The differential expression of GLUT4 and glycogen levels on cells of liver and muscle tissues in hyperglycemic and normoglycemic conditions

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ABSTRACT

Background: Under normal physiological conditions, the glucose uptake into the cell is regulated by glucose transporter (GLUT4) which is stimulated by insulin to be afterward metabolized into energy and/or stored as glycogen. The research aimed to determine the expression of glucose transporter (GLUT4), glycogen levels on cells liver and muscle tissues in hyperglycemic and normoglycemic conditions on rats.

Method: A total of 20 rats of Sprague Dawley strain were grouped into two treatment groups. The normal group and the hyperglycemia group. The observations were performed on blood glucose level by glucose oxidase biosensor method by using the Blood Glucose Test Meter of GlucoDr™, liver and muscle glycogen levels by spectrophotometry and GLUT4 analysis on cells of liver and muscles tissues by using the immunohistochemical technique.

Results: The results showed that 80% of sucrose administration had caused hyperglycemic rats with the blood glucose levels of 131.2 g/dl. In hyperglycemic conditions, the levels of liver glycogen (9.4 µg/mg) and muscle (8.68 µg/mg) were significantly higher (P < 0.05) than in the liver (8.54 µg/mg) and muscle (7.87 µg/mg) in normoglycemic. Meanwhile, the liver glycogen levels were significantly higher (P < 0.05) when compared with the muscle glycogen levels in both hyperglycemic and normoglycemic conditions. The expression of GLUT4 in hyperglycemia was significantly higher (P < 0.05) in both liver (64.05%) and muscle (56.15%) when compared with the liver (53.21%) and the muscle (48.67%) in normoglycemic. Between the liver and muscle tissues, the expression of GLUT4 was significantly higher (P < 0.05) in the liver when compared with muscle tissue in both hyperglycemic and normoglycemic conditions.

Conclusion: Expression of GLUT4 and levels of glycogen in the hyperglycemic liver is higher compared to the muscles.

Keywords: glucose, glycogen, hyperglycemia, GLUT4


INTRODUCTION

Carbohydrates play a fundamental role in life, not only as the primary source of energy for living things but also as compounds that store chemical energy. The glucose uptake into the cell is regulated by a transporter and distributed to various tissues in the body. The distribution process involves a family of transport proteins known as GLUT. Glucose transporter (GLUT) works as a carrier to move glucose through the cell membrane.1 Glucose that enters the cell undergoes a series of metabolic processes converted into energy or stored as energy reserves in the form of glycogen.2

Glucose is a very important universal biomolecule as a biological fuel for mammalian cells. Correspondingly, every cell in the body expresses at least one type of glucose transporter.3 According to Thorens and Mueckler,4 there is 14 GLUT proteins work as carriers. However, the ones that have been known and well known as the glucose transporters are the forms of GLUT 1 to GLUT 4. Glucose transporters of 1 to 4 have been known for different regulatory and kinetic properties in glucose homeostasis in the body.

Based on biochemical properties, GLUT 1-4 is distributed in various tissues.5 GLUT 1 to GLUT 4 are the glucose transporters for glucose. GLUT 1 is spread in the brain and erythrocytes, GLUT 2 is located in the liver cell membranes, pancreas, intestine and kidneys, GLUT 3 is located in the brain, and GLUT 4 is a common transporter of glucose in the skeletal muscle tissue, brain, heart, and adipose tissue.

GLUT 4 is a specific protein that facilitates glucose transport. GLUT 4 is a glucose transporter that is responsive to insulin in muscle and adipose tissue in both humans and rodents. GLUT4 translocates to the plasma membrane in response to insulin. GLUT4 is known as a major glucose transporter and can regulate insulin-stimulated glucose secreted by beta cells as a glucose sensor.6
GLUT 4 is located at the Golgi apparatus and serves for uptake of glucose-stimulated insulin or insulin sensitive. Meanwhile, GLUT1 and GLUT3 in normal physiological conditions have been associated with the plasma membrane so that the mechanism of action of glucose uptake is not dependent on insulin.

According to Watson and Pessin, there are five forms of the glucose transporter, namely GLUT1 to GLUT5. GLUT1 to GLUT4 serves as the glucose transporters whereas GLUT5 is a fructose transporter. GLUT1 to GLUT5 are spread across multiple tissues. In muscle cells and adipose tissues, insulin stimulates the delivery of GLUT4 glucose transporter from the intracellular site to the cell surface, where GLUT4 facilitates plasma glucose uptake into cells. The primary function of insulin is to stimulate the transport of glucose into cells, especially muscle and adipose cells which are then used as energy sources or stored in glycogen.

