GLUT 4 and Insulin Resistance

A thesis submitted in partial fulfillment for the requirements for the
degree of Master of Science

By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Salmin F. Atia Ali ENTITLED GLUT 4 and Insulin Resistance BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

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Five types of glucose transporters stimulate the transport of glucose from the bloodstream into cells such as muscle cells and adipocytes in response to insulin signaling or exercise. Skeletal muscle and adipose tissue are the main sites for glucose uptake and dispose in insulin action dependent pathway. In non-insulin stimulated condition, the GLUT4 storage vesicles stay away from the plasma membrane, so there is no GLUT4 translocation or GLUT4 mediated glucose uptake. However, in insulin stimulated state, insulin initiates insulin signaling cascade which results in rapid GLUT4 translocation and increased glucose uptake at the cell surface. Failure of the translocation of GLUT4 storage compartments into the plasma membrane in response to insulin stimulation or exercise may reduce the GLUT4 mediated glucose uptake and thus insulin resistance and type 2 DM development.
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<td>Diabetes Mellitus</td>
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<td>IDDM</td>
<td>Insulin Dependent Diabetes Mellitus</td>
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<tr>
<td>NIDDM</td>
<td>Non- Insulin Dependent Diabetes Mellitus</td>
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<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-Like Receptor</td>
</tr>
<tr>
<td>PAMPs</td>
<td>Pathogen-associated Molecular Patterns</td>
</tr>
<tr>
<td>GLUT2</td>
<td>Glucose transporter 2</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Highly glycated hemoglobin A1c</td>
</tr>
<tr>
<td>UBC9</td>
<td>Ubiquitin-Conjugating Enzyme 9</td>
</tr>
<tr>
<td>MSG</td>
<td>Monosodium Glutamate</td>
</tr>
<tr>
<td>PBMCs</td>
<td>Peripheral Blood Mononuclear Cells</td>
</tr>
<tr>
<td>RYGB</td>
<td>Roux-en-Y Gastric bypass</td>
</tr>
<tr>
<td>MGB</td>
<td>Mini Gastric bypass</td>
</tr>
<tr>
<td>BPD</td>
<td>Biliopancreatic bypass</td>
</tr>
<tr>
<td>DJB</td>
<td>Duodenal Jejunal bypass</td>
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<tr>
<td>LSG</td>
<td>Laparoscopic Sleeve gastrectomy</td>
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<tr>
<td>AGB</td>
<td>Adjustable Gastric banding</td>
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**Introduction**

Diabetes mellitus (DM) is a group of metabolic diseases in which a patient has high blood sugar levels, either due to impaired insulin secretion by pancreas, or impaired response of cells to the insulin that is produced. There are two types of diabetes mellitus. Type 1 DM, which is called juvenile diabetes or as insulin-dependent diabetes mellitus (IDDM), results from failure of pancreas to produce insulin and the patient requires treating with insulin injection. Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly. It is referred to as non-insulin-dependent diabetes mellitus (NIDDM) or adult onset diabetes (White, 2006).

Type 2 diabetes is the most predominant form that usually develops at middle age. It is not common among children and adolescents. The defect in insulin signaling stimulated by chronic hyperglycemia enhances a group of systemic disorders such as hypertension, hyperlipidemia, cardiovascular disorder, infertility and recurrent miscarriages in women. Diet and regular exercise reduces insulin resistance. Consequently, this can lessen the incidence of type 2 DM (White, 2006).

