Proceedings of the International Conference on Nursing and Health Sciences
Volume 1 No 1, November 2020
http://jurnal.globalhealthsciencegroup.com/index.php/PICNHS
Global Health Science Group

UPREGULATION OF MRNA TNF-α IN SKELETAL MUSCLE TISSUE OF STREPTOZOTOCIN-INDUCED DIABETIC RAT

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ABSTRACT
Inflammation is a molecular mechanism that linking obesity and ageing with insulin resistance in type 2 diabetes mellitus (DM). Although type 1 DM is primarily caused by insulin deficiency, but insulin resistance also prominent feature in this disease. It is not fully understood whether inflammation also contribute in insulin resistance phenotype in type 1 DM. This study aimed to assess mRNA TNF-α expression in the skeletal muscle tissue of type 1 diabetes mellitus rat model.This in-vivo study used 18 adults male wistar rats. The study conducted at Department of Anatomy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada from July to October 2020. Male Wistar rats divided into control group (CDM, n=6), and diabetes mellitus group which is divided into 1-month DM group (DM1M, n=6), and 2-month DM group (DM2M, n=6). The DM model was conducted through single intraperitoneal injection of Streptozotocin 60 mg/kg Body Weight (BW). At the end of study, rats were sacrificed and the gastrocnemius muscle was harvested. The expression of mRNA TNF-α was measured by RT-PCR. Statistical analysis was conducted using One Way ANOVA test. Blood glucose level were significantly higher in DM groups compared to control group ($p<0.05$). The body weight of DM groups was significantly lower after 1 month and 2 months DM period compared to control group ($p<0.05$). DM groups demonstrated upregulation of mRNA TNF-α compared to control group ($p<0.05$). Type 1 diabetes mellitus model demonstrated upregulation of mRNA TNF-α in skeletal muscle tissue.

Keyword: diabetes mellitus; TNF-α; inflammation; skeletal muscle

INTRODUCTION
Diabetes mellitus and its complications is major health problems in the world because of its high morbidity and mortality (Holman, Young, & Gadsby, 2015; Saeedi et al., 2019). In 2010, diabetes mellitus and its complications caused 3.9 million deaths (6.8% global mortality) and increased to 5 million deaths in 2015 (Global Burden of Disease Study 2013 Collaborators, 2013). In 2019, a total of 463 million people are living with diabetes, representing 9.3% of the global adult population (20–79 years) and this number is projected to increase to 578 million (10.2%) in 2030 and 700 million (10.9%) in 2045 (Saeedi et al., 2019).

Diabetes mellitus (DM) described as a glucose metabolism disorder characterized by chronic hyperglycemia that develops as a consequence of defects in insulin secretion, insulin action, or both. The three main types of diabetes are type 1 diabetes (T1D), type 2 diabetes (T2D), and gestational diabetes mellitus (GDM) (Saeedi et al., 2019). Mechanism underlying hyperglycemia in type 2 diabetes is insulin resistance in insulin target cell (Al-Goblan, Al-Alfi, & Khan, 2014; Association, 2014). More than 90% of diabetes mellitus cases are type 2 diabetes mellitus (T2DM) (Holman et al., 2015). Chronic hyperglycemia condition in diabetes mellitus is associated with long-term damage and failure of various organ systems mainly affecting the eyes, nerves, kidneys, and the heart (Chawla, Chawla, & Jaggi, 2016). Diabetic complications are divided into microvascular complications (retinopathy, nephropathy, neuropathy) and macrovascular complications (cardiovascular disease and stroke). Complications of T2DM are very common, with half of patients with T2DM presenting with microvascular complications and 27% with macrovascular complications (Zheng, Ley, & Hu, 2017).

Previous study found that insulin resistance is a prominent feature of patients with type 1 diabetes mellitus and involves hepatic, peripheral and adipose tissues insulin resistance (Donga, Dekkers, Corssmit, & Romijn, 2015). Impaired insulin action has been described in both poorly and adequately controlled patients with type 1 diabetes mellitus (Donga et al., 2015; Kacerovsky et al., 2011). In patients with type 1 diabetes mellitus, insulin resistance is an important risk factor for development of micro-and macrovascular complications (Chaturvedi et al., 2001; Orchard, Olson, Erbey, & Williams, 2003).

Type 1 diabetes mellitus is characterized by both insulin deficiency and insulin resistance. Insulin resistance is defined as a condition in which normal insulin levels and functions in the blood cannot work to influence the phenotype of the target cells (adipocyte, hepatocyte, skeletal muscle cells), hence causing hyperglycemia (Al-Goblan et al., 2014). One of the molecular mechanisms of insulin resistance in target cells is a disruption in the Insulin-Insulin Receptor signaling pathway, especially on the IR-IRS-PI3K-Akt-GLUT4 axis which causes a decrease in the translocation of GLUT 4 protein from endosomal vesicles in the cytoplasm to the cell membrane and decreased glucose diffusion into the cytoplasm of target cells (Taniguchi, Emanuelli, & Kahn, 2006).

