Effects of Methimazole on the Onset of Type 2 Diabetes in Leptin Receptor-Deficient Rats

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ABSTRACT. We investigated the effects of methimazole, an anti-thyroid drug, on the onset of type 2 diabetes in Zucker diabetic fatty (ZDF) rats. For this, 0.03% methimazole was administered to 7-week-old, pre-diabetic ZDF rats in drinking water for 5 weeks and the animals were sacrificed at 12 weeks of age. Methimazole treatment to ZDF rats significantly reduced blood glucose levels, food intake, body weight, and serum T3 levels. Hepatocytes in ZDF-methi rats were more densely stained with eosin than those in ZDF rats because of low fat accumulation in ZDF-methi hepatocytes. The pancreatic islet in ZDF-methi rats was normal compared to that in ZDF rats. Glucagon, not insulin, immunoreactivity in ZDF-methi rats was significantly higher than that in ZDF-methi rats. These suggest that methimazole treatment may delay the onset of type 2 diabetes in leptin receptor-deficient rats and also suggests that thyroid hormones may be necessary for the onset of diabetes.

KEY WORDS: blood glucose, methimazole, thyroid hormones, type II diabetes, Zucker diabetic rat.

FULL PAPER

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Thyroid hormones, such as triiodothyronine (T3) and thyroxine (T4), are important for the growth, development and metabolism of humans and animals. In addition, thyroid hormones have important effects on energy balance, since they influence both energy intake and expenditure. Hypothyroidism has been reported to reduce the weight of the adrenal gland and the plasma concentration of corticosterone in rats [15, 18] and quail [34], indicating that hypothyroidism is associated with adrenal dysfunction.

Type II diabetes (T2DM) is a metabolic disorder that is primarily characterized by insulin resistance, relative insulin deficiency and hyperglycemia [20]. Diabetes shows hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in elevated circulating cortisol [6, 17] in human and glucocorticoids in rodents [2, 16, 33]. The lack of leptin response is well described in Zucker diabetic fatty (ZDF) rats [26], which bear a mutation (fa) in the leptin receptor gene [30, 35]. This model is somewhat different from the diet-induced diabetic model because ZDF rat was derived from the Zucker obese rat by inbreeding for the hyperglycemia phenotype. It has been reported that there are some connections between thyroid hormones and diabetes. Serum leptin concentrations significantly increased in hypothyroid rats [13]. In addition, thyroid hormones are important in maintaining cardiac function in diabetes, and hypothyroidism reduces the cardiac contractility seen in the diabetic, renovascular hypertensive rat [28]. Although some researchers have shown correlations between thyroid hormones and diabetes [5, 13, 17], there are no studies about their correlation in ZDF rats. Therefore, in the present study, we investigated the effects of hypothyroidism on the progress of diabetes in ZDF (fa/fa) rats.

MATERIALS AND METHODS

Experimental animals: Male and female heterozygote ZLC (fa/+ ) rats were purchased from Genetic Models (Indianapolis, IN, U.S.A.) and mated each other. They were housed in a conventional state under adequate temperature (23°C) and humidity (60%) control with a 12-hr light/12-hr dark cycle, and free access to food and water. Purina 5008 rodent diets (7.5% fat) were provided as recommended by Genetic Models. The procedures for handling and caring for the animals adhered to the guidelines that are in compliance with the current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85–23, 1985, revised 1996). All of the experiments were conducted to minimize the number of animals used and the suffering caused by the procedures used in the present study.

Genotyping of fa gene and experimental design: Genotype of fa gene herein was determined with the strategy described previous our study [12, 23, 36]. Ten male ZDF rats were randomly divided into 2 groups (n=5 per group) with control ZDF and hypothyroid-ZDF group. At 7 weeks of age, hypothyroidism was induced by the administration...
of 0.03% 2-mercapto-1-methyl-imidazole (methimazole, Sigma, St. Louis, MO, U.S.A.) in drinking water for 5 weeks. ZLC rats (n=5) were served as the control. All animals were euthanized at 12 weeks of age.

**Food intake, body weight, and blood glucose sampling:** Total food/water intake and body weight over the course of the study was also determined for each animal by summing the weekly averages. To measure blood glucose concentration, blood was sampled each morning (9:00 am) by “tail nick” using a 27 G needle and analyzed by using a blood glucose monitor (Ascensia Elite XL Blood Glucose Meter, Bayer, Toronto, ON, Canada).

**Measurement of serum levels of thyroid hormones and insulin levels:** To confirm the hypothyroid state, blood specimens were drawn from the euthyroid and hypothyroid rats upon killing the age of 12 weeks for analysis of serum total and free tri-iodothyronin (T3) levels to determine thyroid function in these rats using commercial assay kits (Monobind, Inc., Lake Forest, CA, U.S.A.). In addition, we also measured the serum insulin levels to determine the insulin resistance in these rats using a commercially available assay kit (Mercodia, Uppsala, Sweden).

