Gender Difference in Structure and Function of Pancreatic Islet Cells in Prolonged Liquid Fructose Ingestion in Rats

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Abstract

Daily consumption of high fructose in soft drinks and other processed foods is progressively rising in both adults and children, and it becomes a predisposing factor for the development of insulin resistance and type 2 diabetes. We have previously demonstrated that prolonged ingestion of liquid fructose (10% fructose solution) led to insulin resistance and hyperinsulinemia only in male but not female rats. However, it remains unknown whether ingestion of liquid fructose would lead to morphological alteration and function of the pancreatic islet cells. Thus, the present study examined the architecture and distribution of islets from liquid fructose-fed male and female rats. Male and female Sprague-Dawley rats were given either reverse osmosis water or liquid fructose for six weeks. The histological changes in size and number of pancreatic islets were assessed by hematoxylin-eosin staining. Insulin resistance and β-cell function were demonstrated by homeostasis model assessment index of insulin resistance (HOMA-IR) and β-cell function (HOMA-β), respectively. We found that a significant increase in plasma insulin levels observed in fructose-fed male rats was positively correlated with a higher number of oversized islets. Our current findings indicate that there is sex dimorphism in pancreatic islet structure and function in response to prolonged fructose ingestion. The present work provides further understanding in gender differences in the context of the pancreas relevant to fructose-induced metabolic dysfunctions.

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Introduction

The intake of processed foods and beverages containing high sugar content has continuously risen. Particularly, excessive fructose consumption appears to be a key driver of many serious health problems, including obesity, type 2 diabetes and metabolic syndrome.[1-3] Interestingly, diet-induced metabolic disorders are more prominent in males than females,[4-8] thereby indicating sex differences in the development of metabolic abnormalities. We have previously demonstrated that ingestion of liquid fructose for six weeks impaired insulin-stimulated glucose transport and its signaling molecules in skeletal muscle of male but not female rats.[9]

The pancreatic islet β-cell is the source where insulin is synthesized and secreted, and dysfunction of islet β-cells can interrupt glucose homeostasis.[10] Alterations in pancreatic islet size, number and composition are linked with the progression of type 1 and type 2 diabetes.[11,12] However, no study thus far has reported whether the pancreatic islet structure is influenced in insulin-resistant state caused by prolonged fructose consumption. Thus, the present study was designed to investigate the effect of liquid fructose ingestion on pancreatic islet structure and function. We hypothesized that the pancreatic islet structure would be affected by prolonged ingestion of liquid fructose and the effect of liquid fructose on pancreatic islet structure would be different between sexes.

Materials and Methods

Ethics statement

Animal experimental procedures (Protocol no. MUSC56-017-279 and MUSC60-032-382) have been approved by the Institutional Animal Care and Use committee of the Faculty of Science, Mahidol University, in accordance with the International Guiding Principles for Biomedical Research Involving Animals of the Council for International Organizations of Medical Sciences.

Animals

Eight-week-old male and female Sprague-Dawley rats, weighing 260-290 g and 180-210 g, respectively, were obtained from the National Laboratory Animal Center, Thailand. All animals were acclimatized for one week prior to initiating experiments. Rats were housed in the animal facility under controlled temperature at 22°C ± 2°C with a 12-hour light-dark cycle (lights on from 6:00 am to 6:00 pm), and

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allowed free access to standard pellet chow and water *ad libitum*. For the experiment, male and female rats were randomly given either reverse osmosis water or liquid fructose (10% w/v) for six weeks. All animals were randomly divided into four groups with six animals per group as follows: 1) male rats fed with a normal diet + reverse osmosis water; 2) male rats fed with a normal diet + liquid fructose; 3) female rats fed with a normal diet + reverse osmosis water; and 4) female rats fed with a normal diet + liquid fructose. Each animal was initially provided with 50 g of pellet rat chow and 200 ml of water or liquid fructose.

Blood and tissue collection

After a period of treatment, all rats were food restricted (4 g of chow), and the fructose solution was replaced with water. On the next day (9:00 am), tail blood was collected. The blood sample was mixed with anticoagulant (18 mM final concentration of EDTA) and centrifuged at 13,000g, 4°C, for 1 min. Plasma was kept at −80°C and used for the determination of glucose (Gesellschaft für Biochemica und Diagnostica, Wiesbaden, Germany) and insulin (Linco Research, St. Charles, MO). For harvesting pancreatic tissues, animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal; 75 mg/kg body weight). The pancreas was rapidly removed and divided into two parts at the middle of its length. The tail part of the pancreas was fixed in 10% neutral buffered formalin for 24 hours at room temperature for further histomorphological analysis.