The pathophysiology of type 2 diabetes is not confined to insulin production or insulin effects on the liver, muscle and adipose tissue. Innate immune system may have a role in developing type 2 DM. Genetic variations leading to the altered production or function of circulating innate immune proteins, cellular pattern recognition receptors and inflammatory cytokines are associated with insulin resistance and type 2 DM. The accumulation of activated innate immune cells in muscle and adipose tissue results in release of inflammatory mediators such as IL-1β and TNFα which promote insulin resistance and B-cell damage (Fernandez & Pickup, 2011).
The Obesity and Type 2 Diabetes Mellitus

Obesity plays a significant role in developing metabolic disorders such as insulin resistance and type 2 DM. In an obese person, adipose tissue releases high amounts of pro-inflammatory cytokines and other factors that participate in insulin resistance development. Insulin resistance associated with pancreatic islet β cell dysfunction can lead to uncontrolled blood glucose levels. Knowledge of the molecular and genetic basis of the disease could promote new approaches to its treatment and prevention (Kahn, et al., 2006).

Obesity and type 2 diabetes mellitus are lifestyle-related conditions; changes in physical activity and diet may reduce the diabetes risk both directly and indirectly through obesity. The relationship between degree of obesity and diabetes risk might be aggravated by other factors like duration of obesity, body fat distribution, physical activity, age, diet content, genetic susceptibility to type 2 diabetes and obesity. Many of these factors can be considered as risk factors for both obesity and type 2 diabetes mellitus. Therefore, the degree of obesity will determine other risk factors for type 2 DM (Hodge, et al., 2001).
Immune Dysfunction in Patients with Diabetes Mellitus

Patients with DM are more prone to infections than those without DM, perhaps because of a defect in the immune system. The immune system is a divided into innate and adaptive immune systems. The innate immunity represents the first line of defense against microorganisms, and is characterized by rapid response to stimuli. The main components of innate immunity are dendritic cells, macrophages, neutrophils (phagocytes), and natural killer cells, as well as the complement system and the major histocompatibility complex (MHC). Pathogen-associated molecular patterns (PAMPs) stimulate the innate immune response by interaction with pattern recognition receptors (PRR) through Toll-Like receptors (TLRs). The TLR binds to pathogens and initiates the inflammatory response by releasing inflammatory.
cytokines such as IL-1 and TNF-α from phagocytic cells. Defects in innate immunity such as low complement factor 4 and decreased cytokine response after stimulation have been observed in diabetic patients. Most studies of cellular innate immunity demonstrated reduced level of chemotaxis, phagocytosis, and the complement system of diabetic cells compared to cells of non-diabetic controls. In addition, a high glucose environment promotes growth of some microorganisms. Another cause that can lead to increased infections in diabetic patients is an increased adherence of microorganisms to diabetic cells such as increased adherence of E. coli to the epithelial cells of urinary tract system compared to non-diabetic cells. Therefore, many studies conclude that regulation of the blood glucose level leads to an improvement of the cellular innate immune system (Geerlings & Hoepelman, 1999).

![Figure 2](image-url)  
**Figure 2** Different influences on innate immunity lead to type 2DM

(Adapted from Fernandez & Pickup, 2011).
The Liver Role in Glucose Homeostasis

The liver plays an essential role in glucose homeostasis by stabilizing the glucose uptake and storage through the metabolic pathway called gluconeogenesis and the glucose release through glycogenolysis. These metabolic pathways maintain the blood glucose levels for demands of the body, especially the brain tissue. Glucose-6-phosphatase is a gluconeogenic enzyme abundant in liver and kidney that catalyzes the final step in both the gluconeogenesis and glycogenolysis pathways. Glucokinase is glycolytic enzyme has a high specificity for glucose; it catalyzes the conversion of glucose to glucose-6-P and then to pyruvate. In addition, the GLUT2 (Glucose transporter 2) enhances glucose transport from the liver into the bloodstream (Nordlie, et al., 1999).