It is well known that metabolic inflammation (Meta-inflammation) induced insulin resistance and one of the molecular pathway that linking the obesity and insulin resistance in type 2 diabetes (Tanti, Ceppo, Jager, & Berthou, 2013). Previous study demonstrated that non-esterified fatty acid (NEFA) stimulated metabolis inflammation. NEFA can be produced in adipocyte through lipolysis or the breakdown of Triacylglycerol (TAG) in Lipid Dropets. Basal lipolysis in adipocyte is elevated during obesity resulting in incerased NEFA level and is tightly associated with insulin resistance (Karpe, Dickmann, & Frayn, 2011; Morigny, Houssier, Mouisel, & Langin, 2016). In addition, the uptake of NEFA in tissue also comes from the hydrolysis of the TAG component from VLDL (Very-Low Density Lipoprotein) and chylomicron in the blood circulation which is catalyzed by the LPL (Lipoprotein Lipase) enzyme (Ingerslev et al., 2017; Mead, Irvine, & Ramji, 2002). In type 1 diabetes, the decreased of insulin mediated inhibition of lipolysis contributed in increased lipolysis and NEFA level (Donga et al., 2015).

Evidence has been provided that increase of plasma concentrations of NEFA decreases skeletal muscle glucose uptake and glycogen synthesis (Roden et al., 1996). Excessive NEFA level can activate TLR-4 in macrophage cells and its downstream causes NFƙB activation. NF-kB has often been termed a central mediator of the immune response because active NF-kB act as transcription factor that regulates the expression of infammatory cytokines, chemokines, immunoreceptors, and cell adhesion molecules including TNF-α (Pahl, 1999). The active NFƙB is found attached to the DNA sequence NFkB binding sites, which are the promoter or enhancer sequences of the target genes that will activate the transcription of the genes (Liu et al., 2002; Pahl, 1999). In Type 2 Diabetes, there is upregulation of TNF-α expression in skeletal muscle tissue (Steinberg et al., 2006). TNF-α stimulate insulin resistance phenotype and its effect mediated by activation of JNK and IKKß. JNK-1 and IKKß can phosphorylate IRS-1 on serine 307 residu, leading to its inactivation and subsequent disruption of insulin signaling (Nguyen et al., 2005).

While the association between metabolic inflammation and insulin resistance is well understood in type 2 diabetes, but it is not clear whether inflammation also contribute in the molecular mechanism of insulin resistance in type 1 diabetes. We aimed to evaluate the effect

of 1 month and 2 months diabetes mellitus duration on the mRNA expression of pro-inflammatory cytokine TNF-α in skeletal muscle tissue of type 1 diabetes mellitus rat model. This study can provide new insight about chronic inflammation in skeletal muscle tissue during type 1 diabetes mellitus condition.

METHOD

Animal experiment and diabetes mellitus model

Male Wistar rats (3 months-old) weighing 160-270 grams were obtained from Experimental Animal Care Unit of Universitas Gadjah Mada, Yogyakarta, Indonesia. Rats were acclimatized for 7 days and maintained in the standardize cage with temperature 18-22°C, humidity 50-70%, and dark: light cycle 12 hours. Rats were fed with AIN-93A and water ad libitum. The animal studies were approved by the Ethical Committee of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada with the approval number KE/FK/1211/EC/2019.

