ROLE OF PHOSPHOGLUCOMUTASE IN TYPE 2 DIABETES MELLITUS: AN INTEGRATED BIOCHEMICAL, GENETIC AND GENE EXPRESSION STUDY

Thesis submitted for the degree of

Doctor of Philosophy

in

Cell Biology and Molecular Genetics

Under the faculty of Allied Health and Basic Sciences

by

Praveen Kumar K. S.

(Reg No. 18PY7011)

Under the supervision of

Dr. Sharath B.

Department of Cell Biology and Molecular Genetics

Sri Devaraj Urs Academy of Higher Education and Research

Kolar, Karnataka, India

March 2022

Declaration by the candidate

I, **Praveen Kumar K.S.**, hereby declare that this thesis entitled **"Role of Phosphoglucomutase in Type 2 Diabetes Mellitus: An integrated biochemical, genetic and gene expression study"** is a bonafide and genuine research work carried out by me in the Department of Cell Biology and Molecular Genetics Sri Devaraj Urs Academy of Higher Education and Research Tamaka, Kolar, under the supervision of **Dr. Sharath B.,** Associate Professor, Department of Cell Biology and Molecular Genetics, **Dr. Prabhakar K.,** Professor, Department of General Medicine. No part of this thesis has formed the basis for the award of any degree or fellowship previously. Plagiarism check was carried out on the thesis and the report was found to be satisfactory.

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Supervisor

Dr. Sharath B.
Associate Professor
Department of Cell Biology and Molecular Genetics

Date: 31/3/22

Dr. Sharath B. Ph.D
Associate Professor
Cell Biol & Mol Genetics
SDUAHER-Kolar

Co- Supervisor

Professor of Medicine
SDUMC, Tamaka, Kolar.

Dr. Prabhakar K.
Professor
Department of General Medicine

Date: 31/03/2022

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Head
Department of Cell Biology and Molecular Genetics

Date: 31/3/22

Head of Dept.
Cell Biology & Molecular Genetics
SDUAHER - Kolar

Dean
Faculty of Allied Health and Basic Sciences

Date: 31-03-2022

Dean
Faculty of Allied Health Sciences
Sri Devaraj Urs Academy of
Higher Education & Research
Tamaka, Kolar-563 101

Sri Devaraj Urs Academy of Higher Education and Research

Author Name	Mr. PRAVEEN KUMAR K. S
Course of Study	Doctor of Philosophy
Name of Guide	Dr. Sharath B.
Department	Cell Biology and Molecular Genetics
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Signature of Supervisor
Associate Professor
Cell Biol & Mol. Genetics
SDUAHER, Kolar
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Mr. Praveen Kumar K. S.

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ABBREVIATIONS

T2DM	Type 2 Diabetes Mellitus
PGM	Phosphoglucomutase
PBMC	Peripheral Blood Mononuclear Cells
DNA	Deoxyribonucleic Acid
mRNA	Messenger RNA
ATP	Adenosine triphosphate
PCR	Polymerase Chain Reaction
SDS	Sodium Dodecyl Sulphate
RBC	Red Blood Cell
ELB	Erythrocyte Lysis Buffer
WBC	White Blood Cell
RPMI	Roswell-Prank Memorial Institute
FBS	Fetal Bovine Serum
PAS	Periodic Acid-Schiff stain
RIPA	Radioimmunoprecipitation assay
EDTA	Ethylenediaminetetraacetic acid

gm – grams, mU/mg –Milliunits per milligram, mM – millimolar,

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder that arises due to the disruption of glucose homeostasis. In addition to other mechanisms, the glycogen pathway also plays a major role in glucose homeostasis. The phosphoglucomutase (PGM) enzyme catalyzes the key reaction that connects the glycogen pathway with glucose metabolism. Studies have shown that the glycogen pathway is abnormal in T2DM. The purpose of this study was to determine the role of PGM in T2DM by a combination of biochemical, genetic, and gene expression studies.

This was a case–control study comprising T2DM patients (*n* = 63) and healthy volunteers (*n* = 63). All experiments were carried out using peripheral blood mononuclear cells (PBMCs). PGM enzyme activity was found to be reduced in T2DM patients compared to healthy controls (0.9 fold; p = 0.043; Student's t test). Furthermore, PGM enzyme activity showed a reciprocal relationship with the indices of glycemic controls such as fasting blood sugar (r = -0.36; *p* = 0.016; Pearson's correlation test), random blood sugar (r = -0.39; *p* = 0.019; Pearson's correlation test), postprandial blood sugar (r = -0.41; *p* = 0.011; Pearson's correlation test), and glycated hemoglobin (r = -0.35; *p* = 0.028; Pearson's correlation test). Next, gene expression and genetic variation were explored as potential sources for reduced PGM enzyme activity. *PGM1* gene expression was found to be downregulated in T2DM patients compared to healthy subjects (fold difference = 0.59; *p* = 0.032; unpaired t test). Furthermore, PGM enzyme activity showed a positive correlation with *PGM1* gene expression (r = 0.35; p = 0.016; Pearson's correlation test). In addition, a common genetic variation in the *PGM1* gene (SNP rs11208257) was associated with T2DM. Furthermore, a reduction in PGM enzyme activity was linked to the genotype combination *PGM1* SNP rs11208257 (p = 0.018; multiple logistic regression).

Together, these results indicate that PGM enzyme activity is suboptimal in T2DM, probably due to downregulated gene expression and genetic variation.

The physiological impact of reduced PGM enzyme activity on cell viability under glucose-limiting conditions was evaluated. This is based on the assumption that reduced PGM activity may compromise glucose mobilization from glycogen and reduce cell viability under glucose limiting conditions. PBMC viability was reduced in PBMCs of T2DM patients compared to healthy subjects ($p = 0.001$; Student's t test). There was a positive correlation between PGM activity and PBMC viability ($r = 0.35$; $p = 0.001$; Pearson's correlation test). This relationship indicates that reduced PGM enzyme activity impairs PBMC viability under glucose-limiting conditions. In addition, It was found that glycogen degradation in PBMCs was reduced in T2DM patients compared to healthy subjects ($p = 0.001$; Student's t test). Glycogen degradation showed a reciprocal relationship with PBMC viability, and the effect was moderate ($r = -0.46$; $p = 0.012$; Pearson's correlation test). This relationship indicates that glycogen degradation is one of the several factors responsible for PBMC viability under glucose-limiting conditions.

This study shows that PGM enzyme activity is compromised in T2DM patients, possibly due to reduced gene expression and genetic variation. This study represents the first attempt to link the PGM enzyme with the pathogenesis of T2DM. This study adds the PGM enzyme to the list of defects that impair the glycogen pathway in T2DM.

INTRODUCTION

T2DM is a common metabolic disorder that constitutes a major public health burden. It affects approximately 12.1% of the population in India and 9.3% of the population worldwide [Saeedi et al., 2019; IDF 2017]. T2DM is the ninth leading cause of mortality, accounting for over 3.8 million deaths annually in India [Anjana et al., 2018]. T2DM mostly develops after 40 years of age [Wu et al., 2014]. Poor management of T2DM eventually leads to fatal complications such as end-stage renal disease and micro and macrovascular complications [Nasri et al., 2015, Liu et al., 2010]. Therefore, understanding the molecular underpinnings of T2DM is essential to uncover novel drug targets for its management.

Hyperglycemia is the cardinal biochemical hallmark of T2DM. This condition arises due to the reduced capacity of the cells to absorb glucose [Bouche et al., 2004]. Insulin is the main driver of cellular glucose utilization through the enhancement of membrane permeability for glucose [McConell et al., 2020]. Insulin signaling mobilizes glucose transporters from the cytoplasm to the cell membrane [Chadt et al., 2020]. Defects in insulin signaling are considered to play a major role in impairing cellular glucose utilization in T2DM [Samovski et al., 2018]. In addition to insulin signaling, the glycogen pathway also plays an important role in regulating cellular glucose utilization [Favaro et al., 2012]. Glycogen is the metabolic storehouse for glucose. Excess glucose is stored as glycogen and used when it is needed [Adeva-Andany et al., 2016].

The major steps involved in the glycogen pathway are schematically represented in Figure 1.1. Extracellular glucose enters the cytoplasm of the target cell through active transport mediated by the membrane-bound glucose transporter. The hexokinase enzyme in the cytosol catalyzes the conversion of glucose into glucose 6-phosphate, which further enters the glycolytic pathway. During excess, glucose 6-phosphate is

converted into glucose 1-phosphate, which then enters the glycogen pathway. This isomerization of glucose 6-phosphate into glucose 1-phosphate is catalyzed by the phosphoglucomutase (PGM) enzyme.

Several studies have shown that the glycogen pathway is abnormal in T2DM [Krssak et al., 2004, Soares et al., 2019]. The abnormality has been linked to defects in the enzymes involved in the glycogen pathway, such as glycogen synthase, glycogen phosphorylase, and protein phosphatase 1[Krssak et al. 2004; Pandey and Damsbo 1991; Kumar et al. 2018]. PGM is an important enzyme in the glycogen pathway, and its role in the pathogenesis of T2DM is not known. This study was undertaken to fill this gap. The aim of this study was to evaluate the role of PGM in T2DM by a combination of biochemical, gene expression, and genetic studies.

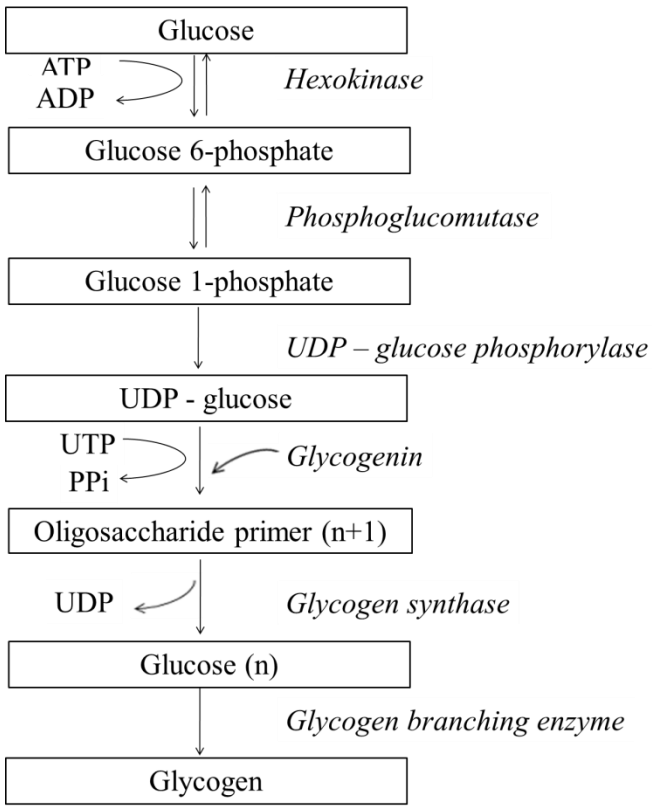

Figure 1.1: Schematic representation of the glycogen pathway.

AIM, OBJECTIVES AND RATIONALE

2.1 Aim of the study:

The aim of this study was to evaluate the role of PGM in T2DM by a combination of biochemical, gene expression, and genetic studies.

2.2. Objectives of the study:

1. To compare the enzymatic activity of the PGM enzyme in cell-free lysates of peripheral blood mononuclear cells (PBMCs) derived from T2DM patients and healthy controls.
2. To compare the gene expression profile of *PGM1* in PBMCs in T2DM patients and healthy controls.
3. To determine the association pattern of a selected genetic variation (rs11208257) within the *PGM1* gene in T2DM patients and healthy controls.
4. To compare the magnitude of glucose deprivation-induced glycogen degradation and cell viability in the PBMCs of T2DM patients and healthy controls.

2.3. Rationale:

Glucose homeostasis is disrupted in T2DM [Galicia-Garcia et al., 2020]. The glycogen pathway plays an important role in glucose homeostasis by converting excess glucose into glycogen. Studies have shown that the glycogen pathway is abnormal in T2DM. This study aimed to evaluate the role of the PGM enzyme in T2DM. The PGM enzyme plays a key role in the glycogen pathway. This enzyme catalyzes the bidirectional conversion of glucose 6-phosphate into glucose 1-phosphate, which is the starting intermediate in the glycogen pathway. Therefore, the PGM enzyme controls the entry of glucose into the glycogen pathway. Reduction of PGM activity is therefore likely to affect the functioning of the glycogen pathway. This study hypothesized that

PGM activity was lower in T2DM patients than in healthy subjects. This hypothesis was tested in the first objective.

The potential sources for the reduction in PGM activity were tested in the second and third objectives. The hypothesis behind the second objective was that PGM activity may be reduced due to the downregulation of the corresponding gene. The hypothesis behind the third objective was that PGM activity is reduced due to genetic variations in the corresponding gene. There are five PGM isozymes in the human genome: PGM1, PGM2, PGM2L1, PGM3, and PGM5. Of these five, PGM1 is the predominant isoenzyme expressed in most cell types, including PBMCs [Stiers et al. 2017]. The PGM1 isoenzyme is encoded by the *PGM1* gene. Therefore, gene expression and genetic variation studies were carried out with the *PGM1* gene.

There are over 24505 SNPs according to the single nucleotide polymorphism database (dbSNP) [Wheeler et al., 2007]. Of these, only three SNPs were missense variations with a global minor allele frequency of more than 5%. The three SNPs are rs1126728, rs11208257, and rs6676290. Among these three SNPs, rs6676290 is absent in the South Asian population (1000 Genomes Project) [1000 Genomes Project Consortium, 2015]. Functional analysis of these two SNPs using the Sorting Intolerant From Tolerant (SIFT) program [Sim et al., 2012] showed that the amino acid change due to SNP rs11208257 is deleterious, whereas the change due to SNP rs1126728 is tolerated. Therefore, SNP rs11208257 was chosen for the genetic association study. The hypothesis of the third objective was that the minor allele of SNP rs11208257 would be more common among T2DM patients than in healthy subjects.

The physiological impact of PGM activity on glycogen utilization was evaluated in the fourth objective. The hypothesis was that reduced PGM activity would compromise glucose mobilization from glycogen under conditions of glucose limitation. The resulting glucose shortage would compromise PBMC viability.

The glycogen pathway is most active in the liver and skeletal muscle cells [Krssak et al., 2004; Jensen et al., 2011]. However, the presence of glycogen has been demonstrated in several other cells, such as red blood cells, astrocytic glial cells, cardiomyocytes, renal tubular cells, Schwann cells, and adipocytes [Miwa et al., 2002, Wiesinger et al., 1997, Milutinovic et al., 2012, Tsuchitani et al., 1990, Brown et al., 2012, Ceperuelo-Mallafre et al., 2015]. This study was carried out using PBMCs. The presence of glycogen has been demonstrated in PBMCs. Furthermore, the transcriptional profiles of PBMCs in T2DM patients have been shown to correlate with the pathophysiology of the disease [Manoel-Caetano et al. 2012].

2.4. Significance of the study:

T2DM is a multifactorial disease with a genetic component, and less is known about the pathophysiological origin of T2DM. Additionally, there are no specific treatments to cure T2DM. Therefore, understanding the pathophysiological basis of T2DM is necessary to uncover novel therapeutic targets. The results of this study will contribute to the understanding of the molecular mechanisms by which PGM in the glycogen pathway plays a role in the pathophysiology of T2DM. If PGM is found to be altered in the glycogen pathway, then it can be taken as a drug target. This study provides the mechanistic role of PGM1 in the glycogen pathway. The knowledge obtained can be

helpful in developing therapeutic strategies that can help in a better understanding of the disease.

REVIEW OF LITERATURE

3.0. Clinical aspects of diabetes:

3.0.1. Signs and Symptoms

The common symptoms of diabetes mellitus include polyphagia, polydipsia, weight loss, polyuria, fatigue, and blurred vision [Kharroubi et al., 2015]. The common signs of diabetes mellitus are elevated sugar levels in the blood (hyperglycemia) and urine (glycosuria).

3.0.2. Disease burden

Diabetes mellitus affects 8.7% of the Indian population (46.3 million) in the age group of 10 – 70 years. It is expected to rise to 10.9% (77 million) by 2045. The frequency is higher in urban regions (10.8 million) than in rural areas (7.2 million). According to the International Diabetes Federation (IDF), diabetes accounts for 6.7 million deaths in India [Cho et al., 2018]. These figures show that diabetes is a major public health burden in India.

3.1. Types of diabetes mellitus

Diabetes mellitus is classified into four types as follows [Kaul et al., 2012]:

a) Type 1 diabetes mellitus

b) Type 2 diabetes mellitus

c) Gestational diabetes

d) Maturity onset diabetes mellitus

a) Type 1 diabetes mellitus

Type 1 arises due to the diminished production of insulin. Insulin production is diminished because the beta cells that produce insulin are destroyed by the

autoantibodies. It is also called juvenile diabetes since it develops in children in the age group of 0 to 15 years [Das et al., 2015].

b) Type 2 diabetes mellitus (T2DM)

Type 2 arises due to insulin resistance, which involves the failure of cells to take up glucose from the blood despite the presence of insulin. Type 2 develops mainly in adults in the age group of 30-80 years [Atre et al., 2020].

c) Gestational diabetes mellitus:

This type of diabetes develops transiently during pregnancy. Placental hormones produced during pregnancy, such as human chorionic gonadotropin hormone and human placental lactogen hormone, have a blocking effect on insulin action. In normal pregnancy, the reduced action of insulin is compensated for by increased insulin secretion from the pancreas. However, such compensatory increases do not occur in women who develop gestational diabetes [Lende et al., 2020].

d) Maturity onset diabetes mellitus (MODY):

MODY is a type of diabetes caused by mutation of genes such as hepatocyte nuclear factor and glucokinase. The proteins encoded by these genes play an important role in the homeostasis and metabolism of glucose. Mutations in these genes are inherited in an autosomal dominant pattern. MODY usually develops before the age of 25 years [Hoffman et al., 2021; Naylor et al., 2018].

Among the four types described above, type 2 is the most common type, which is seen in approximately 60 to 80% of diabetic patients. This is followed by type 1 (8 to 12% of diabetic patients) and gestational diabetes (10% of diabetic patients). MODY is

quite rare, as it is seen in only approximately 1 to 5% of diabetic patients. T2DM is the main focus of this study.

3.2. Glucose metabolism

3.2.1. The biological significance of glucose:

Glucose is the main source of energy for all cellular functions. Glucose is utilized for the production of ATP in the mitochondria [Bonora et al., 2012].

3.2.2. Sources of glucose:

Glucose is mainly derived from the diet. Starch is the main dietary source of glucose. Other sugars in the diet are fructose, sucrose, maltose, lactose, etc. Other forms of carbohydrates are eventually converted into glucose before entering energy metabolism. During starvation, lipids and proteins are broken down and converted into glucose for use in energy production [Chen et al., 2015].

3.2.3. Absorption of dietary sugars:

Dietary sugars are absorbed mainly in the small intestine by enterocytes. These are epithelial cells that line the small intestine. Glucose cannot cross the cell membrane since it is polar in nature. Therefore, the diffusion of glucose is facilitated by proteins called transporters. There are two types of transporters: sodium-glucose cotransporter 1 (SGLT1) and glucose transporters (GLUTs) [Holesh et al., 2021; Navale et al., 2016]. There are six types of glucose transporters, which are summarized in **Table 3.1.**

Table 3.1: Types of transporters for dietary sugar absorption

Type of transporter	Location	Function
SGLT1	Intestinal epithelium and renal tubules	Acts as cotransporter of glucose and galactose with Na^+ requires ATP for the transport of glucose along its concentration gradient
GLUT1	Placenta, skeletal muscle, adipose tissue, liver hepatocytes, RBCs	Essential for glucose sensing by the pancreas, important feedback mechanism for glucose homeostasis with endogenous insulin
GLUT2	Pancreatic beta cells, hepatocytes, renal tubular cells, intestinal epithelium, proximal tubule	Glucose sensor in trans epithelium for glucose and fructose. Important for glucose metabolism in the liver
GLUT3	Central nervous system and small intestine	High affinity for glucose, a scavenger for cells with the high rate of glucose demands
GLUT4	Skeletal muscle cells, enterocytes, cardiomyocytes, brain tissue, and adipocytes	Insulin responsive isoform, translocate into plasma membrane upon insulin stimulation
GLUT5	The small intestine, brain, muscle, and adipose tissue	Fructose transporter
GLUT6	Ubiquitous, present in all the cells	Transporter present in pseudo genes, nonfunctional

Sodium-glucose cotransporter 1 (SGLT1) is the main transporter responsible for glucose absorption in the small intestine. This protein functions as a cotransporter. The cotransporter facilitates the movement of glucose along with sodium from the

intestinal lumen to the enterocyte through active transport via symport, as shown in **Figure 3.1**.

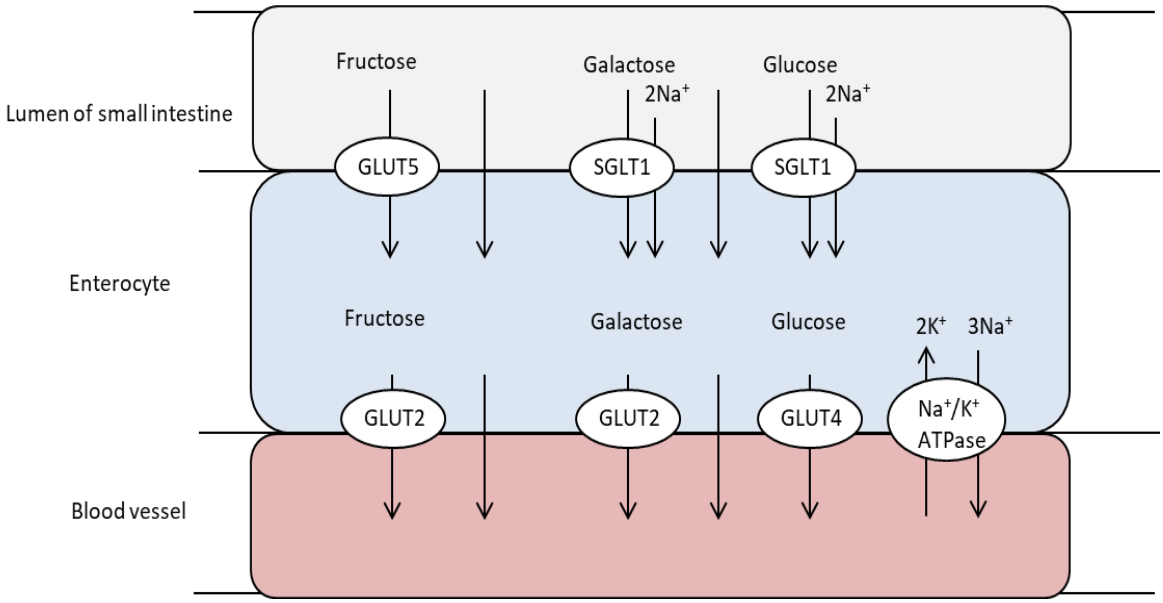

Figure 3.1: Transport of glucose from the small intestine to blood vessels

3.2.4. Circulation of glucose:

From the enterocyte, sugars enter the capillaries through glucose transporters. From here, sugars are transported through the circulatory system for utilization by all the cells of the human body. Again, glucose transporters are responsible for absorbing glucose from the blood.

Transport of glucose through the cell membrane requires the utilization of glucose in most tissue cells, and the transport of glucose from the blood via the cell membrane into the cytoplasm is necessary. Glucose cannot pass through easily due to its membrane polarity. However, cells absorb glucose from the blood with the help of membrane-bound glucose transporters. Some glucose transporters require insulin for

their action. Based on this requirement, glucose transporters are classified into three types [Panahi et al., 2020]. They are as follows:

1. Sodium-dependent glucose transporters, e.g., SGLT1
2. Insulin-independent glucose transporters, e.g., GLUT1, GLUT2, GLUT3
3. Insulin-dependent glucose transporters, e.g., GLUT4

3.2.5. Cellular respiration:

After entering the cytoplasm, glucose undergoes phosphorylation to form glucose-6-phosphate. Glucokinase in the liver and hexokinase in most other cells are involved in this process. The phosphorylation process ensures that glucose is trapped inside the cell. It is typically irreversible, except for liver cells, intestinal epithelial cells, and renal tubular epithelial cells, which have reversible glucose phosphatase [Nakrani et al., 2021].

There are two fates for glucose 6-phosphate. It will either enter the glycolysis pathway for energy release or is converted into glycogen and stored for energy [Bonora et al., 2012]. Glucose 6-phosphate is converted to pyruvate under anaerobic conditions and may become lactate or enter the citric acid cycle for energy release. The aerobic respiration by oxidative phosphorylation takes place by the electron transport chain with the release of ATP, which will be utilized for energy needs (**Figure 3.2**) [Rajas et al., 2019; Yetkin-Arik et al., 2019; Choudhry et al., 2021].

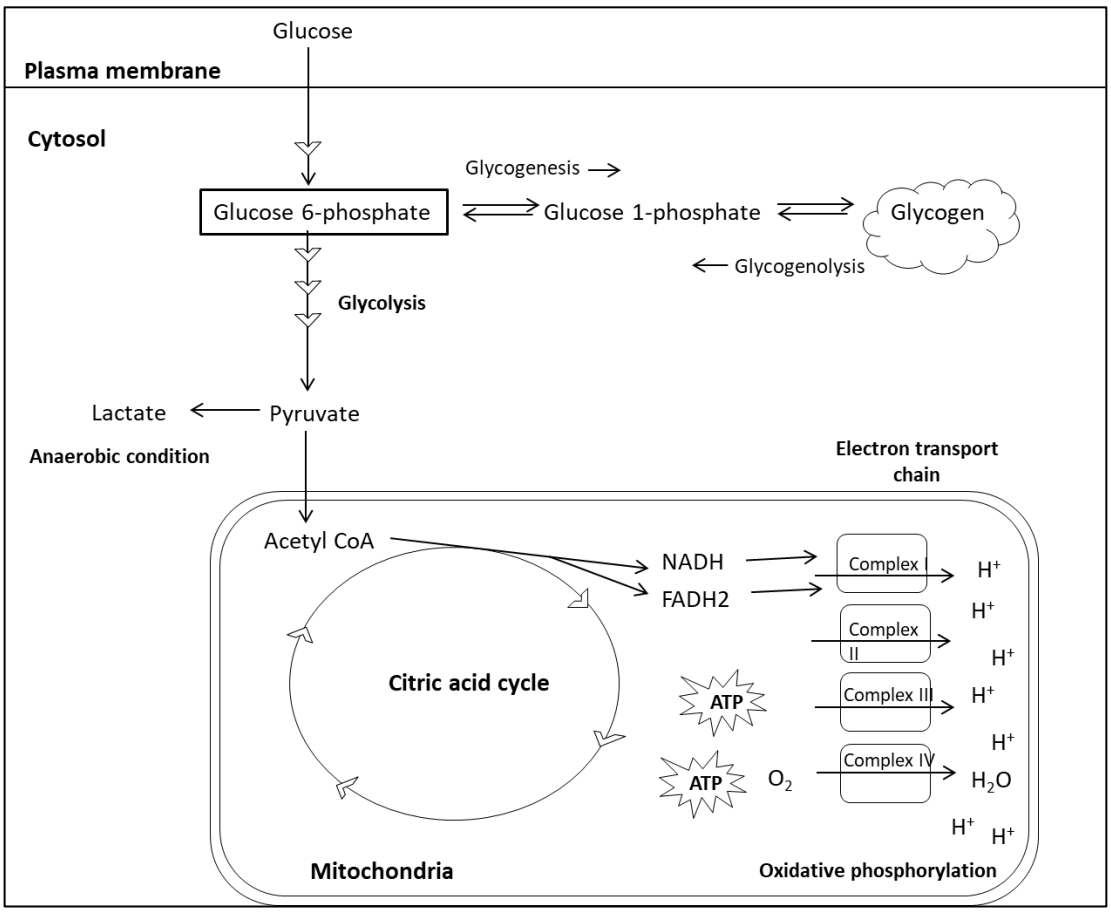

Figure 3.2: The fate of glucose 6-phosphate in cellular respiration

3.2.6. Role of insulin in glucose absorption:

The presence of excess nutrients in the diet results in excess glucose in the blood. This triggers pancreatic beta cells to produce insulin. Insulin itself cannot enter the cells because of its hydrophilic nature. Hydrophobic cell membranes do not allow insulin to enter the cell. Therefore, insulin binds to its receptor on the cell membrane and activates it. The protein tyrosine kinase in the beta subunit attaches to the insulin receptor substrate (IRS). Phosphorylation of the tyrosine residue of IRS upon kinase activity takes place and forms two domains with different transduction pathways. The attachment of tyrosine-protein kinase Src to one of the domains upon phosphorylation activates phosphatidylinositol 3-kinase, which converts phosphatidylinositol 4,5-bis-phosphate to phosphatidylinositol (3,4,5)-triphosphate. This results in the recruitment

of protein kinase B, also called Akt, which is a serine/threonine-specific protein kinase. Membrane-bound cyclin-dependent kinases and DNA-activated protein kinases phosphorylate Akt and are released into the cytoplasm, where they have diverse functions. Upon phosphorylation, Akt attracts vesicles containing glucose transporter 4 and facilitates its fusion to the cell membrane. Transfer of glucose transporters from the cytosol to the cell membrane facilitates the entry of glucose into the cell [Arneth et al., 2019; De Meyts et al., 2016]. The schematic representation is shown in **Figure 3.3**.

