Basic Medical Research

Effect of hyperglycemia on fertility in streptozotocin-induced diabetic male Wistar rats: focus on glucose transporters and oxidative stress

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ABSTRACT

BACKGROUND Glucose transporters (GLUTs) and oxidant metabolism are associated with the mechanism of infertility. This study evaluated the impact of hyperglycemia on glucose and oxidant metabolisms of Sertoli cells (SCs).

METHODS This study was an animal study to investigate the expression of messenger RNA monocarboxylate transporter 4 (MCT4), GLUT1, GLUT3, nuclear factor erythroid 2-related factor 2 (Nrf2), glutathione peroxidase, catalase (CAT), and lactate dehydrogenase A (LDHA) of Wistar rats testes that were induced hyperglycemia. Reverse transcription polymerase chain reaction analysis was used. Hyperglycemic state in the Wistar rats was induced by streptozotocin. 24 rats were divided into 3 groups: non-hyperglycemia (control), 2-week, and 4-week hyperglycemic state. All data were collected and analyzed using SPSS version 15.0 (IBM Corp., USA).

RESULTS The expression of glucose transporter (GLUT1 and GLUT3), lactate transporter (MCT4), and cellular defense protein against oxidant (Nrf2 and CAT) was significantly increased in the 2-week and 4-week hyperglycemic state groups with $p<0.01$, respectively.

CONCLUSIONS Hyperglycemic state affects the metabolism of SCs. Alteration of GLUTs and oxidative metabolism may indicate metabolic alterations by a prolonged exposure to hyperglycemia that may be responsible for diabetes-related male infertility.

KEYWORDS GLUT1, GLUT3, glutathione peroxidase, infertility, MCT4, Nrf2

The risen prevalence of diabetic mellitus (DM) in recent years has been predicted, especially in developing countries. DM is characterized as a metabolic disease that results in hyperglycemia, which eventually would induce damage, dysfunction, and failure of various organs including abnormalities in the male reproductive system.[1–4]

Sertoli cells (SCs) play a vital role in producing metabolic precursors which are essential for the development, proliferation, and maturation of germinal cells during spermatogenesis.[5–9] SCs uptake extracellular glucose via specific high-affinity glucose transporters (GLUTs), which are then oxidized to pyruvate and directly reduced to lactate by lactate dehydrogenase (LDH) with concomitant oxidation of nicotinamide adenine dinucleotide and hydrogen (NADH). Next, lactate is exported to the intratubular fluid through monocarboxylate transporter 4 (MCT4) to develop germinal cells.[10,11] Thus, a clear effect of diabetes on testicular glucose metabolism, especially alteration of expression of MCT4, GLUT1, GLUT3, and LDHA in SCs, is important to understand the mechanisms of diabetes-related infertility.

Excessive advanced glycation end products caused by diabetes can trigger oxidative stress, leading to the activation of mitochondria or endoplasmic reticulum (ER) stress-related mechanisms of cell death and causing sperm reduction.[12,13] Antioxidant genes, such as catalase (CAT), glutathione peroxidase (GPX), and nuclear factor erythroid 2-related factor 2 (Nrf2), are prominent in spermatogenesis and normal sperm function.[14] This was supported by studies using Nrf2, a superoxide dismutase, and GPX gene knockout in animal models, which led to mild or severe male infertility. Therefore, further study on the antioxidant deficiency of spermatozoa related to diabetes is crucial to identify the mechanism associated with male infertility. Thus, this study aimed to evaluate the expression of MCT4, GLUT1, GLUT3, and LDHA of SCs and the antioxidant status of spermatozoa in diabetic streptozotocin (STZ)-induced hyperglycemic rats.