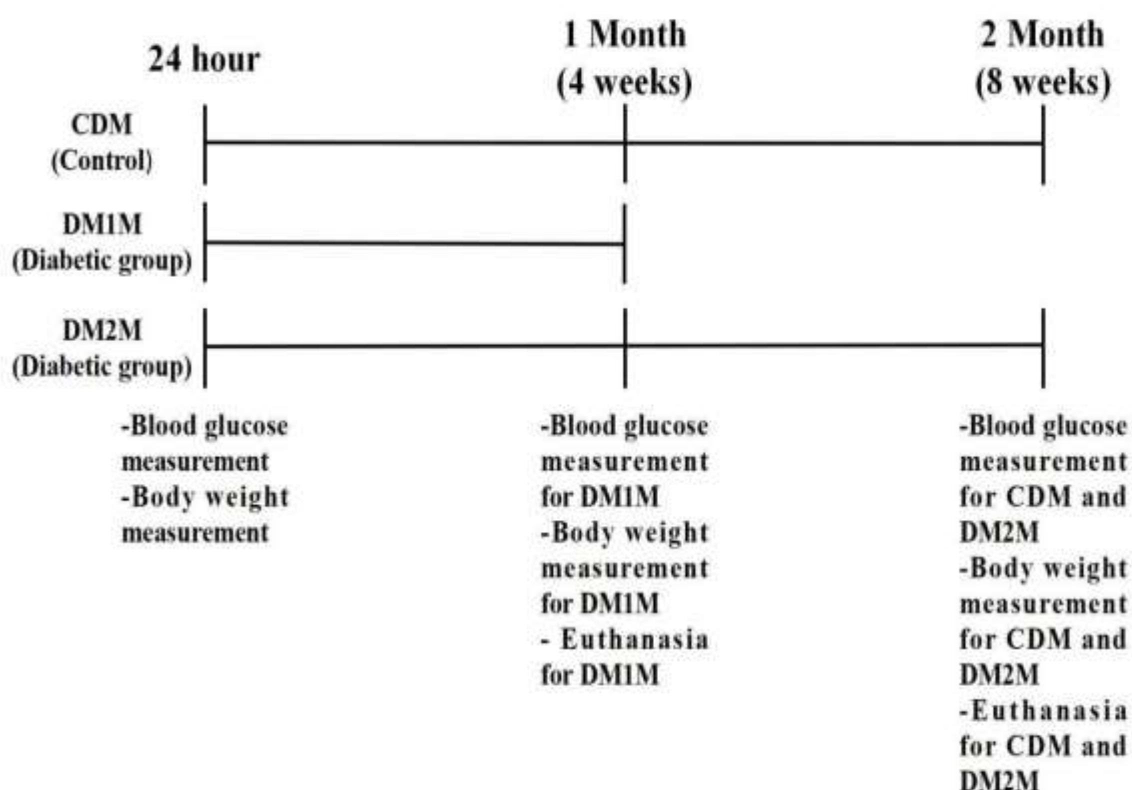

Figure 1. Study design

Eighteen rats were divided into 3 groups consisted of the control group (CDM, n=6), diabetes mellitus for a month period (DM1M, n=6), and diabetes mellitus for two months period (DM2M, n=6). Diabetes mellitus was performed with single dose injection of streptozotocin (Nacalai, Cat. No. 32238-91) 60 mg/kgBW dissolved in 0.1 M citrate buffer pH 4.5 administered by intraperitoneal injection. Rats were allowed to drink 10% of glucose solution to counteract hypoglycemia. At the following day, blood glucose level was measured to assure the diabetes mellitus (blood glucose level>300 mg/dL).

Body Weight Measurement and Blood Glucose Level Measurement

Rats body weight were measured 3 times : before streptozotocin injection to calculate streptozotocin dose required, 1 month diabetes mellitus period, and 2 month diabetes mellitus

period. Random blood glucose level was measured 3 times : one day after streptozotocin injection, 1 month diabetes mellitus period, and 2 months diabetes period. Random blood glucose level measurement was done using EasyTouch® GCU Multi-Function Monitoring System by taking ±100 µl of blood through the tail vein.

Euthanasia and Skeletal Muscle Tissue Collection

All rats (Σn = 18) were euthanized with intraperitoneal injection of combination cocktail ketamine 50 mg/kgBW and xylazine 2 mg/kgBW. Skeletal muscle tissue (musculus gastrocnemius) were collected and preserved using RNA later solution in temperature -20°C before RNA isolation.

Total RNA Isolation from Skeletal Muscle Tissue and Reverse Transcription

Total RNA isolation from skeletal muscle tissue was performed using Genezol RNA Solution (GENEzol™, Cat. No. GZR100) according to the manufacture instruction. After isolation, the concentration and purity of RNA were assessed using a nanodrop spectrophotometer. Then total RNA solution was stored in a refrigerator −80°C for reverse transcription reaction. Total RNA isolated from skeletal muscle tissue was reverse transcribed to obtain cDNA using ExcelRT™ Reverse Transcription Kit II (Smobio, Cat. No. RP1400) according to the manufacture instruction. The cDNA was stored in a refrigerator at −20°C.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was performed to quantify the relative expression of mRNA TNF-α in skeletal muscle tissue. PCR was performed by mixing 3 µl cDNA, 12,5 µl GoTaq® Green Master Mix (Promega, USA, Cat. No. M7122), 0.8 µl primer Forward, 0.8 µl primer Reverse, and 7.9 µl nuclease free water for a total volume 25 µl. PCR was performed with the following conditions : 94°C early denaturation for 2 min, 94°C denaturation for 10 s, the annealing temperature for each target mRNA was shown at table 1 for 30 sec, and extension 72°C for 1 min, final extension phase end with the conditions of 72°C for 10 minutes. To assess the specificity of PCR product, 2% agarose gel electrophoresis was performed along with a 100bp DNA ladder (Bioron, Germany, Cat. No. 306009). Densitometry analysis using the ImageJ software was used to quantify the expression of mRNA TNF-α. mRNA β-actin was used as housekeeping gene to normalize the relative expression.