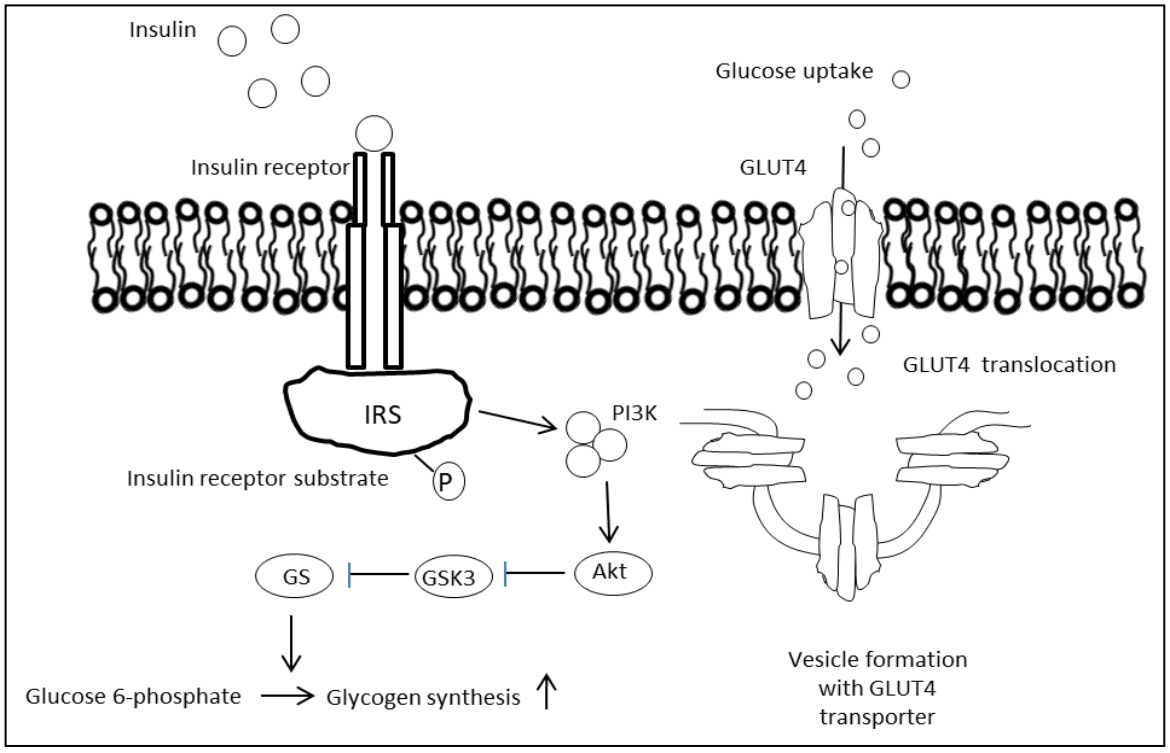

Figure 3.3: Role of insulin in glucose absorption by the signal transduction pathway

3.2.7. Hormonal regulation of blood glucose:

Blood glucose levels were maintained under homeostatic conditions (80-120 mg/dl) for two reasons. First, low levels (hypoglycemia) will result in reduced glucose supply to the cells to meet their energy requirement. Second, higher levels (hyperglycemia) will result in long-term microvascular complications such as diabetic retinopathy,

nephropathy, peripheral neuropathy, and macrovascular complications, viz., ischemic heart disease, and peripheral vascular disease [Chawla et al., 2016].

Hormones are involved in the homeostasis of blood glucose levels. The two main hormones involved in this process are insulin and glucagon. These two hormones function in opposite directions. Insulin serves to reduce blood glucose levels, whereas glucagon serves to increase it. Insulin reduces blood glucose levels by promoting its absorption by insulin-dependent cells of the liver and skeletal muscle. Furthermore, insulin also promotes glycogen synthesis. The mechanism by which insulin promotes glucose absorption is described in the previous section.

Glucagon serves to increase blood glucose levels by promoting the conversion of glycogen to glucose. The major stores of glycogen are present in the liver and skeletal muscle.

Both insulin and glucagon are produced by the pancreas. Insulin is produced from the beta cells of pancreatic islets, and glucagon is produced from the alpha cells of the pancreas. Insulin has a positive feedback mechanism to regulate glucose homeostasis, and glucagon has a negative feedback mechanism and regulates the release of glucose into the blood for normal glucose homeostasis [Kulina et al., 2016; Godoy-Matos et al., 2014]. Shown in **Figure 3.4.**

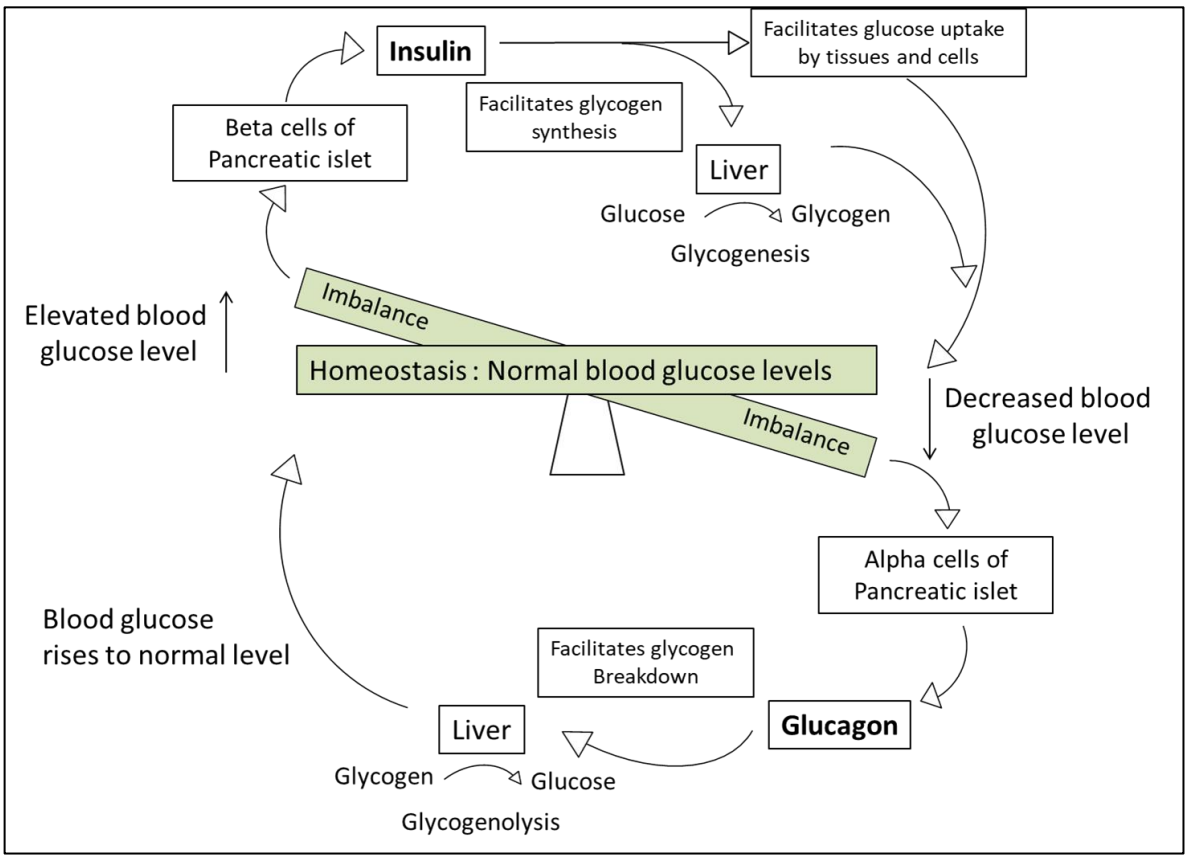

Figure 3.4: Hormonal regulation of blood glucose levels

3.3. Pathogenesis of T2DM:

Insulin resistance plays a major role in the pathogenesis of T2DM. Insulin resistance involves the inability of cells to absorb glucose despite the presence of sufficient levels of insulin in the plasma. Biochemically, insulin resistance is seen as a decreased ratio of blood glucose and blood insulin levels. It is measured using an index called the homeostasis model assessment for insulin resistance (HOMA-IR) [Gayoso-Diz et al., 2013].

In T2DM, because of insulin resistance, the cell is unable to bind to the insulin receptor and affects signal transduction for the absorption of glucose into the cell. Phosphorylation is diminished for IRS and produces inactive phosphatidylinositol 3-kinase. Phosphatidyl 3-kinase will not participate in the conversion of phosphatidyl

inositol 4,5 diphosphate to phosphatidyl inositol 4,5,6-triphosphate and produces inactive protein kinase B (Akt). Therefore, Akt is not involved in the vesicle formation of glucose transporter 4, resulting in reduced glucose uptake into the cell. As a result, the translocation of glucose was diminished. The blood glucose levels were increased, resulting in the production of more insulin. Furthermore, Akt will not phosphorylate glycogen synthase kinase 3, resulting in phosphorylation of glycogen synthase and becoming inactive. Therefore, glycogen synthesis from glucose 6-phosphate was reduced, and gluconeogenesis was increased in the cytosol. A schematic representation is shown in **Figure 3.5.**

Furthermore, insulin resistance is compensated by enhanced insulin secretion leading to hyperinsulinemia [Taylor et al., 2012; Freeman et al., 2020]. However, prolonged compensatory hyperinsulinemia eventually leads to the impairment of pancreatic beta cells. This results in the development of hyperinsulinemia, and T2DM patients become dependent on supplementary insulin.

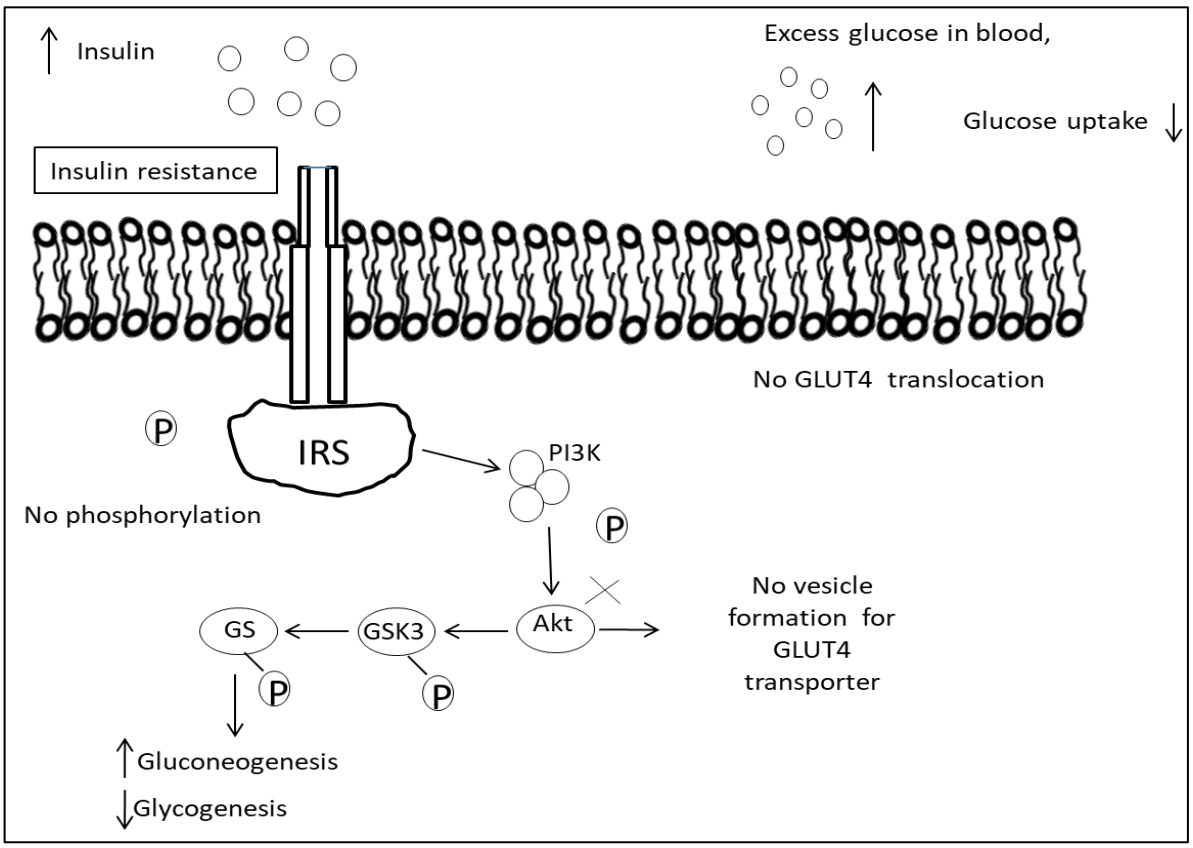

Figure 3.5: Role of insulin resistance in T2DM

3.4. Role of glycogen pathway in glucose metabolism:

The glycogen pathway plays an important role in regulating glucose levels. When the glucose level is above the cellular energy requirement, excess glucose is converted into glycogen through glycogenesis [Jin et al., 2018]. When the cellular glucose level is less than the metabolic requirement, extra glucose is mobilized through the degradation of glycogen, a process referred to as glycogenolysis [Jensen et al., 2011]. Shown in Figure 3.6.

The first step in glycogen synthesis is the conversion of glucose 6-phosphate into glucose 1-phosphate. Glucose 6-phosphate is the intermediate molecule linking glycolysis and the glycogen pathway. This isomerization of glucose 6-phosphate into glucose 1-phosphate and vice versa is catalyzed by the phosphoglucomutase enzyme.

Furthermore, glucose 1-phosphate is converted into uridine diphosphate glucose by the pyrophosphate enzyme. This is the starting point of making the linear glycogen molecule by adding multiple glucose monomers with each other to form a long chain of oligosaccharides. Glycogenin subunits continue to add glucose residues, and the attached glucose then serves as a primer for the glycogen synthase enzyme to add more glucose. Glycogen synthase is the major enzyme involved in glycogen synthesis. Involved in the conversion of glycosylated UDP-glucose, it is converted into glycogen [Han et al., 2016]. The glycogen synthase enzyme catalyzes the conversion of uridine diphosphate glucose to terminal glucose on glycogenin, forming a long chain of linear glycogen with alpha 1,4 glycosidic bonds. Finally, the glycogen branching enzyme catalyzes the conversion of alpha 1,4, glycosidic bonds to alpha 1,6 glycosidic bonds and converts long-chain linear glycogen to branched glycogen molecules **(Fiure 3.6)** [Adeva-Andany et al., 2016].

Glycogen degradation takes place when the extracellular supply of glucose is limited. Glycogen phosphorylase enzyme catalyzes the conversion of glycogen to glucose 1-phosphate. Furthermore, glucose 1-phosphate is converted to glucose 6-phosphate by the phosphoglucomutase enzyme. The resulting glucose 6-phosphate is then utilized in the glycolysis pathway for energy needs [Adeva-Andany et al., 2016; Jensen et al., 2011].

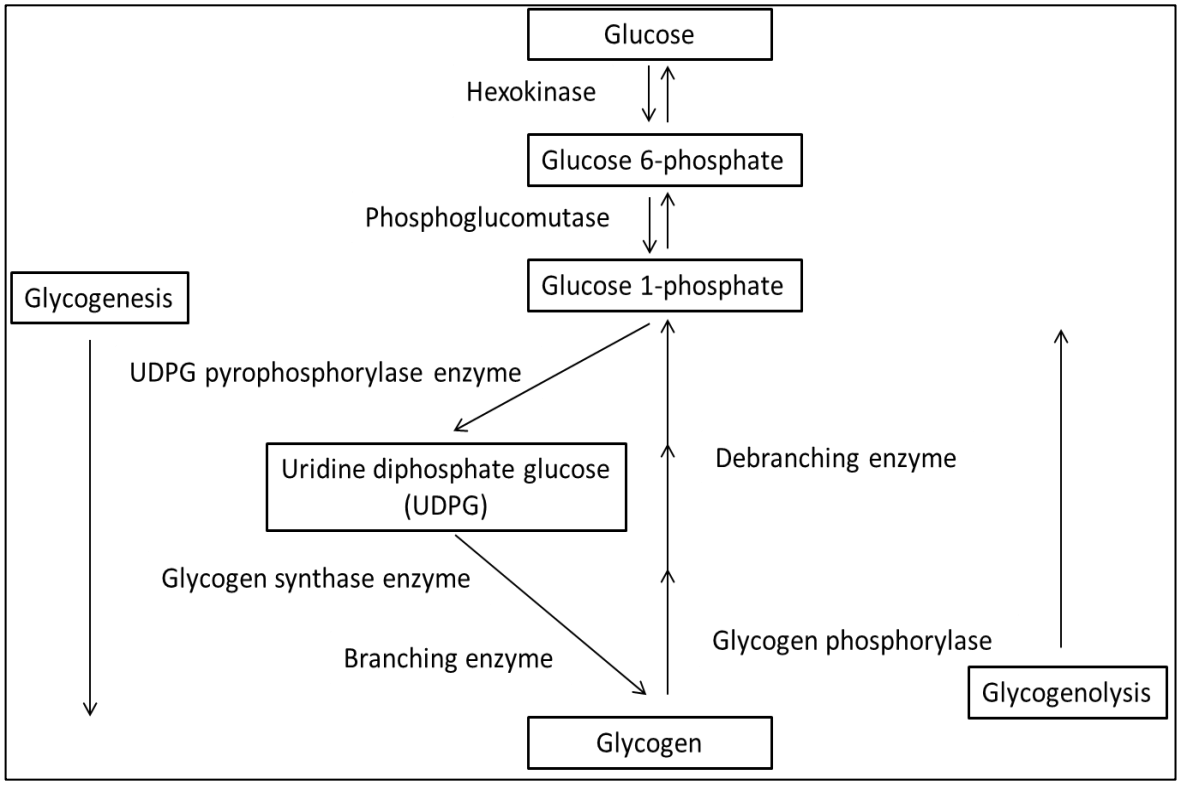

Figure 3.6: Role of the glycogen pathway in glucose metabolism

3.5. Role of the glycogen pathway in the pathogenesis of T2DM:

The glycogen pathway plays an important role in regulating blood glucose levels. Several studies have shown that the glycogen pathway is impaired in T2DM.

Del Prato and coworkers studied glycogen synthesis and degradation in noninsulin-dependent diabetes mellitus using the clamp technique [Del Prato et al., 1994]. Hepatic glycogen synthesis and degradation were measured by using ^{14}C glucose infusion and indirect calorimetry. Insulin-mediated glucose uptake in the liver was reduced in insulin-dependent diabetes mellitus. Furthermore, insulin-mediated glycogen synthesis was also reduced in the liver and muscle. Based on these observations, the authors concluded that glycogen synthesis is defective in insulin-dependent diabetes mellitus.

Krssak and coworkers evaluated hepatic glycogen synthesis and degradation in T2DM patients and healthy volunteers under hyperinsulinemic-euglycemic clamp [Krssak et al., 2004]. The hepatic glycogen concentration was measured by using ^{13}C-labeled nuclear magnetic resonance spectroscopy. Hepatic glycogen synthesis was reduced under postprandial conditions. Furthermore, glycogen degradation was also reduced under prolonged fasting conditions. These observations motivated the authors to conclude that hepatic glycogen metabolism may be defective in T2DM.

Efforts have been made to explore the causes of glycogen pathway impairment in T2DM. Current evidence points to enzyme defects as the potential source. Glycogen synthase and glycogen synthase kinase are implicated in this direction.

Damsbo and coworkers evaluated glycogen synthase activity in T2DM. Glycogen synthase activity was measured in cultured muscle cells obtained from skeletal muscle biopsies cultured under in vitro conditions by insulin stimulation [Damsbo et al., 1991]. Glycogen synthase activities in response to insulin resistance have been found to be reduced in skeletal muscle biopsy samples of T2DM. These observations revealed that insulin-stimulated glycogen synthase activity may be impaired in T2DM.

Glycogen synthase activity is regulated by phosphorylation. The active enzyme is phosphorylated, whereas the inactive enzyme is dephosphorylated. These two processes are catalyzed by glycogen synthase kinase and protein phosphatase 1. Studies by Nikoulina and coworkers showed that the expression of glycogen synthase kinase was elevated in the skeletal muscle of T2DM patients [Nikoulina et al., 2000]. Additionally, insulin-stimulated glycogen synthase activity was reduced in T2DM. Furthermore, a reciprocal relationship was observed between glycogen synthase

activity and glycogen synthase kinase expression. These results suggest that abnormal reduction of glycogen synthase activity in T2DM may arise due to downregulation of glycogen synthase kinase. The role of glycogen synthase phosphorylation in T2DM was further explored by Hojlund and coworkers using the euglycemic hyperinsulinemic clamp technique [Hojlund et al., 2003]. Both phosphorylation and glycogen synthase activity were reduced in the skeletal muscle of T2DM patients. The schematic representation is shown in **Figure 3.7.**

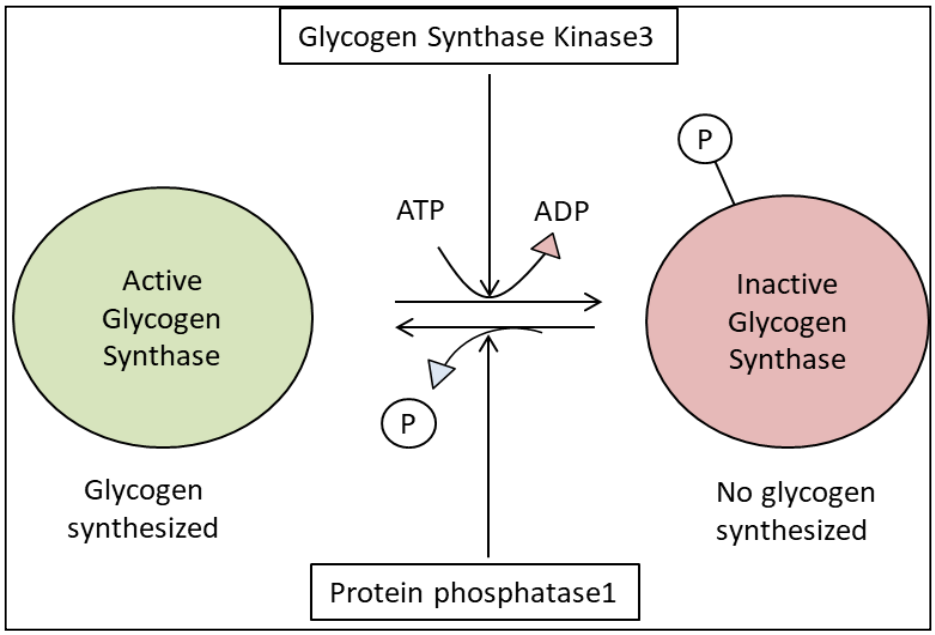

Figure 3.7: Schematic representation of glycogen synthase phosphorylation

3.6. Lacunae in knowledge:

The review of the literature shows that glycogen metabolism is abnormal in T2DM. This appears to arise due to abnormalities in the enzymes involved in the glycogen pathway. Glycogen synthase and glycogen synthase kinase are involved in disrupting the glycogen pathway in T2DM. Phosphoglucomutase (PGM) is a key enzyme in the glycogen pathway that serves as a connecting link with the glucose pathway. Abnormal glycogen metabolism can also arise due to the abnormal functioning of the

phosphoglucomutase enzyme. However, there is no literature on the functional status of the PGM enzyme in T2DM. Hence, this study is planned.

PGM is a group of isozymes of the phosphohexose mutase family. There are four different phosphoglucomutase isozymes in human beings viz., PGM1, PGM2, PGM3, and PGM5. The PGM1 enzyme is expressed in most tissues, whereas PGM2 is expressed predominantly in RBCs. PGM3 is seen mostly in prostate and placental tissue, and PGM5 is seen mostly in the myocardium [Stiers et al., 2017].

Among these four PGM isoforms, the PGM1 isoform is the predominant isoform, which is expressed in most of the tissue types, including hepatocytes, skeletal myocytes, and PBMCs.

MATERIALS AND METHODS

4.1. Study Design:

The study was carried out by using a case–control design. The case group comprised patients diagnosed with T2DM. The control group will comprise age and gender-matched healthy individuals. T2DM patient's blood samples were collected from the participants, and the following parameters will be evaluated: glycogen degradation and PBMC viability under glucose limiting conditions, PGM enzyme activity, *PGM1* gene expression, and frequency of SNP rs11208257 in genomic DNA. The results of the two groups will be compared by statistical methods.

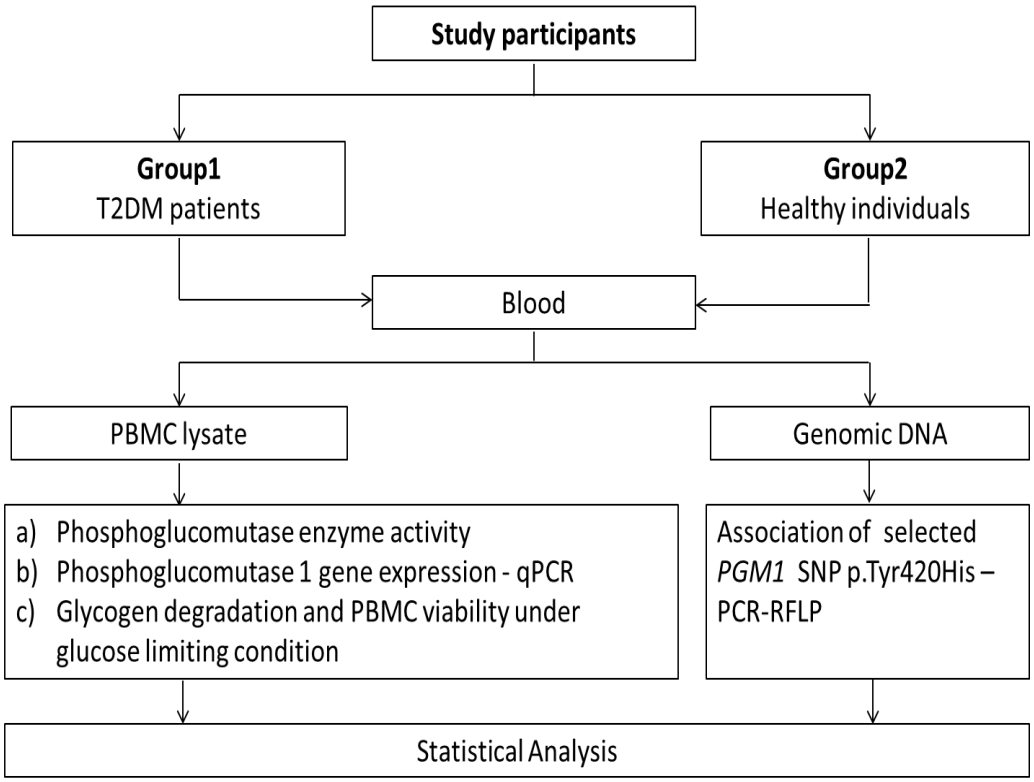

Figure 4.1: Schematic representation of the study design

4.2. Ethical issues:

The study was conducted after obtaining approval from the institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar, India **(Ref.no: SDUMC/KLR/IEC/30/2019-20 dated 06-June-2019).** T2DM patients and healthy

controls were recruited from 2019 to 2020. Informed consent was obtained in writing before enrolment in the present study.

4.3. Selection of study participants:

The study participants were recruited from the Department of General Medicine, R. L. Jalappa Hospital and Research Centre, attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India. T2DM patients were enrolled upon satisfaction of the inclusion and exclusion criteria.

4.4. Inclusion criteria:

The inclusion criteria for the selection of the T2DM group were based on the following criteria of the Indian Council of Medical Research htttp://icmr.nic.in/guidelines_ diabetes/guide_diabetes.htm]:

a) Patients of both genders

b) between the ages 30 - 80 years

c) fasting blood glucose ≥ 126 mg/dL

d) HbA1c > 6.5% in the last test performed in the 12 months before the study

The inclusion criteria for the selection of control group subjects were as follows:

a) healthy individuals of both gender and age, between the ages 30 – 80 years

b) no known history of any chronic disease

c) HbA1c < 6.5% in the last test performed in the 12 months before the study

4.5. Exclusion criteria:

The exclusion criteria for the patient selection were

a) Microvascular complications

b) Chronic comorbidity

4.6. Sample size:

The sample size for the present study was calculated based on the mean difference in hepatic glycogen concentrations observed between the T2DM and control groups (Martin et al., 2004). The sample size was calculated by considering the difference of a 3% increase in hepatic glycogen concentration in the T2DM group compared to the control group. The sample size required with a 95% confidence interval and 90% power was 63 per group. However, the study groups were analyzed for genetic variation in the *PGM1* SNP rs11208257. Based on the preliminary findings of the data (pre hoc power: 45.3%), post hoc power analysis was carried out. The sample size with 80% power is estimated to be 225 per group.

4.7. Clinical sample collection and processing:

5 ml of venous blood will be collected from the T2DM patients and healthy controls. PBMCs were prepared by using Ficoll-histopaque medium and divided into two parts. The first half will be used for cell culture experiments and RNA preparation. The remaining PBMCs were stored as PBMC lysate with radioimmunoprecipitation assay buffer (Himedia, India) supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany) and were stored at -80°C for further use.

4.8. PBMC isolation:

PBMCs were isolated from whole blood using Ficoll-Histopaque (Merck, Darmstadt, Germany) [Mallone et al., 2011]. Briefly, 1 ml of anticoagulated blood was layered on 1 ml of Ficoll-histopaque and centrifuged at 3000 rpm for 30 min without break. The PBMC layer was collected in a 15 ml Falcon tube and washed twice with 10 ml of 1X phosphate-buffered saline (PBS). The number of PBMCs was counted using a hemocytometer and used for *in vitro* culture.

4.9. PBMC culture and glucose treatment

Glucose availability in the PBMC culture was altered in two phases. The initial culture condition was glucose-proficient, whereas the subsequent condition was glucose-limited. PBMCs (1 x 10^5 cells) were seeded into 5 ml of glucose-free RPMI 1640 medium (Cat # 11879020, Gibco, New York, USA). The medium was supplemented with 10 mM glucose, 20% heat-inactivated fetal bovine serum, 300 µl phytohemagglutinin (30 µg/mL final), and 1% antibiotics. The cultures were incubated at 37°C for 48 hours in a 5% CO_2 atmosphere. PBMCs were harvested after incubation by centrifugation for 10 min at 2000 rpm and washed with 1X PBS. The pellet was then cultured under glucose-limited conditions. The culture conditions were as before but without supplementation with 10 mM glucose. The cultures were incubated for 24 hours, and the PBMCs were harvested by centrifugation for 10 min at 2000 rpm. The pellet was washed with 1X PBS and used to measure viability under the glucose-limited conditions.

4.10. Cell viability assay:

The viability of the cultured PBMCs was determined by the Trypan-Blue assay [Strober et al., 2001]. Briefly, the cell suspension (1:1 ratio) was mixed with 0.4% Trypan Blue (Gibco, New York, USA) and loaded into a hemocytometer. Unstained cells were recorded as viable, and blue-stained cells were scored as non-viable. The magnitude of cell viability with induction of glucose deprivation was determined as below:

$$Viability(\%) = \begin{bmatrix} Viable\ cells \\ prior\ to\ glucose \\ deprivation\ (\%) \end{bmatrix} - \begin{bmatrix} Viable\ cells \\ post\ glucose \\ deprivation\ (\%) \end{bmatrix}$$

4.11. Determination of glycogen degradation

The Periodic Acid-Schiff (PAS) staining technique was used to evaluate the amounts of glycogen in the cultured PBMCs [Tabatabaei Shafiei., 2014]. Briefly, 1X PBS-washed slides were treated with fixative (37% formaldehyde and 99% ethanol). PBMCs were then added to the slides and left for 1 min. Then, the slides were treated for 5 min with 1% periodic acid and 15 min with Schiff's reagent (SRL Biolabs, Maharashtra, India). Then, the slides were counterstained for 30-45 sec with hematoxylin. Approximately 80-85% of PBMCs were scored. The percentage of glycogen-positive cells was used as the measure of glycogen levels. The levels of glycogen degradation were estimated by using the following formula:

$$\begin{matrix} Glycogen \\ degradation = \\ (\%) \end{matrix} \left[\begin{matrix} Glycogen\ levels \\ prior\ to\ glucose \\ deprivation\ (\%) \end{matrix} \right] - \left[\begin{matrix} Glycogen\ levels \\ upon\ glucose \\ deprivation\ (\%) \end{matrix} \right]$$

4.12. PBMC lysate preparation and protein estimation

PBMCs were isolated from whole blood using Ficoll-Histopaque (Merck, Darmstadt, Germany). Briefly, 1 ml of anticoagulated blood was added to 1 ml of Ficoll-histopaque and centrifuged for 30 min at 3000 rpm. The PBMC layer was separated in a 15 ml falcon tube, and 1X phosphate-buffered saline (PBS) was used to wash twice. The number of viable PBMCs was counted using a hemocytometer and used for the PGM1 enzyme assay [Mallone et al., 2011]. Approximately 1 x 10^5 PBMCs were considered from the study participants. Approximately 50 µl PBMCs were collected with radioimmunoprecipitation assay (RIPA) buffer (Himedia, Nashik, India) supplemented with the protease inhibitor cocktail phenylmethlsulfonyl fluoride (PMSF) (Roche, Mannheim, Germany), brought to a final volume of 50 µl and stored at -20^0 C for further use. Total protein in PBMC cell lysates was determined by the

bicinchoninic acid (BCA) method [Smith PK et al., 1985]. Briefly, the BCA protein assay was used for the quantitation of total protein in a sample. The principle of this method is that proteins can reduce Cu^{+2} to Cu^{+1} in an alkaline solution (the biuret reaction) and result in a purple colour formation by BCA.