![Figure 3 Regulation of gene expression by insulin through a MAPK cascade (Adapted from Nelson & Cox, 2008).](image)

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**Figure 3** Regulation of gene expression by insulin through a MAPK cascade (Adapted from Nelson & Cox, 2008).
The Glucose Transporters

Five types of glucose transporters stimulate the transport of glucose from the bloodstream into cells such as muscle cells and adipocytes in response to insulin signaling or exercise. GLUT1 is found in human erythrocytes and in the endothelial cells lining the blood vessels of the brain tissue. GLUT2 is abundant in liver, kidney, and pancreatic β cells, and has a low-affinity (high Km) to glucose. GLUT3 is expressed in neurons, and acts with GLUT1 to enable glucose to cross the blood-brain barrier and enter neurons. GLUT5 is a fructose transporter. GLUT4 isoform is the major glucose transporter that is mostly confined to striated muscle and adipocyte. GLUT4 transporter proteins are insulin dependent transporters which are stored inside the specialized storage vesicles called (GSVs GLUT4 storage vesicles) that stay inside the cells under inactive conditions. When postprandial glucose levels increase, the subsequent elevation in insulin stimulates intracellular signaling cascades that eventually result in the translocation of the GLUT4 storage compartments into the plasma membrane and then GLUT4 mediated glucose uptake. This process is reversible when circulating insulin levels decrease; GLUT4 transporters are removed from the plasma membrane by endocytosis and are recycled back to their GLUT4 storage vesicles (GSVs). Failure of the translocation of GLUT4 storage compartments into the plasma membrane in response to insulin stimulation or exercise may reduce the GLUT4 mediated glucose uptake and thus insulin resistance and type 2 DM development (Watson & Pessin, 2005).
Figure 4 Diagram illustrates the insulin regulated intracellular signal transduction cascade

(Adapted from Frojdo, et al., 2008).

Figure 5 Regulation of GLUT4 exocytosis in response to insulin signaling (Adapted from Leto & Saltiel, 2012).
This review will examine the following areas of research:

- GLUT4 leads to decreased blood glucose levels.
- In the metabolic syndrome GLUT4 content decreases along with insulin resistance and high levels of inflammatory markers.
- GLUT4 expression in human muscle fibers is not associated with intracellular TG content.
- The HbA1c (Highly glycated hemoglobin A1c) may decrease in type 2 DM in response to different treatments such as surgical treatment.

GLUT4 Is Decreased in Slow Skeletal Muscle Fibers of Type 2 Diabetic Patients

The aim of Gaster, et al., (2001) was to test the effect of obesity and type 2 diabetes mellitus on GLUT4 immunoreactivity in slow and fast-twitch fibers by utilizing immunohistochemistry and morphometry, and to evaluate what might occur if GLUT4 content decreases in skeletal muscle fibers. This study concentrated on sedentary men only. Eight obese type 2 diabetic patients, nine obese control subjects, and nine young slim control subjects participated in this study. The obesity of subjects was indicated by their high measurements of waist to hip ratio. GLUT4 density (GLUT4 expression) was higher in slow fibers compared with fast fibers in both lean and obese subjects. In contrast, GLUT4 density was markedly lower in slow fibers compared with fast fibers in type 2 diabetic patients. The authors also showed that the slow fiber portion which represent by dark bars was decrease to 86% in the obese subjects and to 75% in the diabetic patients compared with control group as shown in figure 6 and 7 (adapted from
Gaster, et al., 2001). Therefore, the authors suggested that the decreased GLUT4 from the more insulin-sensitive slow-switch fibers may participate in the reduced insulin-stimulated glucose uptake in skeletal muscle in type 2 diabetes due to insulin resistance. Therefore, they concluded that decreased GLUT4 mediated glucose uptake in response to insulin signaling in slow fibers lead to the development of skeletal muscle insulin resistance. They did not demonstrate the role of GLUT4 in the development of type 2 DM by investigating whether a decrease in GLUT4 content or translocation leads to insulin resistance and increase the extracellular glucose levels (Gaster, et al., 2001).