**Tissue processing for histology:** For the histological analysis, animals at each group were anesthetized with sodium pentobarbital and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The liver and pancreas were removed and postfixed in the same conditions in each group in order to examine whether the immunostaining. The negative control resulted in the absence of immunoreactivity in any structures.

**Quantification of data:** All measurements were performed in order to ensure objectivity in blind conditions, by 2 observers for each experiment, carrying out the measures of experimental samples under the same conditions.

In order to quantitatively analyze glucagon and insulin immunoreactivity, the corresponding areas of the pancreatic islet were measured from 10 sections per animal. Images of all glucagon and insulin immunoreactive structures were taken from randomly selected islet through a BX51 light microscope (Olympus, Tokyo, Japan) equipped with a digital camera (DP71, Olympus) connected to a PC monitor. Images were calibrated into an array of 512 × 512 pixels corresponding to a tissue area of 140 × 140 μm (40 × primary magnification). Each pixel resolution was 256 gray levels. The staining intensity of all glucagon and insulin immunoreactive structures was evaluated on the basis of a relative optical density (ROD), which was obtained after the transformation of the mean gray level using the formula: ROD=log (256/mean gray level). The ROD of the complete field was measured, and the brightness and contrast of each image file were calibrated using Adobe Photoshop version 8.0 and then analyzed using NIH ImageJ 1.59 software. Values of background staining were obtained and subtracted from the immunoreactive intensities.

**Statistical analysis:** The GraphPad Prism (Ver 4.03) statistical analysis software was used for all data analysis. The data shown here represent the means of experiments performed for each experimental area. Differences among the means were statistically analyzed by 2-tailed Student t-test.

**RESULTS**

**Genotyping of the fa gene and changes in food intake, body weight and blood glucose level:** We confirmed the genotype of the fa gene in order to identify the homozygote (ZDF) and others (ZLC), as in the previous study [11, 35]. Blood glucose level increased in an age-dependent manner and was 369.4 mg/dl in 12-week-old ZDF rats. However, the blood glucose level in the ZDF-methi group increased until 8 weeks of age and was thereafter maintained until 12 weeks of age. In this group, the blood glucose level was 226 mg/dl at 12 weeks of age (Fig. 1A).

Food intake in the ZDF-methi group was significantly decreased compared to that in the ZDF group (Fig. 1B). Similarly, body weight was significantly higher in the ZDF group at 10 weeks of age and this pattern was maintained until 12 weeks of age (Fig. 1C).

**Serum levels of total/free T3 and insulin:** At 12 weeks of age, total and free T3 levels in ZDF rats were 183.06 ng/ml and 530.2 pg/ml, respectively. In ZDF-methi rats, the total and free T3 levels were significantly decreased by 75.5% (t=18.247, df=4, P<0.05) and 77.8% (t=15.351, df=4, P<0.05) compared to those in the ZDF group, respectively. In this group, total and free T3 levels were 44.75 ng/ml and 117.55 pg/ml, respectively (Fig. 2A and 2B).

At 12 weeks of age, serum insulin level in ZDF and ZDF-methi rats was 6.26 ng/ml and 4.26 ng/ml, respectively. In the ZDF-methi rats, the serum insulin levels were signifi-
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Changes in blood glucose levels (A), food intake (B) and body weight (C) in vehicle-treated ZDF and methimazole-treated ZDF (ZDF-methi) rats. Blood glucose concentration, food intake and body weight are significantly higher in ZDF rats compared to those in ZDF-methi rats. Differences between means were analyzed using the student’s t-test (n=5 per group; * P<0.05, significantly different from vehicle-treated ZDF group). The bars indicate the means ± SE.

(A) Blood glucose

(B) Food intake

(C) Body weight

Changes in morphology of liver and pancreas: In ZDF rats, hepatocytes hypertrophied and the cytoplasm was not stained with eosin dye, presumably because of fat accumulation (Fig. 3A and 3B). However, in ZDF-methi rats, the cytoplasm of hepatocytes was slightly hypertrophied and the cytoplasm was stained with eosin dye (3C and 3D).

In ZDF rats, the pancreatic duct did not show any morphological changes, but the pancreatic islet was elongated or often bilobed (Fig. 3E and 3F). In ZDF-methi rats, the histological findings in the islet were similar to those in the ZDF rats, but some eosinophilic blood vessels were observed in the pancreatic ducts (Fig. 3G and 3H).

