). Low density lipoprotein-cholesterol (LDL-C) concentrations estimated according to the formula specified by Friedwald et al.[22] as follows:

$$\text{LDL} - \text{cholesterol} = \text{Total cholesterol} - [\text{HDL} - \text{cholesterol} + \text{TG}/5)].$$

2.10. Measurement of cytokines

ELISA performed to measure serum concentrations of TNF-α (CAT # K0331196), IL1β (CAT # K0331212), IL4 (CAT # K0332133) and IL10 (CAT # K0332134) using KOMA Biotech commercial kits and following the instructions supplied with each kit.

2.11. Measurement of serum concentrations of malondialdehyde and total thiol

Serum concentration of malondialdehyde (MDA) estimated by the method of Uchiyama and Mihara.[23] Calculation of concentrations = A × 3.84 where A = absorbance.