4.13. PGM enzyme assay:

PGM enzyme activity was measured using the PBMC lysate. The lysate was made by resuspending approximately 50 µl of PBMC preparation in RIPA buffer (Himedia, India) with protease inhibitor cocktail (Roche, Mannheim, Germany). The lysate was stored at -20°C until further use. The total protein present in the PBMC lysate was determined by the bicinchoninic acid method [Smith, 1985]. The protein concentration of the lysate was used for the normalization of the enzyme activity. PGM1 activity was measured in the PBMC lysate by the colorimetric method [Najjar, 1955] by using a commercial kit (Cat # K774-100, BioVision, Milpitas, CA). Briefly, diluted PBMC cell lysates (1:10 dilution) from the study participants were resuspended in assay buffer and then processed according to the manufacturer's protocol. One unit of PGM1 activity was defined as the amount of enzyme that generated 1.0 µmol of NADH per min at pH = 8.0 at 37°C. PGM enzyme activity was expressed as milliunits per milligram of lysate protein (mU/mg of lysate protein).

4.14. *PGM1* gene expression analysis:

PGM1 gene expression was quantified by using the quantitative reverse transcription-polymerase chain reaction (qRT–PCR) method. Total RNA from PBMCs was prepared using a commercial kit (Cat # 15596018, Thermo Scientific, Waltham, USA) according to the TRIzol method [Chomczynski et al., 1993]. The cDNA preparation from total RNA was carried out by the *in vitro* reverse transcription method using a

commercial kit according to the manufacturer's instructions (Cat # 1708891, Bio–Rad, Hercules, CA). A positive control (pooled sample) was used for interplate adjustment. The reaction mixture composition for each 20 µl reaction was as follows: 14 µl of total RNA, 1 µl of reverse transcriptase enzyme, and 6 µl reaction mixture (4 µl of 5X script reaction mix and 1 µl of nuclease-free water). The primers used for cDNA synthesis are shown in **Table 4.1.**

Table 4.1. The PCR conditions for cDNA synthesis:

PCR conditions	Temperature
➢ PCR priming	25°C for 5 min
➢ Reverse transcription	46°C for 20 min
➢ Reverse transcriptase inactivation	95°C for 1 min

The primers used for amplification of the *PGM1* gene are shown in **Table 4.2.** The *GAPDH* gene was used as the internal reference. The 10 µl reaction mixture for qRT–PCR contained 2 µl of cDNA, 1 µl of primers (40 nM final), 5 µl of SYBR green (Cat #1725271, Bio–Rad, Hercules, CA), and 2 µl of nuclease-free water.

Table 4.2: qRT–PCR primers used for the quantification of the *PGM1* gene

Gene	Primers	*PGM1* primers
PGM1	Forward primer	5' TAA TGG AGG TCC TGC TCC AG 3'
	Reverse primer	5' TTT CCC AGA ACA CCA AGG TC 3'
GAPDH	Forward primer	5' GAT CAT CAG CAA TGC CTC CT 3'
	Reverse primer	5' GAC TGT GGT CAT GAG TCC TTC 3'

The thermal program for the qRT–PCR run is shown in **Table 4.3.** The reactions were carried out in duplicate, and the average ΔCt was determined for both the *GAPDH* and *PGM1* genes. The comparative Ct method [Livak, 2001] was used to determine the fold change ($2^{-\Delta\Delta Ct}$) in *PGM1* gene expression.

Table 4.3: Thermal parameters for qRT–PCR

Parameter	**Temperature**
➢ Initial denaturation ➢ Cycle denaturation ➢ Annealing and extension + Plate Read	95^0 C for 10 min 95^0 C for 15 sec 60.6^0 C for 30 sec
Thermal cycles (Go to step 2, 35X)	
➢ Melt curve For 0.05 + Plate Read	55^0 C to 95^0 C increments of 0.5^0 C

4.15. DNA extraction

Genomic DNA was isolated by the salting-out method (Miller *et al.* 1988). Approximately 2 ml of the blood sample was collected in an EDTA vacutainer, which was vortexed and then transferred into a sterile 15 ml falcon tube. Erythrocyte lysis buffer (ELB) was added to the Falcon tube containing the blood sample at a ratio of 1:4, followed by thorough mixing. The sample was then incubated for approximately 30-45 min on ice to induce hemolysis. The hemolysed sample was then centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and 10 ml of ELB was added to the pellet. The suspension was subjected to centrifugation at 3000 rpm for 10 min. The supernatant was discarded, and the pellet was resuspended in 5 ml of ELB. The suspension was then supplemented with 270 µl of 20% SDS and 30 µl of proteinase K.

The suspension was incubated at 37°C overnight in a water bath. The next day, 500 µl of 5 M sodium chloride was added to the samples, and an equal volume of 100% isopropyl alcohol was added to the Falcon tube to precipitate the DNA. The DNA was then transferred to a 1.5 ml micro centrifuge tube containing freshly prepared 500 µl of 80% ethanol. The sample was incubated for 15 min at room temperature and centrifuged at 12,000 rpm for 5 min. The supernatant was discarded, and the pellet was washed with 80% ethanol thrice. The pellet was air-dried and then resuspended in 500µl of Tris-EDTA buffer. The sample was then incubated at 65°C in a water bath for 30 min. Following this, the sample was kept in a rotator overnight to dissolve the DNA completely. The sample was then stored at -80°C until further analysis.

4.16. DNA quantification and purity analysis:

The DNA concentration and purity were determined by spectrophotometry. Measurements were carried out on a UV–Vis spectrophotometer (Perkin Elmer model Lambda 35, Waltham, MA, USA) to check the concentration and purity of the DNA. The amount of DNA was estimated using the formula dsDNA concentration = 50µg/ml x OD260 x dilution factor. The ratio of absorbance at 260 and 280 nm in the range of 1.7 to 2.0 was regarded as pure.

4.17. Genotyping of *PGM1* SNP rs11208257:

Genomic DNA was prepared from peripheral blood samples by using the salting-out method [Miller et al., 1988]. The concentration and purity of the genomic DNA were determined by UV spectrophotometry (Perkin Elmer model Lambda 35, Waltham, USA). PCRs were performed on a gradient thermal cycler (Bio–Rad, California, USA). The 20 µl reaction mixture included 1X assay buffer, PCR mix comprising 10pmol of each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 150 – 300ng of genomic

DNA, and 1 unit of Taq DNA polymerase (Bangalore Genei, India) in a final volume of 25µl. PCR primers used for the genotyping of c.1258 T > C SNP alleles are summarized in **Table 4.4.** The PCR parameters employed for PCR are summarized in **Table 4.5.**

Table 4.4: PCR primers used for genotyping of the *PGM1* gene

Parameters	***PGM1* primers**
Forward primer (5' – 3')	5' CCC TCC CTC AAC ATG AGA TTT G 3'
Reverse primer (3' – 5')	5' CAA TTG AGA GAG GCT GGA TGA C 3'

Table 4.5: PCR parameters used for genotyping the *PGM1* gene

Parameter	**Temperature**
➢ Initial denaturation	95^0 C for 3 min
➢ Cycle denaturation	95^0 C for 30 sec
➢ Annealing	60.6^0 C for 30 sec
➢ Extension	72^0 C for 1 min
Thermal cycles (35X)	
➢ Final extension	72^0 C for 7 min
PCR amplicon size (bp)	375 bp

The genotyping was performed by the PCR-RFLP method. The PCR amplicon was analyzed by electrophoresis on a 2% agarose gel. The restriction digestion was carried out for the amplicon with 5 units of NlaIII (New England Biolabs, Ipswich, USA) at 37°C for 8 hours and analyzed on a 2% agarose gel with ethidium bromide staining. The 'C' allele was cleaved, resulting in two fragments of sizes 226 bp and 149 bp,

while the T allele was visible as an uncut fragment of size 375 bp. The CC genotype was used as the positive control, which is a Sanger sequenced sample, and the results are summarized in **Table 4.6.**

Table 4.6: PCR-RFLP band pattern of *PGM1* SNP rs11208257:

Genotype	Band pattern
Major allele (TT)	375 bp
Heterozygous allele (TC)	375 bp, 226 bp, 149 bp
The minor allele (CC)	226 bp, 149 bp

4.18. Statistical analysis:

Statistical analysis was carried out using SPSS Statistics *V24.0* (International Business Machine Corporation, Armonk, New York). Quantitative variables are represented as the mean and standard deviation. Qualitative variables are represented as percentages. The Shapiro–Wilk test was performed with Q–Q plots and normality plots. The mean was determined if the data showed a normal distribution; otherwise, the median was calculated. The means of the two groups were compared using Student's t test, while the medians of the two groups were compared using the Mann–Whitney U test. Pearson's correlation test was used to assess the correlation between the variables. The difference was statistically significant if the p value was less than 0.05.

RESULTS

5.1. PGM enzyme activity was reduced in T2DM:

PGM enzyme activity was measured in PBMCs from T2DM patients (*n* = 63) and healthy subjects (*n* = 63). The PGM enzyme activity between the study groups is graphically represented in **Figure 5.1.** PGM enzyme activity showed a normal distribution. Therefore, the mean and standard deviation (Mean ± S.D) were calculated for both study groups. The PGM enzyme activity in the T2DM group was 0.7 ± 0.2 mU/mg of protein lysate, and the healthy control group showed 0.9 ± 0.3 mU/mg of lysate protein. The PGM enzyme activity was 0.9 times lower in the T2DM group than in the healthy control group. The difference in the PGM enzyme activity between the two groups was statistically significant ($p = 0.043$; Student's t test).

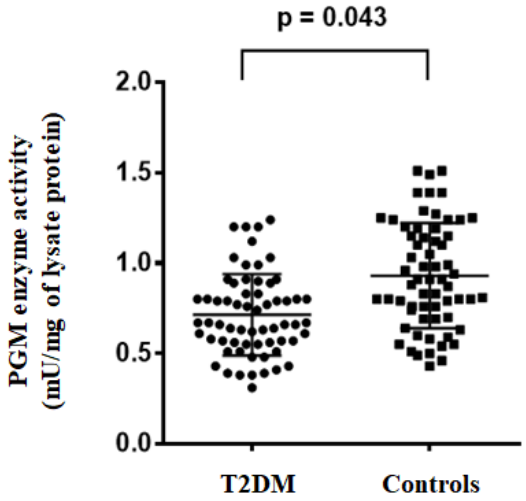

Figure 5.1: PGM enzyme activity in the PBMC protein lysate of the study groups

The impact of clinico-biochemical variables on PGM enzyme activity was checked by correlation analysis. The data showed a normal distribution; therefore, Pearson's correlation test was used. The data are presented in **Figure 5.2.** Individually, **A)** the correlation between PGM enzyme activity and fasting blood sugar levels was negatively correlated, and the effect was moderate (p = -0.36; r = 0.016; Pearson's

correlation test). **B.** The correlation between PGM enzyme activity and random blood sugar levels was negatively correlated, and the effect was moderate (p = -0.39; r = 0.019; Pearson's correlation test). **C.** The correlation between PGM enzyme activity and postprandial blood sugar levels was negatively correlated, and the effect was moderate (p = -0.41; r = 0.011; Pearson's correlation test). **D.** The correlation between PGM enzyme activity and glycated hemoglobin A1c levels was negatively correlated, and the effect was moderate (p = -0.35; r = 0.028; Pearson's correlation test).

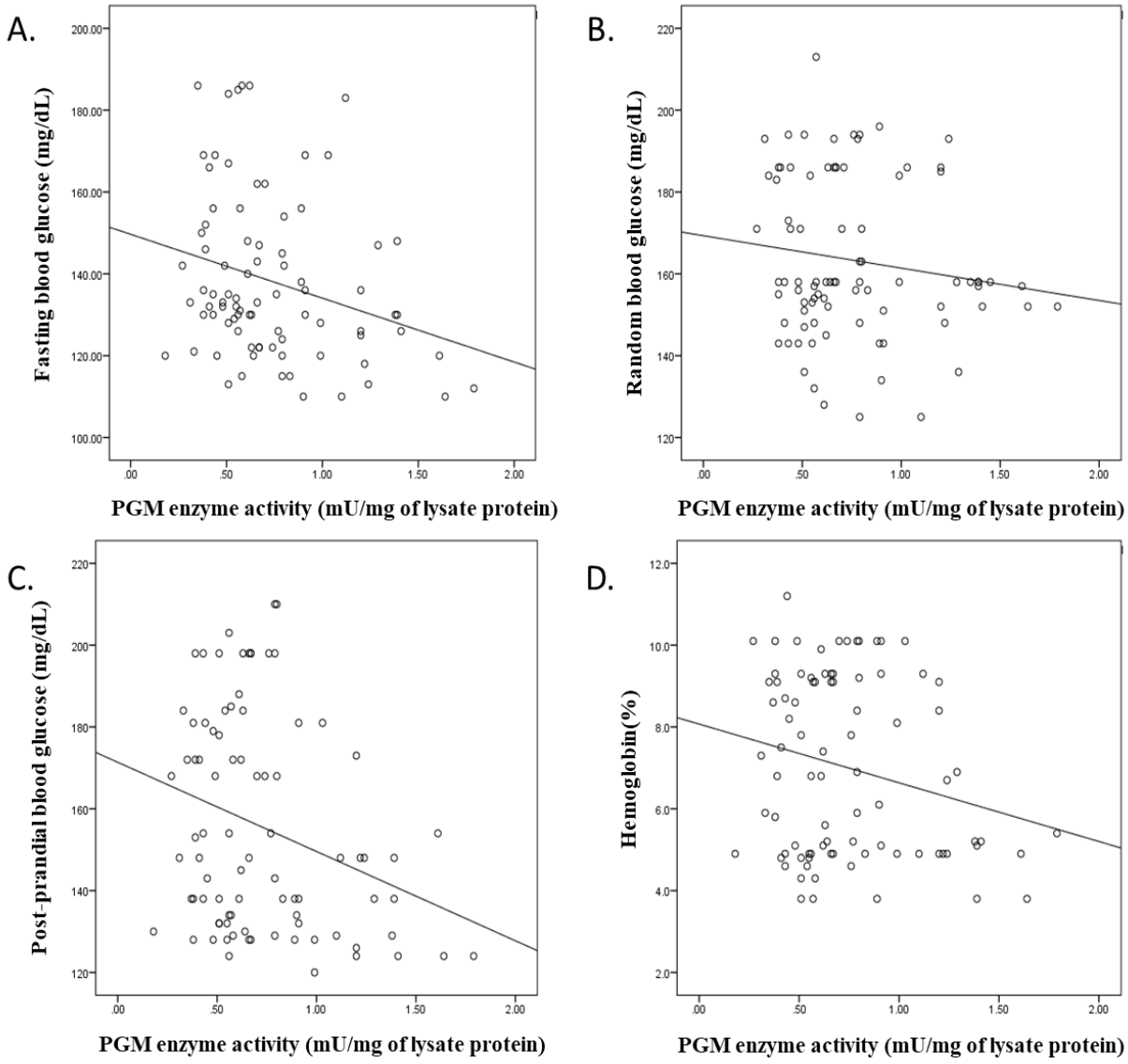

Figure 5.2: Correlation between PGM enzyme activity and clinico-biochemical variables

5.2. *PGM1* gene expression is downregulated in T2DM:

PGM1 gene expression was measured in the PBMCs of both study groups (n = 63). The *PGM1* gene expression in both groups is graphically represented in **Figure 5.3.** *PGM1* gene expression showed a normal distribution. Therefore, the mean and standard deviation (Mean ± S. D) were calculated for both study groups. Therefore, the mean delta Ct (ΔCt) was calculated for both groups. The fold change in *PGM1* gene expression was calculated by following the comparative Ct method. The fold change ($2^{-\Delta\Delta Ct}$) of *PGM1* gene expression was 0.34 in the T2DM group and 0.57 in the control group. The results are presented in **Table 5.1.** The fold change in the T2DM group was calculated by considering the control group as the reference. *PGM1* gene expression was downregulated in T2DM patients compared to healthy subjects (fold difference = 0.59; $p = 0.032$; unpaired t test).

Table 5.1: *PGM1* gene expression in the study groups

Study group	ΔCt	ΔΔCt	Fold change ($2^{-\Delta\Delta Ct}$)
T2DM	3.18 ± 1.7	2.11	0.34
Control	3.23 ± 1.0	1.25	0.57

$\Delta\Delta Ct_{(T2DM)} = \Delta Ct_{(T2DM)} - \text{Average } \Delta Ct_{(Control)}$

$\Delta\Delta Ct_{(Controls)} = \Delta Ct_{(Control)} - \text{Average } \Delta Ct_{(Control)}$

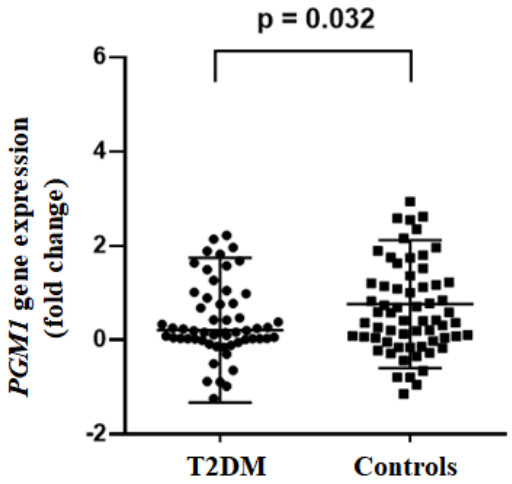

Figure 5.3: *PGM1* gene expression in the PBMCs of the study groups

Correlation analysis was carried out between PGM enzyme activity and *PGM1* gene expression in both study groups. The data showed a normal distribution; therefore Pearson's correlation test was used to measure the levels of both study groups. The results are graphically represented in **Figure 5.4.** The two parameters showed statistically significant moderate positive correlations (r = 0.35; p = 0.016; Pearson's correlation test).

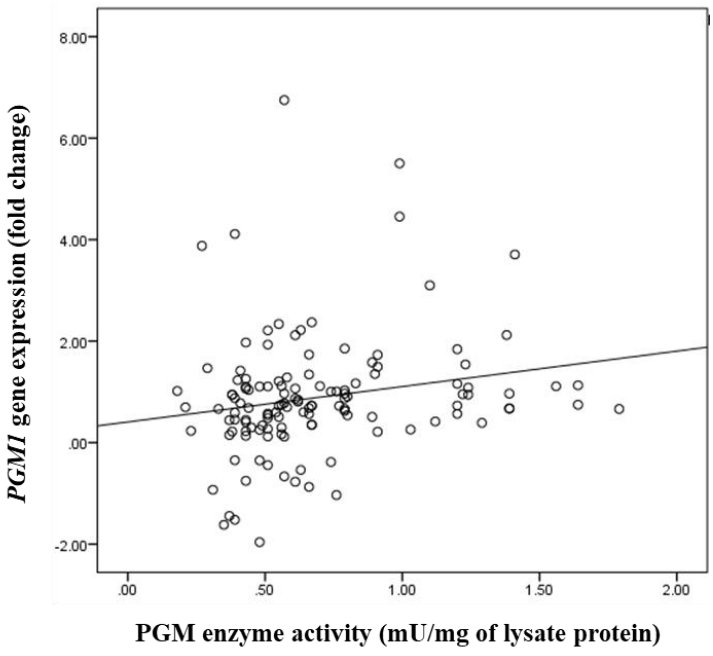

Figure 5.4: Correlation between *PGM1* gene expression and PGM enzyme activity

5.3. SNP rs11208257 associated with T2DM:

The distribution of both alleles and the genotypic frequency of the *PGM1* SNP rs11208257 were determined in the genomic DNA in both study groups. Hardy-Weinberg equilibrium for the genotype frequency was carried out for control samples ($\chi 2 = 6.75$). The frequency of 32.4% was seen with the minor allele 'C', which is common among the control group. The distribution of both genotype and allele frequencies showed a statistically significant difference between the two groups. The frequency of the minor allele 'C' was 44.8% in T2DM patients and 32.4% in the healthy controls. The frequency of minor alleles was 1.3 times higher in T2DM patients. The results are summarized in **Table 5.2.**

Table 5.2: Distribution of SNP rs11208257 in the study groups

Genotype/ Allele	Controls (n = 225)	T2DM (n = 225)	P- value*	OR# (0.95 CI)
TT	155	134	**0.036**	NA
TC	67	81		
CC	3	10		
T	377	349	**0.018**	**1.5 (1.07 - 2.08)**
C	73	101		

* Chi-square, two-tailed (Fisher's exact test)
# OR: Odds ratio; CI: confidence intervals; NA: Not applicable

The association of genetic variation of SNP rs11208257 with various genetic models showed that the highest difference in terms of odds ratio was observed in the case of the additive genetic model. A schematic representation showing the genetic model is shown in **Table 5.3.**

Table 5.3: Evaluation of the association between *PGM1*p. Tyr420His SNP and T2DM under different genetic models

Model	Genotype	*P* value	Odds Ratio #
Dominant	TC + CC vs. TT	**0.028**$	2.4 (0.93 - 12.6)
Recessive	CC vs. TC + TT	0.24	-
Additive	TT *vs.* CT *vs.* CC	**0.012**&	1 < 1.4 < 2.86

$ Chi-square, two-tailed (Fisher's exact test)

& Mantel–Haenszel Chi-square test for linear trend

Parentheses with 95% confidence intervals

The representative band patterns of PCR-RFLP genotyping of the *PGM1* gene variant p. Tyr420His SNP are shown in **Figure 5.5.**

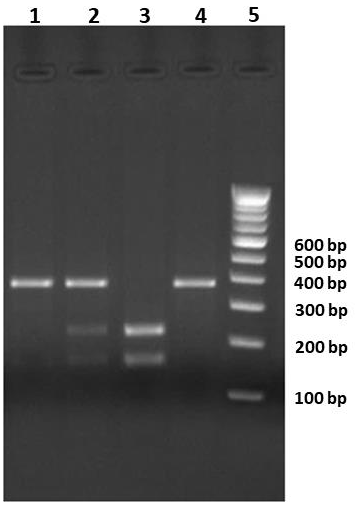

Figure 5.5: Representative PCR-RFLP band pattern of the *PGM1* rs11208257 SNP

Lane 1 represents the TT genotype (375 bp). **Lane 2** represents the TC genotype (375 bp, 226 bp, and 149 bp). **Lane 3** represents the CC genotype (226 bp and 149 bp). **Lane 4** represents an undigested PCR amplicon (375 bp). **Lane 5** represents 100 bp ladder

The association of the genotypic frequency of SNP rs11208257 with PGM enzyme activity was carried out between the study groups, i.e., irrespective of the study groups (n = 126), and the analysis was also carried out for each study group separately (n = 63). The average PGM enzyme activity showed a normal distribution. The data are presented as the mean ± SD. The SNP rs11208257 shows TT, TC, and CC genotypes with PGM enzyme activities of 0.71, 0.54, and 0.15 mU/mg of protein lysate, respectively. The difference between the groups was statistically significant (p = 0.023; Multiple logistic regression). The difference between the groups was statistically significant (p = 0.016; Multiple logistic regression). The data are presented in **Table 5.4.** The effect of SNP rs11208257 on PGM enzyme activity is graphically represented in **Figure 5.6.**

Table 5.4: The association of SNP rs11208257 with PGM enzyme activity

Genotype	**Irrespective of study groups (n = 126)**	**PGM enzyme activity (mU/mg of protein lysate) (n = 126)**	***p* value***
TT	83	0.71 ± 0.21	0.023
TC	39	0.52 ± 0.09	
CC	4	0.15 ± 0.05	

* Multiple logistic regression

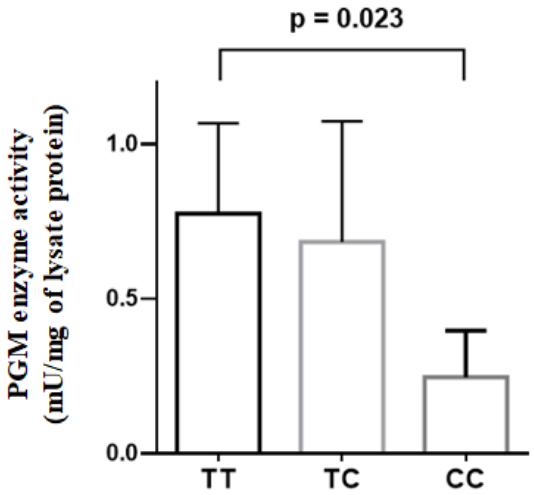

Figure 5.6: Effect of SNP rs11208257 on PGM enzyme activity

Furthermore, the association of SNP rs11208257 and PGM enzyme activity showed a statistically significant difference in the T2DM group (p = 0.017; Multiple logistic regression) and the control group (p = 0.001; Multiple logistic regression). The data are presented in **Table 5.5.** The effect of SNP rs11208257 on enzyme activity in the T2DM and control groups is separately and graphically represented in **Figure 5.7 (a) and (b).**

Table 5.5: The association of SNP rs11208257 with PGM enzyme activity in the study groups

Genotype	T2DM (n = 63)	Enzyme activity (T2DM)	p value*	Controls (n = 63)	Enzyme activity (Controls)	p value*
TT	43	0.74 ± 0.21	0.017	31	0.68 ± 0.20	0.001
TC	17	0.55 ± 0.20		31	0.66 ± 0.17	
CC	3	0.17 ± 0.04		01	0.46 ± 0	

* Multiple logistic regression

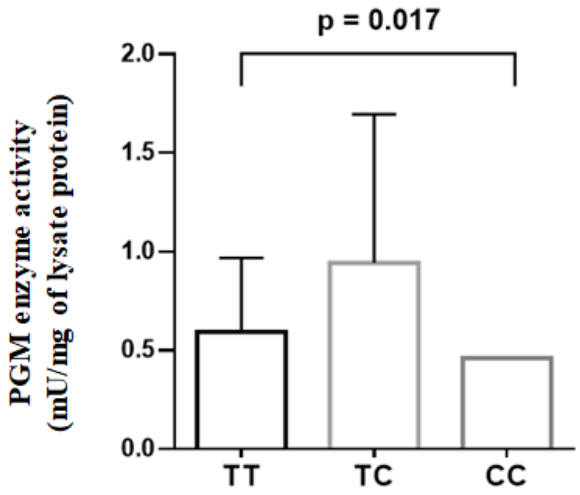

Figure 5.7 (a): Effect of the *PGM1* SNP rs11208257 on PGM enzyme activity in the T2DM group

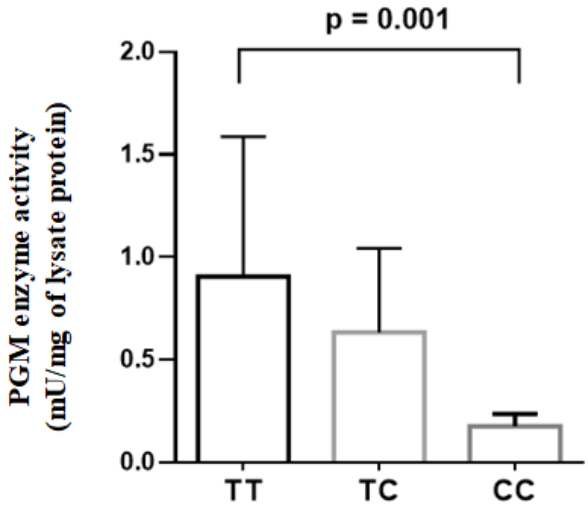

Figure 5.7 (b): Effect SNP rs11208257 on PGM enzyme activity in the control group

5.6. Relationship between PGM enzyme activity, gene expression, and SNP rs11208257:

Stepwise regression was used to analyze the combinatorial impact of *PGM1* gene expression and SNP rs11208257 on PGM enzyme activity. The R-square value was used to check the predictive power of independent variables (*PGM1* gene expression and SNP rs11208257) in determining the dependent variable (PGM enzyme activity).

The results are presented in **Table 5.6.** *PGM1* gene expression was found to determine PGM enzyme activity with a power of 6%, and the power was statistically significant (p = 0.007). Furthermore, SNP rs11208257 was found to determine PGM enzyme activity with a power of 11%, and the power was statistically significant (p = 0.012). Together, *PGM1* gene expression and SNP rs11208257 were capable of determining PGM enzyme activity with a power of 21%, and the determining power was statistically significant (p = 0.001).

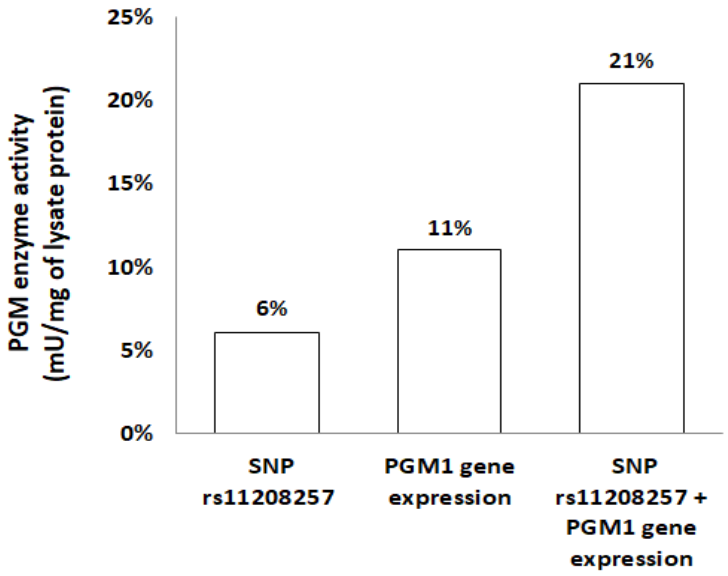

Figure 5.8: Stepwise regression analysis for the determinant of PGM enzyme activity

5.4. PBMC viability under glucose-limiting conditions was reduced in T2DM:

The PBMC viability was measured under glucose-limiting conditions. The first measurement was carried out before glucose deprivation (i.e., under glucose-proficient conditions), and the second measurement was carried out under glucose-limiting conditions.