Insulin sensitivity

Homeostasis model assessment of insulin resistance (HOMA-IR) was used as an index of insulin sensitivity. HOMA-IR = (fasting plasma insulin concentration (mU/l) × fasting plasma glucose (mmol/l) / 22.5.[13] HOMA-β was used to estimate β-cell function. HOMA-β = [20 × fasting plasma insulin concentration (mU/l)] / [fasting plasma glucose (mmol/l) - 3.5].[13]

Islet histomorphology

Formalin-fixed pancreas was then embedded in paraffin, sectioned at 5 μm thickness (Leica Biosystems, Singapore) and processed for hematoxylin and eosin (H&E) staining to evaluate the morphology of pancreatic islets. The islet size was determined from measurements of the cross-sectional area of the entire of the islet for different slices throughout the islet using the Olympus CellSens Dimension software (Olympus). The islet size was represented by the total area in islets and categorized depending on its maximal diameter as small (S), < 10,000 μm^2; medium (M), 10,000-50,000 μm^2; large (L), 50,000-100,000 μm^2; or extra large (XL), > 100,000 μm^2. The islet density was defined as the number of islets per pancreatic section and calculated as follows: (number of each islet size / total number of islets) × 100. Pancreatic tissue slides were examined and imaged using a Nikon Eclipse E600 microscope fitted with Nikon digital camera DXM1200 with the 10x objective lens (Nikon Inc., Melville, NY). All images were captured using the same settings.

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM) of six animals per group. Data analysis was performed by using GraphPad Prism 6.0 Software (GraphPad, La Jolla, CA, USA). Statistical analyses were performed by unpaired Student's *t*-test. *P* value of less than 0.05 was considered statistically significant. Pearson's correlation coefficient was used to determine the correlation between the islet size and plasma insulin level.

Results

Prolonged consumption of liquid fructose induces hyperinsulinemia in male but not female rats

No significant difference in the fasting glucose levels was observed between male and female rats fed with liquid fructose (Figure 1A). However, the fasting insulin levels were higher in fructose-fed males than fructose-fed females (Figure 1B). Compared with control rats, prolonged ingestion of liquid fructose

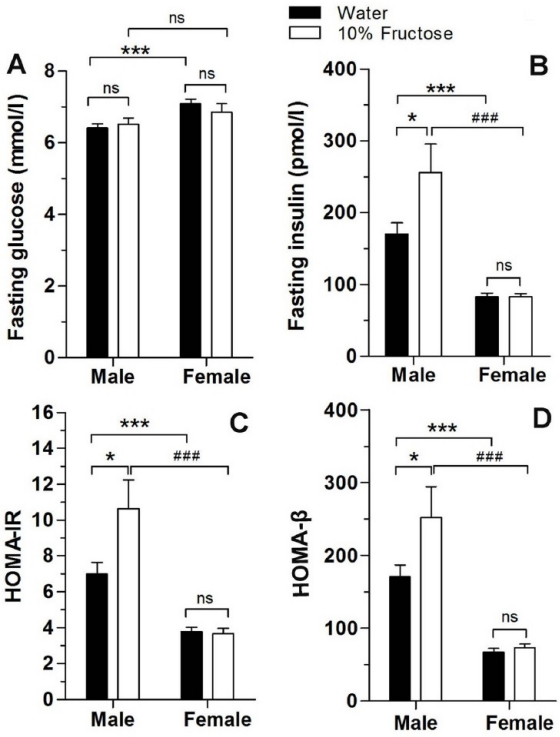

Figure 1 Prolonged consumption of liquid fructose exhibits insulin resistance in male rats. (*A*) Fasting glucose, (*B*) Fasting insulin, (*C*) HOMA-IR, and (*D*) HOMA-β of male and female rats receiving either reverse osmosis drinking water or 10% fructose in the drinking water (n = 6 rats in each group). **P* < 0.05 and ****P* < 0.001 compared with male rats receiving reverse osmosis water; ###*P* < 0.001 compared with male rats receiving 10% fructose in the drinking water.

