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Estrogen Reduced Blood Glucose in High Fat-Fed Mice: An Animal Model of Type 2 Diabetes

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ABSTRACT

Objective: This study aims to produce a mouse model of type 2 diabetes by using high fat diet. The C57BL/6J mouse strain can develop type 2 diabetes by putting on high fat diet.
Methods: A group of C57BL/6J male mice were fed with a high fat diet (53% energy by fat) while another group was fed with normal diet (4.5% energy by fat).
Results: At the 16th week of feeding study, the high fat-fed mice developed type 2 diabetes and had higher fat-pad weight than the normal diet-fed mice. However, plasma triglyceride (TG) levels of the two groups were not different. High fat-induced diabetic mice were administered 0.2 μg/g body weight of 17-β estradiol for 2 weeks. Their fasting blood levels were reduced to become lesser than the levels in high-fat fed mice without estrogen. A trend of decrease in plasma TG level of 17-β estradiol treated mice was observed.
Conclusion: This study demonstrated that high fat diet could induce type 2 diabetes in a mouse model and that estrogen could reduce the fasting blood glucose in these mice.

Keywords: Blood glucose; C57BL/6J mice; estrogen; high fat diet; type 2 diabetes

This study aims to develop a mouse model of type 2 diabetes induced by high fat diet. Previously, a mouse model of type 2 diabetes that was used in Thailand was obtained by streptozotocin (STZ) injection. STZ is a chemical agent that causes pancreatic β cell death.[1] High doses of STZ destroyed total pancreatic β cells and the animal became diabetic.[2] According to the definition of diabetes, this animal was categorized as type 1 DM.[3] Whereas, small doses of STZ partially damaged pancreatic β cells in rodents and the animal was claimed as a model of type 2 DM.[4,5] STZ-treated mouse developed type 2 DM due to an insulin insufficiency without insulin resistance, which is one characteristic of type 2 DM. A high-fat induced diabetic mouse was introduced by Surwit et al. as a model of type 2 DM 20 years ago.[6] This model of mouse carried both insulin resistance and insufficient islet compensation to the insulin resistance.[7,8] In this report, a local high-fat diet recipe was established and given to the C57BL/6J mice. They developed obesity and subsequently hyperglycemia.

Estrogen replacement studies showed both improvement.[9,10] and impairment of[11] glucose metabolism. Although, the effect of estrogen on glucose metabolism is still controversial, estrogen seems to have a protective effect on the development of type 2 diabetes in humans[10-13] and animals[14,15]. In this study estrogen was administered to a high-fat induced type 2 diabetic animal model. Plasma glucose and TG were measured to determine the effect of estrogen.

The model of diet-induced type 2 diabetic mouse can be used to study the effect of herbs in type 2 diabetes as well as the effect of estrogen as shown in this study. Also, it can be used for studying the molecular pathological aspects of type 2 diabetes, which cannot be studied in humans.

MATERIALS AND METHODS

Mice and diet
Male wild-type C57BL/6J mice aged 12 weeks old were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. Twenty mice fed with normal diet were divided into two groups.

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During 16 weeks, one group was still fed ad libitum with a normal diet containing 4.5% fat, 71.5% carbohydrate and 24% protein. Another group was fed with a high fat diet comprised of 53% fat, 28% carbohydrate and 19% protein. Mice were housed 5 per cage in a temperature-controlled room at 25-30°C, with a 12h light/dark cycle. The mice were weighed weekly.

Plasma and tissue samples

Fasting blood glucose was taken at 12 weeks old and after 16 weeks on the high fat diet. Blood was drawn by retro-orbital puncture after an 8-hour fast. Blood glucose was measured by a glucometer (Onetouch® Ultra™, Lifescan Inc, USA). Blood samples for TG measurement were collected by the same procedure before all mice were sacrificed on the 24th week of study. Blood was centrifuged at 3,000 rpm for 5 min. Plasma was separated and kept at -20°C until measurement of TG. The animals were killed by overdose of ether inhalation. Epididymal fat pads were dissected out and weighed.

Estrogen administration

17-β -estradiol (Sigma, USA) was diluted in corn oil. The estrogen-treated mice were injected subcutaneously with 17-β -estradiol at a dose of 0.2 μg/g body weight on the 22nd week every weekday for 2 weeks. Control mice were injected with corn oil.

Statistical analyses

Body weight, fasting blood glucose, plasma triglyceride and weight of epididymal fat pads between the two different groups were compared using paired Student's t test. Significant difference was set at $P < 0.05$. Data in all figures are presented as means ± SEM.