The percentage of viable cells was used as the measure of cell viability. Cell viability levels showed a normal distribution. Therefore, the mean and standard deviation were

calculated for both groups. Before the induction of glucose deprivation, the mean cell viability levels were 81% in the T2DM group and 83% in the healthy control group. There was no statistically significant difference between the two groups (p = 0.144; Student's t test). This indicates that the baseline levels of cell viability in the two groups were comparable before the induction of glucose deprivation. After inducing glucose deprivation, the mean cell viability levels were 72.3 ± 5.3 in the T2DM group and 87.1 ± 5.7 in the healthy control group. The difference in the cell viability levels of the two groups was statistically significant (p = 0.001; Student's t test). The results are graphically represented in **Figure 5.9.**

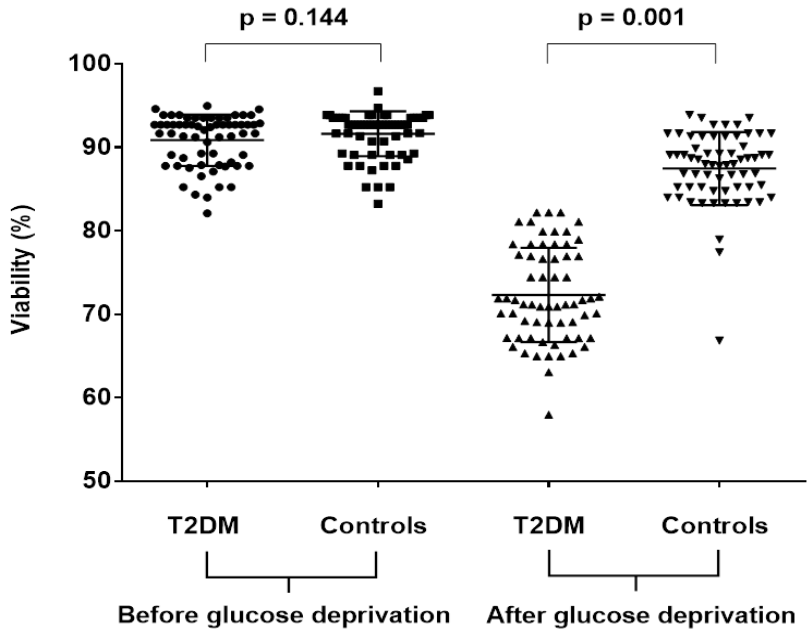

Figure 5.9: PBMC viability under glucose-limiting conditions

The percentage of cell viability in the T2DM group was 70.6 ± 4.7 and 86.8 ± 5.2 in the healthy control group. The percentage of cell viability was 0.81 times lower in the T2DM group than in the healthy control group. The difference in the levels of the two groups was statistically significant (p = 0.001; Student's t test). This indicates that the cell viability levels under glucose limiting conditions were different in the two groups.

The percentage of cell viability in the two groups is graphically represented in **Figure 5.10.**

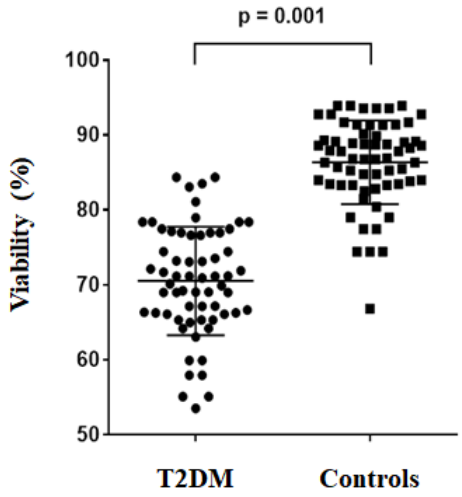

Figure 5.10: Glucose deprivation-induced viability of PBMCs from T2DM and healthy subjects

5.5 Glycogen degradation under glucose-limiting conditions is reduced in T2DM:

The glycogen levels were measured in PBMCs at two time intervals in T2DM and healthy subjects. The first measurement was carried out before glucose deprivation (i.e., under glucose-proficient conditions), and the second measurement was carried out under glucose-deprived conditions.

The glycogen levels showed a normal distribution. Therefore, the mean and standard deviation were calculated for both study groups. Before the induction of glucose deprivation, the mean glycogen levels were 81% in the T2DM group and 83% in the healthy control group. There was no statistically significant difference between the two groups (p = 0.432; Student's t test). This indicates that the baseline levels of glycogen in the two groups were comparable before the induction of glucose deprivation. After inducing glucose deprivation, the mean glycogen levels were 27.5 ± 7.2 in the T2DM group and 14.8 ± 5.6 in the healthy control group. There was a statistically significant

difference in the levels between the two groups (p = 0.001; Student's t test). The results are graphically represented in **Figure 5.11.**

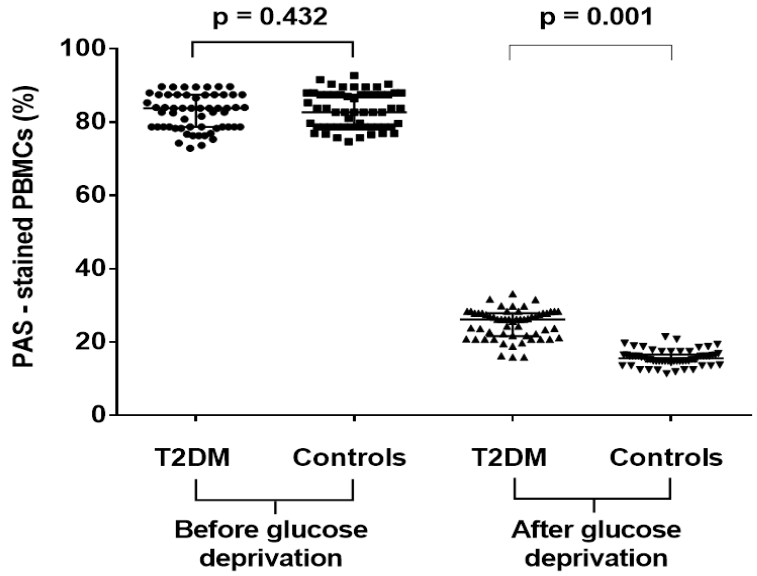

Figure 5.11: Glycogen levels in PBMCs under glucose limiting conditions

The glycogen degradation levels were 55.4 ± 3.5 in the T2DM group and 69.5 ± 4.2 in the healthy control group. The levels of glycogen degradation were 0.8 times lower in the T2DM patients than in the healthy control group. The difference between the two groups was found to be statistically significant (p = 0.001; Student's t test). This indicates that the levels of glycogen degradation under glucose limiting conditions were different in the two groups. The levels of glycogen degradation in the two groups are graphically represented in **Figure 5.12.**

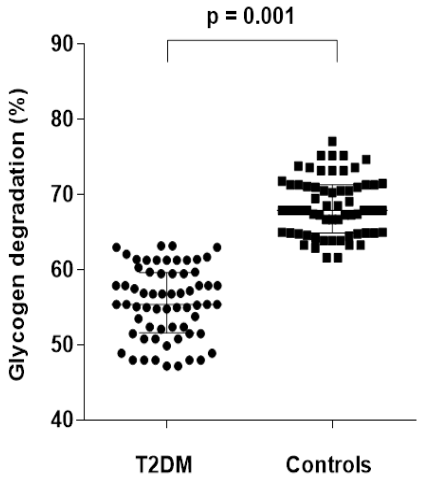

Figure 5.12: Glycogen degradation in PBMCs from T2DM and healthy controls

Correlation analysis was carried out between PBMC viability and glycogen degradation after combining the data from both study groups. Pearson's correlation test was used, as the data showed a normal distribution. The graphical representation of the results is given in **Figure 5.13.** The two parameters showed statistically significant correlation (r = - 0.46; p = 0.012; Pearson's correlation test). The correlation was negative, i.e., reciprocal in the relationship and the magnitude of the correlation was moderate. Furthermore, correlation analysis was also carried out for each study group separately. The correlation was statistically significant in both the T2DM (r = - 0.41; p = 0.008; Pearson's correlation test) and control (r = - 0.36; p = 0.013; Pearson's correlation test) groups. However, the correlation between the two groups was statistically significant, and the magnitude of the correlation was moderate.

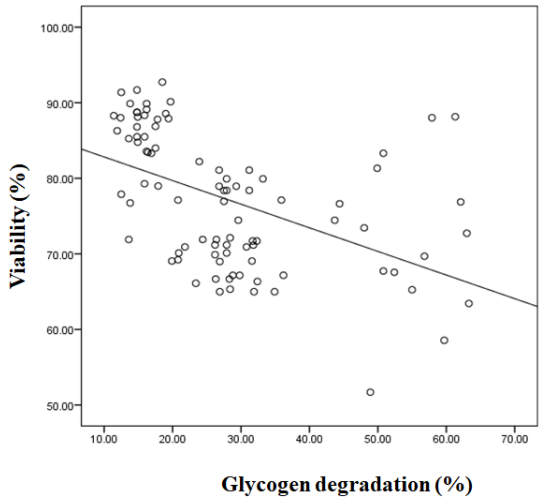

Figure 5.13: Correlation between glycogen degradation and viability in PBMCs under glucose limiting conditions

Correlation analysis was carried out between PBMC viability and PGM enzyme activity after combining the data from both study groups. Pearson's correlation test was used, as the levels of both study parameters showed a normal distribution. The graphical representation of the results is given in **Figure 5.14.** The two parameters showed statistically significant moderate positive correlations (r = 0.37; p = 0.019; Pearson's correlation test).

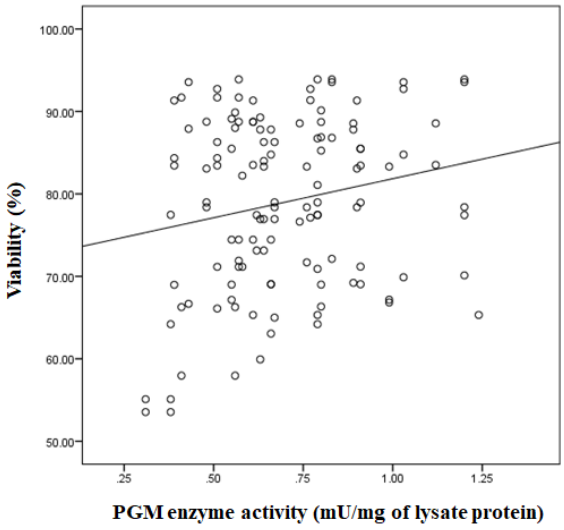

Figure 5.14: Correlation between PBMC viability and PGM enzyme activity under glucose-limiting conditions

5.6. Relationship between PGM enzyme activity, PBMC viability, and glycogen degradation:

Stepwise regression was used to analyze the combinatorial effect of PGM enzyme activity and glycogen degradation on PBMC viability. The results are presented in **Figure 5.15**. The R-square value was used to check the power of the independent variables (PGM enzyme activity and glycogen degradation) in determining the dependent variable (PBMC viability). PGM enzyme activity could determine PBMC viability with a power of 12%, and the power was statistically significant (p = 0.015). Furthermore, glycogen degradation could determine PBMC viability with a power of 16%, and the power was statistically significant (p = 0.001). Together, PGM enzyme activity and glycogen degradation were capable of determining PBMC viability with a power of 23%, and the power was statistically significant (p = 0.001).

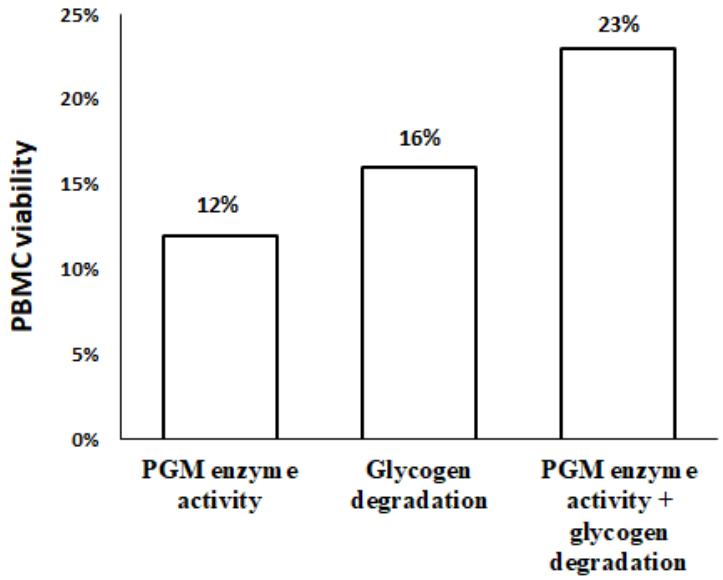

Figure 5.15: Stepwise regression analysis for the determinant of PBMC viability.

DISCUSSION

The purpose of this study was to evaluate the role of PGM in T2DM by a combination of biochemical, genetic, and gene expression studies. The main findings of this study are as follows:

a) PGM enzyme activity is reduced in T2DM
b) *PGM1* gene expression is downregulated in T2DM
c) *PGM1* rs11208257 SNP is associated with T2DM
d) Cell viability and glycogen degradation under glucose-limiting conditions are reduced in T2DM

The inference and significance of each finding are discussed individually below:

Inference 1: PGM enzyme activity is suboptimal in T2DM

PGM enzyme activity was found to be lower in T2DM patients than in healthy subjects. This indicates that suboptimal PGM enzyme activity is associated with T2DM. Furthermore, PGM enzyme activity also showed a correlation with the indices of glycemic control, viz., fasting blood glucose, random blood glucose, postprandial blood glucose, and glycated hemoglobin (figure 5.2). The correlation was negative or reciprocal in nature. This relationship indicates that the PGM enzyme may be involved in regulating blood glucose levels. PGM enzymes are involved in regulating blood glucose levels by facilitating glycogen synthesis. Blood glucose can be lowered only when it is absorbed by the cells. Furthermore, the cells can absorb glucose only if they can metabolize the excess glucose or store it in the form of glycogen. PGM is an important enzyme involved in the storage of excess blood sugar in the form of glycogen inside cells. The observation of the negative relationship between parameters of blood sugar regulation and the PGM enzyme is in conformity with the mechanism

by which the PGM enzyme contributes to its regulation. However, the effect size of the correlation between PGM enzyme activity and indices of glycemic control was moderate. The limited effect size indicates that the PGM enzyme may not be the principal factor but one of the several factors involved in regulating blood glucose levels.

The current understanding is that the impairment of the glycogen pathway in T2DM arises mainly due to defects in enzymes such as glycogen synthase and glycogen phosphorylase [Hojlund et al., 2009; Damsbo et al., 1991]. The results of this study support the inclusion of PGM in the list of enzymes responsible for the impairment of the glycogen pathway in T2DM.

Inference 2: Suboptimal PGM enzyme activity linked to reduced gene expression

PGM1 gene expression was found to be lower in the PBMCs of T2DM patients than in those of healthy controls. This indicates that the *PGM1* gene is downregulated in T2DM (figure 5.3). Furthermore, a positive correlation was found between PGM1 enzyme activity and *PGM1* gene expression. This relationship indicates that suboptimal PGM enzyme activity may arise due to the downregulation of the corresponding *PGM1* gene. However, the effect size of the correlation was moderate. The limited effect size indicates that downregulated gene expression may not be the major factor but one of the several factors responsible for the reduction in PGM enzyme activity.

Inference 3: Suboptimal PGM enzyme activity linked to gene polymorphism

The minor allele frequency of SNP rs11208257 was significantly higher among T2DM patients than among healthy volunteers (Table 5.2). This indicates that SNP

rs11208257 is associated with the risk of T2DM. This relationship is expected since this SNP has been shown to reduce the structural stability of the PGM enzyme.

Further analysis was carried out to determine whether this SNP may be responsible for the reduction in PGM enzyme activity in T2DM. This SNP results in c.1258 T>C substitution in the cDNA, which then leads to p. Y420H substitution in the protein chain. The protein chain with histidine at position 420 in the protein chain is thermodynamically less stable than the protein chain with tryptophan [Cheng et al., 2006]. Therefore, the C allele of this SNP is the risk allele. The average PGM enzyme activity was highest in the PBMCs of individuals who were homozygous for the major allele (TT) and lowest in individuals who were homozygous for the minor allele (CC). Intermediate levels were found in the individuals who were heterozygous (TC). The difference in the average PGM enzyme activity among the three groups was statistically significant. This relationship indicates that SNP rs11208257 may contribute to the reduction of PGM enzyme activity.

The association between SNP rs11208257 and T2DM was evaluated by using various genetic models (Table 5.3). Statistically, a significant association was seen in the case of dominant and additive genetic models but not with recessive models. This indicates that a single copy of the risk allele is sufficient to predispose the individual to develop T2DM. Furthermore, the additive model agrees with the progressive reduction of the PGM enzyme activity with increasing copy number of the risk alleles.

The role of genetic variation in the *PGM1* gene in the pathogenesis indicated by this study also agrees with a previous report. Inshaw and coworkers showed that T2DM is associated with SNP rs2269247 located in the intergenic region 0.5 Mb from the

PGM1 gene [Inshaw et al., 2021]. The implications of this report were limited since a tag SNP was discovered. In contrast, this study provides evidence for a functional SNP in the *PGM1* gene.

Reduction in PGM enzyme activity was linked to both downregulation of *PGM1* gene expression and SNP rs11208257. Stepwise regression analysis showed that *PGM1* gene expression and SNP rs11208257 had an additive effect in determining PGM enzyme activity (figure 5.8).

Inference 4: Suboptimal PGM enzyme activity reduces cell viability under glucose-limiting conditions

Experiments were carried out to determine whether PGM enzyme activity affects cell physiology. Cytoplasmic glycogen becomes the main source of glucose when the extracellular supply is limited. The PGM enzyme plays a key role in mobilizing glucose from glycogen. Therefore, a reduction in PGM enzyme activity may compromise glucose mobilization from glycogen and reduce cell viability under glucose-limiting conditions. In agreement with this hypothesis, a positive correlation was observed between PGM enzyme activity and cell viability (figure 5.14). However, the effect size was moderate. This indicates that PGM enzyme activity may not be the only factor responsible for cell viability under glucose-limiting conditions. The role of the PGM enzyme in cell viability indicated by this study agrees with similar observations in tumor cells [Jin et al., 2018]. Under low nutritional conditions, knockdown of the *PGM1* gene in HEK293K, MCF-7, and HeLa cell lines was found to reduce cell proliferation [Bae et al., 2014].

Next, the role of glycogen degradation as an additional factor determining cell viability was checked. Glycogen degradation was reduced in the PBMCs of T2DM patients (figure 5.13). Additionally, glycogen degradation showed a negative correlation with PBMC viability. However, the effect size was moderate, indicating that glycogen degradation may be one of the several factors responsible for cell viability under glucose-limiting conditions. The reduction in PBMC viability was linked to both reduced PGM enzyme activity and glycogen degradation. Stepwise regression analysis showed that PGM enzyme activity and glycogen degradation have an additive effect in determining PBMC viability (figure 5.15). This study shows that PGM enzyme activity is suboptimal in T2DM, probably due to downregulated gene expression and genetic variation. Furthermore, suboptimal PGM enzyme activity has been shown to impair cell viability under glucose-limiting conditions.

SUMMARY AND CONCLUSION

T2DM is a common metabolic disorder that arises due to the disruption of glucose homeostasis. In addition to other mechanisms, the glycogen pathway regulates glucose homeostasis by converting excess glucose into glycogen. The phosphoglucomutase (PGM) enzyme catalyzes the key reaction that links the glycogen pathway and glucose metabolism. Studies have shown that the glycogen pathway is abnormal in diabetes. The purpose of this study was to determine the role of PGM in T2DM by a combination of biochemical, genetic, and gene expression studies.

This study was carried out by following the case–control design. The case group (n = 63) comprised T2DM patients, while the control group (n = 63) comprised healthy individuals. Experimental studies were carried out using PBMCs since the transcriptional profile of PBMCs in T2DM patients has been shown to correlate with the pathophysiology of the disease.

PGM enzyme activity was measured in the PBMC lysate. PGM enzyme activity was found to be comparatively reduced in T2DM patients compared to healthy controls (p = 0.043; Student's t test). Furthermore, PGM enzyme activity showed a reciprocal relationship with the indices of glycemic controls. Then, *PGM1* gene expression and a common functional variation in the *PGM1* gene (SNP rs11208257; p. Tyr420His) were explored as the likely sources for the reduced PGM enzyme activity. *PGM1* gene expression was downregulated in T2DM patients compared to healthy subjects (fold difference = 0.59; p = 0.032; unpaired t test). A positive correlation was observed between PGM enzyme activity and *PGM1* gene expression (r = 0.35; p = 0.016; Pearson's correlation test). Furthermore, SNP rs11208257 was found to be associated with T2DM (p = 0.001; Student's t test). The PGM enzyme activity was relatively

lower in samples with the TC and CC genotypes (0.54 mU/mg of protein lysate) than in samples with the TT genotype (0.69 mU/mg of protein lysate) (p = 0.018; Multiple logistic regression).

The physiological impact of reduced PGM enzyme activity on cell viability was evaluated under glucose-limiting conditions. It was found that PBMC viability was reduced in T2DM patients compared to healthy subjects (p = 0.001; Student's t test). The glycogen degradation showed a reciprocal relationship with PBMC viability, and the effect was moderate (r = -0.46; p = 0.012; Pearson's correlation test). This relationship indicates that glycogen degradation is one of the several factors responsible for PBMC viability under glucose-limiting conditions. There was a positive correlation between PGM enzyme activity and PBMC viability under glucose-limiting conditions (p = 0.001, r = 0.35; Pearson's correlation test). This indicates that reduced PGM enzyme activity affects PBMC viability under glucose-limiting conditions.

This study shows that PGM enzyme activity is suboptimal in T2DM, probably due to downregulated gene expression and genetic variation. Furthermore, suboptimal PGM enzyme activity is shown to impair cell viability under glucose-limiting conditions. This study adds the PGM enzyme to the list of defects that are responsible for impairing the glycogen pathway in T2DM. This is the first attempt to link the PGM enzyme with the pathogenesis of T2DM.

LIMITATIONS OF THE STUDY

The glycogen pathway is directional. It involves both synthesis and degradation. However, only the degradation component was measured. The PGM enzyme does not have a direct role in glycogen degradation. Instead, it is necessary for channelling glucose produced from glycogen degradation to glycolysis. However, the PGM enzyme is necessary for glycogen synthesis. Excess glucose can be converted into glycogen only if the PGM enzyme can channel it into the glycogen pathway. Future studies should check for the relationship between PGM enzyme activity and glycogen synthesis. This aspect will confirm the role of PGM enzyme activity in impairing the glycogen pathway in T2DM.

NEW KNOWLEDGE GENERATED

a) This study provides the first evidence to link PGM with T2DM through a combination of biochemical, gene expression, genetic and cellular studies.

b) This is the first study to explore the status of glycogen degradation in PBMCs. The results of this study establish the use of PBMCs as surrogate cells for studying glycogen metabolism in T2DM.

RECOMMENDATIONS

Overall, the results of this study support the conclusion that phosphoglucomutase is suboptimal in type 2 diabetes mellitus, probably due to downregulated gene expression and destabilizing genetic variation. Furthermore, the suboptimal activity of phosphoglucomutase appears to impair PBMC viability under glucose-limiting conditions.

- This study highlights the importance of phosphoglucomutase in the pathogenesis of type 2 diabetes mellitus.
- Phosphoglucomutase may be considered a target for developing anti-diabetic drugs.

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LIST OF PRESENTATIONS AND PUBLICATIONS

PRESENTATIONS:

1. Praveen Kumar KS, Prabhakar K, Sharath B. Novel association of *PGM1* gene variant with type 2 diabetes mellitus. Poster session presented at 88th Annual meeting of the Society of biological chemists, India (SBCI-2019) and conference on Advances at the Interface of Biology & Chemistry; 2019 Oct 31 to Nov 3; DAE Convention Centre, Anushaktinagar, Mumbai

2. Praveen Kumar KS, Prabhakar K, Sharath B. Enzyme activity of phosphoglucomutase1 in type 2 diabetes mellitus. Oral session presented at ABGCON 2021, National virtual conference and workshop; 2021 Aug 27 to Aug 28; Sri Balaji Medical College and Hospital, Chennai

PUBLICATIONS:

1. Praveen Kumar K S, Kamarthy P, Balakrishna S, Manu M S, Ramaswamy S. Association between phosphoglucomutase-1 gene Y420H polymorphism and type 2 diabetes mellitus: A Case-control study. Arch Med Health Sci. 2021; 9: 225-228

2. Praveen Kumar K S, Prabhakar K, Balakrishna S. Glucose deprivation-induced glycogen degradation and viability are altered in peripheral blood mononuclear cells of type 2 diabetes patients. Ukrainian Biochemical Journal. (Manuscript accepted)

3. Praveen Kumar K S, Prabhakar K, Balakrishna S. Reduced phosphoglucomutase1 activity impairs the survival of peripheral blood mononuclear cells under glucose-limiting conditions in diabetic patients. Manuscript submitted.

MASTER CHART

Master chart: T2DM patients

Sl. No	code	(M/F)	FBS	PPBS	HbA_{1C}	Genotype	Cell Viability	Enzyme activity	*PGM1* gene expression	Gly deg T2DM
1	DC1	F	131	185	9.1	TT	71.9	0.57	0.95	61.7
2	DC2	M	125	173	9.1	TT	70.1	1.20	7.22	55.4
3	DC3	M	124	210	10.1	TT	70.9	0.79	3.25	51.5
4	DC4	F	169	181	11.2	TT	76.6	0.74	3.15	47.2
5	DC5	M	135	198	7.8	TT	66.1	0.51	4.25	56.8
6	DC6	M	186	172	9.1	TC	69.0	0.39	7.04	61.3
7	DC7	F	142	168	10.1	TC	74.4	0.57	3.25	51.5
8	DC8	F	122	198	9.3	TC	65.0	0.67	2.96	47.2
9	DC 9	M	133	148	9.1	TT	63.1	0.66	0.54	56.8
10	DC10	F	128	128	8.1	TC	67.2	0.99	6.74	61.3
11	DC11	M	136	138	9.3	TT	78.9	0.91	5.42	61.4
12	DC12	F	186	138	10.1	TT	69.2	0.89	8.68	63.2
13	DC13	F	206	126	8.4	TT	78.4	1.20	5.34	59.5
14	DC14	M	156	138	10.1	TC	66.7	0.43	6.02	56.9
15	DC15	F	130	138	8.6	CC	77.1	0.77	5.34	55.1
16	DC16	M	154	210	9.2	TT	66.3	0.80	5.16	57.9
17	DC17	M	169	181	10.1	TC	69.9	1.03	4.09	55.3
18	DC18	M	135	198	8.7	TT	72.1	0.83	8.06	55.4
19	DC19	F	162	198	9.3	TT	69.0	0.66	6.22	48.9
20	DC20	F	169	181	10.1	TC	71.2	0.91	8.19	50.8
21	DC21	M	135	198	7.8	TT	71.7	0.76	5.18	48.0
22	DC22	F	186	172	9.1	TC	82.2	0.58	8.78	59.7
23	DC23	M	142	168	10.1	TT	81.1	0.79	4.88	55.0
24	DC24	M	122	198	9.3	TT	59.9	0.63	2.90	52.4
25	DC25	F	186	172	9.1	TC	67.1	0.55	6.63	63.0
26	DC26	F	142	168	10.1	TT	77.0	0.64	7.06	52.4
27	DC27	M	148	188	9.9	TT	65.3	0.61	2.31	52.1
28	DC28	M	184	178	9.3	TT	71.2	0.51	3.22	50.8
29	DC29	F	132	179	8.6	TC	78.4	0.48	3.67	48.0
30	DC30	M	185	203	9.2	TT	58.0	0.56	5.98	49.9
31	DC31	F	136	138	9.3	TT	64.2	0.38	0.11	61.3
32	DC32	M	133	148	7.3	TC	55.1	0.31	4.29	53.8
33	DC33	F	169	181	10.1	TC	53.5	0.38	6.88	57.9
34	DC34	M	135	198	6.9	TC	77.5	0.79	5.20	59.5
35	DC35	M	186	172	7.5	TC	66.3	0.41	3.05	55.0
36	DC36	F	142	168	10.1	CC	83.1	0.90	1.16	56.8
37	DC37	F	122	198	6.8	TT	84.3	0.39	4.47	53.5
38	DC38	M	133	148	9.3	CC	83.5	1.12	6.45	57.9
39	DC39	F	186	172	7.4	TT	73.1	0.62	2.09	48.0

40	DC40	M	142	168	10.1	TT	69.0	0.80	5.69	52.4
41	DC41	F	122	198	9.1	TT	77.0	0.8	3.09	54.8
42	DC42	M	133	148	6.7	TT	65.3	0.7	3.05	57.9
43	DC43	F	186	172	9.3	TC	71.2	1.0	1.16	48.0
44	DC44	F	162	198	10.1	TC	78.4	1.7	4.47	60.3
45	DC45	M	133	148	7.8	TT	69.0	1.4	5.20	55.4
46	DC46	M	133	169	7.6	TT	76.6	0.99	6.02	51.5
47	DC47	F	128	178	7.6	TT	66.1	0.91	5.34	57.2
48	DC48	F	136	163	7.3	TT	69.0	0.89	5.16	54.8
49	DC49	M	152	198	6.8	TT	74.4	1.20	4.09	61.3
50	DC50	F	142	176	8.1	TT	65.0	0.43	8.06	61.4
51	DC51	F	132	184	8.1	TT	63.1	0.77	6.22	63.2
52	DC52	F	130	195	8.3	TC	67.2	0.80	8.19	59.5
53	DC53	M	132	176	7.8	TC	78.9	1.03	5.18	56.9
54	DC54	M	138	184	7.9	TT	69.2	0.83	2.31	62.1
55	DC55	M	126	176	7.3	TT	78.4	0.79	3.22	57.9
56	DC56	M	128	162	7.9	TT	66.7	0.63	3.67	61.3
57	DC57	M	132	183	7.2	TT	71.2	0.55	5.98	55.4
58	DC58	F	120	194	8.1	TC	78.4	0.64	0.11	48.9
59	DC59	F	128	178	7.9	TC	58.0	0.61	7.29	50.8
60	DC60	F	128	168	8	TT	64.2	0.51	6.88	48.0
61	DC61	F	136	187	7.7	TC	55.1	0.48	5.2	59.7
62	DC62	F	138	198	8	TT	53.5	0.56	3.05	55.0
63	DC63	F	142	173	6.8	TT	78.9	0.80	5.42	57.5