Changes in glucagon and insulin immunoreactivity in pancreas: In ZDF rats, glucagon immunoreactivity was detected in the periphery of pancreatic islet (Fig. 4A), while insulin immunoreactivity was only observed in the median portion of islet (Fig. 4B). However, in ZDF-methi rats, glucagon immunoreactivity was significantly higher than that in ZDF rats (Fig. 4C). However, insulin immunoreactivity was similar in ZDF and ZDF-methi rats (Fig. 4B, 4D and 4E).

DISCUSSION

In this study, we observed the effects of hypothyroidism...
on diabetic onset. We used the anti-thyroid drug methimazole, which is able to decrease thyroid hormone production [4]. In addition, we used the genetically engineered ZDF rats because these rats are characterized by the progressive induction of diabetes from 7 to 12 weeks of age [9, 32]; on a Purina diet, these animals can be maintained for at least 6 months.

Methimazole treatment in ZDF rats reduced T3 levels, body weight as well as the blood glucose levels in serum compared to age-matched, vehicle-treated ZDF rats. Total and free T3 levels may be associated with the disruption of follicles of thyroid hormone because it has been reported that dwindled follicles and increased numbers of follicular epithelial cells in the thyroid glands were found in the methimazole-treated group [34]. However, there are some contradictory reports that body weight and blood glucose levels increase in hypothyroid animals. The differences between our and these studies may be associated with the animal models used in the studies. In our study, we used genetically engineered ZDF rats, which exhibit progressive induction of diabetes. In normal animals, hypothyroidism is not only known to induce hypercholesterolemia and obesity, but also to diminish insulin secretion [7, 8, 13]. Similar to our study, serum glucose concentrations in methimazole-treated SD rats were not significantly different from controls [38].

In this study, we also observed the changes in insulin levels and glucagon and insulin immunoreactivity in the pancreas of ZDF and methi-ZDF rats. Hypothyroidism ameliorated the reduction of glucagon immunoreactivity in the ZDF rats. It has been reported that insulin and glucagon immunoreactivity was significantly decreased in ZDF rats compared to that in ZLC [27]. In addition, the pancreatic insulin reservoir of ZDF rats is depleted by 12 weeks of age, and plasma insulin levels are reduced to the low-normal range, with concomitant exacerbation of hyperglycemia consistent with end-stage type II diabetes [19, 21, 24]. In contrast, ZDF rats are hyperinsulinemic at 10–13 wk of age and serum insulin levels decline to below levels of insulin in age-matched lean control rats by 22–42 wk of age [25]. It has been reported that thyroid hormone is closely related with α-cells in the pancreas because thyroid hormone receptors were mainly detected in the glucagon positive cells in

Fig. 3. The photomicrographs of H&E staining in liver (A-D) and pancreas (E-H) in vehicle-treated ZDF and methimazole-treated ZDF (ZDF-methi) rats. Note that hepatocytes in ZDF rats are faintly stained with eosin dye compared to those in ZDF-methi rats. In addition, the morphology of the pancreatic islet is irregular in ZDF rats compared to that in ZDF-methi rats. Bar=100 μm (A, C, E-H), 50 μm (B and D).
the pancreas [39]. The decrease of thyroid hormones induced by methimazole treatment may increase the glucagon in the pancreas which causes the liver to convert stored glycogen into glucose and release it into bloodstream.

Hepatic lipogenic enzyme gene expression is regulated by the opposing effects of insulin and leptin [14]. Hepatocytes from ZDF rats exhibit increased lipogenesis, glycogen synthesis, and glycolysis and decreased gluconeogenesis, glycogenolysis, and fatty acid oxidation [11]. Hypothyroidism increases the lipid gain and decreases the fatty acid oxidation activity [13, 22]. In addition, overt hypothyroidism is widely recognized as a risk factor for atherosclerosis by its association with different factors, including lipid disturbances [8, 10]. However, in our study, hypothyroidism reduced diabetes-induced changes (hypertrophied cytoplasm and decreased eosinophilic cytoplasm) of hepatocytes [37]. These discrepancies may be associated with the action of methimazole in the animals. Since thyroid hormone regulates mitochondrial gene expression and function in skeletal muscle, reductions in T3-mediated transcription may contribute to diabetes-related impairments in oxidative metabolism [5]. It has been reported that methimazole has immunosuppressive effects by decreasing the production of free oxygen radicals in neutrophils and monocytes [1, 29]. In addition, methimazole alleviates hepatic encephalopathy in bile duct-ligated cirrhotic rats [3] and protects lungs during hepatic ischemia-reperfusion in rats [31].

In conclusion, methimazole administered to 7-week-old, pre-diabetic ZDF rats ameliorates the diabetes-induced changes in pancreas and liver as well as in blood glucose levels. These effects may be associated with the low levels of thyroid hormone or the direct anti-oxidant effects of methimazole.

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REFERENCES