RESULTS

Body weight and visceral fat-pad weight

The percent increment of body weight in high fat-fed
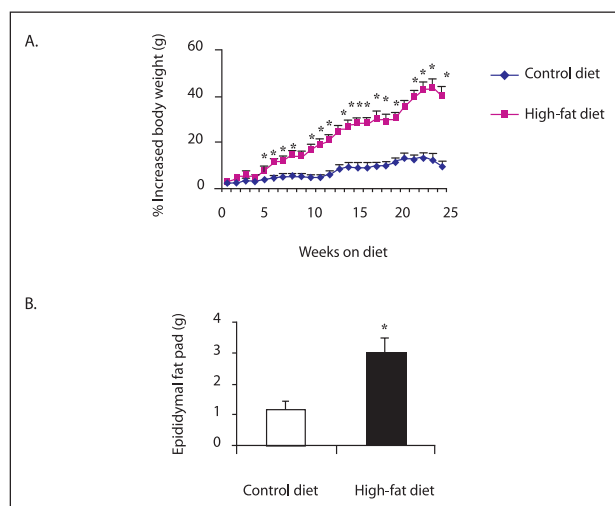
Fig 1. Effect of high-fat diet on body weight and weight of epididymal-fat pad. A. Increase in body weight of male C57BL/6J mice maintained on a control diet and a high fat diet for 25 weeks. Mice were weighed weekly. B. Weight of epididymal fat pad of mice fed with a control diet (□) and a high fat diet (■) were measured at the end of experiments. Values were expressed as means ± SEM (n =8-10). * $P < 0.05$ from comparison between a control diet and a high fat diet.
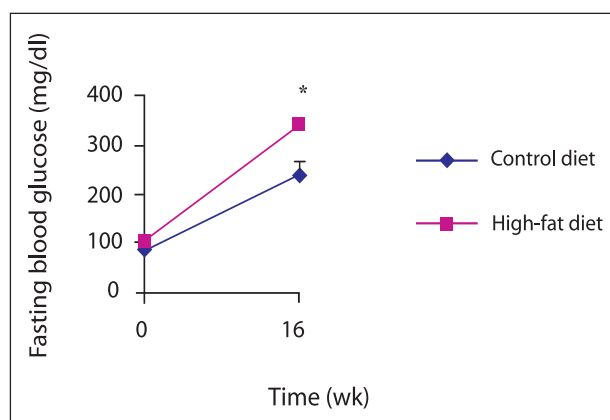
Fig 2. Effect of high fat diet on fasting blood glucose. Mice were fasted for 8 hours, then blood samples were taken from retro-orbital sinus and blood glucose was measured by glucometer. Values were expressed as means ± SEM, n =8-10. * $P < 0.05$ from comparison between a control diet and a high fat diet.

mice was significantly higher than normal fat-fed mice after 4 weeks of study (Fig 1A). After 22 weeks on a high-fat diet, body weight of high fat-fed mice increased around 40 percent from an initial body weight while 10 percent increment was detected in mice fed with a normal diet. Two weeks after administration with 17-β -estradiol (the 24th week of study), 2 percent and 4 percent decrement
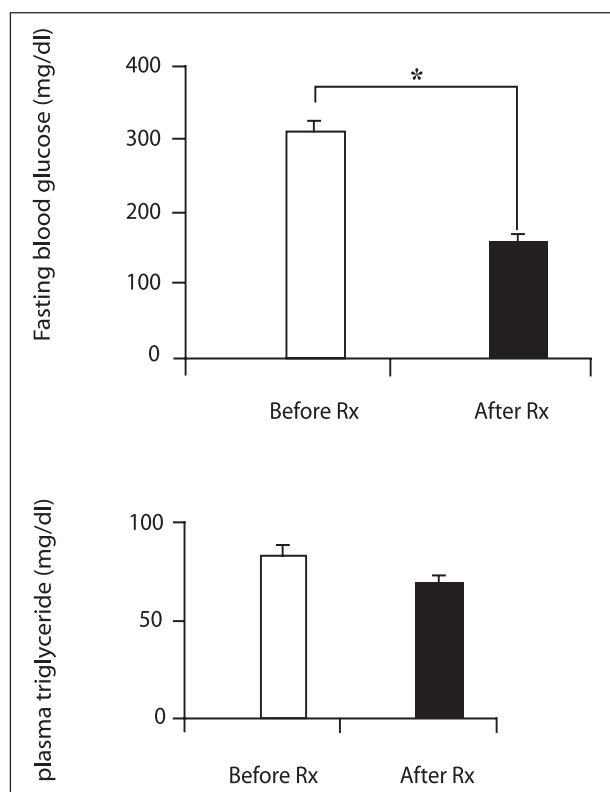
Fig 3. Effect of 17-β-estradiol administration on fasting blood glucose and plasma TG. High fat diet-fed mice were subcutaneously injected with 0.2 μg 17-β-estradiol per gram of body weight every weekday for 2 weeks. Blood samples were taken after administrating 17-β-estradiol. Fasting blood glucose (A) Plasma TG (B) were measured at the end of experiments. Values were expressed as mean ± SEM, n =4-5. * $P < 0.05$ from comparison between before (□) and after (■) treatment.