This study aims to determine the levels of glucose, glycogen levels of liver and muscle as well as profiles of glucose transporter (GLUT4) in various organs (brain, heart, muscle, liver, kidney, and pancreas) of the hyperglycemic rats.

**METHOD**

**Preparation of experimental animals**

A total of 20 rats of Spraque Dawley strain with body weight between 200-225 g were used in this study. The rats were divided into two treatment groups with each group consisted of 10 rats, i.e. the group of normal rats and the group of hyperglycemic rats. Before treatments, the experimental rats were adapted to laboratory conditions for seven days with commercial ration and drinking water ad libitum. Treatment of hyperglycemia was by the administration of 80% sucrose solution (b/v) 2 cc orally twice daily for three weeks. Blood glucose levels were measured at the beginning and at the end of treatment. At the end of the study, all rats were sacrificed by anesthesia with ketamine-HCl. Rats were dissected, then the gastrocnemius muscles and liver tissue were taken for analysis of GLUT4 expression in the liver and muscle.

**Analysis of blood glucose levels**

Measurement of blood glucose levels on day 0 (initial data base) and at the end of treatment was carried out. Blood glucose levels were determined by the glucose oxidase biosensor method, using the Blood glucose Test Meter GlucoDr™ of AGM-2100 Model (manufactured by Allmedicus Co Ltd., Korea). Blood was taken from the tip of the tails of the rats that had previously been cleaned with 70% alcohol, then rubbed slowly before the tail tip was punctured with a small needle. The blood that came out was then placed on a glucometer strip. Blood glucose levels will be read on GlucoDr™ screen after 11 seconds, and blood glucose levels are expressed in mg/dl.

**Analysis of glycogen levels of liver and muscle**

Glycogen analysis was conducted by using the method according to Peungvicha et al. Each 5 g of liver and muscle (M. gastrocnemius) were taken and then dried in an oven at 50°C for one night, then crushed into flour. Each sample was taken 25 mg and extracted with one mL of 30% KOH solution and incubated in a boiling water bath for 20 minutes, then cooled. It was added 1.5 ml of cold ethanol 95% to the sample tubes and stored at 4°C for 30 minutes. To separate the glycogen deposits, the sample was centrifuged at 2500 rpm for 20 minutes. The sample deposition was diluted with 1 ml of aquadest. Into the filled tubes of each 100 μl muscle sample and 100 μl liver sample was added 3 ml of 0.2% (w/v) anthrone-sulfuric acid. The green color that appears indicates that the sample of solution contains glycogen. The absorbance value was measured at a wavelength of 620 nm. The glycogen levels were calculated by comparing with standard glycogen levels.

**Immunohistochemical analysis on glucose transporter 4 (GLUT4)**

Glucose transporter GLUT4 was immunohistochemically analyzed by using methods according to Beesley. The tissue was fixed for 24 hours in 10% formalin solution and then processed by a standard method using paraffin. The tissue was incubated with H2O2 in methanol for 15 minutes. Further, it was dropped with a 10% bovine serum albumin (BSA) for 45 minutes at 37°C. After washing; the tissue was spilled with antibody primary monoclonal anti-GLUT 4 and left at room temperature for 1 hour. Furthermore, the tissue was dropped with biotinylated IgG for 30 minutes at room temperature. The next stage was spilled with avidin biotin HRP for 30 minutes. The antigen-antibody reaction results were visualized by using diaminobenzidine (DAB) at room temperature for 5 minutes, then counterstained with HE. In each new treatment, the tissue was washed with PBS. The observations were performed under a light microscope of 20 time-magnifications. The results were positively showed GLUT4 if the tissue indicated brown color.

**Calculating GLUT4 expression**

GLUT4 expression in the liver and muscle is characterized by a brown color indicating that there is GLUT4 in the cell’s membrane and its intensity...
depends on the concentration of GLUT4. How to calculate GLUT4 expression on cells using H-score IHC. The IHC score was calculated using a modification of cancer cell count according to Cohen. The intensity of the color expression is calculated in five different fields of view with 400X magnification. Color intensity with score: 1+: weak; 2+: medium; and 3+: strong. The number of cell expression in percentage (%) is calculated by the formula: \((1 \times \%\text{cell } 1+) + (2 \times \%\text{cell } 2+) + (3 \times \%\text{cell } 3+)/3\).

**Experiment design and data analysis**

This study used unpaired design (independent) with the treatment I was a normal rat and treatment II of the hyperglycemic rat. The data of glycogen levels and GLUT4 expression obtained were analyzed by using unpaired t-test.

**RESULTS**

**Analysis of blood glucose levels**

The findings of blood glucose level analysis in rats for three weeks of treatment are shown in Figure 1.