Table 1.
Primer sequence used in this study

Gene	Primer sequence	Product length (bp)	Annealing temperature (^{o}C)
TNF-α	F : 5'-GTAGCCCACGTCGTAGCAAA-3'	192	52
	R : 5'-GGTGAGGAGCACGTAGTCG-3'		
β-Actin	F : 5'-GCAGATGTGGATCAGCAAGC-3'	100	54
	R : 5'-GGTGTAAAACGCAGCTCAGTAA-3'		

Statistical Analysis

Statistical analyses conducted using SPSS 24 (IBM Corp., Chicago). All variables were reported as mean ± standard deviation (SD). Shapiro-wilk test was used to asses normality of the data, and p>0.05 considered as normal distribution. The differences in blood glucose

level, body weight, and expression level of mRNA TNF-α between groups was assessed using One-Way ANOVA test and p<0.05 was considered as significant.

RESULTS AND DISCUSSION

Blood Glucose Level Significantly Increased in Diabetes Mellitus Group

The blood glucose levels of the CDM group was 83.25±7.23 mg/dL at 24 hour, 85.50±7.59 mg/dL at 1 month, and 82.25±5.32 mg/dL at 2 months. This result suggesting that blood glucose level of CDM group was in normal value throughout the study. In other hand, The DM1M and DM2M groups showed hyperglycemia conditions 24 hours after streptozotocin injection as indicated by a blood glucose level of ≥ 300 mg/dL. The blood glucose level of DM1M group and DM2M group was significantly elevated compared to CDM group (*p*<0.05). The blood glucose level of DM1M group was 444±28.59 mg/dL at 24 hours after STZ injection, and increased to 507.25±32.17 mg/dL after 1month DM duration. The Blood glucose level of DM2M was 467.75±11.05 mg/dL at 24 hours after STZ injection, and increased to 550.25±27.93 mg/dL after 1month DM duration, and further increased to 566.75 after 2 months DM duration.

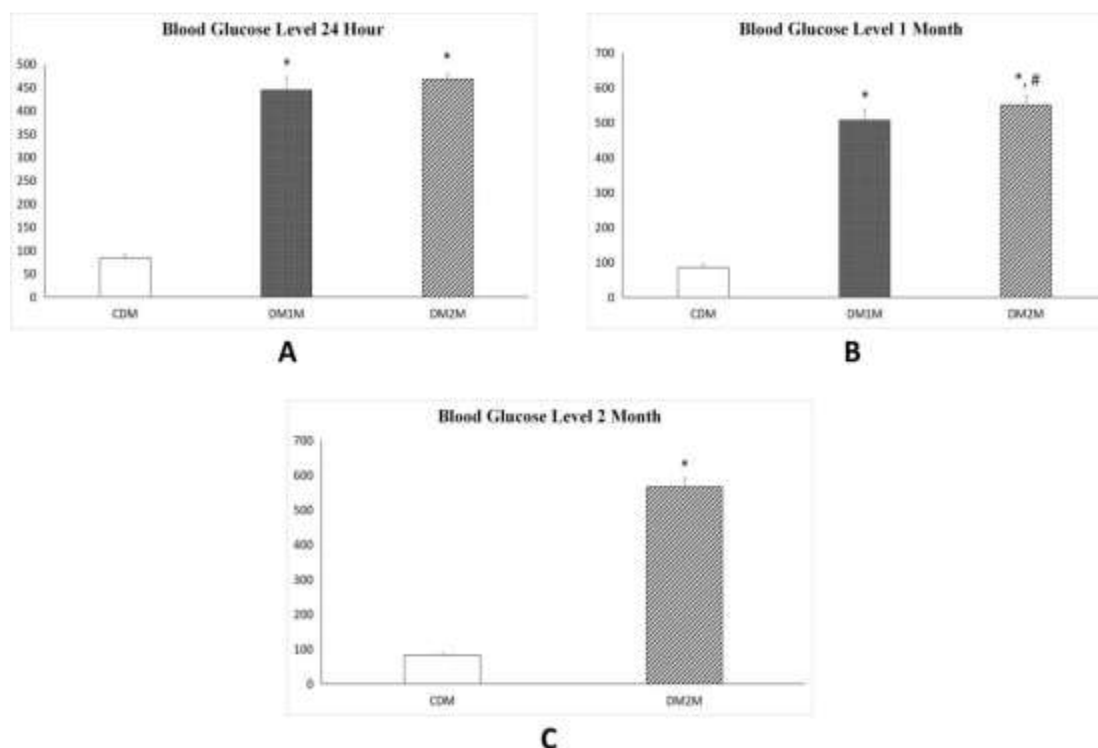

Figure 1. Blood glucose level in Diabetes Mellitus. A. Comparison of blood glucose level between groups at 24 hours diabetes mellitus duration. B. Comparison of blood glucose level between groups at 1month diabetes mellitus duration. C. Comparison of blood glucose level between groups at 2 months diabetes mellitus duration. *p<0.05 vs CDM, #p<0.05 vs DM1M

Diabetes Mellitus Stimulated Body Weight Loss

During the study, the rats' body weight in the CDM group increased from 218±11.52 grams to 257.3±14.3 grams at the end of the study. On the other hand, diabetes mellitus group demonstrated significant body weight loss throughout the study. The rat's body weight in the DMIM group decreased from 225.25±13.72 grams to 181.75±13.72 grams after 1 month diabetes mellitus duration. While body weight of DM1M and CDM group did not differ significantly at 24 hours after STZ injection, the body weight of DM1M group after 1 month

diabetes mellitus duration significantly lower compared to CDM group ($p<0.05$). The rat's body weight in the DM2M group decreased from 231.25±21.65 grams to 143±9.38 grams after 2 months diabetes mellitus duration. After 2 months diabetes mellitus period, the body weight of DM2M group significantly lower compared to CDM group ($p<0.05$).