Master chart: Healthy controls

Sl. No	patient code	(M/F)	FBS	PPBS	HbA_{1C}	Genotype	Cell Viability	Enzyme activity	*PGM1* gene expression	Gly deg Ctrl
1	NDC1	F	120	134	4.9	TT	66.8	0.80	3.62	61.7
2	NDC2	F	110	153	4.8	TT	83.4	0.59	4.34	55.4
3	NDC3	F	120	143	2.8	TT	88.6	0.32	4.95	51.5
4	NDC4	M	100	154	2.8	TC	77.4	0.51	3.79	47.2
5	NDC5	M	140	130	4.8	TT	87.9	1.24	4.18	56.8
6	NDC6	M	110	132	5	TT	91.4	0.74	6.86	61.3
7	NDC7	M	110	138	4.9	TT	85.3	0.88	3.04	51.5
8	NDC8	M	130	124	4.9	TC	92.7	0.69	3.98	47.2
9	NDC9	M	120	128	4.9	TC	93.6	1.51	1.42	56.8
10	NDC10	F	115	184	4.9	TT	93.9	1.39	2.16	61.3
11	NDC11	F	120	143	5.2	TT	87.8	0.49	2.98	61.4
12	NDC12	F	110	138	4.9	TT	85.5	0.91	4.56	63.2
13	NDC13	M	126	138	4.9	TC	83.3	0.79	5.85	59.5
14	NDC14	M	130	129	3.8	TT	74.4	0.63	3.89	56.9
15	NDC15	M	136	154	5.1	TT	86.3	0.54	5.16	55.1
16	NDC16	M	138	154	4.9	TT	88.8	1.05	4.02	57.9
17	NDC17	F	130	124	4.9	TC	89.9	0.64	5.54	55.3
18	NDC18	F	120	128	4.9	TT	90.1	0.32	1.41	55.4
19	NDC19	F	120	132	3.8	TC	86.9	0.80	4.05	48.9
20	NDC20	M	110	120	5.1	TT	88.0	0.81	4.41	50.8
21	NDC21	M	130	128	4.8	TT	79.0	1.24	2.73	48.0
22	NDC22	M	110	128	5.1	TT	91.7	0.83	1.57	59.7
23	NDC23	M	120	134	3.8	TC	91.3	0.69	4.78	55.0
24	NDC24	F	110	138	4.9	TT	84.0	0.60	3.56	52.4
25	NDC25	M	120	148	5.1	TT	89.1	1.15	2.77	63.0
26	NDC26	F	110	134	4.6	TC	89.3	0.46	3.78	52.4
27	NDC27	F	130	148	5.2	TC	86.7	0.80	3.27	52.1
28	NDC28	F	140	148	8	TT	86.8	1.25	3.51	50.8
29	NDC29	F	126	145	5	TT	84.8	0.76	-3.06	48.0
30	NDC30	F	115	184	4.8	TT	88.7	0.98	5.31	49.9
31	NDC31	M	130	124	5.2	TT	92.7	0.70	1.68	61.3
32	NDC32	F	130	129	4.9	TT	93.6	0.43	2.65	53.8
33	NDC33	F	130	128	5.1	TT	93.9	0.55	2.12	57.9
34	NDC34	M	126	138	3.8	CC	87.8	0.87	3.04	59.5
35	NDC35	F	130	129	4.9	TT	85.5	1.19	4.06	55.0
36	NDC36	F	130	124	5.2	TC	83.3	0.50	5.06	56.8
37	NDC37	F	120	128	4.9	TC	74.4	1.27	1.60	53.5

38	NDC38	M	120	132	4.9	TC	86.3	0.80	3.94	57.9
39	NDC39	M	100	154	5	TT	88.8	1.10	4.93	48.0
40	NDC40	F	140	130	4.3	TT	79.0	0.58	-1.71	52.4
41	NDC41	F	110	132	4.6	TT	91.7	0.76	0.65	54.8
42	NDC42	F	115	184	4.9	TT	91.3	0.70	2.12	57.9
43	NDC43	F	130	124	5.1	TT	83.4	0.98	3.04	48.0
44	NDC44	M	130	129	3.8	TT	88.6	1.70	1.06	60.3
45	NDC45	F	120	134	4.9	TC	77.4	1.43	3.94	55.4
46	NDC46	M	100	148	3.8	TT	88.8	1.15	2.77	51.5
47	NDC47	F	150	138	4.3	TT	89.9	0.96	3.78	57.2
48	NDC48	F	120	132	4.9	TT	90.1	0.80	3.27	54.8
49	NDC49	M	115	132	4.8	TT	86.9	1.25	4.41	61.3
50	NDC50	F	121	158	4.8	TT	88.0	0.76	2.73	61.4
51	NDC51	F	110	141	4.6	TT	79.0	0.98	1.57	63.2
52	NDC52	F	115	148	5	TC	91.7	1.12	4.78	59.5
53	NDC53	M	120	151	4.9	TC	91.3	1.03	3.56	56.9
54	NDC54	M	115	138	3.8	TT	84.8	1.55	2.77	62.1
55	NDC55	M	110	158	4.9	TT	88.7	1.51	3.78	57.9
56	NDC56	M	120	132	5.1	TT	92.7	1.39	3.27	61.3
57	NDC57	M	120	138	4.9	TT	93.6	1.14	3.51	55.4
58	NDC58	F	110	124	5.1	TC	93.9	0.91	2.16	48.9
59	NDC59	F	120	128	4.9	TC	87.8	0.79	0.98	50.8
60	NDC60	F	100	132	4.9	TT	85.5	1.10	4.56	48.0
61	NDC61	F	140	120	4.8	TC	77.4	0.94	5.85	59.7
62	NDC62	F	110	128	3.8	TT	87.9	0.91	3.89	55.0
63	NDC63	F	110	128	4.9	TT	87.8	1.49	0.98	57.5

APPENDICES

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SDUAHER

SRI DEVARAJ URS MEDICAL COLLEGE

Tamaka, Kolar

INSTITUTIONAL ETHICS COMMITTEE

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1. **Dr. D.E.Gangadhar Rao, (Chairman)** Prof. & HOD of Zoology, Govt. Women's College, Kolar,
2. **Dr. Sujatha.M.P, (Member Secretary),** Assoc. Prof. of Anesthesia, SDUMC,
3. Dr. C.S.Babu Rajendra Prasad, Prof. of Pathology, SDUMC
4. Dr. Srinivasa Reddy.P, Prof. & HoD of Forensic Medicine, SDUMC
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11. Dr. Talasila Sruthi, Assoc. Prof. of OBG, SDUMC
12. Dr. Mahendra.M , Asst. Prof. of Community Medicine, SDUMC
13. Dr. Mamata Kale, Asst. Professor of Microbiology, SDUMC

No. SDUMC/KLR/IEC/30/2019-20 **Date:06-06-2019**

PRIOR PERMISSION TO START OF STUDY

The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Ph.D study entitled **"Role of phosphoglucomutase 1 in type 2 diabetes mellitus an integrated biochemical, genetic and gene expression study"** being investigated by **Mr. Praveen Kumar.K.S,** Dr. Sharath.B & Dr. Prabhakar.K[1] in the Departments of Cell Biology and Molecular Genetics, & Medicine[1] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

Sujatha .M.P

Member Secretary

Member Secretary
Institutional Ethics Committee
Sri Devaraj Urs Medical College
Tamaka, Kolar.

Chairman

CHAIRMAN
Institutional Ethics Committee
Sri Devaraj Urs Medical College.
Tamaka, Kolar

PATIENT PROFORMA

Topic: "Role of phosphoglucomutase 1 in Type 2 Diabetes Mellitus: An Integrated Biochemical, Genetic and Gene Expression study"

Date:

Patient Data			
Patient's Name			
IP/OP Number			
Age			
Gender		□M □F	
Contact number			
Address			
Clinical Signs And Symptoms			
Hypertension		□Yes □ No □ Unknown	
Hyperglycemia/ Euglycemia		□Yes □ No □ Unknown	
Weight loss/ Fatigue		□Yes □ No □ Unknown	
Polyuria		□Yes □ No □ Unknown	
Polyphagia		□Yes □ No □ Unknown	
Polydipsia		□Yes □ No □ Unknown	
Systolic		Diastolic	
Laboratory Investigations			
RBS (mg/dL)		HDL(mg/dL)	
FBS (mg/dL)		LDL(mg/dL)	
PPBS (mg/dL)		HbA1c	
Serum Creatinine (mg/dL)		Urine sugar	
Other complications/ Remarks			

PART I: PATIENT INFORMATION SHEET

Name of the project :	Role of phosphoglucomutase 1 in Type 2 Diabetes Mellitus: An Integrated Biochemical, Genetic and Gene Expression study
Name of the Research Scholar :	Mr. Praveen Kumar K.S.
Name of Organization :	Sri Devaraj Urs Academy of Higher Education and Research. Tamaka, Kolar.

Purpose of the study: Diabetes is a disease involving an abnormal increase in blood sugar levels. One of the complications of high blood sugar levels is impaired glucose, malfunctioning of glycogenesis, and gluconeogenesis in the liver, which is a major storage organ, where the entire process takes place. The enzyme involved in the process is Phosphoglucomutase1, which is the catalytic enzyme that plays a key regulatory role in maintaining glucose homeostasis and glycogen metabolism in the cell. Abnormal functioning of the PGM enzyme leads to the disease condition. One such complication of glucose metabolism in Type 2 Diabetes Mellitus. The purpose of this study is to evaluate the role of PGM1 enzyme activity in the development of T2DM. The results obtained from this study will contribute towards the development of therapies that can halt the development of T2DM.

P**articipant selection:** Individuals aged ≥18 years, who are not suffering from diabetes and also do not have a history of any known chronic diseases will be included in this study.

Voluntary Participation: Your participation in this study is entirely voluntary. There is no compulsion to participate in this study. You will be in no way affected if you do not wish to participate in the study. You are required to sign only if you voluntarily agree to participate in this study. Further, you are at liberty to withdraw from the study at any time. We assure you that your withdrawal will not affect your treatment by the concerned physician in any way.

Procedure: We will be collecting a small volume of blood (3 ml) from your arm. The sample will be used for genomic analysis. Clinical and family history is also necessary.

Duration: The research will take place for about 3 years.

Risks: No drug will be tested on you. 3 ml of blood will be collected using a sterile and disposable needle and syringe. Standard of care for the treatment of Type 2 diabetes will be given to you irrespective of your decision to enroll in the project.

Benefits: This study will be useful to understand the role of PGM1 in the development of Type 2 diabetic patients. The results gathered from this study will be beneficial in the management of

diseases. Participation in this study does not involve any cost for you. Also, no monetary compensation will be paid for your participation in this study.

Storage of samples: The samples collected from you may be stored at -80^0 C for future research projects on molecular studies on PGM1. In such an event, ethics clearance will be obtained.

Confidentiality: All information collected from you will be strictly confidential & will not be disclosed to anyone except if it is required by the law. This information collected will be used only for research. This information will not reveal your identity.

Sharing of Results: The results obtained from this study will be published in scientific/Medical Journals/Medical conferences.

For any information, you are free to contact the investigator. This study has been approved by the Institutional Ethics Committee & has been started only after their formal approval. The sample collected will be stored in the institute and I request you to permit us to store and use this sample for any future study. This document will be stored in the safe locker & a copy given to you for information. Person in charge of providing a standard of care to the patients:

Dr. Prabhakar K. Professor,
Dept. of General Medicine
Sri Devaraj Urs Medical College, Tamaka, Kolar.
Mob: 9845209858Mob: 9845209858

HEALTHY CONTROL INFORMATION SHEET

Name of the project :	Role of phosphoglucomutase 1 in Type 2 Diabetes Mellitus: An Integrated Biochemical, Genetic and Gene Expression study
Name of the Research Scholar :	Mr. Praveen Kumar K.S.
Name of Organization :	Sri Devaraj Urs Academy of Higher Education and Research. Tamaka, Kolar.

Purpose of the study: Diabetes is a disease involving an abnormal increase in blood sugar levels. One of the complications of high blood sugar levels is impaired glucose, malfunctioning of glycogenesis and gluconeogenesis in the liver results in causing disease. The enzyme involved in the process is Phosphoglucomutase 1, which is the catalytic enzyme that plays a key regulatory role in maintaining glucose homeostasis and glycogen metabolism in the cell. Abnormal functioning of the PGM1 enzyme leads to the disease condition. One such complication of glucose metabolism in Type 2 Diabetes mellitus. The purpose of this study is to evaluate the role of PGM1 enzyme activity in the development of T2DM. The results obtained from this study will contribute towards the development of therapies that can halt the development of T2DM.

P**articipant selection:** Individuals aged ≥18 years, who are not suffering from diabetes and also do not have a history of any known chronic diseases will be included in this study.

Voluntary Participation: Your participation in this study is entirely voluntary. There is no compulsion to participate in this study. You will be in no way affected if you do not wish to participate in the study. You are required to sign only if you voluntarily agree to participate in this study. Further, you are at liberty to withdraw from the study at any time. We assure you that your withdrawal will not affect your treatment by the concerned physician in any way.

Procedure: We will be collecting a small volume of blood (3 ml) from your arm. The sample will be used for immunological analysis. Clinical and family history is also necessary.

Duration: The research will take place for about 3 years.

Risks: No drug will be tested on you. 3 ml of blood will be collected using a sterile and disposable needle and syringe. Standard of care for the treatment of Type 2 diabetes will be given to you irrespective of your decision to enroll in the project.

Benefits: This study will be useful to understand the role of PGM1 in the development of Type 2 diabetic patients. The results gathered from this study will be beneficial in the management of diseases. Participation in this study does not involve any cost for you. Also, no monetary compensation will be paid for your participation in this study.

Storage of samples: The samples collected from you may be stored at -80^0 C for future research projects on molecular studies on PGM1. In such an event, ethics clearance will be obtained.

Confidentiality: All information collected from you will be strictly confidential & will not be disclosed to anyone except if it is required by the law. This information collected will be used only for research. This information will not reveal your identity.

Sharing the Results: The results obtained from this study will be published in scientific/Medical Journals/Medical conferences

For any information, you are free to contact the investigator. This study has been approved by the Institutional Ethics Committee & has been started only after their formal approval. The sample collected will be stored in the institute and I request you to permit us to store and use this sample for any future study.

This document will be stored in the safe locker & a copy given to you for information. For any further clarification you are free to contact:

Dr. Prabhakar K. Professor,
Dept. of General Medicine
Sri Devaraj Urs Medical College, Tamaka, Kolar.
Mob: 9845209858Mob: 9845209858

PART II:

INFORMED CONSENT FORM (Patients)

Note: Consent for the case group (patients with Type 2 Diabetics) should be obtained from the participant.

Name of Participant (Patient/Volunteer): _____

- This research study has been explained to me; I have been given the chance to discuss it and ask questions. All of my questions have been answered to my satisfaction.
- I have read each page of the Patient Information Sheet or it has been read to me.
- I agree to allow access to my health information as explained in the patient information sheet. (In case of the patient only)
- I agree to allow the collection of 3ml blood samples and health data for the research purposes explained in the Patient Information Sheet.
- I voluntarily consent to the storage of my sample for future research projects.
- I understand that all the information collected will be kept confidential.
- I voluntarily consent to take part in this research study.

Participant's signature or thumb impression

	Name	**Signature**	**Date**
Participant			
Witness 1			
Witness 2			
The person taking consent*			

Date: _____

If illiterate: Two literate witnesses must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should include their thumb-print as well.

PART II:

INFORMED CONSENT FORM (Healthy controls)

Note: Consent for the control group (Healthy individuals without Type 2 Diabetics) should be obtained from the participant.

Name of Participant (Healthy control /Volunteer): _____

- This research study has been explained to me; I have been given the chance to discuss it and ask questions. All of my questions have been answered to my satisfaction.
- I have read each page of the Patient Information Sheet or it has been read to me.
- I agree to allow access to my health information as explained in the patient information sheet. (In case of the patient only)
- I agree to allow the collection of 3ml blood samples and health data for the research purposes explained in the Patient Information Sheet.
- I voluntarily consent to the storage of my sample for future research projects.
- I understand that all the information collected will be kept confidential.
- I voluntarily consent to take part in this research study.

Participant's signature or thumb impression

	Name	**Signature**	**Date**
Participant			
Witness 1			
Witness 2			
The person taking consent*			

Date: _____

If illiterate: Two literate witnesses must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should include their thumb-print as well.

ಭಾಗ 1: ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿ ಪತ್ರ
ರೋಗಿಯ ಮಾಹಿತಿ ಪತ್ರ

ಯೋಜನೆಯ ಹೆಸರು	ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್‌ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್‌ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ
ಸಂಶೋಧಕಹೆಸರು	ಪ್ರವೀಣ್ ಕುಮಾರ್ ಕೆ.ಎಸ್.
ಸಂಸ್ಥೆಯ ಹೆಸರು	ಶ್ರೀ ದೇವರಾಜ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಸಂಸ್ಥೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಟಮಕ, ಕೋಲಾರ.

ಭಾಗವಹಿಸುವವರು ಆಯ್ಕೆ : ≥18 ವರ್ಷಕ್ಕಿಂತ ಮೇಲ್ಪಟ್ಟ ಟೈಪ್ 2 ಮಧುಮೇಹದಿಂದ ಬಳಲುತ್ತಿರುವ ರೋಗಿಗಳು. ಎಲ್ಲಾ ಡಯಾಬಿಟಿಕ್ ರೋಗಿಗಳಿಗೆ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವ ತಮ್ಮ ನಿರ್ಧಾರವನ್ನು ಮಾತ್ರ ಪರಿಗಣಿಸದೆ ಆರ್. ಎಲ್. ಜಾಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರಕ್ಕೆ ಬರುವ ಮಧುಮೇಹ ಕ್ಲಿನಿಕ್ ಮೂಲಕ ಚಿಕಿತ್ಸೆಯ ಪ್ರಮಾಣಿತ ಆರೈಕೆ ನೀಡಲಾಗುವುದು. ಆಸ್ಪತ್ರೆಯ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರದಲ್ಲಿನ ಮಧುಮೇಹ ಕ್ಲಿನಿಕ್ನ ಉಸ್ತುವಾರಿ ವೈದ್ಯರನ್ನು ಅಧ್ಯಯನದಲ್ಲಿ ಸಹ-ಮಾರ್ಗದರ್ಶಿಯಾಗಿ ಸೇರಿಸಲಾಗಿದೆ. ಅವರು ತಮ್ಮ ತೊಡಕುಗಳನ್ನು ಲೆಕ್ಕಿಸದೆಯೇ ಎಲ್ಲ ಮಧುಮೇಹ ರೋಗಿಗಳ ಎಲ್ಲಾ ಪರೀಕ್ಷೆಗಳು ಮತ್ತು ಕ್ಲಿನಿಕಲ್ ನಿರ್ವಹಣೆಯನ್ನು ಸಹಕರಿಸುತ್ತಾರೆ.

ಸ್ವಯಂ ಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ: ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂ ಪ್ರೇರಿತವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ನಿರ್ಬಂಧವಿಲ್ಲ. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ, ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನೀವು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದರೆ ಮಾತ್ರ ನೀವು ಸಹಿ ಮಾಡಬೇಕಾಗುತ್ತದೆ. ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಸ್ವಾತಂತ್ರ್ಯವಾಗಿರುತ್ತೀರಿ. ನಿಮ್ಮ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆಯು ವೈದ್ಯರನ್ನು ಯಾವುದೇ ರೀತಿಯಲ್ಲಿ ಯಾವುದೇ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ ಎಂದು ನಾವು ಭರವಸೆನೀಡುತ್ತೇವೆ.

ವಿಧಾನ: ನಾವು ನಿಮ್ಮ ಕೈಯಿಂದ ಸಣ್ಣ ಪ್ರಮಾಣದ ರಕ್ತವನ್ನು (5 ಮಿ.ಲೀ) ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. ಈ ಮಾದರಿಯನ್ನು ರೋಗ ನಿರೋಧಕ ವಿಶ್ಲೇಷಣೆಗಾಗಿ ಬಳಸಲಾಗುತ್ತದೆ. ಕುಟುಂಬದ ಕ್ಲಿನಿಕಲ್ ಇತಿಹಾಸವೂ ಅವಶ್ಯಕವಾಗಿರುತ್ತದೆ.

ಅವಧಿ : ಸಂಶೋಧನೆ ಸುಮಾರು 3 ವರ್ಷಗಳ ಕಾಲ ನಡೆಯುತ್ತದೆ.

ಅಪಾಯಗಳು: ನಿಮ್ಮ ಮೇಲೆ ಯಾವುದೇ ಔಷಧಿ ಪರೀಕ್ಷಿಸಲಾಗುವುದಿಲ್ಲ. 3 ಮಿ. ಲೀ ರಕ್ತವನ್ನು ಸೂಜಿ ಮತ್ತು ಸಿರಿಂಜ್ ಬಳಸಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಯೋಜನೆಯಲ್ಲಿ ತೊಡಗಿಸಿಕೊಳ್ಳುವ ನಿಮ್ಮನಿರ್ಧಾರವನ್ನುಹೊರತುಪಡಿಸಿ ಟೈಪ್ 2 ಮಧುಮೇಹ ಹೊಂದಿರುವ ರೋಗಿಗಳಿಗೆ ಗುಣಮಟ್ಟದ ಚಿಕಿತ್ಸೆಯನ್ನು ನೀಡಲಾಗುತ್ತದೆ.

ಪ್ರಯೋಜನಗಳು: ಡಯಾಬಿಟಿಕ್ ರೋಗಿಗಳಲ್ಲಿ ಫಾಸ್ಫೊಗ್ಲೂಕೊಮ್ಯುಟೇಸ್ ಬೆಳವಣಿಗೆಯನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ಈ ಅಧ್ಯಯನವು ಉಪಯುಕ್ತವಾಗಿದೆ. ಈ ಅಧ್ಯಯನದಿಂದ ಸಂಗ್ರಹಿಸಿದ ಫಲಿತಾಂಶಗಳು ರೋಗಗಳ ನಿರ್ವಹಣೆಗೆ ಅನುಕೂಲಕರವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ

ಭಾಗವಹಿಸುವಿಕೆಯು ನಿಮಗೆ ಯಾವುದೇ ವೆಚ್ಚವನ್ನುಒಳಗೊಂಡಿರುವುದಿಲ್ಲ. ಅಲ್ಲದೆ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗೆ ಯಾವುದೇ ವಿತ್ತೀಯ ಪರಿಹಾರವನ್ನು ಪಾವತಿಸಲಾಗುವುದಿಲ್ಲ.

ಮಾದರಿಗಳಶೇಖರಣೆ: ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗಾಗಿ ನಿಮ್ಮ ರಕ್ತದ ಮಾದರಿಯಿಂದ ವಂಶವಾಹಿ ವಸ್ತುಗಳನ್ನು ಸಂಗ್ರಹಿಸಬಹುದು. ಅಂತಹ ಸಂದರ್ಭದಲ್ಲಿ, ಎಥಿಕ್ಸ್ಕಮಿಟಿಯಿಂದ ಅನುಮತಿ ಪಡೆಯುವ ಮೊದಲು ಅದನ್ನು ಪಡೆಯಬಹುದು.

ಗೋಪ್ಯತೆ:ವೈಯಕ್ತಿಕ ಗುರುತಿನ ಬಗ್ಗೆ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನೂ ಗೌಪ್ಯವಾಗಿರಿಸಲಾಗುವುದು ಮತ್ತು ಕಾನೂನಿನ ಅಗತ್ಯವನ್ನು ಹೊರತು ಪಡಿಸಿ ಯಾರಿಗೂ ಅದನ್ನು ಬಹಿರಂಗ ಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಯೋಜನೆಯ ಶೋಧಕರು ಮಾತ್ರ ಗುರುತಿನ ವಿವರಗಳಿಗೆ ಪ್ರವೇಶವನ್ನುಹೊಂದಿರುತ್ತಾರೆ.

ಫಲಿತಾಂಶಗಳನ್ನುಹಂಚಿಕೆ: ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಫಲಿತಾಂಶಗಳು ವೈಜ್ಞಾನಿಕ / ವೈದ್ಯಕೀಯ ಜರ್ನಲ್ / ಮೆಡಿಕಲ್ ಸಮ್ಮೇಳನಗಳಲ್ಲಿ ಪ್ರಕಟವಾಗುತ್ತವೆ.

ಯಾವುದೇ ಮಾಹಿತಿಗಾಗಿ ನೀವು ತನಿಖೆದಾರರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ. ಈ ಅಧ್ಯಯನವು ಸಾಂಸ್ಥಿಕ ನೀತಿ ಶಾಸ್ತ್ರ ಸಮಿತಿಯಿಂದ ಅನುಮೋದಿಸಲ್ಪಟ್ಟಿದೆ ಮತ್ತು ಅವರ ಔಪಚಾರಿಕ ಅನುಮೋದನೆಯ ನಂತರ ಮಾತ್ರ ಪ್ರಾರಂಭಿಸಲ್ಪಟ್ಟಿದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾದರಿಯನ್ನು ಇನ್ಸ್ಟಿಟ್ಯೂಟ್ನಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುವುದು ಮತ್ತು ಭವಿಷ್ಯದ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಈ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸಲು ಮತ್ತು ಬಳಸಲು ನಮಗೆ ಅನುಮತಿಸಲು ನಾನು ವಿನಂತಿಸುತ್ತೇನೆ.

ಈ ಡಾಕ್ಯುಮೆಂಟ್ ಅನ್ನುಸುರಕ್ಷಿತ ಲಾಕರ್ನಲ್ಲಿ ಮತ್ತು ಮಾಹಿತಿಗಾಗಿ ನಿಮಗೆ ನೀಡಿದ ಪ್ರತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಈ ಕೆಳಗಿನ ಸಂಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ:

ಡಾ. ಪ್ರಭಾಕರ್ ಕೆ.
ಪ್ರೊಫೆಸರ್ , ಸಾಮಾನ್ಯ ಔಷಧ ವಿಭಾಗ
ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು,
ತಮಕಾ, ಕೋಲಾರ.
ಮೊಬೈಲ್: 9845209858

1: ಸಾಮಾನ್ಯ ಗುಂಪಿನ ಮಾಹಿತಿ ಪತ್ರ

ಯೋಜನೆಯ ಹೆಸರು	ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್‌ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್‌ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ
ಸಂಶೋಧಕಹೆಸರು	ಪ್ರವೀಣ್ ಕುಮಾರ್ ಕೆ.ಎಸ್.
ಸಂಸ್ಥೆಯ ಹೆಸರು	ಶ್ರೀ ದೇವರಾಜ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಸಂಸ್ಥೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಟಮಕ, ಕೋಲಾರ.

ಭಾಗವಹಿಸುವವರು ಆಯ್ಕೆ : ≥ 18 ವರ್ಷ ವಯಸ್ಸಿನ ವ್ಯಕ್ತಿಗಳು, ಮಧುಮೇಹದಿಂದ ಬಳಲುತ್ತಿರುವವರು ಮತ್ತು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಯಾವುದೇ ದೀರ್ಘಕಾಲೀನ ರೋಗಗಳ ಇತಿಹಾಸವನ್ನು ಹೊಂದಿಲ್ಲದಿರುವವರು.

ಸ್ವಯಂ ಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ: ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂ ಪ್ರೇರಿತವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ನಿರ್ಬಂಧವಿಲ್ಲ. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ, ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನೀವು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದರೆ ಮಾತ್ರ ನೀವುಸಹಿಮಾಡಬೇಕಾಗುತ್ತದೆ. ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಸ್ವಾತಂತ್ರ್ಯವಾಗಿರುತ್ತೀರಿ. ನಿಮ್ಮ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆಯು ವೈದ್ಯರನ್ನು ಯಾವುದೇ ರೀತಿಯಲ್ಲಿ ಯಾವುದೇ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ ಎಂದು ನಾವು ಭರವಸೆನೀಡುತ್ತೇವೆ.

ವಿಧಾನ: ನಾವು ನಿಮ್ಮ ಕೈಯಿಂದ ಒಂದು ಸಣ್ಣ ಪ್ರಮಾಣದ ರಕ್ತವನ್ನು (3 ಮಿ.ಲೀ) ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. ಈ ಮಾದರಿಯನ್ನು ರೋಗ ನಿರೋಧಕ ವಿಶ್ಲೇಷಣೆಗಾಗಿ ಬಳಸಲಾಗುತ್ತದೆ. ವೈದ್ಯಕೀಯ ಇತಿಹಾಸವೂ ಅವಶ್ಯಕ.

ಅವಧಿ : ಸಂಶೋಧನೆ ಸುಮಾರು 3 ವರ್ಷಗಳ ಕಾಲ ನಡೆಯುತ್ತದೆ.

ಅಪಾಯಗಳು: ನಿಮ್ಮ ಮೇಲೆ ಯಾವುದೇ ಔಷಧಿ ಪರೀಕ್ಷಿಸಲಾಗುವುದಿಲ್ಲ. 3 ಮಿ.ಲೀ ರಕ್ತವನ್ನು ಸೂಜಿ ಮತ್ತು ಸಿರಿಂಜ್ ಬಳಸಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಯೋಜನೆಯಲ್ಲಿ ತೊಡಗಿಸಿಕೊಳ್ಳುವ ನಿಮ್ಮನಿರ್ಧಾರವನ್ನುಹೊರತುಪಡಿಸಿ ಟೈಪ್ 2 ಮಧುಮೇಹ ಹೊಂದಿರುವ ರೋಗಿಗಳಿಗೆ ಗುಣಮಟ್ಟದ ಚಿಕಿತ್ಸೆಯನ್ನು ನೀಡಲಾಗುತ್ತದೆ.

ಪ್ರಯೋಜನಗಳು: ಡಯಾಬಿಟಿಕ್ ರೋಗಿಗಳಲ್ಲಿ ಫಾಸ್ಫೊಗ್ಲೂಕೊಮ್ಯುಟೇಸ್ ಬೆಳವಣಿಗೆಯನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ಈ ಅಧ್ಯಯನವು ಉಪಯುಕ್ತವಾಗಿದೆ. ಈ ಅಧ್ಯಯನದಿಂದ ಸಂಗ್ರಹಿಸಿದ ಫಲಿತಾಂಶಗಳು ರೋಗಗಳ ನಿರ್ವಹಣೆಗೆ ಅನುಕೂಲಕರವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವಿಕೆಯು ನಿಮಗೆ ಯಾವುದೇ ವೆಚ್ಚವನ್ನು ಒಳಗೊಂಡಿರುವುದಿಲ್ಲ. ಅಲ್ಲದೆ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗೆ ಯಾವುದೇ ವಿತ್ತೀಯ ಪರಿಹಾರವನ್ನುಪಾವತಿಸಲಾಗುವುದಿಲ್ಲ.

ಮಾದರಿಗಳ ಶೇಖರಣೆ: ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗಾಗಿ ನಿಮ್ಮ ರಕ್ತದ ಮಾದರಿಯಿಂದ ವಂಶವಾಹಿ ವಸ್ತುಗಳನ್ನು ಸಂಗ್ರಹಿಸಬಹುದು. ಅಂತಹ ಸಂದರ್ಭದಲ್ಲಿ, ನೈತಿಕ ಸಮಿತಿಯಿಂದ ಅನುಮತಿ ಪಡೆಯುವ ಮೊದಲು ಅದನ್ನು ಪಡೆಯಬಹುದು.