The average blood glucose level in the normal group on day 0 was 93.6 mg/dl and on the 21st day was 95.1 mg/dl. In the treatment of hyperglycemia, the average initial glucose level (day 0) was 91.5 mg/dl and on the 21st day after the treatment, the average of blood glucose level was 131.2 mg/dl. The initial average of blood glucose level (day 0) in both the normal group and the hyperglycemic group was not significantly different (P> 0.05), in other words, it was still within the normal range.

**Analysis of glycogen levels**

The findings of glycogen level analysis on the liver and the muscle of rats during the 21 days of treatment are presented in Table 1.

The average levels of liver glycogen of hyperglycemia group reached 9.40 µg/mg, was significantly higher (P<0.05) compared with the average of the normal group glycogen levels of 8.54 µg/mg. The glycogen levels in muscle tissues in hyperglycemia group amounted to 8.68 µg/mg was significantly higher (P<0.05) compared with the average of the control group glycogen levels of 7.87 µg/mg. The levels of liver glycogen in both normoglycemic and hyperglycemic states were significantly higher (P<0.05) compared with muscle (Table 1, Figure 2 and Figure 3).

The glycogen levels between the liver and the muscle tissues also show different levels. Levels of liver glycogen in the normal group were significantly higher (P <0.05) when compared with glycogen levels of muscle tissues. The same thing happened in the treatment group of hyperglycemia. Levels of liver glycogen in the treatment of hyperglycemia were significantly higher (P <0.05) when compared with glycogen levels of muscle tissues.

**The results of immunohistochemical staining of GLUT4 in liver and muscle**

The glucose transporter is a family of proteins embedded in cell membranes that serve to mediate the absorption of glucose from the surrounding environment into cells. The results of immunohistochemical staining and percentage of GLUT4 expression in liver and muscle in hyperglycemic and normoglycemic conditions are presented in Table 2 and Figure 4.

Immunohistochemical staining results show that the intensity of GLUT4 expression is stronger in hyperglycemic liver and muscles compared to the normoglycemic. Qualitatively, the expression of GLUT4 in hyperglycemic conditions is indicated by the strong brown intensity in the liver and muscle cells (Figure 4).

Based on quantitative results, the average GLUT4 expression in hyperglycemic liver was significantly higher (P <0.05) than with normoglycemic liver. Similarly, the expression of GLUT4 in hyperglycemic muscles are also significantly higher than that of normoglycemic muscles. GLUT4 expression in the liver was significantly higher (P <0.05) compared to the muscles in both hyperglycemic and normoglycemic conditions (Table 2).

The results immunohistochemical staining of GLUT4 expression on cells of liver and muscles...
tissues in normoglycemic and hyperglycemic conditions are presented in Figure 4.

GLUT4 is expressed and spread to the liver, brain, muscle, heart, kidneys, and pancreas with varying intensity in each organ. Expressions with strong intensity are seen in the organs of the heart, brain, muscles, then on the liver and kidneys. Descriptively, GLUT4 expression in hyperglycemic conditions showed stronger intensity in all organs or tissues compared to the normal conditions (Figure 4).

In the liver, GLUT4 is expressed in the cell nucleus, cytoplasm, and plasma membrane. On the brain, it is seen in the Purkinje layer, the granular cell layer, the pyramidal cell layer, and the myelin fibers. In the heart muscle, it is spread on the cytoplasm and nucleus. On the muscle, it is seen in the cytoplasm and nucleus of the cell. In the kidneys, it is spread on glomerular cells, and on the distal tubules. In Pancreas, it is expressed in endocrine cells and on the islet of Langerhans.

**DISCUSSION**

Some researchers reported that the normal blood glucose levels of rats are 74.35 to 84.85 mg/dl, and between 99 to 127 mg/dl. Blood glucose levels of normal rats vary widely depending on the physiological conditions, sex, feed, and metabolic processes. The group of hyperglycemic treatment until day 21 showed significantly higher glucose levels (P <0.05) than on day 0, and this indicated that experimental rats had experienced hyperglycemia.

Glucose concentrations in the blood will stimulate the secretion of the insulin hormone to induce glucose transport into the cell. Liver and muscle cells play an important role as a buffering of post-prandial hyperglycemia by involving mechanisms of energy synthesis and converted into energy stores in the form of glycogen.

The ability to take up glucose at the cellular level are traits possessed by most organisms. Most mammalian cells enter glucose through a facilitated diffusion process that is mediated by GLUT members (glucose transporter) which are a group of membrane transport proteins. Physiologically, there are 14 GLUTs, but half of them are well known. Glucose transporter 4 (GLUT 4) is the primary insulin-responsive glucose transporter in skeletal muscle and adipose tissue in both humans and rodents.