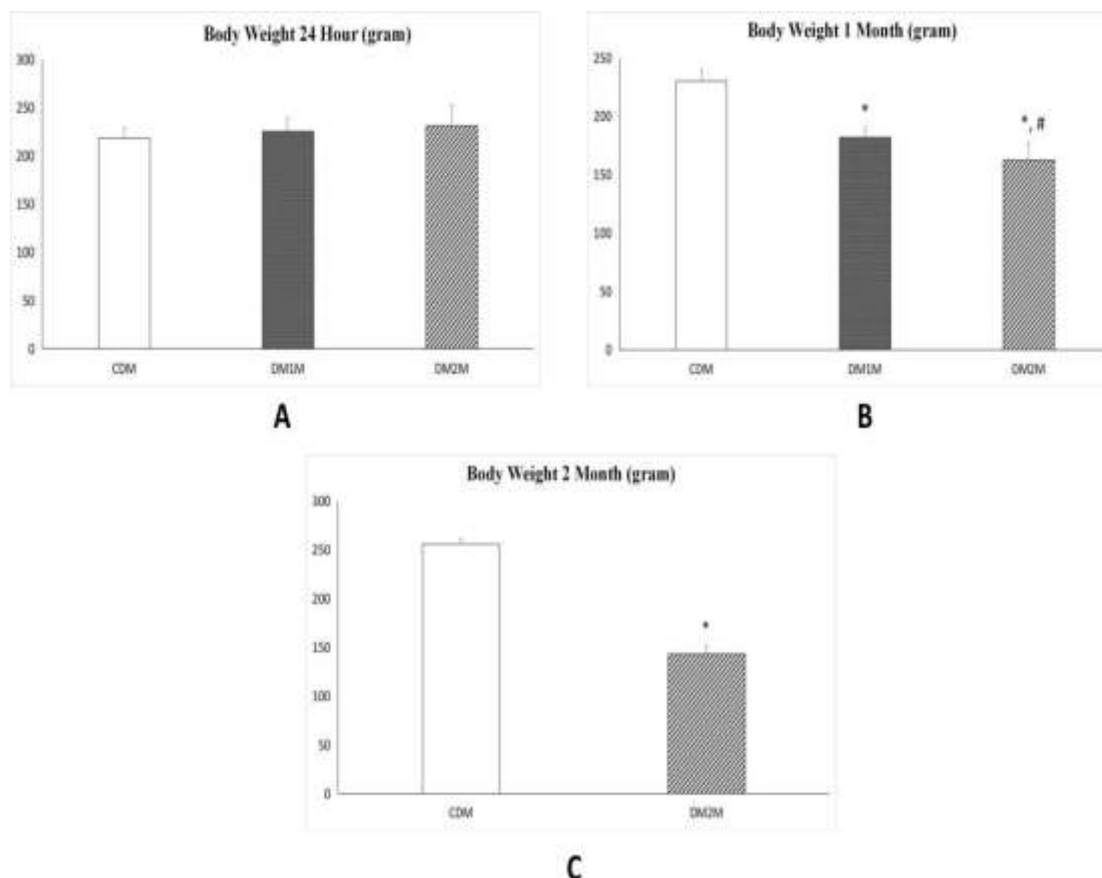

Figure 2. Body weight in Diabetes Mellitus. A. Comparison of body weight between groups at 24 hours diabetes mellitus duration. B. Comparison of body weight between groups at 1 month diabetes mellitus duration. C. Comparison of body weight between groups at 2 months diabetes mellitus duration. *$p<0.05$ vs CDM, #$p<0.05$ vs DM1M

Upregulation of mRNA TNF-α in Skeletal Muscle Tissue During Diabetes Mellitus Condition

Finally, we quantify the expression of mRNA TNF-α in skeletal muscle tissue. Polymerase Chain Reaction (PCR) was performed, then followed with electrophoresis and densitometry analysis to quantify the relative expression of mRNA TNF-α. mRNA β-actin was used as housekeeping gene to normalize the relative expression. The relative expression of mRNA TNF-α in skeletal muscle tissue showed at figure 3. Density of mRNAβ-actin did not differ significantly between group suggesting that the expression of mRNA β-actin in skeletal muscle tissue is stable between group. Our study demonstrated that 1 month diabetes mellitus period (DM1M) and 2 month diabetes mellitus period (DM2M) significantly upregulated the expression of mRNA TNF-α compared to CDM group ($p<0.05$). This result suggesting that type 1 diabetes mellitus for 1 month and 2 months period stimulated inflammation phenotype in skeletal muscle tissue.