**Figure 6** GLUT4 densities in slow and fast skeletal muscle fibers (Adapted from Gaster, et al., 2001).
Expression of GLUT4 and UBC9 Protein Is Diminished in Skeletal Muscle of Type2 Diabetes Patients

Kampmann, et al., (2011) demonstrated the relationship between the severity of insulin resistance with GLUT4 expression and associated proteins such as UBC9 protein in skeletal muscle of type 2 diabetic patients. Type 2 diabetic patients with extreme insulin resistance (they were on daily insulin doses) with same age matched type 2 diabetic patients who did not need insulin treatment participated in this study. GLUT4 content was lower in skeletal muscle fibers and fat tissue of type 2 diabetic patients who are on daily insulin dose compared with type 2 diabetic patients that were not on insulin treatment. They also showed reduced expression of ubiquitin-conjugating enzyme 9 (UBC9) (for GLUT4 degradation) in combination with the reduced GLUT4 expression in skeletal muscle of same type 2 diabetic subjects who were on
insulin, as shown in figure 8 and 9 (adapted from Kampmann, et al., 2011). The data from this study suggest that GLUT4 has a role in type 2 diabetes mellitus development because GLUT4 decreased among type 2 diabetic patients with high insulin resistance. Small sample size is used in this study so it would be helpful to increase the sample size in order to clarify the mechanism behind the role of GLUT4 in insulin resistance. The authors did not mention the level of HbA1c of patients which would demonstrate whether these patients had normal blood glucose levels in these patients who required or did not require insulin treatment (Kampmann, et al., 2011).

**Figure 8** Total protein content (GLUT4, UBC9) in muscle biopsies was assessed by Western blotting analysis. TBC1D1 and AS160: Rab-GTPase activating proteins. UBC9: ubiquitin conjugating enzyme 9 regulates GLUT4 degradation. B-actin: loading control (Adapted from Kampmann, et al., 2011).
Increase GLUT4 Translocation could Enhance Insulin Sensitivity of Muscle Glucose Transport in Response to Exercise

Hansen, et al., (1998) investigated that increasing in insulin sensitivity of skeletal muscle glucose transport in response to a single bout of exercise caused by increasing glucose uptake through GLUT4 translocation. The rate of glucose transport induced by efficient concentration of insulin (30μU/ml) in order to show whether increase GLUT4 translocation led to increase insulin sensitivity of glucose transport. GLUT4 translocation was assessed by using the ATB-[2-H]BMPA exofacial photo labeling technique (insulin stimulation lead to 2 fold increase in D-mannose-4-yloxy uptake, and increase in GLUT4 content). The results of this study shown in

Figure 9 Western blotting for GLUT4 content in fat biopsy from type 2 diabetic patients

(Adapted from Kampmann, et al., 2011).
Figure 10 (Hansen, et al., 1998) indicate that the major increase in muscle glucose transport in response to sub maximal insulin injection following single bout of exercise is promoted by translocation of a higher number of GLUT4 to the plasma membrane as evaluated by exofacial photo labeling technique. Increase in insulin sensitivity of muscle glucose transport in response to sub maximal insulin and single bout of exercise is caused by increase GLUT4 translocation to the cell surface which eventually leads to decreased insulin resistance and then glucose homeostasis (Hansen, et al., 1998).
Expression of GLUT4 Is not associated With Intracellular Triglyceride (TG) Content in Human Skeletal Muscle Fibers

Gaster, et al., (2002) asked whether GLUT4 expression has a direct relationship with triglyceride content in the same skeletal muscle fibers from obese and obese type 2 diabetic subjects by utilizing histochemistry and stereology. As shown in figures 11 and 12 (Gaster, et al., 2002), they found that an elevated triglyceride level in slow fibers of diabetic patients is associated with decreased GLUT4 expression. However, the intracellular triglyceride densities in slow and fast fibers in the same study group were not associated with GLUT4 density. In addition, they found a high amount of triglyceride in slow twitch fibers of obese diabetic subjects compared to obese and lean controls. Also, plasma lipid levels such as cholesterol, triglyceride and HDL did not correspond with GLUT4 expression in either slow or fast fibers. As a whole, triglyceride content was significantly higher in slow muscle fibers of diabetic patients with GLUT4 expression reduction. Therefore, intracellular triglyceride and plasma lipids such as cholesterol may not have effect on muscle glucose transport and may not contribute to the development of skeletal muscle insulin resistance (Gaster, et al., 2002).