ಗೋಪ್ಯತೆ: ವೈಯಕ್ತಿಕ ಗುರುತಿನ ಬಗ್ಗೆ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನೂ ಗೌಪ್ಯವಾಗಿರಿಸಲಾಗುವುದು ಮತ್ತು ಕಾನೂನಿನ ಅಗತ್ಯವನ್ನು ಹೊರತು ಪಡಿಸಿಯಾರಿಗೂ ಅದನ್ನು ಬಹಿರಂಗ ಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಯೋಜನೆಯ ಶೋಧಕರು ಮಾತ್ರ ಗುರುತಿನ ವಿವರಗಳಿಗೆ ಪ್ರವೇಶವನ್ನುಹೊಂದಿರುತ್ತಾರೆ.

ಫಲಿತಾಂಶಗಳನ್ನುಹಂಚಿಕೆ: ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಫಲಿತಾಂಶಗಳು ವೈಜ್ಞಾನಿಕ / ವೈದ್ಯಕೀಯ ಜರ್ನಲ್ / ಮೆಡಿಕಲ್ ಸಮ್ಮೇಳನಗಳಲ್ಲಿ ಪ್ರಕಟಿಸಲಾಗುತ್ತದೆ.

ಯಾವುದೇ ಮಾಹಿತಿಗಾಗಿ ನೀವು ತನಿಖೆದಾರರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ. ಈ ಅಧ್ಯಯನವು ಸಾಂಸ್ಥಿಕ ನೀತಿಶಾಸ್ತ್ರ ಸಮಿತಿಯಿಂದ ಅನುಮೋದಿಸಲ್ಪಟ್ಟಿದೆ ಮತ್ತು ಅವರ ಔಪಚಾರಿಕ ಅನುಮೋದನೆಯ ನಂತರ ಮಾತ್ರ ಪ್ರಾರಂಭಿಸಲ್ಪಟ್ಟಿದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾದರಿಯನ್ನು ಸಂಸ್ಥೆಯಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುವುದು ಮತ್ತು ಭವಿಷ್ಯದ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಈ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸಲು ಮತ್ತು ಬಳಸಲು ನಮಗೆ ಅನುಮತಿಸಲು ನಾನು ವಿನಂತಿಸುತ್ತೇನೆ.

ಈ ಪ್ರತಿಯನ್ನು ಸುರಕ್ಷಿತ ಲಾಕರ್ನಲ್ಲಿ ಮತ್ತು ಮಾಹಿತಿಗಾಗಿ ನಿಮಗೆ ನೀಡಿದ ಪ್ರತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ.ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಈ ಕೆಳಗಿನ ಸಂಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ:

ಡಾ. ಪ್ರಭಾಕರ್ ಕೆ.
ಪ್ರೊಫೆಸರ್ , ಸಾಮಾನ್ಯ ಔಷಧ ವಿಭಾಗ
ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು,
ತಮಕಾ, ಕೋಲಾರ.
ಮೊಬೈಲ್: 9845209858

ತಿಳಿವಳಿಕೆಯ ಸಮ್ಮತಿ ನಮೂನೆ (ರೋಗಿಗಳು)

ವಿಷಯ: ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ

ಗಮನಿಸಿ: ರೋಗಿಯ ಗುಂಪು (ಟೈಪ್ 2 ಮಧು ಮೇಹ ರೋಗಿಗಳು) ಒಪ್ಪಿಗೆ ಪಡೆಯುವವರು ಭಾಗವಹಿಸುವವರಿಂದ ಪಡೆಯಬೇಕು.

ಪಾಲ್ಗೊಳ್ಳುವವರ ಹೆಸರು (ರೋಗಿಯ / ಸ್ವಇಚ್ಛೆಯಿಂದ ಪಾಲ್ಗೊಳ್ಳುವವರು) : _____

- ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನವನ್ನು ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ; ಇದನ್ನು ಚರ್ಚಿಸಲು ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶ ನೀಡಲಾಗಿದೆ. ನನ್ನ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರ ನೀಡಲಾಗಿದೆ.
- ನಾನು ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯ ಪ್ರತಿ ಪುಟವನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ನನಗೆ ಓದಲಾಗಿದೆ.
- ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿದಂತೆ ನನ್ನ ಆರೋಗ್ಯ ಮಾಹಿತಿಯನ್ನು ಪಡೆಯಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ. (ರೋಗಿಗೆಮಾತ್ರ)
- ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿರುವ ಸಂಶೋಧನಾ ಉದ್ದೇಶಗಳಿಗಾಗಿ 3 ಮಿ. ರಕ್ತ ಮಾದರಿ ಮತ್ತು ಆರೋಗ್ಯದ ಡೇಟಾ ಸಂಗ್ರಹಣೆಯನ್ನು ಕೂಡಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.
- ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುವುದು ಎಂದು ನಾನು ಅರ್ಥ ಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.
- ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗೆ ನನ್ನ ಮಾದರಿ ಸಂಗ್ರಹಿಸುವುದಕ್ಕೆ ನಾನು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೇನೆ.
- ನಾನು ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸುತ್ತೇನೆ
- ಭಾಗವಹಿಸುವವರ ಸಹಿ ಅಥವಾ ಹೆಬ್ಬೆರಳು ಗುರುತು

ದಿನಾಂಕ: _____

ಅನಕ್ಷರಸ್ಥನಾಗಿದ್ದರೆ: ಎರಡು ಸಾಕ್ಷರತಾ ಸಾಕ್ಷಿಗಳು ಸಹಿಹಾಕಬೇಕು (ಸಾಧ್ಯವಾದರೆ,ಈ ವ್ಯಕ್ತಿಯನ್ನು ಸಹಭಾಗಿಯು ಆರಿಸಬೇಕು ಮತ್ತು ಸಂಶೋಧನಾತಂಡಕ್ಕೆಯಾವುದೇಸಂಪರ್ಕವನ್ನುಹೊಂದಿಲ್). ಅನಕ್ಷರಸ್ಥರು ಭಾಗವಹಿಸುವವರು ತಮ್ಮಹೆಬ್ಬೆರಳು–ಮುದ್ರಣವನ್ನೂ ಸಹ ಒಳಗೊಂಡಿರಬೇಕು. ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಸಮ್ಮತಿರೂಪದ ನಿಖರವಾದ ಓದುವಿಕೆಗೇ ನಾನು ಸಾಕ್ಷಿಯಾಗಿದ್ದೇನೆ ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ವ್ಯಕ್ತಿಗೆ ಅವಕಾಶವಿದೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾನೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

	ಸಾಕ್ಷಿ 1	ಸಾಕ್ಷಿ 2
ಹೆಸರು		
ಸಹಿ		
ದಿನಾಂಕ		

ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ನಾನು ಓದಿದ್ದೇನೆ ಮತ್ತು ವಿವರಿಸಿದ್ದೇನೆ ಮತ್ತು ಈ ಪ್ರತಿಗಳ ಪ್ರತಿಯನ್ನು ಅವರಿಗೆ ನೀಡಲಾಗಿದೆ.

ಸಮ್ಮತಿಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಹೆಸರು _____
ಒಪ್ಪಿಗೆ ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಸಹಿ _____ ದಿನಾಂಕ: _____

ತಿಳಿವಳಿಕೆಯ ಸಮ್ಮತಿ ನಮೂನೆ (ಆರೋಗ್ಯಕರ ವ್ಯಕ್ತಿಗಳು)

ವಿಷಯ: ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ

ಗಮನಿಸಿ: ಆರೋಗ್ಯಕರ ವ್ಯಕ್ತಿಗಳ ಒಪ್ಪಿಗೆ ಪಡೆಯುವವರು ಭಾಗವಹಿಸುವವರಿಂದ ಪಡೆಯಬೇಕು.

ಪಾಲ್ಗೊಳ್ಳುವವರ ಹೆಸರು (ಆರೋಗ್ಯಕರ ವ್ಯಕ್ತಿ): _____

- ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನವನ್ನು ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ; ಇದನ್ನು ಚರ್ಚಿಸಲು ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶ ನೀಡಲಾಗಿದೆ. ನನ್ನ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನತೃಪ್ತಿಗೆ ಉತ್ತರ ನೀಡಲಾಗಿದೆ.
- ನಾನು ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯ ಪ್ರತಿಪುಟವನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ನನಗೆ ಓದಲಾಗಿದೆ. .
- ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿದಂತೆ ನನ್ನ ಆರೋಗ್ಯ ಮಾಹಿತಿಗೆ ಪ್ರವೇಶವನ್ನು ಅನುಮತಿಸಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.
- ರೋಗಿಯ ಮಾಹಿತಿಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿರುವ ಸಂಶೋಧನಾ ಉದ್ದೇಶಗಳಿಗಾಗಿ 3 ಮಿ.ಲೀ ರಕ್ತ ಮಾದರಿ ಮತ್ತು ಆರೋಗ್ಯದ ಡೇಟಾ ಸಂಗ್ರಹಣೆಯನ್ನು ಅನುಮತಿಸಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.
- ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗೆ ನನ್ನ ಮಾದರಿ ಸಂಗ್ರಹಿಸುವುದಕ್ಕೆ ನಾನು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೇನೆ.
- ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನುಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುವುದು ಎಂದು ನಾನು ಅರ್ಥ ಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.
- ನಾನು ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರ ಸಹಿ ಅಥವಾ ಹೆಬ್ಬೆರಳು ಗುರುತು

ದಿನಾಂಕ: _____

ಅನಕ್ಷರಸ್ಥನಾಗಿದ್ದರೆ: ಎರಡು ಸಾಕ್ಷರತಾ ಸಾಕ್ಷಿಗಳು ಸಹಿ ಹಾಕಬೇಕು (ಸಾಧ್ಯವಾದರೆ, ಈ ವ್ಯಕ್ತಿಯನ್ನು ಸಹಭಾಗಿಯು ಆರಿಸಬೇಕು ಮತ್ತು ಸಂಶೋಧನಾ ತಂಡಕ್ಕೆ ಯಾವುದೇ ಸಂಪರ್ಕವನ್ನು ಹೊಂದಿಲ್ಲ. ಅನಕ್ಷರಸ್ಥರು ಭಾಗವಹಿಸುವವರು ತಮ್ಮ ಹೆಬ್ಬೆರಳು-ಮುದ್ರಣವನ್ನೂ ಸಹ ಒಳಗೊಂಡಿರಬೇಕು. ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಸಮ್ಮತಿ ರೂಪದ ನಿಖರವಾದ ಓದುವಿಕೆಗೇ ನಾನು ಸಾಕ್ಷಿಯಾಗಿದ್ದೇನೆ ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ವ್ಯಕ್ತಿಗೆ ಅವಕಾಶವಿದೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾನೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

	ಸಾಕ್ಷಿ 1	ಸಾಕ್ಷಿ 2
ಹೆಸರು		
ಸಹಿ		
ದಿನಾಂಕ		

ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ನಾನು ಓದಿದ್ದೇನೆ ಮತ್ತು ವಿವರಿಸಿದ್ದೇನೆ ಮತ್ತು ಈ ಒಂದು ಪ್ರತಿಯನ್ನು ಅವರಿಗೆ ನೀಡಲಾಗಿದೆ.

ಸಮ್ಮತಿಯನ್ನುತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಹೆಸರು _____
ಒಪ್ಪಿಗೆ ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಸಹಿ _____ ದಿನಾಂಕ: _____

ABBREVIATIONS

T2DM	Type 2 Diabetes Mellitus
PGM	Phosphoglucomutase
PBMC	Peripheral Blood Mononuclear Cells
DNA	Deoxyribonucleic Acid
mRNA	Messenger RNA
ATP	Adenosine triphosphate
PCR	Polymerase Chain Reaction
SDS	Sodium Dodecyl Sulphate
RBC	Red Blood Cell
ELB	Erythrocyte Lysis Buffer
WBC	White Blood Cell
RPMI	Roswell-Prank Memorial Institute
FBS	Fetal Bovine Serum
PAS	Periodic Acid-Schiff stain
RIPA	Radioimmunoprecipitation assay
EDTA	Ethylenediaminetetraacetic acid

gm – grams, mU/mg –Milliunits per milligram, mM – millimolar,

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder that arises due to the disruption of glucose homeostasis. In addition to other mechanisms, the glycogen pathway also plays a major role in glucose homeostasis. The phosphoglucomutase (PGM) enzyme catalyzes the key reaction that connects the glycogen pathway with glucose metabolism. Studies have shown that the glycogen pathway is abnormal in T2DM. The purpose of this study was to determine the role of PGM in T2DM by a combination of biochemical, genetic, and gene expression studies.

This was a case–control study comprising T2DM patients (*n* = 63) and healthy volunteers (*n* = 63). All experiments were carried out using peripheral blood mononuclear cells (PBMCs). PGM enzyme activity was found to be reduced in T2DM patients compared to healthy controls (0.9 fold; p = 0.043; Student's t test). Furthermore, PGM enzyme activity showed a reciprocal relationship with the indices of glycemic controls such as fasting blood sugar (r = -0.36; *p* = 0.016; Pearson's correlation test), random blood sugar (r = -0.39; *p* = 0.019; Pearson's correlation test), postprandial blood sugar (r = -0.41; *p* = 0.011; Pearson's correlation test), and glycated hemoglobin (r = -0.35; *p* = 0.028; Pearson's correlation test). Next, gene expression and genetic variation were explored as potential sources for reduced PGM enzyme activity. *PGM1* gene expression was found to be downregulated in T2DM patients compared to healthy subjects (fold difference = 0.59; *p* = 0.032; unpaired t test). Furthermore, PGM enzyme activity showed a positive correlation with *PGM1* gene expression (r = 0.35; p = 0.016; Pearson's correlation test). In addition, a common genetic variation in the *PGM1* gene (SNP rs11208257) was associated with T2DM. Furthermore, a reduction in PGM enzyme activity was linked to the genotype combination *PGM1* SNP rs11208257 (p = 0.018; multiple logistic regression).

Together, these results indicate that PGM enzyme activity is suboptimal in T2DM, probably due to downregulated gene expression and genetic variation.

The physiological impact of reduced PGM enzyme activity on cell viability under glucose-limiting conditions was evaluated. This is based on the assumption that reduced PGM activity may compromise glucose mobilization from glycogen and reduce cell viability under glucose limiting conditions. PBMC viability was reduced in PBMCs of T2DM patients compared to healthy subjects ($p = 0.001$; Student's t test). There was a positive correlation between PGM activity and PBMC viability ($r = 0.35$; $p = 0.001$; Pearson's correlation test). This relationship indicates that reduced PGM enzyme activity impairs PBMC viability under glucose-limiting conditions. In addition, It was found that glycogen degradation in PBMCs was reduced in T2DM patients compared to healthy subjects ($p = 0.001$; Student's t test). Glycogen degradation showed a reciprocal relationship with PBMC viability, and the effect was moderate ($r = -0.46$; $p = 0.012$; Pearson's correlation test). This relationship indicates that glycogen degradation is one of the several factors responsible for PBMC viability under glucose-limiting conditions.

This study shows that PGM enzyme activity is compromised in T2DM patients, possibly due to reduced gene expression and genetic variation. This study represents the first attempt to link the PGM enzyme with the pathogenesis of T2DM. This study adds the PGM enzyme to the list of defects that impair the glycogen pathway in T2DM.

INTRODUCTION

T2DM is a common metabolic disorder that constitutes a major public health burden. It affects approximately 12.1% of the population in India and 9.3% of the population worldwide [Saeedi et al., 2019; IDF 2017]. T2DM is the ninth leading cause of mortality, accounting for over 3.8 million deaths annually in India [Anjana et al., 2018]. T2DM mostly develops after 40 years of age [Wu et al., 2014]. Poor management of T2DM eventually leads to fatal complications such as end-stage renal disease and micro and macrovascular complications [Nasri et al., 2015, Liu et al., 2010]. Therefore, understanding the molecular underpinnings of T2DM is essential to uncover novel drug targets for its management.

Hyperglycemia is the cardinal biochemical hallmark of T2DM. This condition arises due to the reduced capacity of the cells to absorb glucose [Bouche et al., 2004]. Insulin is the main driver of cellular glucose utilization through the enhancement of membrane permeability for glucose [McConell et al., 2020]. Insulin signaling mobilizes glucose transporters from the cytoplasm to the cell membrane [Chadt et al., 2020]. Defects in insulin signaling are considered to play a major role in impairing cellular glucose utilization in T2DM [Samovski et al., 2018]. In addition to insulin signaling, the glycogen pathway also plays an important role in regulating cellular glucose utilization [Favaro et al., 2012]. Glycogen is the metabolic storehouse for glucose. Excess glucose is stored as glycogen and used when it is needed [Adeva-Andany et al., 2016].

The major steps involved in the glycogen pathway are schematically represented in Figure 1.1. Extracellular glucose enters the cytoplasm of the target cell through active transport mediated by the membrane-bound glucose transporter. The hexokinase enzyme in the cytosol catalyzes the conversion of glucose into glucose 6-phosphate, which further enters the glycolytic pathway. During excess, glucose 6-phosphate is

converted into glucose 1-phosphate, which then enters the glycogen pathway. This isomerization of glucose 6-phosphate into glucose 1-phosphate is catalyzed by the phosphoglucomutase (PGM) enzyme.

Several studies have shown that the glycogen pathway is abnormal in T2DM [Krssak et al., 2004, Soares et al., 2019]. The abnormality has been linked to defects in the enzymes involved in the glycogen pathway, such as glycogen synthase, glycogen phosphorylase, and protein phosphatase 1[Krssak et al. 2004; Pandey and Damsbo 1991; Kumar et al. 2018]. PGM is an important enzyme in the glycogen pathway, and its role in the pathogenesis of T2DM is not known. This study was undertaken to fill this gap. The aim of this study was to evaluate the role of PGM in T2DM by a combination of biochemical, gene expression, and genetic studies.

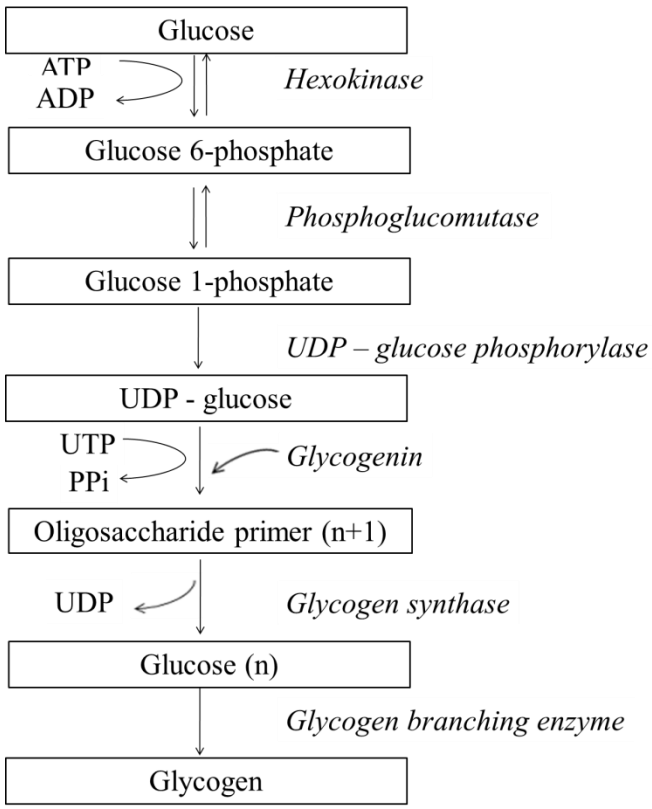

Figure 1.1: Schematic representation of the glycogen pathway.

AIM, OBJECTIVES AND RATIONALE

2.1 Aim of the study:

The aim of this study was to evaluate the role of PGM in T2DM by a combination of biochemical, gene expression, and genetic studies.

2.2. Objectives of the study:

1. To compare the enzymatic activity of the PGM enzyme in cell-free lysates of peripheral blood mononuclear cells (PBMCs) derived from T2DM patients and healthy controls.
2. To compare the gene expression profile of *PGM1* in PBMCs in T2DM patients and healthy controls.
3. To determine the association pattern of a selected genetic variation (rs11208257) within the *PGM1* gene in T2DM patients and healthy controls.
4. To compare the magnitude of glucose deprivation-induced glycogen degradation and cell viability in the PBMCs of T2DM patients and healthy controls.

2.3. Rationale:

Glucose homeostasis is disrupted in T2DM [Galicia-Garcia et al., 2020]. The glycogen pathway plays an important role in glucose homeostasis by converting excess glucose into glycogen. Studies have shown that the glycogen pathway is abnormal in T2DM. This study aimed to evaluate the role of the PGM enzyme in T2DM. The PGM enzyme plays a key role in the glycogen pathway. This enzyme catalyzes the bidirectional conversion of glucose 6-phosphate into glucose 1-phosphate, which is the starting intermediate in the glycogen pathway. Therefore, the PGM enzyme controls the entry of glucose into the glycogen pathway. Reduction of PGM activity is therefore likely to affect the functioning of the glycogen pathway. This study hypothesized that

PGM activity was lower in T2DM patients than in healthy subjects. This hypothesis was tested in the first objective.

The potential sources for the reduction in PGM activity were tested in the second and third objectives. The hypothesis behind the second objective was that PGM activity may be reduced due to the downregulation of the corresponding gene. The hypothesis behind the third objective was that PGM activity is reduced due to genetic variations in the corresponding gene. There are five PGM isozymes in the human genome: PGM1, PGM2, PGM2L1, PGM3, and PGM5. Of these five, PGM1 is the predominant isoenzyme expressed in most cell types, including PBMCs [Stiers et al. 2017]. The PGM1 isoenzyme is encoded by the *PGM1* gene. Therefore, gene expression and genetic variation studies were carried out with the *PGM1* gene.

There are over 24505 SNPs according to the single nucleotide polymorphism database (dbSNP) [Wheeler et al., 2007]. Of these, only three SNPs were missense variations with a global minor allele frequency of more than 5%. The three SNPs are rs1126728, rs11208257, and rs6676290. Among these three SNPs, rs6676290 is absent in the South Asian population (1000 Genomes Project) [1000 Genomes Project Consortium, 2015]. Functional analysis of these two SNPs using the Sorting Intolerant From Tolerant (SIFT) program [Sim et al., 2012] showed that the amino acid change due to SNP rs11208257 is deleterious, whereas the change due to SNP rs1126728 is tolerated. Therefore, SNP rs11208257 was chosen for the genetic association study. The hypothesis of the third objective was that the minor allele of SNP rs11208257 would be more common among T2DM patients than in healthy subjects.

The physiological impact of PGM activity on glycogen utilization was evaluated in the fourth objective. The hypothesis was that reduced PGM activity would compromise glucose mobilization from glycogen under conditions of glucose limitation. The resulting glucose shortage would compromise PBMC viability.

The glycogen pathway is most active in the liver and skeletal muscle cells [Krssak et al., 2004; Jensen et al., 2011]. However, the presence of glycogen has been demonstrated in several other cells, such as red blood cells, astrocytic glial cells, cardiomyocytes, renal tubular cells, Schwann cells, and adipocytes [Miwa et al., 2002, Wiesinger et al., 1997, Milutinovic et al., 2012, Tsuchitani et al., 1990, Brown et al., 2012, Ceperuelo-Mallafre et al., 2015]. This study was carried out using PBMCs. The presence of glycogen has been demonstrated in PBMCs. Furthermore, the transcriptional profiles of PBMCs in T2DM patients have been shown to correlate with the pathophysiology of the disease [Manoel-Caetano et al. 2012].

2.4. Significance of the study:

T2DM is a multifactorial disease with a genetic component, and less is known about the pathophysiological origin of T2DM. Additionally, there are no specific treatments to cure T2DM. Therefore, understanding the pathophysiological basis of T2DM is necessary to uncover novel therapeutic targets. The results of this study will contribute to the understanding of the molecular mechanisms by which PGM in the glycogen pathway plays a role in the pathophysiology of T2DM. If PGM is found to be altered in the glycogen pathway, then it can be taken as a drug target. This study provides the mechanistic role of PGM1 in the glycogen pathway. The knowledge obtained can be

helpful in developing therapeutic strategies that can help in a better understanding of the disease.

REVIEW OF LITERATURE

3.0. Clinical aspects of diabetes:

3.0.1. Signs and Symptoms

The common symptoms of diabetes mellitus include polyphagia, polydipsia, weight loss, polyuria, fatigue, and blurred vision [Kharroubi et al., 2015]. The common signs of diabetes mellitus are elevated sugar levels in the blood (hyperglycemia) and urine (glycosuria).

3.0.2. Disease burden

Diabetes mellitus affects 8.7% of the Indian population (46.3 million) in the age group of 10 – 70 years. It is expected to rise to 10.9% (77 million) by 2045. The frequency is higher in urban regions (10.8 million) than in rural areas (7.2 million). According to the International Diabetes Federation (IDF), diabetes accounts for 6.7 million deaths in India [Cho et al., 2018]. These figures show that diabetes is a major public health burden in India.

3.1. Types of diabetes mellitus

Diabetes mellitus is classified into four types as follows [Kaul et al., 2012]:

a) Type 1 diabetes mellitus

b) Type 2 diabetes mellitus

c) Gestational diabetes

d) Maturity onset diabetes mellitus

a) Type 1 diabetes mellitus

Type 1 arises due to the diminished production of insulin. Insulin production is diminished because the beta cells that produce insulin are destroyed by the

autoantibodies. It is also called juvenile diabetes since it develops in children in the age group of 0 to 15 years [Das et al., 2015].

b) Type 2 diabetes mellitus (T2DM)

Type 2 arises due to insulin resistance, which involves the failure of cells to take up glucose from the blood despite the presence of insulin. Type 2 develops mainly in adults in the age group of 30-80 years [Atre et al., 2020].

c) Gestational diabetes mellitus:

This type of diabetes develops transiently during pregnancy. Placental hormones produced during pregnancy, such as human chorionic gonadotropin hormone and human placental lactogen hormone, have a blocking effect on insulin action. In normal pregnancy, the reduced action of insulin is compensated for by increased insulin secretion from the pancreas. However, such compensatory increases do not occur in women who develop gestational diabetes [Lende et al., 2020].

d) Maturity onset diabetes mellitus (MODY):

MODY is a type of diabetes caused by mutation of genes such as hepatocyte nuclear factor and glucokinase. The proteins encoded by these genes play an important role in the homeostasis and metabolism of glucose. Mutations in these genes are inherited in an autosomal dominant pattern. MODY usually develops before the age of 25 years [Hoffman et al., 2021; Naylor et al., 2018].

Among the four types described above, type 2 is the most common type, which is seen in approximately 60 to 80% of diabetic patients. This is followed by type 1 (8 to 12% of diabetic patients) and gestational diabetes (10% of diabetic patients). MODY is

quite rare, as it is seen in only approximately 1 to 5% of diabetic patients. T2DM is the main focus of this study.

3.2. Glucose metabolism

3.2.1. The biological significance of glucose:

Glucose is the main source of energy for all cellular functions. Glucose is utilized for the production of ATP in the mitochondria [Bonora et al., 2012].

3.2.2. Sources of glucose:

Glucose is mainly derived from the diet. Starch is the main dietary source of glucose. Other sugars in the diet are fructose, sucrose, maltose, lactose, etc. Other forms of carbohydrates are eventually converted into glucose before entering energy metabolism. During starvation, lipids and proteins are broken down and converted into glucose for use in energy production [Chen et al., 2015].

3.2.3. Absorption of dietary sugars:

Dietary sugars are absorbed mainly in the small intestine by enterocytes. These are epithelial cells that line the small intestine. Glucose cannot cross the cell membrane since it is polar in nature. Therefore, the diffusion of glucose is facilitated by proteins called transporters. There are two types of transporters: sodium-glucose cotransporter 1 (SGLT1) and glucose transporters (GLUTs) [Holesh et al., 2021; Navale et al., 2016]. There are six types of glucose transporters, which are summarized in **Table 3.1.**

Table 3.1: Types of transporters for dietary sugar absorption

Type of transporter	Location	Function
SGLT1	Intestinal epithelium and renal tubules	Acts as cotransporter of glucose and galactose with Na^+ requires ATP for the transport of glucose along its concentration gradient
GLUT1	Placenta, skeletal muscle, adipose tissue, liver hepatocytes, RBCs	Essential for glucose sensing by the pancreas, important feedback mechanism for glucose homeostasis with endogenous insulin
GLUT2	Pancreatic beta cells, hepatocytes, renal tubular cells, intestinal epithelium, proximal tubule	Glucose sensor in trans epithelium for glucose and fructose. Important for glucose metabolism in the liver
GLUT3	Central nervous system and small intestine	High affinity for glucose, a scavenger for cells with the high rate of glucose demands
GLUT4	Skeletal muscle cells, enterocytes, cardiomyocytes, brain tissue, and adipocytes	Insulin responsive isoform, translocate into plasma membrane upon insulin stimulation
GLUT5	The small intestine, brain, muscle, and adipose tissue	Fructose transporter
GLUT6	Ubiquitous, present in all the cells	Transporter present in pseudo genes, nonfunctional

Sodium-glucose cotransporter 1 (SGLT1) is the main transporter responsible for glucose absorption in the small intestine. This protein functions as a cotransporter. The cotransporter facilitates the movement of glucose along with sodium from the

intestinal lumen to the enterocyte through active transport via symport, as shown in **Figure 3.1**.

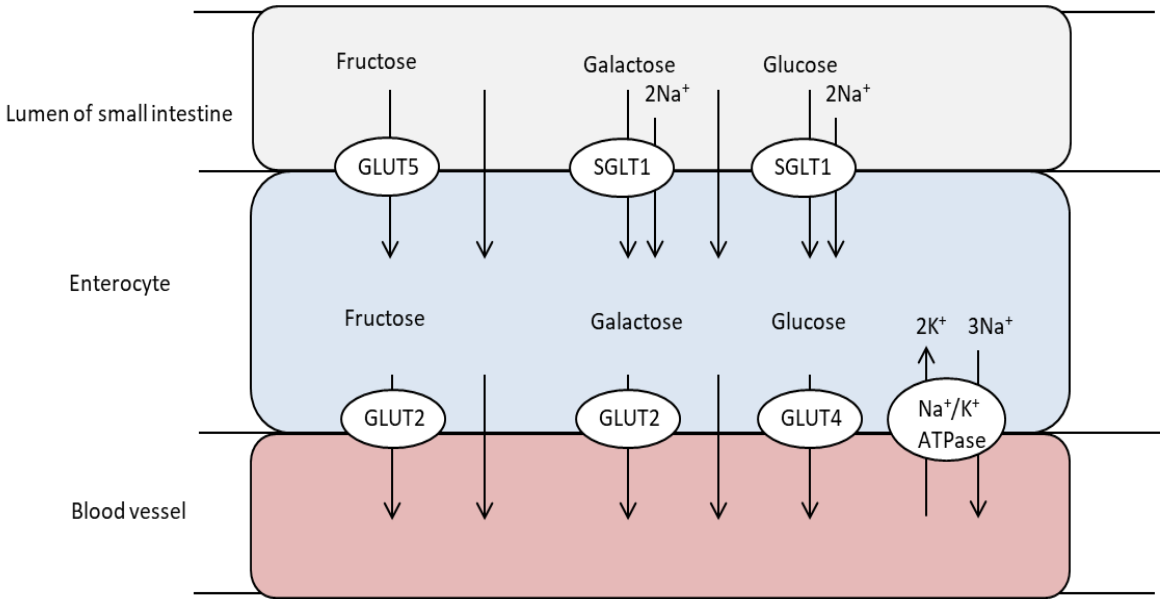

Figure 3.1: Transport of glucose from the small intestine to blood vessels

3.2.4. Circulation of glucose:

From the enterocyte, sugars enter the capillaries through glucose transporters. From here, sugars are transported through the circulatory system for utilization by all the cells of the human body. Again, glucose transporters are responsible for absorbing glucose from the blood.