METHODS

Experimental animals and protocol design

Twenty four adult Wistar male rats of 5–8 weeks, weighing 160–200 g were obtained from the *Lembaga Penelitian dan Pengujian Terpadu* (LPPT) Faculty of Medicine, Universitas Gadjah Mada. All rats were housed in a standard animal facility with room temperature and light/dark cycle of 12/12 hours in the Department of Anatomy, Faculty of Medicine, Universitas Gadjah Mada. Water and food (supplemented with 18% proteins, 3% lipids, and 10% fibers) were provided with *ad libitum* and had undergone an acclimatization period of 7 days.[15] The animal procedure protocols were approved by the Animal Care and Welfare Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia (Ref. No.: KE/FK/0628/EC/2019) and followed the regulatory animal care guidelines of the U.S. National Institutes of Health.

This study is an experimental study. Hyperglycemia was induced using multiple low doses as described by Deeds et al.[16] The administration of intraperitoneal injection of 60 mg/kg STZ was freshly dissolved in 0.05 M chilled sodium citrate buffer at pH 4.5, and the STZ was injected within 5 min. Controls were injected with the equivalent volume of citrate buffer after 12 hours of fasting. Rats were randomly divided into three groups: non-hyperglycemia (control), 2-week, and 4-week hyperglycemic state. Only rats with fasting blood glucose more than 200 mg/dl were included in this study.

Gene expression on rats testicular

Testicular tissues were extracted from rats and mechanically homogenized in TRIzol (Invitrogen, Thermo Fisher, USA). The total RNA was isolated from tissue samples using the RNeasy Mini Kit (Qiagen, USA). A total of 0.5 µg RNA was used to produce complementary DNA (cDNA) using LongRange 2Step reverse transcription polymerase chain reaction (RT-PCR) Kit (Catalog no. 205920) (Qiagen), and 5 µl of total cDNA was mixed with 12.5 µl of 2x SYBR® Green PCR (Bio-Rad Laboratories, Inc., USA) and ROX (Bio-Rad Laboratories, Inc.), with 10 pmol/µl of each was the forward and reverse primer for the measured genes. MCT4, LDHA, GLUT1, GLUT3, Nrf2, and GPx gene expressions were quantified using a real time PCR. The PCR primer pairs used are shown in Table 1.[2,17–21] For the relative quantification, the target gene expression was determined by the (2−ΔΔCt) method after a normalization to the gene of glyceraldehyde 3-phosphate dehydrogenase cycle threshold values.

Table 1. The target gene primers sequences

First author, year	Gene expression	Primer sequence
Chidlow,[17] 2005	MCT4	5'-TGCGGCCCTACTCTGTCTAC-3' 5'-TCTTCCCGATGCAGAAGAAG-3'
Ma,[18] 2013	Nrf2	5'-AGCACATCCAGACAGACACCA-3' 5'-TATCCAGGGCAAGCGACTC-3'
Singh,[19] 2011	LDHA	5'-GACTTGGCAGATGAACTTACC-3' 5'-CACAAGGAACACTTAGGAAGAC-3'
Rato,[2] 2015	GLUT1	5'-TCCGGCGGGAGACGCATAGT-3' 5'-CCCGCATCATCTGCCGACCC-3'
	GLUT3	5'-GCGCAGCCCTTCCGTTTTGC-3' 5'-CCCCTCGAAGGCCCGGGTAA-3'
Afifi,[20] 2015	GPx	5'-GCTCACCCGCTCTTTACCTT-3' 5'-AATGGACGAGAACATGCCCA-3'
Almeer,[21] 2018	CAT	5'-CCGACCAGGGCATCAAAA-3' 5'-GAGGCCATAATCCGGATCTTC-3'

CAT=catalase; GLUT=glucose transporter; GPx=glutathione peroxidase; LDHA=lactate dehydrogenase A; MCT4= monocarboxylate transporter 4; Nrf2=nuclear factor erythroid 2-related factor 2

Statistical analysis

Data were presented as mean (standard error of the mean). SPSS version 15.0 (IBM Corp., USA) was used to calculate the statistical significance between groups, which was determined using one-way analysis of variance.