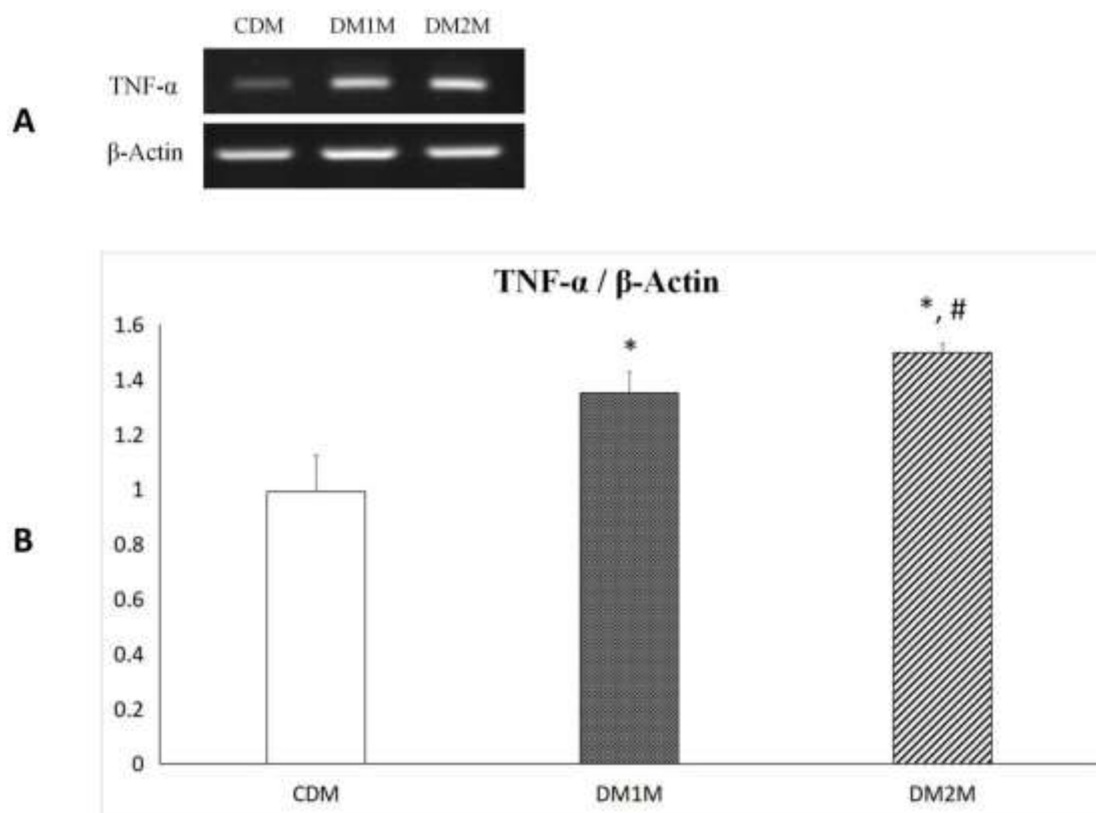

Figure 3. Diabetes Mellitus stimulated upregulation of mRNA TNF-α in skeletal muscle tissue. A. The representative figures of mRNA TNF-α according to the RT-PCR. B. The bar charts of mRNA TNF-α expression. *$p<0.05$ vs CDM, #$p<0.05$ vs DM1M

Diabetes mellitus and the complications are one of the major health problems and contribute to an increase of morbidity and mortality rates (Saeedi et al., 2019). Diabetes mellitus (DM) described as a glucose metabolism disorder characterized by chronic hyperglycemia that develops as a consequence of defects in insulin secretion, insulin action, or both. The three main types of diabetes are type 1 diabetes (T1D), type 2 diabetes (T2D), and gestational diabetes mellitus (GDM) (Saeedi et al., 2019). absolute insulin deficiency. Type 1 *Diabetes mellitus* results from the cell-mediated autoimmune destruction of the β-cells of the pancreas leading to absolute insulin deficiency (Damasceno et al., 2014; Saeedi et al., 2019). Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65), and auto antibodies to the tyrosine phosphatases IA-2 and IA-2b (Association, 2014) .