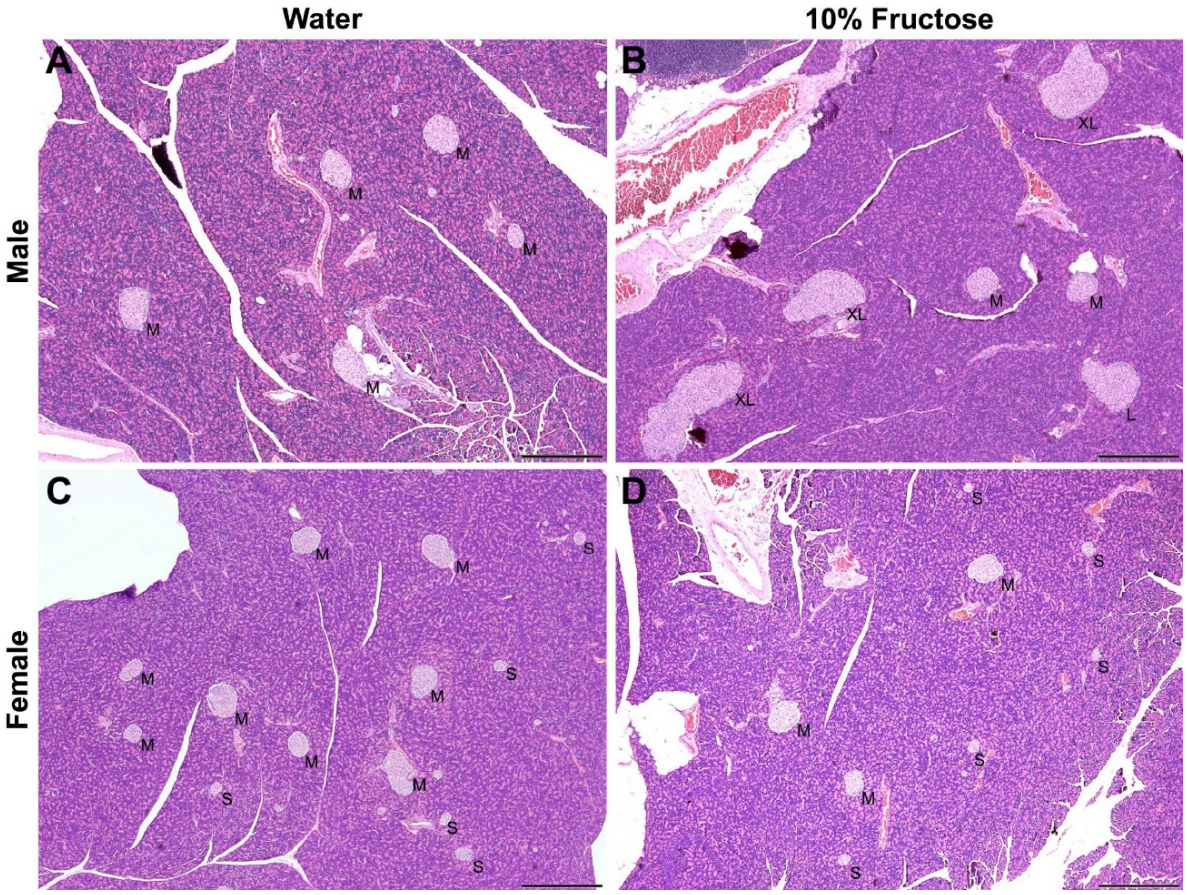

Figure 2 Male Sprague Dawley rats ingesting liquid fructose for six weeks demonstrates increased number of extra large-sized pancreatic islets. Representative photomicrographs of pancreas stained with hematoxylin and eosin (H&E) from (*A*) male rats receiving reverse osmosis water, (*B*) male rats receiving 10% fructose in the drinking water, (*C*) female rats receiving reverse osmosis water, (*D*) female rats receiving 10% fructose in the drinking water (n = 6 rats in each group). S, small-sized islets; M, medium-sized islets; L, large-sized islets; XL, extra large-sized islets. Scale bar = 500 μm.

significantly increased HOMA-IR and HOMA-β only in male rats (Figure 1C-D). These results indicate that liquid fructose induced insulin resistance and impaired β-cell function only in males.

Increased density of enlarged pancreatic islets exhibits in fructose-fed male rats

The islet morphology in both sexes in the absence or presence of high fructose condition was assessed. Histological examination revealed enlarged islets in fructose-fed male rats, whereas this manifestation was not seen in fructose-fed female rats (Figure 2A-D).