![Figure 11](image)

**Figure 11** Triglyceride densities in slow fibers (hatched bars) and fast fibers (open bars) of obese and diabetic patients (Adapted from Gaster, et al., 2002).
Figure 12 GLUT4 densities in slow fibers cross cholesterol, triglyceride, and HDL. A: solid line for controls. B: dashed line for obese. C: dotted line for diabetics (Adapted from Gaster, et al., 2002)

Syntaxin 4 Heterozygous Knockout Mice have Skeletal Muscle Insulin Resistance

[Syntaxin is a family of membrane integrated proteins participating in exocytosis, and mediating docking of transport vesicles necessary for the translocation]. Yang, et al., (2001) studied the effect of syntaxin 4 on the regulation of GLUT4 storage vesicles (GSVs) which are necessary for GLUT4 translocation. They generated syntaxin 4 knockout mice by homologous recombination. The data demonstrated that homozygotic disturbance of the syntaxin 4 gene leads to early death of embryo with growth defect, while heterozygous knockout mice (syn4+/-) did not have early fetal death and no significant growth and development abnormalities, as shown in figure 13 (Yang, et al., 2001). In addition, heterozygous syntaxin 4 mutant mice lack syntaxin 4 protein activity. Since syntaxin 4 have a role in insulin stimulated glucose transport in skeletal
muscle and GLUT4 translocation, these mice may have significant decreasing in insulin stimulated glucose transport in skeletal muscle and GLUT4 translocation which end up with insulin resistance. However, heterozygous knockout mice syn4+/– mice showed normal insulin stimulated glucose transport and uptake in adipose tissue as shown in figure 13 (Yang, et al., 2001). The data suggest that syntaxin 4 is necessary for muscle glucose transport and GLUT4 translocation while it may not have a role in glucose transport and uptake in adipose tissue (Yang, et al., 2001).

![Figure 13](image_url)

**Figure 13** Stimulation of insulin in the skeletal muscle led to GLUT4 translocation in wild type syn4+/+, but not heterozygotic syn4+/– mice (Adapted from Yang, et al., 2001).

**Rats with Metabolic Syndrome characterized by Reduction in GLUT4 Content along with Insulin Resistance and elevated levels of Inflammatory Markers**

Leguisamo, et al., (2012) focused on GLUT4 protein expression, insulin resistance, and inflammatory cytokines levels over specific periods of time in an animal model of metabolic syndrome SHR (spontaneously hypertensive). They used (MSG) monosodium glutamate to increase lipid levels and develop insulin resistance in these rats. This study showed significant
decrease in GLUT4 expression, accompanied by insulin resistance, and increased concentration of inflammatory cytokines such as IL-6 and TNF-α in obese hypertensive rats that received MSG treatment (MetS), as shown in figures 14 and 15 (Leguisamo, et al., 2012). These findings characterized the metabolic syndrome which has low levels of GLUT4 that mediate glucose uptake at cell surface, and thus insulin resistance development. Thus, decrease the GLUT4 protein is associated with high inflammatory markers and insulin resistance in metabolic syndrome (Leguisamo, et al., 2012).

**Figure 14** Total GLUT4 expressions in three different tissues A. heart, B. gastrocnemius muscle, C. white adipose tissue (Adapted from Leguisamo, et al., 2012).
The Relationship between the Innate Immunity and Glucose Concentration in the Oldest Old

Wijsman, et al., (2012) tested the relationship between innate immune response and glucose levels in the oldest old (85 years old) who are at high risk of fluctuating blood glucose levels and more prone to have infection. They found decreases in inflammatory cytokines such as TNF-α, IL-6, IL-1β and IL-10, but not IL1-RA, with increasing blood glucose levels, as shown in figure 16 (Wijsman, et al., 2012). They also found increasing levels of CRP with high
glucose levels and HbA1c. These results demonstrated that elderly non diabetic patients are more prone to have infectious diseases in response to high blood glucose levels. So, there may be a strong or direct relationship between glucose concentration and innate immune response. In this study the authors did not measure insulin sensitivity (Wijsman, et al., 2012).