This study found that administration of STZ (60 mg/kg) to diabetic control group stimulated significantly increase in blood glucose level as compared to normal control (>300 mg/dL), suggesting that STZ injection induced diabetes mellitus phenotype. Hyperglycemia phenotype in diabetes mellitus group persisted at 1 month and 2 month after STZ administration. Previous research also demonstrated that single intraperitoneal injection of streptozotocin 60 mg/kgBW induced diabetes mellitus phenotype in rat, indicated by increased of blood glucose level (Fitriawan, Widayati, Setyaningsih, Arfian, & Sari, 2019; Mali, Dias, Havaldar, & Yadav, 2017). Depending on the animal strain, dose, route of drug administration, and the life period in which STZ is administered in rats, severe diabetes (blood glucose superior to 200/300 mg/dL) or mild diabetes (glycemia between 120 and 200/300 mg/dL) are generated. For severe diabetes induction, STZ is administered at 40–50 mg/kg body weight intravenously

or intraperitoneally during adulthood. After approximately three days, these animals present blood glucose levels greater than 300 mg/dL (Damasceno et al., 2014). In this study, diabetic groups demonstrated blood glucose level >300 mg/dL throughout the study, suggesting severe diabetes mellitus.

Experimental diabetes mellitus has been induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. However, to induce a notable form of diabetes, at least 90-95% of the pancreas has to be removed. Otherwise, the Langerhans islets in the remaining pancreas may undergo hypertrophy and secrete a sufficient amount of insulin for fulfilling the natural metabolic needs. The second method for creating diabetes in animals is injecting drugs such as alloxan or Streptozotocin (Akbarzadeh et al., 2007).

Experimentally induced diabetes through the administration of β-cytotoxic drugs such as streptozotocin (STZ) is well characterized. Streptozotocin is an antibiotic and has also been used as a chemotherapeutic alkylating agent (Lenzen, 2008). It contains a glucose molecule (in deoxy form) that is linked to a highly reactive methylnitrosourea moiety that is thought to exert STZ's cytotoxic effects, while the glucose moiety directs the chemical to the pancreatic β cells. STZ recognizes the GLUT2 receptor that is abundant on β cell plasma membranes. Therefore, pancreatic β cell is a specific target of STZ (Wu & Yan, 2015). Streptozotocin diabetes is caused by the specific necrosis of the pancreatic β-cells (Portha, Levacher, Picon, & Rosselin, 1974). Single injection of streptozotocin induced type 1 diabetes in rodent (Wu & Yan, 2015). Streptozotocin prevents DNA synthesis in mammalian and bacterial cells. In bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The biochemical mechanism results in mammalian cell death. Streptozotocin prevents cellular reproduction with a much smaller dose than the dose needed for inhibiting the substrate connection to the DNA or inhibiting many of the enzymes involved in DNA synthesis. Although Streptozotocin prevents entry of cells into mitosis but no special phase of the cellular cycle is especially sensitive to its mortal effects.

These materials inflate and ultimately degenerate the Langerhans islets beta cells (Ikebukuro et al., 2002). A less reliable method for creating diabetes is injection of the anterior hypophysis extract (Rastellini et al., 1997). Streptozotocin injection stimulated pancreas swelling and degeneration of Langerhans islet β cells and induces experimental diabetes mellitus (Akbarzadeh et al., 2007). The final symptoms of insulin deficiency are clearly seen in rats afflicted with diabetes chemically by Streptozotocin (Elias, Prigozin, Polak, Rapoport, & Lohse, 1994). Using 60mg/kg Streptozotocin dose can begin an autoimmune process that results in the destruction of the Langerhans islets beta cells and the 60mg/kg Streptozotocine dose results in the toxicity of beta cells with emergence of clinical diabetes within 2-4 days (Weiss, 1982). Study by Akbarzadeh et.al demonstrated that streptozotocin injection stimulate reduction of plasma insulin and C-peptide levels and significant increase of blood glucose level at adult male wistar rats (Akbarzadeh et al., 2007).

This study showed that diabetes mellitus stimulated significant body weight loss. The clinical manifestation of insulin deficient and hyperglycemia are polyphagia and loss of body weight (Kahanovitz, Sluss, & Russell, 2017). Some research proved that diabetes mellitus enhanced loss of body weight (Fitriawan et al., 2019; Gayathri, Lekshmi, & Padmanabhan, 2011; Kabir et al., 2014; Mali et al., 2017; Naidu, 2019; Pournaghi, Sadrkhanlou, Hasanzadeh, & Foroughi, 2012; Sasikala, Lakshminarasaiah, & Naidu, 2015). Abnormal loss of body weight

caused by diabetes mellitus stimulated by an increase of lipid metabolic conversion into energy (Hackett & Jacques, 2009). In this study, Hyperglycemia accompanied by body weight loss were seen in adult wistar rats within 1 month and 2 months after Streptozotocin treatment, which indicates irreversible destruction of Langerhans islets cells. Researchers around the world have used streptozotocin to create experimental diabetes because it is a simple, inexpensive and available method
In this study, we found that type 1 diabetes mellitus for 1 month period and 2 month period significantly upregulated mRNA TNF-α expression in skeletal muscle tissue. TNFα is a powerful pro-inflammatory mediator that modulate multiple signalling pathways and play important role in inflammation response (Parameswaran & Patial, 2010). TNF is produced primarily by monocytes/macrophages, but a number of other cell types, such as T and B lymphocytes, mast cells, natural killer cells, and neutrophils also produced it (Finkel & Holbrook, 2000). TNF-α binds and activate two type receptors, TNFR1 and TNFR2 with high affinity. Activation from TNFR1 is responsible for a large number of inflammatory responses classically attributed to TNFα (Parameswaran & Patial, 2010). The upregulation of mRNA TNF-α in this study suggesting that type 1 diabetes mellitus induced chronic inflammation condition in skeletal muscle tissue.