Further morphometric analysis showed a significant difference in islet density and size between male and female rats fed with liquid fructose (Figure 3A-B). The distribution of small (S), medium

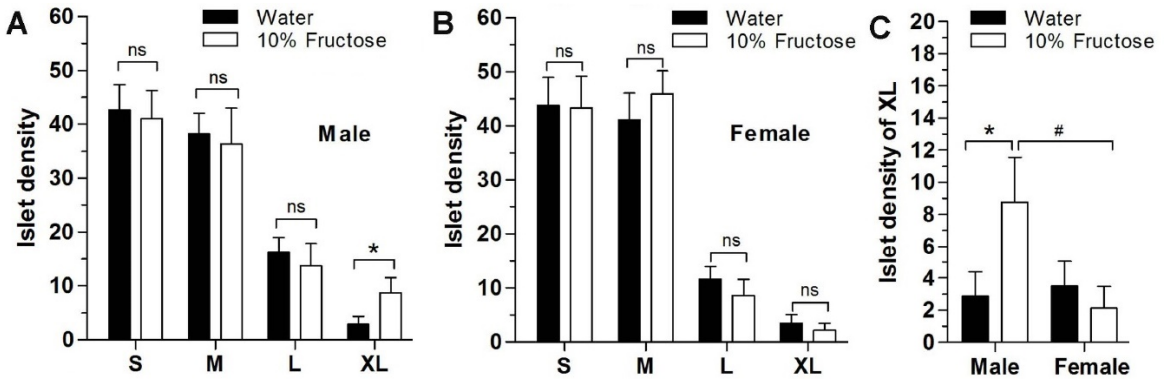

Figure 3 Prolonged liquid fructose ingestion increases number of enlarged islets in male rats. (*A*) islet density of male rats, (*B*) islet density of female rats, and (*C*) Density of XL-sized islets in male and female rats receiving either reverse osmosis water or 10% fructose in the drinking water (n = 6 rats in each group). $^{*}P < 0.05$ compared with reverse osmosis drinking water. $^{\#}P < 0.05$ compared with male rats receiving 10% fructose in the drinking water. S: small-sized islets; M: medium-sized islets; L: large-sized islets; XL: extra large-sized islets.