Transport of glucose through the cell membrane requires the utilization of glucose in most tissue cells, and the transport of glucose from the blood via the cell membrane into the cytoplasm is necessary. Glucose cannot pass through easily due to its membrane polarity. However, cells absorb glucose from the blood with the help of membrane-bound glucose transporters. Some glucose transporters require insulin for

their action. Based on this requirement, glucose transporters are classified into three types [Panahi et al., 2020]. They are as follows:

1. Sodium-dependent glucose transporters, e.g., SGLT1
2. Insulin-independent glucose transporters, e.g., GLUT1, GLUT2, GLUT3
3. Insulin-dependent glucose transporters, e.g., GLUT4

3.2.5. Cellular respiration:

After entering the cytoplasm, glucose undergoes phosphorylation to form glucose-6-phosphate. Glucokinase in the liver and hexokinase in most other cells are involved in this process. The phosphorylation process ensures that glucose is trapped inside the cell. It is typically irreversible, except for liver cells, intestinal epithelial cells, and renal tubular epithelial cells, which have reversible glucose phosphatase [Nakrani et al., 2021].

There are two fates for glucose 6-phosphate. It will either enter the glycolysis pathway for energy release or is converted into glycogen and stored for energy [Bonora et al., 2012]. Glucose 6-phosphate is converted to pyruvate under anaerobic conditions and may become lactate or enter the citric acid cycle for energy release. The aerobic respiration by oxidative phosphorylation takes place by the electron transport chain with the release of ATP, which will be utilized for energy needs (**Figure 3.2**) [Rajas et al., 2019; Yetkin-Arik et al., 2019; Choudhry et al., 2021].

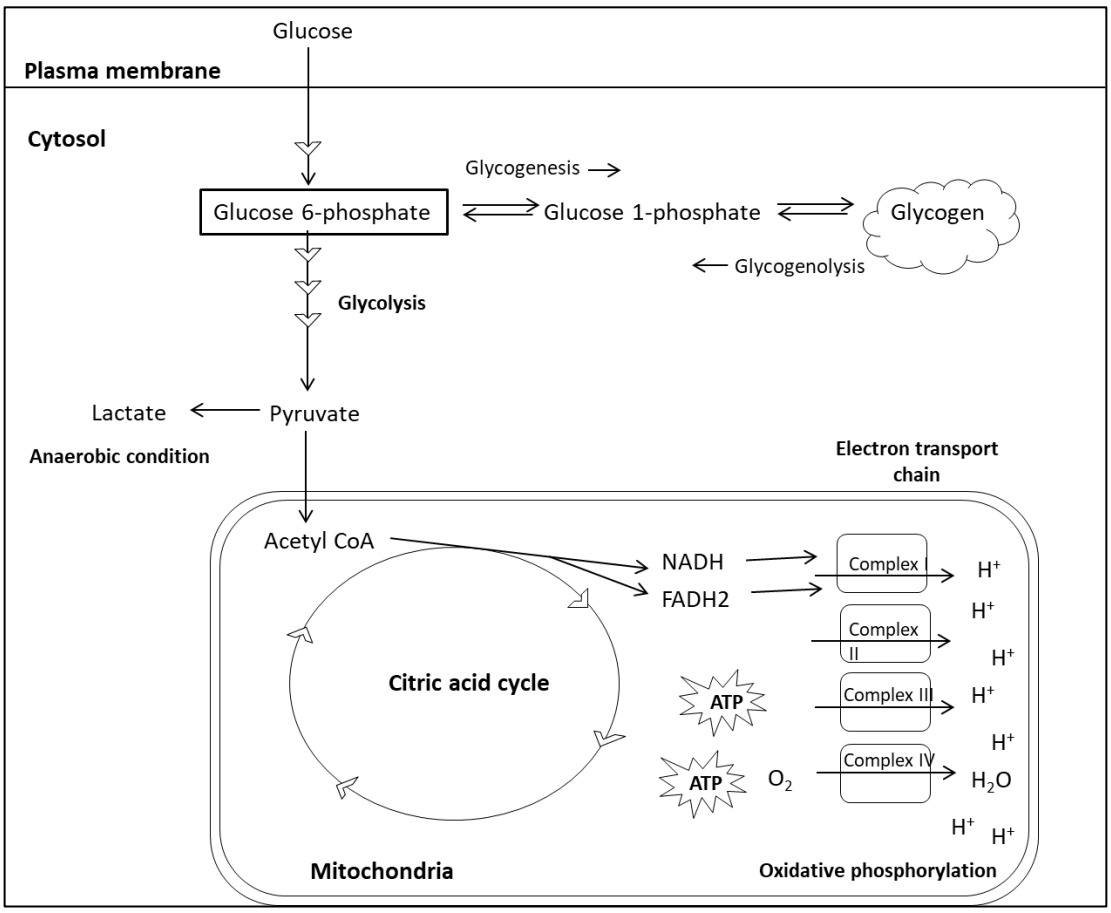

Figure 3.2: The fate of glucose 6-phosphate in cellular respiration

3.2.6. Role of insulin in glucose absorption:

The presence of excess nutrients in the diet results in excess glucose in the blood. This triggers pancreatic beta cells to produce insulin. Insulin itself cannot enter the cells because of its hydrophilic nature. Hydrophobic cell membranes do not allow insulin to enter the cell. Therefore, insulin binds to its receptor on the cell membrane and activates it. The protein tyrosine kinase in the beta subunit attaches to the insulin receptor substrate (IRS). Phosphorylation of the tyrosine residue of IRS upon kinase activity takes place and forms two domains with different transduction pathways. The attachment of tyrosine-protein kinase Src to one of the domains upon phosphorylation activates phosphatidylinositol 3-kinase, which converts phosphatidylinositol 4,5-bis-phosphate to phosphatidylinositol (3,4,5)-triphosphate. This results in the recruitment

of protein kinase B, also called Akt, which is a serine/threonine-specific protein kinase. Membrane-bound cyclin-dependent kinases and DNA-activated protein kinases phosphorylate Akt and are released into the cytoplasm, where they have diverse functions. Upon phosphorylation, Akt attracts vesicles containing glucose transporter 4 and facilitates its fusion to the cell membrane. Transfer of glucose transporters from the cytosol to the cell membrane facilitates the entry of glucose into the cell [Arneth et al., 2019; De Meyts et al., 2016]. The schematic representation is shown in **Figure 3.3**.

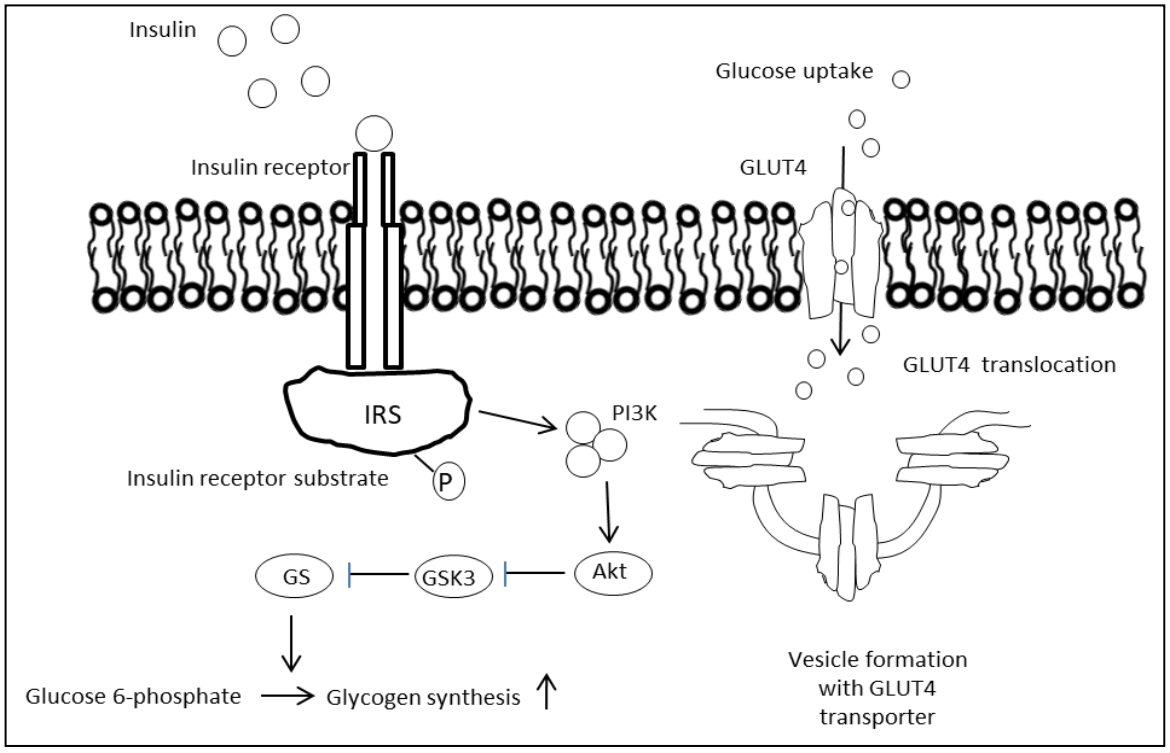

Figure 3.3: Role of insulin in glucose absorption by the signal transduction pathway

3.2.7. Hormonal regulation of blood glucose:

Blood glucose levels were maintained under homeostatic conditions (80-120 mg/dl) for two reasons. First, low levels (hypoglycemia) will result in reduced glucose supply to the cells to meet their energy requirement. Second, higher levels (hyperglycemia) will result in long-term microvascular complications such as diabetic retinopathy,

nephropathy, peripheral neuropathy, and macrovascular complications, viz., ischemic heart disease, and peripheral vascular disease [Chawla et al., 2016].

Hormones are involved in the homeostasis of blood glucose levels. The two main hormones involved in this process are insulin and glucagon. These two hormones function in opposite directions. Insulin serves to reduce blood glucose levels, whereas glucagon serves to increase it. Insulin reduces blood glucose levels by promoting its absorption by insulin-dependent cells of the liver and skeletal muscle. Furthermore, insulin also promotes glycogen synthesis. The mechanism by which insulin promotes glucose absorption is described in the previous section.

Glucagon serves to increase blood glucose levels by promoting the conversion of glycogen to glucose. The major stores of glycogen are present in the liver and skeletal muscle.

Both insulin and glucagon are produced by the pancreas. Insulin is produced from the beta cells of pancreatic islets, and glucagon is produced from the alpha cells of the pancreas. Insulin has a positive feedback mechanism to regulate glucose homeostasis, and glucagon has a negative feedback mechanism and regulates the release of glucose into the blood for normal glucose homeostasis [Kulina et al., 2016; Godoy-Matos et al., 2014]. Shown in **Figure 3.4.**

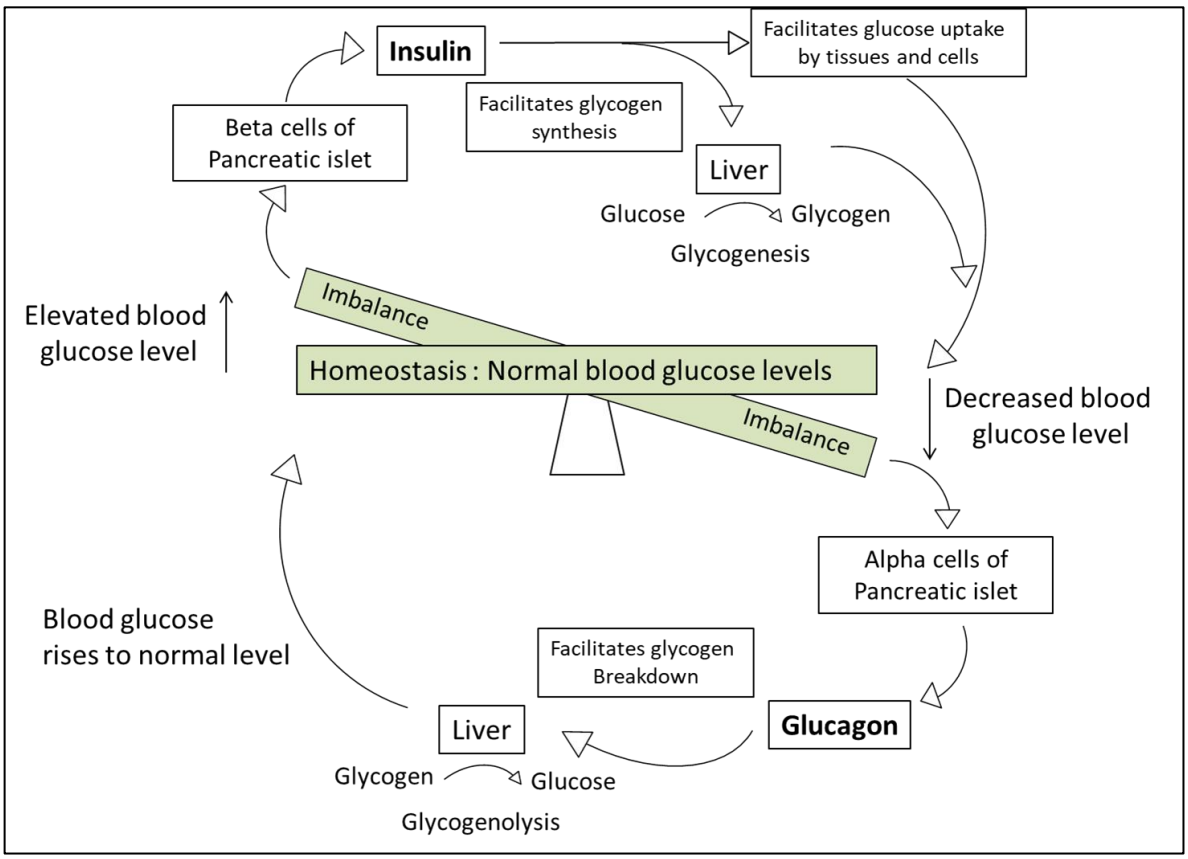

Figure 3.4: Hormonal regulation of blood glucose levels

3.3. Pathogenesis of T2DM:

Insulin resistance plays a major role in the pathogenesis of T2DM. Insulin resistance involves the inability of cells to absorb glucose despite the presence of sufficient levels of insulin in the plasma. Biochemically, insulin resistance is seen as a decreased ratio of blood glucose and blood insulin levels. It is measured using an index called the homeostasis model assessment for insulin resistance (HOMA-IR) [Gayoso-Diz et al., 2013].

In T2DM, because of insulin resistance, the cell is unable to bind to the insulin receptor and affects signal transduction for the absorption of glucose into the cell. Phosphorylation is diminished for IRS and produces inactive phosphatidylinositol 3-kinase. Phosphatidyl 3-kinase will not participate in the conversion of phosphatidyl

inositol 4,5 diphosphate to phosphatidyl inositol 4,5,6-triphosphate and produces inactive protein kinase B (Akt). Therefore, Akt is not involved in the vesicle formation of glucose transporter 4, resulting in reduced glucose uptake into the cell. As a result, the translocation of glucose was diminished. The blood glucose levels were increased, resulting in the production of more insulin. Furthermore, Akt will not phosphorylate glycogen synthase kinase 3, resulting in phosphorylation of glycogen synthase and becoming inactive. Therefore, glycogen synthesis from glucose 6-phosphate was reduced, and gluconeogenesis was increased in the cytosol. A schematic representation is shown in **Figure 3.5.**

Furthermore, insulin resistance is compensated by enhanced insulin secretion leading to hyperinsulinemia [Taylor et al., 2012; Freeman et al., 2020]. However, prolonged compensatory hyperinsulinemia eventually leads to the impairment of pancreatic beta cells. This results in the development of hyperinsulinemia, and T2DM patients become dependent on supplementary insulin.

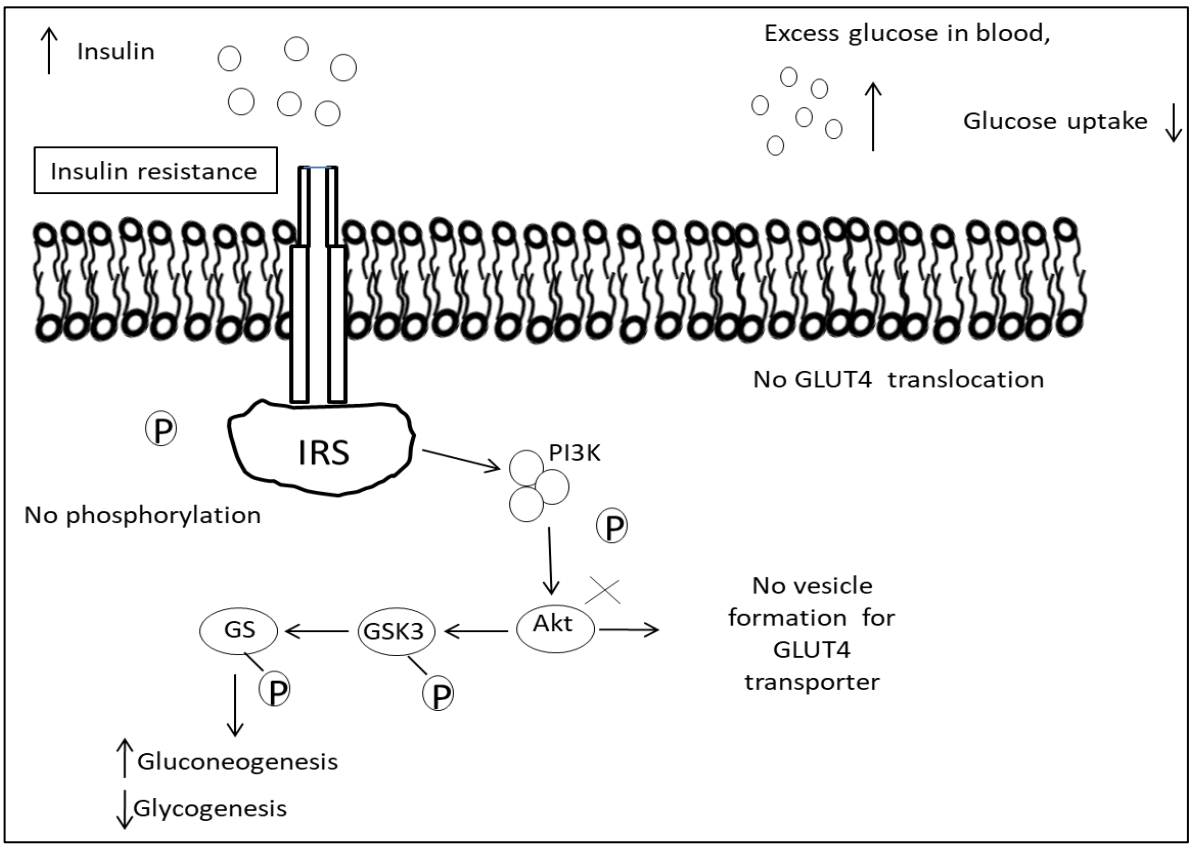

Figure 3.5: Role of insulin resistance in T2DM

3.4. Role of glycogen pathway in glucose metabolism:

The glycogen pathway plays an important role in regulating glucose levels. When the glucose level is above the cellular energy requirement, excess glucose is converted into glycogen through glycogenesis [Jin et al., 2018]. When the cellular glucose level is less than the metabolic requirement, extra glucose is mobilized through the degradation of glycogen, a process referred to as glycogenolysis [Jensen et al., 2011]. Shown in Figure 3.6.

The first step in glycogen synthesis is the conversion of glucose 6-phosphate into glucose 1-phosphate. Glucose 6-phosphate is the intermediate molecule linking glycolysis and the glycogen pathway. This isomerization of glucose 6-phosphate into glucose 1-phosphate and vice versa is catalyzed by the phosphoglucomutase enzyme.

Furthermore, glucose 1-phosphate is converted into uridine diphosphate glucose by the pyrophosphate enzyme. This is the starting point of making the linear glycogen molecule by adding multiple glucose monomers with each other to form a long chain of oligosaccharides. Glycogenin subunits continue to add glucose residues, and the attached glucose then serves as a primer for the glycogen synthase enzyme to add more glucose. Glycogen synthase is the major enzyme involved in glycogen synthesis. Involved in the conversion of glycosylated UDP-glucose, it is converted into glycogen [Han et al., 2016]. The glycogen synthase enzyme catalyzes the conversion of uridine diphosphate glucose to terminal glucose on glycogenin, forming a long chain of linear glycogen with alpha 1,4 glycosidic bonds. Finally, the glycogen branching enzyme catalyzes the conversion of alpha 1,4, glycosidic bonds to alpha 1,6 glycosidic bonds and converts long-chain linear glycogen to branched glycogen molecules **(Fiure 3.6)** [Adeva-Andany et al., 2016].

Glycogen degradation takes place when the extracellular supply of glucose is limited. Glycogen phosphorylase enzyme catalyzes the conversion of glycogen to glucose 1-phosphate. Furthermore, glucose 1-phosphate is converted to glucose 6-phosphate by the phosphoglucomutase enzyme. The resulting glucose 6-phosphate is then utilized in the glycolysis pathway for energy needs [Adeva-Andany et al., 2016; Jensen et al., 2011].

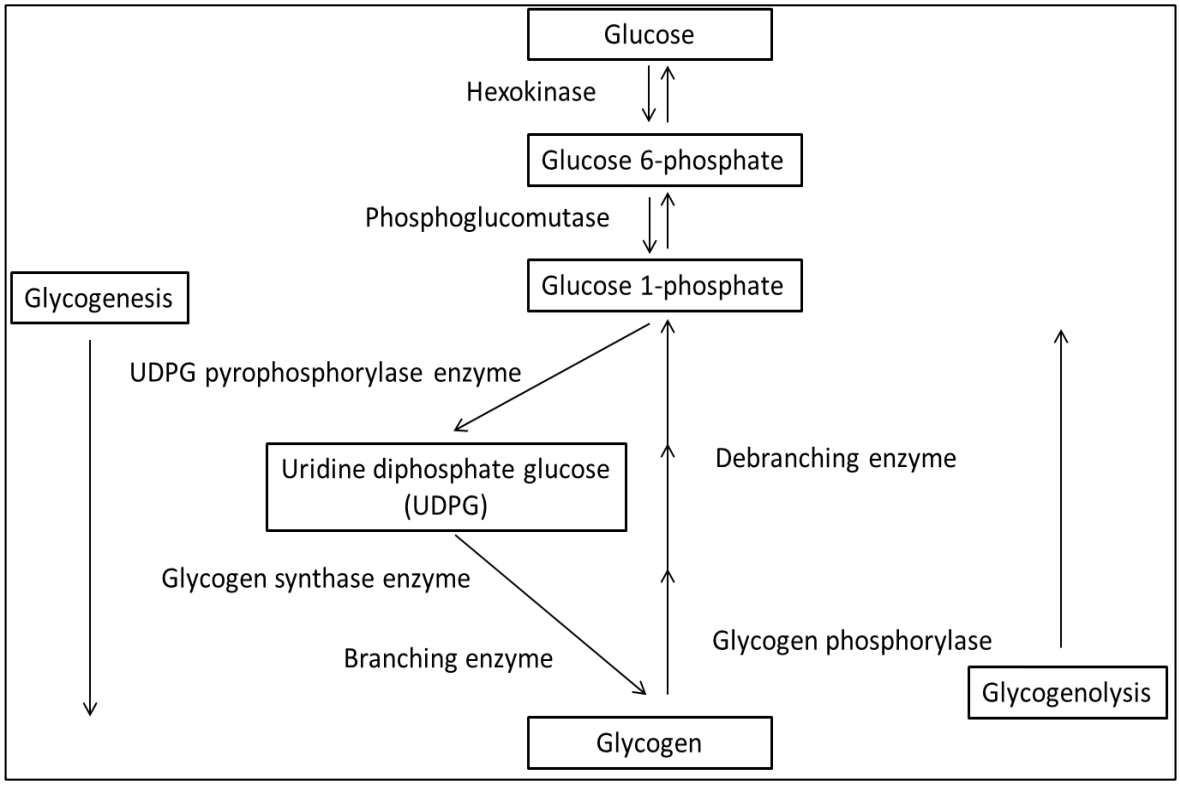

Figure 3.6: Role of the glycogen pathway in glucose metabolism

3.5. Role of the glycogen pathway in the pathogenesis of T2DM:

The glycogen pathway plays an important role in regulating blood glucose levels. Several studies have shown that the glycogen pathway is impaired in T2DM.

Del Prato and coworkers studied glycogen synthesis and degradation in noninsulin-dependent diabetes mellitus using the clamp technique [Del Prato et al., 1994]. Hepatic glycogen synthesis and degradation were measured by using ^{14}C glucose infusion and indirect calorimetry. Insulin-mediated glucose uptake in the liver was reduced in insulin-dependent diabetes mellitus. Furthermore, insulin-mediated glycogen synthesis was also reduced in the liver and muscle. Based on these observations, the authors concluded that glycogen synthesis is defective in insulin-dependent diabetes mellitus.

Krssak and coworkers evaluated hepatic glycogen synthesis and degradation in T2DM patients and healthy volunteers under hyperinsulinemic-euglycemic clamp [Krssak et al., 2004]. The hepatic glycogen concentration was measured by using ^{13}C-labeled nuclear magnetic resonance spectroscopy. Hepatic glycogen synthesis was reduced under postprandial conditions. Furthermore, glycogen degradation was also reduced under prolonged fasting conditions. These observations motivated the authors to conclude that hepatic glycogen metabolism may be defective in T2DM.

Efforts have been made to explore the causes of glycogen pathway impairment in T2DM. Current evidence points to enzyme defects as the potential source. Glycogen synthase and glycogen synthase kinase are implicated in this direction.

Damsbo and coworkers evaluated glycogen synthase activity in T2DM. Glycogen synthase activity was measured in cultured muscle cells obtained from skeletal muscle biopsies cultured under in vitro conditions by insulin stimulation [Damsbo et al., 1991]. Glycogen synthase activities in response to insulin resistance have been found to be reduced in skeletal muscle biopsy samples of T2DM. These observations revealed that insulin-stimulated glycogen synthase activity may be impaired in T2DM.

Glycogen synthase activity is regulated by phosphorylation. The active enzyme is phosphorylated, whereas the inactive enzyme is dephosphorylated. These two processes are catalyzed by glycogen synthase kinase and protein phosphatase 1. Studies by Nikoulina and coworkers showed that the expression of glycogen synthase kinase was elevated in the skeletal muscle of T2DM patients [Nikoulina et al., 2000]. Additionally, insulin-stimulated glycogen synthase activity was reduced in T2DM. Furthermore, a reciprocal relationship was observed between glycogen synthase

activity and glycogen synthase kinase expression. These results suggest that abnormal reduction of glycogen synthase activity in T2DM may arise due to downregulation of glycogen synthase kinase. The role of glycogen synthase phosphorylation in T2DM was further explored by Hojlund and coworkers using the euglycemic hyperinsulinemic clamp technique [Hojlund et al., 2003]. Both phosphorylation and glycogen synthase activity were reduced in the skeletal muscle of T2DM patients. The schematic representation is shown in **Figure 3.7.**

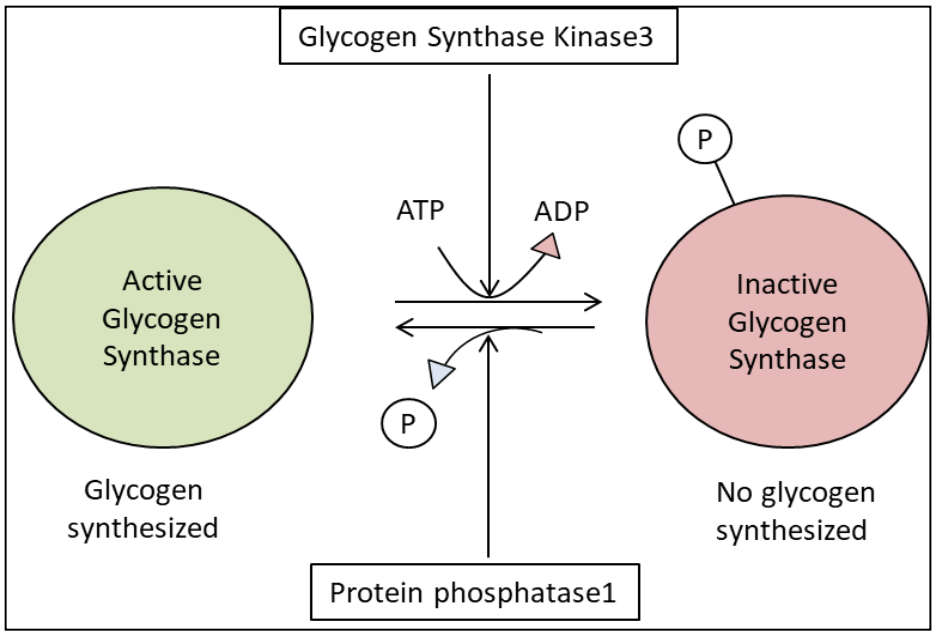

Figure 3.7: Schematic representation of glycogen synthase phosphorylation

3.6. Lacunae in knowledge:

The review of the literature shows that glycogen metabolism is abnormal in T2DM. This appears to arise due to abnormalities in the enzymes involved in the glycogen pathway. Glycogen synthase and glycogen synthase kinase are involved in disrupting the glycogen pathway in T2DM. Phosphoglucomutase (PGM) is a key enzyme in the glycogen pathway that serves as a connecting link with the glucose pathway. Abnormal glycogen metabolism can also arise due to the abnormal functioning of the

phosphoglucomutase enzyme. However, there is no literature on the functional status of the PGM enzyme in T2DM. Hence, this study is planned.

PGM is a group of isozymes of the phosphohexose mutase family. There are four different phosphoglucomutase isozymes in human beings viz., PGM1, PGM2, PGM3, and PGM5. The PGM1 enzyme is expressed in most tissues, whereas PGM2 is expressed predominantly in RBCs. PGM3 is seen mostly in prostate and placental tissue, and PGM5 is seen mostly in the myocardium [Stiers et al., 2017].

Among these four PGM isoforms, the PGM1 isoform is the predominant isoform, which is expressed in most of the tissue types, including hepatocytes, skeletal myocytes, and PBMCs.