of body weight was observed in high-fat fed mice and control mice, respectively. Epididymal fat pad was used as a representative for visceral fat pad. In the high fat-fed mice, an average weight of epididymal fat pads at the 24th week was greater than in the control mice (Fig 1B).

Blood glucose and plasma triglyceride

The two groups of mice had indifferent blood glucose levels when fed with a normal diet at the baseline of the study (Fig 2). Sixteen weeks after changing to the high-fat diet, fasting blood glucose in these obese mice was significantly higher than in the non-obese control mice (Fig 2). Fasting blood glucose in obese mice showed clear-cut development of diabetes. 17-β -estradiol significantly reduced the fasting blood glucose in the high fat group (307 ± 17 vs 160 ± 7 mg/dl) (Fig 3A). There was no significant difference in plasma TG after estradiol treatment in these mice (84 ± 6 vs 69 ± 5 mg/dl) (Fig 3B).

DISCUSSION

A local-made high fat diet was given to male C57BL/6J mice, which is reported as a susceptible strain to develop type 2 diabetes. Mice fed with this diet recipe suffered significant weight gain compared to those having a control diet from the 4th week till the end of the study. Though, plasma TG levels were not different between the two groups of mice, visceral fat pads of obese mice were heavier than in the control group. This suggested that high fat-diet increases the body fat. Several studies showed that high fat-fed mice became insulin resistance and developed hyperglycemia[7,8,16]. Our results confirm the previous finding that obese mice developed diabetes according to a fasting blood glucose level of greater than 240 mg/dl[6] after feeding with a high fat food for 16 weeks.

Male animals were more susceptible to develop type 2 diabetes[17]. Ovariectomized mice had a higher incidence of type 2 diabetes independent of any change in food intake[18]. Also, estrogen reduced hyperglycemia in type 2 diabetes mice expressing human islet amyloid[14]. This may suggest that female sex hormone carries a protective effect against type 2 diabetes[14,15,19]. The present study also showed that when estrogen was administrated subcutaneously for 2 weeks, a significant reduction of fasting blood glucose was detected in this diabetic mouse model.

It is well accepted that obesity can lead to both insulin resistance and a defective insulin secretion[20]. This may result from several adipokines including TNF-α, IL-6 and resistin produced by adipose tissues[21,22]. These adipokines have been shown to reduce the insulin action in various insulin target tissues[23-25]. An elevation of adipokine levels affects the β -cell survival[26] and function[27]. Adipokines promote β -cell apoptosis[22,27] and reduce β -cell mass by induction of an inflammatory process and oxidative stress in pancreatic β -cells[27,28]. Obese individuals usually have high circulating free fatty acids (FFA)[29]. High levels of plasma FFA causes a reduction of insulin-induced glucose uptake in skeletal muscle[30], while increasing hepatic glucose production in the liver[31]. Moreover, elevated plasma FFA decreases glucose-stimulated insulin secretion by both impairing the insulin secretion[32] and insulin synthesis[33].

Recent studies have demonstrated that estrogen increased glucose-stimulated insulin secretion by increasing insulin secretion[34,35] and insulin synthesis[36]. When estrogen is bound to its receptor at the pancreatic β -cell plasma membrane, the cyclic guanosine monophosphate (cGMP) level was increased and lead to activation of the regulatory subunit of the cGMP-dependent protein kinase G (PKG)[37]. Then, the PKG phosphorylated ATP-sensitive K^+ (KATP) channel generated insulin secretion[37]. The mechanism in which estrogen increased insulin gene expression is still unknown. Estrogen has also been shown to have an antioxidative effect in several tissues[38,39]. Moreover, it has been shown that estrogen protected pancreatic β -cells against toxic substances such as amyloid[14] and proinflammatory cytokines[40]. Thus, estrogen may act on several mechanisms to decrease fasting blood glucose in high fat diet-fed mice.

CONCLUSION

In conclusion, this study shows that a high fat diet can induce C57BL/6J mice to develop type 2 diabetes. Administration of 17-β -estradiol can reduced fasting blood glucose in obese mice. To our knowledge, this is the first time to demonstrate the protective effect of estrogen against the deleterious effect of a high fat diet inducing diabetes in a mouse model.

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