![Figure 16](image)

**Figure 16** The association between cytokine production in response to high blood glucose levels

(Adapted from Wijsman, et al., 2012)

**What Is the Influence of Diabetes Mellitus As opposed to in vitro Hyperglycemia on Immune Cell Functions?**

The main objective of Daoud, et al., (2009) study was to determine the effect of acute hyperglycemia (in vitro) on immune cell functions; they compared short term exposure to elevated glucose levels rather than the long term repetitive in vivo exposures that occur in
This study also tested the effectiveness of treatment with either insulin or oral hypoglycemic drugs, and if the type of DM might have different effect on immune system cells. PBMCs (peripheral blood mononuclear cells) were collected from ten diabetic patients (type 1 and type 2 DM) and same number of healthy controls. After that they examined neutrophil (PMNC) for respiratory burst activity (as indicator of innate immune system) by using nitroblue tetrazolium (used as measure the production of reactive oxygen species). There was marked suppression of the proliferative PBMCs responses to mitogens (Con-A), which is selective T lymphocyte mitogen triggering respiratory burst activity. Nevertheless, this suppression was not affected by either the addition of increasing amounts of glucose, the type of diabetes, or whether the patients had been or had not been on insulin treatments, as shown in figure 17 (Daoud, et al., 2009). So, PBMC and neutrophils of diabetic patients have suppressed stimulation responses compared to those from healthy controls. These effects are related to the long-term in vivo exposure to hyperglycemia rather than due to acute in vitro exposure to hyperglycemia only. In addition, there were no differences in this inhibition due to diabetic type or treatment with insulin or with oral hypoglycemic agents (Daoud, et al., 2009).

Figure 17 Proliferation responses of PBMCs to Con-A mitogen (Adapted from Daoud, et al., 2009).
Metabolic Surgery Results in Reduced HbA1c in Type 2 Diabetes Mellitus Patients with BMI <35 kg/m²

Highly glycated hemoglobin A1c (HbA1c) is significantly associated with developing type 2 DM complications. The Ngiam, et al., (2014) study demonstrates the effectiveness of different types of metabolic surgeries in decreasing HbA1c levels in type 2 diabetics with BMI<35 kg/m² (non-morbid obesity) compared to type 2 diabetic patients with morbid obesity (BMI>35 kg/m²). This study described different types of surgery such as Roux-en-Y gastric bypass (RYGB), mini gastric bypass (MGB), Biliopancreatic bypass (BPD), duodenal jejunal bypass (DJB), Laparoscopic sleeve gastrectomy (LSG), and adjustable gastric banding (AGB) as in figure 18 (Ngiam, et al., 2014). The authors concluded that metabolic surgery have significant effect in decreasing HbA1c in type 2 diabetic patients with BMI<35 kg/m². Thus, decrease HbA1c in type 2 diabetic patients enhances the insulin sensitivity, increases GLUT4 translocation and GLUT4 mediated glucose uptake at cell surface which eventually lead to glucose homeostasis and decrease diabetic mellitus complications (Ngiam, et al., 2014).