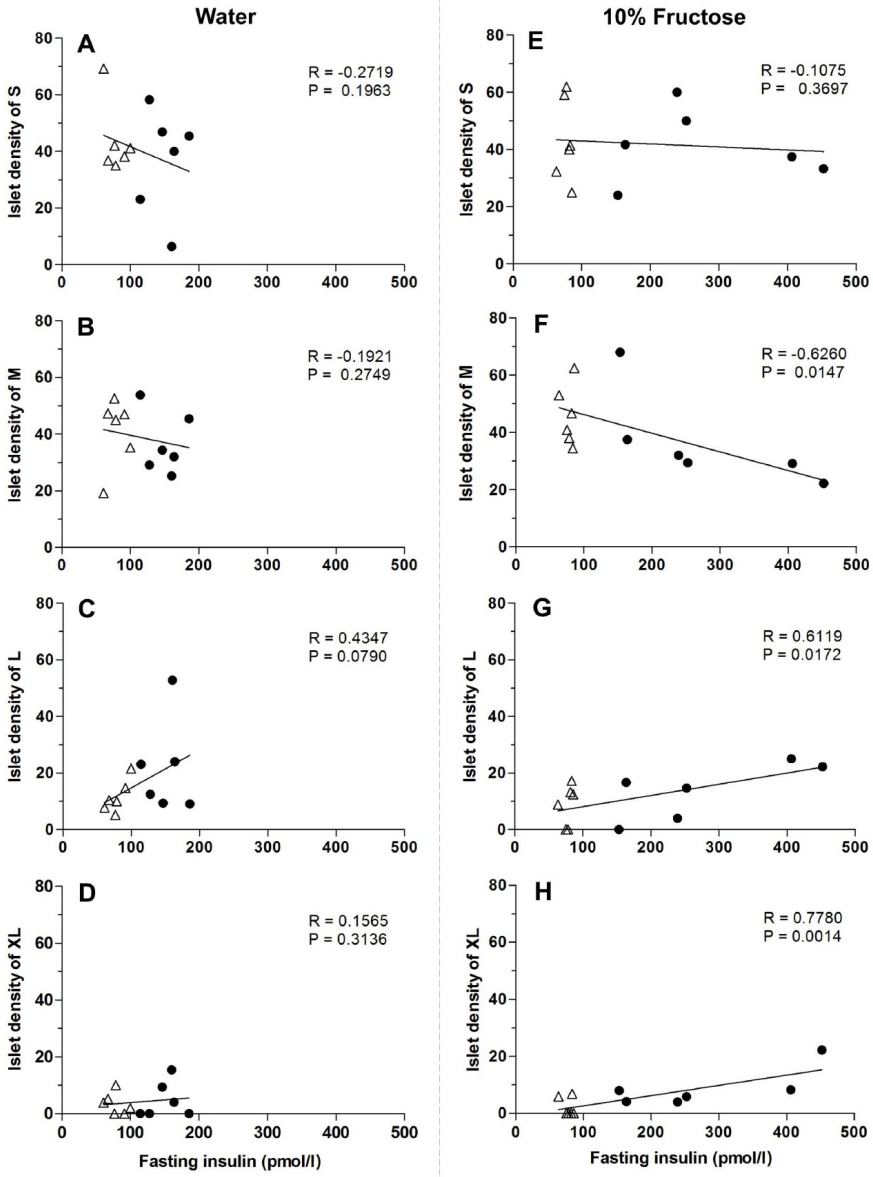

Figure 4 Increased fasting plasma insulin levels positively correlate with increased islet density of outsized islets in male rats consuming liquid fructose. Correlation between fasting plasma insulin levels and islet density of (*A*) small, (*B*) medium, (*C*) large and (*D*) extra large-sized islets in male and female rats receiving reverse osmosis water, and islet density of (*E*) small, (*F*) medium, (*G*) large and (*H*) extra large-sized islets in male and female rats receiving 10% fructose in the drinking water (n = 6 rats in each group). *Abbreviation.* S: small-sized islets; M: medium-sized islets; L: large-sized islets; XL: extra large-sized islets. *Symbol:* Δ = female, ● = male

(M), and large (L) islets was similar between males and females that received water. However, a striking difference in extra large (XL) islets was observed in fructose-fed male rats. The density of XL islets in fructose-fed male rats was approximately two-fold higher than that of control male rats (Figure 3A). In contrast, there was no significant difference in all sizes of islets in the female groups (Figure 3B). Of note, fructose-fed male rats had significantly higher density of XL islets compared to fructose-fed female rats (Figure 3C). Collectively, these data indicate that prolonged ingestion of liquid fructose led to changes in islets, which preferentially occurred in XL islets in male rats.

Extra large islets elicited in fructose-fed male rats relates to insulin resistance status

Because a marked expansion of the pancreatic islets was observed in coordination with increased plasma insulin levels only in fructose-fed male rats, we further assessed whether the change in pancreatic islet size also correlated with insulin resistance status. While there was no correlation between islet size and plasma insulin levels in both male and female rats given water, as well as in fructose-fed female rats (Figure 4A-E), there was a significant inverse correlation between M islets and the plasma insulin levels in fructose-fed male rats (Figure 4F). Moreover, the L and XL islets were significantly

positively associated with the plasma insulin levels in fructose-fed male group (Figure 4G-H). These data indicate that liquid fructose-induced hyperinsulinemia in male rats is associated with enlarged pancreatic islets.

Discussion

We have previously demonstrated that ingestion of liquid fructose for six weeks influenced insulin action and signaling of skeletal muscle glucose uptake differently between males and females.[9] In the present study, we aimed to extend our understanding in regards to the impact of chronic fructose ingestion on pancreatic islet morphology and the role of gender. We have found that persistent consumption of liquid fructose elicited striking changes in the morphology and size of the islets in males, but not in females. Contrary to females, fructose-fed male rats manifested insulin resistance along with increasing density of L and XL islets, which were also significantly correlated with increased plasma insulin levels.

The role of sex differences in the development of metabolic abnormalities remains unclear. Although existing data regarding sex and the development of metabolic dysfunctions are inconsistent, it has been documented that men have lower insulin sensitivity and are more likely to develop type 2 diabetes than women.[14] In addition, a number of studies have addressed the role of female sex hormones in the discrepancy of metabolic dysfunctions between sexes.[9,15] For example, estrogen supplement could improve insulin sensitivity and prevent the development of hypertensive condition in male rats fed liquid fructose,[16] whereas ovarian hormone deprivation by ovariectomy induced glucose intolerance and hypertension in fructose-fed female rats.[9]

In the present study, we observed that liquid fructose significantly increased the plasma insulin levels, HOMA-IR and HOMA-β with a significant change in islet morphology only in fructose-fed male rats. Thus, abnormally enlarged size of islets appeared to be a part of an adaptive mechanism to insulin resistance in fructose-fed male rats. Moreover, a significant positive correlation was observed between islet density (L and XL sizes) and the plasma insulin levels only in fructose ingested rats (Figure 4E-H), indicating that an increase in the number of oversized pancreatic islet could reflect the insulin-resistant state.

In summary, this work provided new findings in which islet adaptive changes to fructose-induced insulin resistance exhibited only in male rats, which accentuates the presence of sex differences in metabolic dysfunctions. Our finding supports the concept that females had greater protection against diet-induced impairment of pancreatic function, which could contribute to a lower risk of type 2 diabetes in women. In addition, the islet-size determining criteria established in this study could be applicable in the prediction of insulin resistance associated with islet hypertrophy.

Acknowledgments

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Conflict of Interest

None to declare.

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