RESULTS

In this study, hyperglycemia significantly upregulated the messenger RNA (mRNA) expression of GLUT1 (Figure 1a) and GLUT3 (Figure 1b), compared with the control group in the rat testes ($p<0.01$). The significant increment of GLUT1 and GLUT3 was observed in the 2-week hyperglycemic state group, and it continued to increase in the 4-week hyperglycemic state group although it was not statistically significant ($p>0.05$). In contrast to the 2-week group (Figure 1c), the 4-week group did not show a significantly upregulated mRNA expression of MCT4 ($p>0.05$). Additionally, hyperglycemia conditions were seen by a significantly upregulated mRNA expression of LDHA in the intervention groups, compared with the control group ($p<0.05$) (Figure 1d).

In the 2-week and 4-week groups, there was a significantly upregulated mRNA expression of Nrf2 by 2.19 fold and 3.88 fold, respectively ($p<0.05$) (Figure 1e). Furthermore, the 2-week and 4-week groups had a significantly upregulated mRNA expression of CAT by 1.67 and 1.87 fold (Figure 1f) and GPX by 1.67 and 1.76 fold, respectively (Figure 1g), compare with the control group.

DISCUSSION

Our present study revealed that hyperglycemia induced a glucose metabolism dysfunction in rat testes by modulating the expression of GLUT1/GLUT3, MCT4, and LDHA. It is suggested that STZ-induced hyperglycemia may affect SCs' metabolism that accounts for diabetes-related infertility. This is supported by a significant increment of the gene expression of GLUT1/GLUT3, MCT4, and LDHA after 2 weeks in hyperglycemic state. Although it was not statistically significant, the trend continued after 4 weeks in hyperglycemic state. It is supported by the recent findings: 1) the ultrastructural changes of mitochondrial and reduction in the smooth ER in the SCs and Leydig cells were observed following STZ-induced diabetes, 2) a vimentin apical extension of SCs was decreased significantly following STZ-induced diabetes, which suggested that SCs were the target of hyperglycemia induced by STZ, and 3) SCs played a vital role to produce metabolic precursors which were essential for germinal cells and spermatogenesis.[8,9,22–25] SCs uptake the extracellular glucose via specific high-
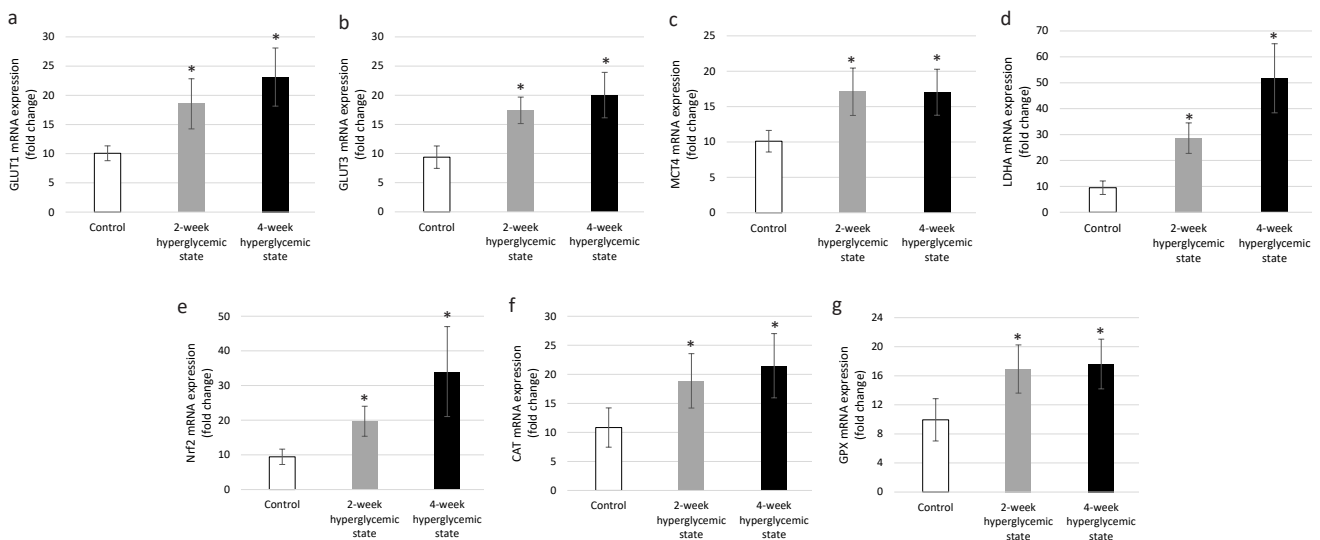