Figure 18 Weighted mean BMI and HbA1c change dependent to surgery type (Adapted from Ngiam, et al., 2014).
Cardiac Insulin Resistance Is Accompanied with a Reduced Recruitment of
Phosphatidylinositol 3-Kinase to (GSVs)

Phosphatidylinositol (PI) 3-kinase has a significant role in transport the insulin signaling to GLUT4 vesicles in insulin signaling cascade manner which eventually leads to enhanced GLUT4 translocation and GLUT4 mediated glucose uptake. The objective of Eckel, et al., (2000) study was to examine the defect in insulin signaling cascade in obese rats that is associated with decreased cardiac GLUT4 translocation. They focused on tyrosine phosphorylation of IRS-1 (Insulin Receptor Substrate-1) and the correlation of the regulatory subunit of PI 3-kinase (p85) with IRS-1 following insulin binding to its receptor. The significant result of this study was insufficient insulin signaling cascade which represents in lack the binding of p85 subunits to IRS-1 and consequently fail the GLUT4 translocation. Thus, variation in insulin signaling leads to insulin resistance and decrease GLUT4 translocation (Eckel, et al., 2000).

Figure 19 GLUT4 and p85 and in cardiac tissue of lean and obese rats (Adapted from Eckel, et al., 2000).
Conclusion and Future Researches

I. Conclusion:

In general, skeletal muscle and adipose tissue are the main places for glucose uptake and dispose in insulin action dependent pathway. In non-insulin stimulated condition, the GLUT4 storage vesicles stay away from the plasma membrane, so there is no GLUT4 translocation or GLUT4 mediated glucose uptake. However, in insulin stimulated state, insulin initiates insulin signaling cascade which results in rapid GLUT4 translocation and increased glucose uptake at the cell surface. Impairment of any step in this pathway leads to decreased GLUT4 translocation and insulin resistance that ultimately results in type 2 diabetes mellitus (Lampson, et al., 2000).

GLUT 4 levels are significantly correlated with insulin sensitivity in humans by reduction in GLUT4 content in response to insulin stimulation results in insulin resistance and type 2 diabetes mellitus development (Kampmann, et al., 2011). Many studies show that exercise plays an important therapeutic role in improving insulin sensitivity of muscle glucose transport and increasing GLUT 4 translocation. High amounts of triglycerides have been found in human skeletal muscle tissue of obese and type 2 diabetic patients with accompanying insulin resistance. Moreover, decreasing the triglyceride level by regular exercise, diet, and weight loss may improve insulin sensitivity (Gaster, et al., 2003).

Some studies showed that metabolic surgery such as AGB, DJB, LSG, and BPD significantly decreases HbA1c in type 2 DM with BMI less than 35 kg/m² and this kind of surgery will lead to control the obesity and then DM. HbA1c plays crucial role in determining the blood glucose levels during specific period of time (from 8 to 12 weeks). HbA1c plays role in decreasing the complications of Diabetes mellitus by controlling the blood glucose levels.
Depending on HbA1c level, insulin or oral hypoglycemic agents can be used in diabetic patients, or to reduce likelihood of developing diabetes in obese individuals.

Sedentary lifestyle and diet have been found to be increased the incidence of type 2 diabetes mellitus in both men and women. Physical activity may decrease type 2 DM development through reduced total body fat especially abdominal distributed fat or through its action in enhancing insulin sensitivity and GLUT4 translocation (Hodge, et al., 2001).

II. Future research:

Determination the mechanism beyond the correlation between cytokines production and glucose concentrations in non-diabetic patients needs further study.

Studying precise mechanisms by which hyperglycemia or diabetes affected cellular immune responses and activity. In addition, the effect of hyperglycemia on signal transduction in the immune cells such as TCR-β chain selection (results in the proliferation of the cell and cytokines production) and MAPK-STAT transduction (has a significant role in immune mediated inflammatory responses), and on cytokine synthesis and secretion such as TNF-α, IL-6, because these inflammatory cytokines have been related to reduce GLUT4 expression, consequently lowering glucose uptake by muscle, need further study.

Knowledge of HbA1c level in each study is important, in order to determine whether the diabetic subjects need insulin or oral hypoglycemic treatment. Furthermore, the relationship between the HbA1c, obesity (BMI), and type 2 DM, and the effect of GLUT 4 in improvement of HbA1c, and in enhancement of insulin sensitivity should be defined in each study.
Many studies demonstrated the decrease in GLUT4 content or translocation associated insulin resistance. However, the role of GLUT 4 in the pathogenesis of type 2 DM stills not understood and needs further research.