In type 1 diabetes, the decreased of insulin mediated inhibition of lipolysis contributed in increased lipolysis and NEFA level (Donga et al., 2015). Evidence has been provided that increase of plasma concentrations of NEFA decreases skeletal muscle glucose uptake and glycogen synthesis (Roden et al., 1996). Elevation of NEFA level can activate TLR-2 (Zhanga et al., 2018) and TLR-4 (Fresno, Alvarez, & Cuesta, 2011) in macrophage and neutrophils and its downstream causes NFƘB activation. TLR-2 and TLR-4 are Toll-like receptors (TLRs) family that can recognize pathogen-associated molecular patterns and are located on the surface of innate immune cells, such as PMNs and macrophages. TLR2 and TLR4 are major pattern recognition receptors that recognize pathogenic microorganisms (Lee & Hwang, 2006). Recently, growing evidence has shown that NEFAs are ligands for TLR4 (Shi et al., 2006). and subsequently induce intracellular signaling, initiating nuclear factor kappa B (NF-κB) signaling pathway. Normally, the transcription factor NF-κB is sequestered in the cytoplasm by binding to its inhibitor IκBα, whose phosphorylation is dependent on IKKβ activity. In response to stimuli, the NF-κB subunit p65 separates from IκBα and translocates into the nucleus where NF-κB can regulate the transcription of several inflammatory cytokine genes (Zhanga et al., 2018).

NF-kB has often been termed a `central mediator of the immune response because active NF-kB act as transcription factor that regulates the expression of infammatory cytokines, chemokines, immunoreceptors, and cell adhesion molecules including TNF-α and IL-6 (Pahl, 1999). Active NFƘB was found to attach to DNA sequences of NFkB binding sites, which are promoter or enhancer sequences of target genes that will activate the transcription of these genes (Liu et al., 2002; Pahl, 1999). Previous study found that high concentrations of NEFA significantly elevated TLR2 and TLR4 expression, IkBα and NF-kB p65 phosphorylation expression, NF-κB p65 transcriptional activity, and IL-6, IL-1β, and TNF-α expression in neutrophils (Zhanga et al., 2018)

Several study confirm that NEFA elevation induced Endoplasmic Reticulum (ER) stress (Chen et al., 2017; Coll, Barroso, & Palomer, 2013). ER stress arises when misfolded proteins accumulate inside the ER lumen, triggering the unfolded protein response (UPR). ER stress markers, activating transcription factor 4 (Atf4) and heat shock protein 5 (Hspa5), but not

Atf6, were significantly up-regulated by NEFA treatment. Several signaling pathways activated by the endoplasmic reticulum (ER) stress response induce sterile inflammation. When activated, all three sensors of the unfolded protein response (UPR), PERK, IRE1, and ATF6, participate in upregulating inflammatory processes (Garg et al., 2012). Study by Hu et.al found that ER stress-induced expression of TNF-α in IRE1α and NF-κB dependent manner (Hu et al., 2006).

Several studies have found that hyperglycemia in DM itself can also induce inflammation (Corrêa-Silva et al., 2018; Lin et al., 2005). Hyperglycemia causes upregulation of proinflammatory cytokine protein expression such as TNF-α (Morey, O'Gaora, Pandit, & Hélary, 2019), IL-1β, IL-6, and MCP-1, which depends on the NFƙB and Inflammasome pathways (Corrêa-Silva et al., 2018; Lin et al., 2005). In addition, hyperglycemia has also been found to cause upregulation of TLR-4 expression, and this contributes to the emergence of an inflammatory phenotype in hyperglycemic conditions (Rajamani & Jialal, 2014). Previous study demonstrated that hyperglycemia induced upregulation of TNF-α, upregulation of 11 anti-apoptosis protein, and downregulation of 3 pro-apoptosis protein in macrophage, suggesting that hyperglycemia stimulated chronic inflammation state (Morey et al., 2019).

CONCLUSION

Streptozotocin-induced diabetes mellitus model demonstrated upregulation of TNF-α mRNA expression in skeletal muscle tissue, suggesting that type 1 diabetes induced inflammation in skeletal muscle tissue.

ACKNOWLEDGEMENTS

The authors would like to thank LPPT Universitas Gadjah Mada for providing wistar rats for this research. Also, we would like to thank all staff of Department of Anatomy, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada who provided support and guidance in doing this research.

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