Figure 1. Glucose transport and oxidative stress in hyperglycemic state. Hyperglycemia significantly upregulated the mRNA expression of (a) GLUT1; (b) GLUT3; (c) MCT4; (d) LDHA; (e) Nrf2; (f) CAT; and (g) GPX in the rat testes. Data are presented as mean (SEM) (n = 8 independent repeats with duplicates). *One-way ANOVA; $p<0.001$ compared with the control group. Control: non-hyperglycemia. ANOVA=analysis of variance; CAT=catalase; GLUT=glucose transporter; GPX=glutathione peroxidase; LDHA=lactate dehydrogenase A; MCT4=monocarboxylate transporter 4; Nrf2=nuclear factor erythroid 2-related factor 2; SEM=standard error of the mean

affinity GLUTs, which are then oxidized to pyruvate and directly reduced to lactate by LDH with concomitant oxidation of NADH. The lactate produced is then exported to the intratubular fluid through the MCT4 to develop germ cells.[10,11] Therefore, alteration in GLUT1/3, MCT4, and LDHA following STZ-induced diabetes may indicate stimulation of glycogen accumulation, which is associated with diabetes-related infertility. However, further study is needed to confirm whether the expression of GLUT1/3, MCT4, and LDHA are SCs' specific.

The signaling Nrf2/antioxidant response element pathway is a significant mechanism for cells to combat oxidative stress. Thus, the effects of hyperglycemia on the Nrf2 signaling pathway are employed. Our study showed that after 2 weeks in hyperglycemic state, Nrf2 gene expression showed a significant upregulation, indicating that hyperglycemia produced excessive amounts of reactive oxygen species (ROS) that were compensated by the antioxidant Nrf2 pathways. A previous study on renal model showed a protective role of Nrf2 against antioxidants in the diabetic mice models.[26]

Testicular CAT and GPX are biomarkers on the other pathways linked to the decrement of oxidative markers. Meanwhile, they also have an important role in scavenging superoxide unions and preventing lipid peroxidation. This mechanism will decrease the risk of DNA damages.[27] The increased activities of Nrf2, CAT, and GPX might be a protective mechanism in response to excessive production of ROS and hydrogen peroxide due to the autoxidation of glucose and non-enzymatic glycation.[28]

The effect of hyperglycemia on diabetes has been reported to cause infertility due to increased oxidative stress.[28–30] Our study found that the intrinsic anti-oxidation mechanisms compensated for the effect of hyperglycemia. Despite this preclinical study showed promising results, a longer time frame is needed to observe the chronic effect of hyperglycemia on diabetic conditions. Thus, this study may be a guide to treat infertility among diabetic patients and evaluate these mRNA biomarkers on human semen samples.

The limitation of this study is the short duration of observation. A longer duration is needed to observe the chronic effect of hyperglycemia on the metabolism of testes. This study did not evaluate the direct effect of hyperglycemia on fertility. Therefore, further studies to confirm the clinical impact of hyperglycemia and its metabolism effects are suggested. In conclusion, alteration of testicular metabolisms in diabetic patients occurs on a testicular level, and testicular metabolic alterations are closely associated with diabetes-related male infertility.

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