Regarding effects of diabetes mellitus on immune cell functions, this literature review does not show the precise mechanisms by which diabetes or hyperglycemia affected cellular immune responses and activity. In addition, whether regulation of glucose levels may improve the immune system in DM, needs further research. For instance, improve the phagocytosis, chemotaxis, and increase cytokine response after stimulation in response to control or normalize blood glucose levels.

High levels of cholesterol are not associated with failure of insulin stimulated glucose uptake and may not be a major player in the regulation of GLUT4 trafficking. Nevertheless, some studies showed that there is relationship between hypercholesterolemia and insulin resistance. Therefore, Future studies are needed to clarify whether differences in plasma cholesterol influence GLUT4 translocation.

1. **Hypothesis:**

Identify the mechanism behind the association between glucose concentrations and innate immune response. It would be nice to know the regulation of blood glucose level might lead to innate immune response improvement by focusing on inflammatory cytokines, M1, and M2 macrophage cells.
Methods:

• Newly diabetic subjects aged between 40-70 years old, and healthy controls participate in this study.

• BMI of diabetic subjects. If the BMI shows significant reduction after treatment compared to before treatment, may lead to reduction in HbA1c and consequently complications of diabetes mellitus may decreased and ultimately the cellular innate immunity will be improved.

• Fasted and postprandial blood glucose levels in order to regulate blood glucose levels, HbA1c and CRP.

• TNF-alpha, IL-6 and IL-10. These inflammatory cytokines are collected from newly diabetic subjects.

• Dividing the subjects into two groups, group one treats with change the life style such as eating balanced diet and regular daily exercise. Group two treats with oral hypoglycemic drugs and insulin injections.

Expected results:

• The level of TNF-alpha and IL-10 start to elevate at the beginning after that it might show decrease in both levels of TNF-alpha and IL-10 in response to keep the blood glucose levels within normal glycemic limit.

• At the beginning of treatment either with change lifestyle or put the patients on medicines, M1 and M2 cells have stimulated and released TNF-alpha and IL-10 which promote Th1 and Th2 response. After that M1 and M2 might show decrease in inflammatory cytokines production such as TNF-alpha and IL10.
Figure 20 The relationship between TNF-α production and glucose concentration.

Figure 21 The relationship between IL-10 production and glucose concentration.
2. **Hypothesis:**

Study the effect of hyperglycemia on M1 and M2 macrophage cells, and on cytokine synthesis and secretion.

**Methods:**

- Number of patients with type 1 and type 2 DM (poorly controlled), non-diabetic controls.
- Fasted and postprandial blood glucose levels, HbA1c and CRP
- TNF-alpha, IL-6 and IL-10 are collected from subjects.

**Expected results:**

- Poorly controlled diabetic patients demonstrate decrease in M1 and M2 inflammatory response which plays a significant role in increase the prevalence of infection in diabetic patients.
- Significant reduction in inflammatory cytokine synthesis and secretion such as TNF-alpha, IL-6, IL-10, and IL-12 in diabetic compared to controls.
- Reduction in Th1 and Th2 response in diabetic compared to controls.
Figure 22 Effects of hyperglycemia on cytokine production.
References


4- Gaster, M., Ottosen, P. D., Vach, W., Christiansen, H., Staehr, P., BECK-NIELSEN, H., & Schrøder, H. D. "GLUT4 expression in human muscle fibers is not correlated with intracellular triglyceride (TG) content. Is TG a maker or a marker of insulin resistance?." Apmis (2003), 111.2: 338-348.


7- Wijsman, C. A., Mooijaart, S. P., Westendorp, R. G., & Maier, A. B.
"Responsiveness of the innate immune system and glucose concentrations in the oldest old." Age (2012), 34.4: 983-986.