**Table 1** Results of the analysis of glycogen levels in liver and muscle tissues in normoglycemic and hyperglycemic conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glycogen levels (µg/mg)</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>8.54 + 0.57aA</td>
<td>7.87 + 0.59aB</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia group</td>
<td>9.40 + 0.43bA</td>
<td>8.68 + 0.66bB</td>
<td></td>
</tr>
</tbody>
</table>

Description: The numbers followed by different (lower) letters towards the columns show significantly different (P <0.05), and the numbers followed by different (upper) letters to the rows indicate significantly different (P <0.05)

**Table 2** Findings of the analysis percentage of GLUT4 expression on liver and muscle tissues in normoglycemic and hyperglycemic conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>GLUT4 expression (% cells)</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>53.21 + 3.16aA</td>
<td>48.67 + 2.91aB</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia group</td>
<td>64.05 + 4.16bA</td>
<td>56.15 + 3.84bB</td>
<td></td>
</tr>
</tbody>
</table>

Description: The numbers followed by different (lower) letters towards the columns show significantly different (P <0.05), and the numbers followed by different (upper) letters to the rows indicate significantly different (P <0.05)
Glucose can regulate gene transcription, enzyme activity, hormone secretion, and activity of glucoregulatory neurons, and possibly can regulate GLUT4 expression. Oleszczak et al., reported that hyperglycemia and hypoglycemia affect the glucose transport and expression of glucose transporters in B and T lymphocytes in humans. Many agents regulate the transport of glucose in lymphocytes. The research findings on lymphocyte of hyperglycemia condition showed GLUT 4 was expressed with higher intensity compared with GLUT 1 and GLUT 3. This study demonstrated that changes in glucose concentration significantly affected the expression of glucose transporter in B and T lymphocytes.

Study by Klip et al. in rats with both IDDM (insulin dependent diabetes mellitus) and non-insulin dependent diabetes mellitus (NIDDM), in which the blood glucose levels are higher, but lower insulin levels, showed the total of GLUT 4 levels and the mRNA levels of GLUT 4 in adipose tissue and the skeletal muscle were decreased. This may be due to insulin induced the GLUT 4 translocation from the intracellular membrane compartment to the surface of the fat cell membrane and the skeletal muscle (skeleton) to incorporate glucose into the cell.

Vannucci et al., reported that GLUT 4 expression in rat brains of cerebral increased in diabetic conditions, as well as hyperinsulinemia and its expression, is higher in female rats compared with male ones. In addition, Choeri et al., in his research of glucose transporters in rat brain immunohistochemically reported that GLUT 1, GLUT 3, and GLUT 4 expression were found to be higher than GLUT 2, GLUT 5, and GLUT 8. It is specifically reported that GLUT 4 expression in the cells of Golgi apparatus and cell layers of Granular of the cerebellum. GLUT 1 is expressed in the front of the motor cortex of the brain, while GLUT 3 is expressed in the body of the Purkinje cell near the axon hill. The presence of GLUT4 in the area strongly supports the role in providing the energy needed for motoric activity control.

In basal conditions, GLUT 4 is mostly located in intracellular organelles, and GLUT 1 is primarily in plasma membranes. Therefore, in order to move to the surface of the cell membrane, GLUT 4 requires signals from insulin. According to Kobayashi et al., the expression of some forms of glucose transporter on the surface of the cell plays a role in glucose uptake, signal generation, and metabolism to maintain the body’s cellular metabolic integrity.

In all cells, the insulin-responsive GLUT 4 pool is localized to the membrane vesicles, i.e. insulin responsive vesicles. GLUT 4 is expressed in insulin-sensitive tissues, e.g. in the fat tissue, skeletal muscle, and some neurons, the only glucose transporter responsible for the effects of insulin on the use of postprandial blood glucose. Similarly, Huang et al., suggests that adipose cells, skeletal muscle cells, and some neurons respond to insulin stimulation by transferring GLUT4 to the plasma membrane of the set.

The results of this study indicate that the hyperglycemic conditions of GLUT4 expression are higher than normoglycemic conditions in both liver and muscles. The increase of GLUT4 expression correlates with the increased levels of glycogen in both the liver and muscle in hyperglycemic conditions. GLUT4 plays an active role in inserting glucose into cells. Inside the cells, glucose undergoes metabolism converted into energy and also glycogen. The resulting glycogen is stored as a reservoir of energy in the cell, both liver cells, and muscle cells.

CONCLUSION

Expression of GLUT4 and levels of glycogen in hyperglycemic liver is higher compared to the muscles.

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