MATERIALS AND METHODS

4.1. Study Design:

The study was carried out by using a case–control design. The case group comprised patients diagnosed with T2DM. The control group will comprise age and gender-matched healthy individuals. T2DM patient's blood samples were collected from the participants, and the following parameters will be evaluated: glycogen degradation and PBMC viability under glucose limiting conditions, PGM enzyme activity, *PGM1* gene expression, and frequency of SNP rs11208257 in genomic DNA. The results of the two groups will be compared by statistical methods.

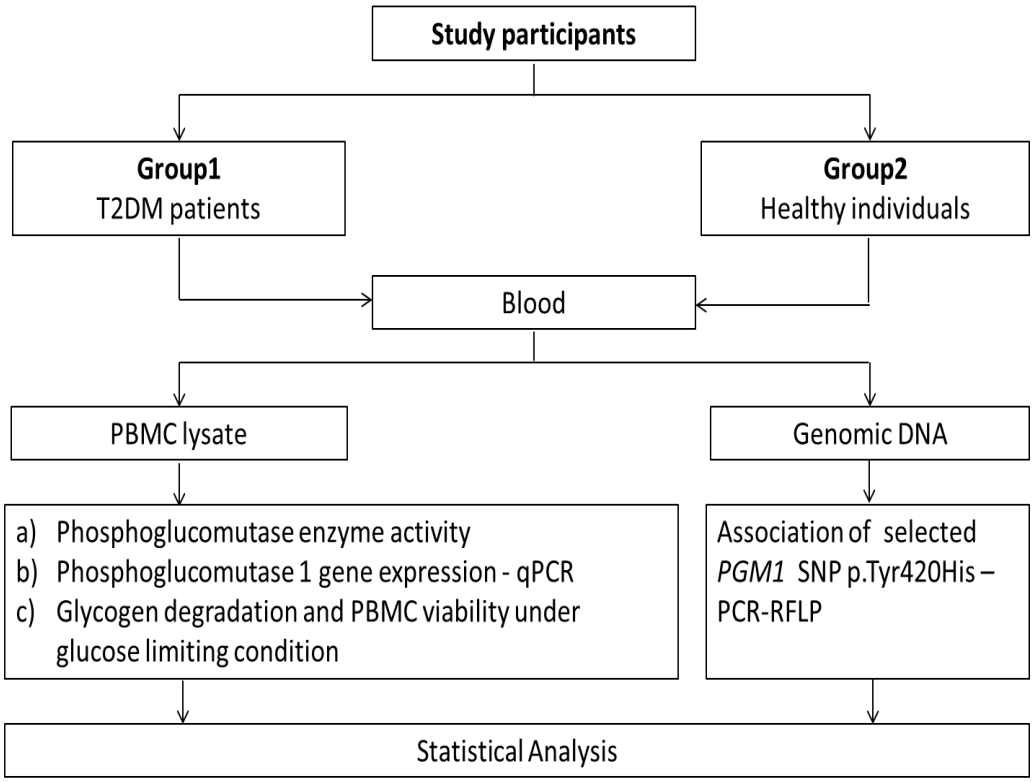

Figure 4.1: Schematic representation of the study design

4.2. Ethical issues:

The study was conducted after obtaining approval from the institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar, India **(Ref.no: SDUMC/KLR/IEC/30/2019-20 dated 06-June-2019).** T2DM patients and healthy

controls were recruited from 2019 to 2020. Informed consent was obtained in writing before enrolment in the present study.

4.3. Selection of study participants:

The study participants were recruited from the Department of General Medicine, R. L. Jalappa Hospital and Research Centre, attached to Sri Devaraj Urs Medical College, Kolar, Karnataka, India. T2DM patients were enrolled upon satisfaction of the inclusion and exclusion criteria.

4.4. Inclusion criteria:

The inclusion criteria for the selection of the T2DM group were based on the following criteria of the Indian Council of Medical Research htttp://icmr.nic.in/guidelines_ diabetes/guide_diabetes.htm]:

a) Patients of both genders

b) between the ages 30 - 80 years

c) fasting blood glucose ≥ 126 mg/dL

d) HbA1c > 6.5% in the last test performed in the 12 months before the study

The inclusion criteria for the selection of control group subjects were as follows:

a) healthy individuals of both gender and age, between the ages 30 – 80 years

b) no known history of any chronic disease

c) HbA1c < 6.5% in the last test performed in the 12 months before the study

4.5. Exclusion criteria:

The exclusion criteria for the patient selection were

a) Microvascular complications

b) Chronic comorbidity

4.6. Sample size:

The sample size for the present study was calculated based on the mean difference in hepatic glycogen concentrations observed between the T2DM and control groups (Martin et al., 2004). The sample size was calculated by considering the difference of a 3% increase in hepatic glycogen concentration in the T2DM group compared to the control group. The sample size required with a 95% confidence interval and 90% power was 63 per group. However, the study groups were analyzed for genetic variation in the *PGM1* SNP rs11208257. Based on the preliminary findings of the data (pre hoc power: 45.3%), post hoc power analysis was carried out. The sample size with 80% power is estimated to be 225 per group.

4.7. Clinical sample collection and processing:

5 ml of venous blood will be collected from the T2DM patients and healthy controls. PBMCs were prepared by using Ficoll-histopaque medium and divided into two parts. The first half will be used for cell culture experiments and RNA preparation. The remaining PBMCs were stored as PBMC lysate with radioimmunoprecipitation assay buffer (Himedia, India) supplemented with protease inhibitor cocktail (Roche, Mannheim, Germany) and were stored at -80°C for further use.

4.8. PBMC isolation:

PBMCs were isolated from whole blood using Ficoll-Histopaque (Merck, Darmstadt, Germany) [Mallone et al., 2011]. Briefly, 1 ml of anticoagulated blood was layered on 1 ml of Ficoll-histopaque and centrifuged at 3000 rpm for 30 min without break. The PBMC layer was collected in a 15 ml Falcon tube and washed twice with 10 ml of 1X phosphate-buffered saline (PBS). The number of PBMCs was counted using a hemocytometer and used for *in vitro* culture.

4.9. PBMC culture and glucose treatment

Glucose availability in the PBMC culture was altered in two phases. The initial culture condition was glucose-proficient, whereas the subsequent condition was glucose-limited. PBMCs (1 x 10^5 cells) were seeded into 5 ml of glucose-free RPMI 1640 medium (Cat # 11879020, Gibco, New York, USA). The medium was supplemented with 10 mM glucose, 20% heat-inactivated fetal bovine serum, 300 µl phytohemagglutinin (30 µg/mL final), and 1% antibiotics. The cultures were incubated at 37°C for 48 hours in a 5% CO_2 atmosphere. PBMCs were harvested after incubation by centrifugation for 10 min at 2000 rpm and washed with 1X PBS. The pellet was then cultured under glucose-limited conditions. The culture conditions were as before but without supplementation with 10 mM glucose. The cultures were incubated for 24 hours, and the PBMCs were harvested by centrifugation for 10 min at 2000 rpm. The pellet was washed with 1X PBS and used to measure viability under the glucose-limited conditions.

4.10. Cell viability assay:

The viability of the cultured PBMCs was determined by the Trypan-Blue assay [Strober et al., 2001]. Briefly, the cell suspension (1:1 ratio) was mixed with 0.4% Trypan Blue (Gibco, New York, USA) and loaded into a hemocytometer. Unstained cells were recorded as viable, and blue-stained cells were scored as non-viable. The magnitude of cell viability with induction of glucose deprivation was determined as below:

$$Viability(\%) = \begin{bmatrix} Viable\ cells \\ prior\ to\ glucose \\ deprivation\ (\%) \end{bmatrix} - \begin{bmatrix} Viable\ cells \\ post\ glucose \\ deprivation\ (\%) \end{bmatrix}$$

4.11. Determination of glycogen degradation

The Periodic Acid-Schiff (PAS) staining technique was used to evaluate the amounts of glycogen in the cultured PBMCs [Tabatabaei Shafiei., 2014]. Briefly, 1X PBS-washed slides were treated with fixative (37% formaldehyde and 99% ethanol). PBMCs were then added to the slides and left for 1 min. Then, the slides were treated for 5 min with 1% periodic acid and 15 min with Schiff's reagent (SRL Biolabs, Maharashtra, India). Then, the slides were counterstained for 30-45 sec with hematoxylin. Approximately 80-85% of PBMCs were scored. The percentage of glycogen-positive cells was used as the measure of glycogen levels. The levels of glycogen degradation were estimated by using the following formula:

$$\begin{matrix} Glycogen \\ degradation = \\ (\%) \end{matrix} \left[\begin{matrix} Glycogen\ levels \\ prior\ to\ glucose \\ deprivation\ (\%) \end{matrix} \right] - \left[\begin{matrix} Glycogen\ levels \\ upon\ glucose \\ deprivation\ (\%) \end{matrix} \right]$$

4.12. PBMC lysate preparation and protein estimation

PBMCs were isolated from whole blood using Ficoll-Histopaque (Merck, Darmstadt, Germany). Briefly, 1 ml of anticoagulated blood was added to 1 ml of Ficoll-histopaque and centrifuged for 30 min at 3000 rpm. The PBMC layer was separated in a 15 ml falcon tube, and 1X phosphate-buffered saline (PBS) was used to wash twice. The number of viable PBMCs was counted using a hemocytometer and used for the PGM1 enzyme assay [Mallone et al., 2011]. Approximately 1 x 10^5 PBMCs were considered from the study participants. Approximately 50 µl PBMCs were collected with radioimmunoprecipitation assay (RIPA) buffer (Himedia, Nashik, India) supplemented with the protease inhibitor cocktail phenylmethlsulfonyl fluoride (PMSF) (Roche, Mannheim, Germany), brought to a final volume of 50 µl and stored at -20^0 C for further use. Total protein in PBMC cell lysates was determined by the

bicinchoninic acid (BCA) method [Smith PK et al., 1985]. Briefly, the BCA protein assay was used for the quantitation of total protein in a sample. The principle of this method is that proteins can reduce Cu^{+2} to Cu^{+1} in an alkaline solution (the biuret reaction) and result in a purple colour formation by BCA.

4.13. PGM enzyme assay:

PGM enzyme activity was measured using the PBMC lysate. The lysate was made by resuspending approximately 50 µl of PBMC preparation in RIPA buffer (Himedia, India) with protease inhibitor cocktail (Roche, Mannheim, Germany). The lysate was stored at -20°C until further use. The total protein present in the PBMC lysate was determined by the bicinchoninic acid method [Smith, 1985]. The protein concentration of the lysate was used for the normalization of the enzyme activity. PGM1 activity was measured in the PBMC lysate by the colorimetric method [Najjar, 1955] by using a commercial kit (Cat # K774-100, BioVision, Milpitas, CA). Briefly, diluted PBMC cell lysates (1:10 dilution) from the study participants were resuspended in assay buffer and then processed according to the manufacturer's protocol. One unit of PGM1 activity was defined as the amount of enzyme that generated 1.0 µmol of NADH per min at pH = 8.0 at 37°C. PGM enzyme activity was expressed as milliunits per milligram of lysate protein (mU/mg of lysate protein).

4.14. *PGM1* gene expression analysis:

PGM1 gene expression was quantified by using the quantitative reverse transcription-polymerase chain reaction (qRT–PCR) method. Total RNA from PBMCs was prepared using a commercial kit (Cat # 15596018, Thermo Scientific, Waltham, USA) according to the TRIzol method [Chomczynski et al., 1993]. The cDNA preparation from total RNA was carried out by the *in vitro* reverse transcription method using a

commercial kit according to the manufacturer's instructions (Cat # 1708891, Bio–Rad, Hercules, CA). A positive control (pooled sample) was used for interplate adjustment. The reaction mixture composition for each 20 µl reaction was as follows: 14 µl of total RNA, 1 µl of reverse transcriptase enzyme, and 6 µl reaction mixture (4 µl of 5X script reaction mix and 1 µl of nuclease-free water). The primers used for cDNA synthesis are shown in **Table 4.1.**

Table 4.1. The PCR conditions for cDNA synthesis:

PCR conditions	Temperature
➢ PCR priming	25°C for 5 min
➢ Reverse transcription	46°C for 20 min
➢ Reverse transcriptase inactivation	95°C for 1 min

The primers used for amplification of the *PGM1* gene are shown in **Table 4.2.** The *GAPDH* gene was used as the internal reference. The 10 µl reaction mixture for qRT–PCR contained 2 µl of cDNA, 1 µl of primers (40 nM final), 5 µl of SYBR green (Cat #1725271, Bio–Rad, Hercules, CA), and 2 µl of nuclease-free water.

Table 4.2: qRT–PCR primers used for the quantification of the *PGM1* gene

Gene	Primers	*PGM1* primers
PGM1	Forward primer	5' TAA TGG AGG TCC TGC TCC AG 3'
	Reverse primer	5' TTT CCC AGA ACA CCA AGG TC 3'
GAPDH	Forward primer	5' GAT CAT CAG CAA TGC CTC CT 3'
	Reverse primer	5' GAC TGT GGT CAT GAG TCC TTC 3'

The thermal program for the qRT–PCR run is shown in **Table 4.3.** The reactions were carried out in duplicate, and the average ΔCt was determined for both the *GAPDH* and *PGM1* genes. The comparative Ct method [Livak, 2001] was used to determine the fold change ($2^{-\Delta\Delta Ct}$) in *PGM1* gene expression.

Table 4.3: Thermal parameters for qRT–PCR

Parameter	**Temperature**
➢ Initial denaturation ➢ Cycle denaturation ➢ Annealing and extension + Plate Read	95^0 C for 10 min 95^0 C for 15 sec 60.6^0 C for 30 sec
Thermal cycles (Go to step 2, 35X)	
➢ Melt curve For 0.05 + Plate Read	55^0 C to 95^0 C increments of 0.5^0 C

4.15. DNA extraction

Genomic DNA was isolated by the salting-out method (Miller *et al.* 1988). Approximately 2 ml of the blood sample was collected in an EDTA vacutainer, which was vortexed and then transferred into a sterile 15 ml falcon tube. Erythrocyte lysis buffer (ELB) was added to the Falcon tube containing the blood sample at a ratio of 1:4, followed by thorough mixing. The sample was then incubated for approximately 30-45 min on ice to induce hemolysis. The hemolysed sample was then centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and 10 ml of ELB was added to the pellet. The suspension was subjected to centrifugation at 3000 rpm for 10 min. The supernatant was discarded, and the pellet was resuspended in 5 ml of ELB. The suspension was then supplemented with 270 µl of 20% SDS and 30 µl of proteinase K.

The suspension was incubated at 37°C overnight in a water bath. The next day, 500 µl of 5 M sodium chloride was added to the samples, and an equal volume of 100% isopropyl alcohol was added to the Falcon tube to precipitate the DNA. The DNA was then transferred to a 1.5 ml micro centrifuge tube containing freshly prepared 500 µl of 80% ethanol. The sample was incubated for 15 min at room temperature and centrifuged at 12,000 rpm for 5 min. The supernatant was discarded, and the pellet was washed with 80% ethanol thrice. The pellet was air-dried and then resuspended in 500µl of Tris-EDTA buffer. The sample was then incubated at 65°C in a water bath for 30 min. Following this, the sample was kept in a rotator overnight to dissolve the DNA completely. The sample was then stored at -80°C until further analysis.

4.16. DNA quantification and purity analysis:

The DNA concentration and purity were determined by spectrophotometry. Measurements were carried out on a UV–Vis spectrophotometer (Perkin Elmer model Lambda 35, Waltham, MA, USA) to check the concentration and purity of the DNA. The amount of DNA was estimated using the formula dsDNA concentration = 50µg/ml x OD260 x dilution factor. The ratio of absorbance at 260 and 280 nm in the range of 1.7 to 2.0 was regarded as pure.

4.17. Genotyping of *PGM1* SNP rs11208257:

Genomic DNA was prepared from peripheral blood samples by using the salting-out method [Miller et al., 1988]. The concentration and purity of the genomic DNA were determined by UV spectrophotometry (Perkin Elmer model Lambda 35, Waltham, USA). PCRs were performed on a gradient thermal cycler (Bio–Rad, California, USA). The 20 µl reaction mixture included 1X assay buffer, PCR mix comprising 10pmol of each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 150 – 300ng of genomic

DNA, and 1 unit of Taq DNA polymerase (Bangalore Genei, India) in a final volume of 25µl. PCR primers used for the genotyping of c.1258 T > C SNP alleles are summarized in **Table 4.4.** The PCR parameters employed for PCR are summarized in **Table 4.5.**

Table 4.4: PCR primers used for genotyping of the *PGM1* gene

Parameters	***PGM1* primers**
Forward primer (5' – 3')	5' CCC TCC CTC AAC ATG AGA TTT G 3'
Reverse primer (3' – 5')	5' CAA TTG AGA GAG GCT GGA TGA C 3'

Table 4.5: PCR parameters used for genotyping the *PGM1* gene

Parameter	**Temperature**
➢ Initial denaturation	95^0 C for 3 min
➢ Cycle denaturation	95^0 C for 30 sec
➢ Annealing	60.6^0 C for 30 sec
➢ Extension	72^0 C for 1 min
Thermal cycles (35X)	
➢ Final extension	72^0 C for 7 min
PCR amplicon size (bp)	375 bp

The genotyping was performed by the PCR-RFLP method. The PCR amplicon was analyzed by electrophoresis on a 2% agarose gel. The restriction digestion was carried out for the amplicon with 5 units of NlaIII (New England Biolabs, Ipswich, USA) at 37°C for 8 hours and analyzed on a 2% agarose gel with ethidium bromide staining. The 'C' allele was cleaved, resulting in two fragments of sizes 226 bp and 149 bp,

while the T allele was visible as an uncut fragment of size 375 bp. The CC genotype was used as the positive control, which is a Sanger sequenced sample, and the results are summarized in **Table 4.6.**

Table 4.6: PCR-RFLP band pattern of *PGM1* SNP rs11208257:

Genotype	Band pattern
Major allele (TT)	375 bp
Heterozygous allele (TC)	375 bp, 226 bp, 149 bp
The minor allele (CC)	226 bp, 149 bp

4.18. Statistical analysis:

Statistical analysis was carried out using SPSS Statistics *V24.0* (International Business Machine Corporation, Armonk, New York). Quantitative variables are represented as the mean and standard deviation. Qualitative variables are represented as percentages. The Shapiro–Wilk test was performed with Q–Q plots and normality plots. The mean was determined if the data showed a normal distribution; otherwise, the median was calculated. The means of the two groups were compared using Student's t test, while the medians of the two groups were compared using the Mann–Whitney U test. Pearson's correlation test was used to assess the correlation between the variables. The difference was statistically significant if the p value was less than 0.05.

RESULTS

5.1. PGM enzyme activity was reduced in T2DM:

PGM enzyme activity was measured in PBMCs from T2DM patients (*n* = 63) and healthy subjects (*n* = 63). The PGM enzyme activity between the study groups is graphically represented in **Figure 5.1.** PGM enzyme activity showed a normal distribution. Therefore, the mean and standard deviation (Mean ± S.D) were calculated for both study groups. The PGM enzyme activity in the T2DM group was 0.7 ± 0.2 mU/mg of protein lysate, and the healthy control group showed 0.9 ± 0.3 mU/mg of lysate protein. The PGM enzyme activity was 0.9 times lower in the T2DM group than in the healthy control group. The difference in the PGM enzyme activity between the two groups was statistically significant ($p = 0.043$; Student's t test).

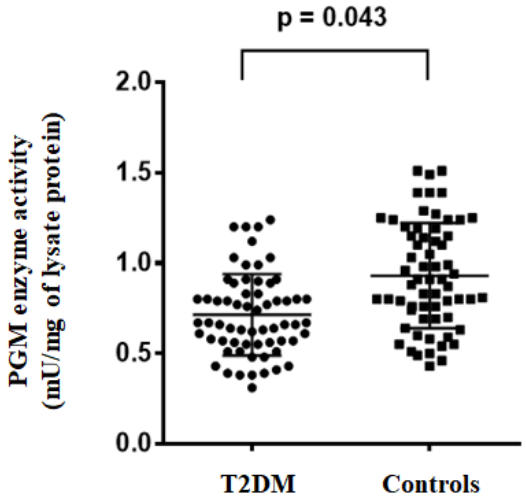

Figure 5.1: PGM enzyme activity in the PBMC protein lysate of the study groups

The impact of clinico-biochemical variables on PGM enzyme activity was checked by correlation analysis. The data showed a normal distribution; therefore, Pearson's correlation test was used. The data are presented in **Figure 5.2.** Individually, **A)** the correlation between PGM enzyme activity and fasting blood sugar levels was negatively correlated, and the effect was moderate (p = -0.36; r = 0.016; Pearson's

correlation test). **B.** The correlation between PGM enzyme activity and random blood sugar levels was negatively correlated, and the effect was moderate (p = -0.39; r = 0.019; Pearson's correlation test). **C.** The correlation between PGM enzyme activity and postprandial blood sugar levels was negatively correlated, and the effect was moderate (p = -0.41; r = 0.011; Pearson's correlation test). **D.** The correlation between PGM enzyme activity and glycated hemoglobin A1c levels was negatively correlated, and the effect was moderate (p = -0.35; r = 0.028; Pearson's correlation test).

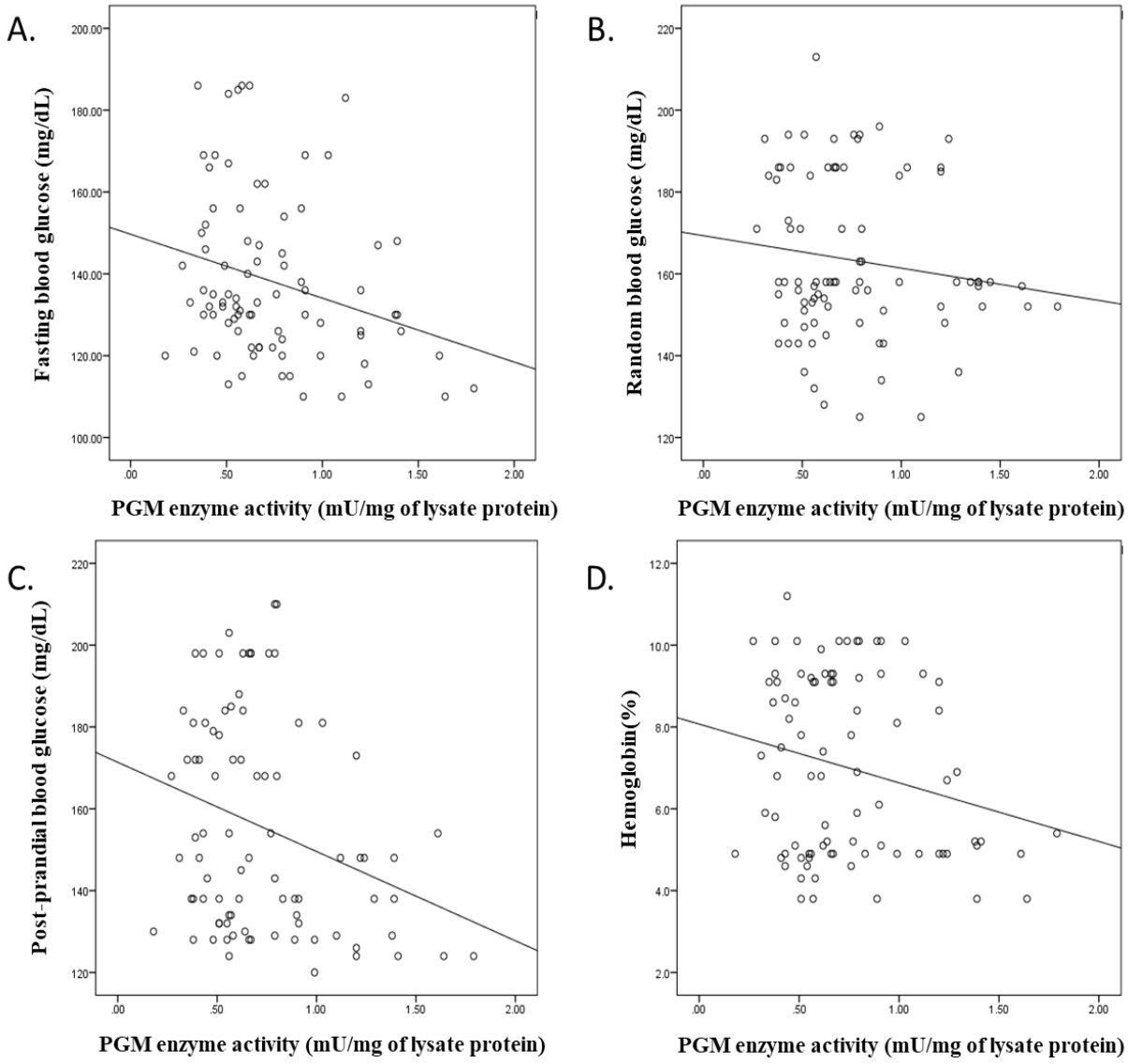

Figure 5.2: Correlation between PGM enzyme activity and clinico-biochemical variables

5.2. *PGM1* gene expression is downregulated in T2DM:

PGM1 gene expression was measured in the PBMCs of both study groups (n = 63). The *PGM1* gene expression in both groups is graphically represented in **Figure 5.3.** *PGM1* gene expression showed a normal distribution. Therefore, the mean and standard deviation (Mean ± S. D) were calculated for both study groups. Therefore, the mean delta Ct (ΔCt) was calculated for both groups. The fold change in *PGM1* gene expression was calculated by following the comparative Ct method. The fold change ($2^{-\Delta\Delta Ct}$) of *PGM1* gene expression was 0.34 in the T2DM group and 0.57 in the control group. The results are presented in **Table 5.1.** The fold change in the T2DM group was calculated by considering the control group as the reference. *PGM1* gene expression was downregulated in T2DM patients compared to healthy subjects (fold difference = 0.59; $p = 0.032$; unpaired t test).

Table 5.1: *PGM1* gene expression in the study groups

Study group	ΔCt	ΔΔCt	Fold change ($2^{-\Delta\Delta Ct}$)
T2DM	3.18 ± 1.7	2.11	0.34
Control	3.23 ± 1.0	1.25	0.57

$\Delta\Delta Ct_{(T2DM)} = \Delta Ct_{(T2DM)} - \text{Average } \Delta Ct_{(Control)}$

$\Delta\Delta Ct_{(Controls)} = \Delta Ct_{(Control)} - \text{Average } \Delta Ct_{(Control)}$

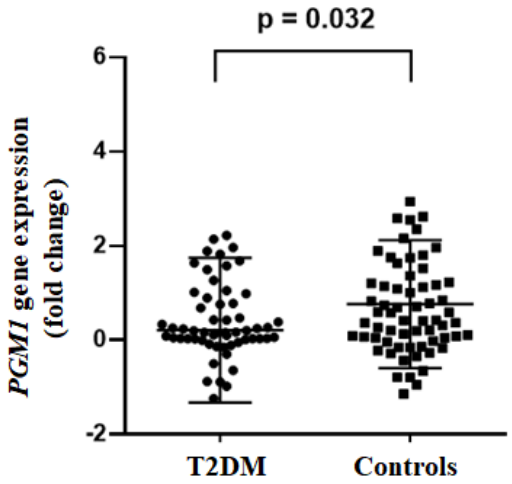

Figure 5.3: *PGM1* gene expression in the PBMCs of the study groups

Correlation analysis was carried out between PGM enzyme activity and *PGM1* gene expression in both study groups. The data showed a normal distribution; therefore Pearson's correlation test was used to measure the levels of both study groups. The results are graphically represented in **Figure 5.4.** The two parameters showed statistically significant moderate positive correlations (r = 0.35; p = 0.016; Pearson's correlation test).

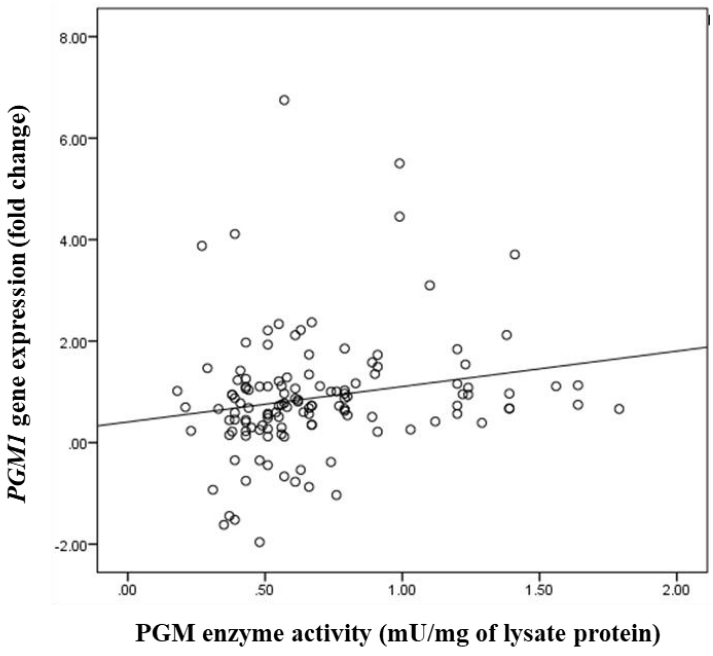

Figure 5.4: Correlation between *PGM1* gene expression and PGM enzyme activity

5.3. SNP rs11208257 associated with T2DM:

The distribution of both alleles and the genotypic frequency of the *PGM1* SNP rs11208257 were determined in the genomic DNA in both study groups. Hardy-Weinberg equilibrium for the genotype frequency was carried out for control samples ($\chi 2 = 6.75$). The frequency of 32.4% was seen with the minor allele 'C', which is common among the control group. The distribution of both genotype and allele frequencies showed a statistically significant difference between the two groups. The frequency of the minor allele 'C' was 44.8% in T2DM patients and 32.4% in the healthy controls. The frequency of minor alleles was 1.3 times higher in T2DM patients. The results are summarized in **Table 5.2.**

Table 5.2: Distribution of SNP rs11208257 in the study groups

Genotype/ Allele	Controls (n = 225)	T2DM (n = 225)	P- value*	OR# (0.95 CI)
TT	155	134	**0.036**	NA
TC	67	81		
CC	3	10		
T	377	349	**0.018**	**1.5 (1.07 - 2.08)**
C	73	101		

* Chi-square, two-tailed (Fisher's exact test)
# OR: Odds ratio; CI: confidence intervals; NA: Not applicable

The association of genetic variation of SNP rs11208257 with various genetic models showed that the highest difference in terms of odds ratio was observed in the case of the additive genetic model. A schematic representation showing the genetic model is shown in **Table 5.3.**

Table 5.3: Evaluation of the association between *PGM1*p. Tyr420His SNP and T2DM under different genetic models

Model	Genotype	*P* value	Odds Ratio #
Dominant	TC + CC vs. TT	**0.028**$	2.4 (0.93 - 12.6)
Recessive	CC vs. TC + TT	0.24	-
Additive	TT *vs.* CT *vs.* CC	**0.012**&	1 < 1.4 < 2.86

$ Chi-square, two-tailed (Fisher's exact test)

& Mantel–Haenszel Chi-square test for linear trend

Parentheses with 95% confidence intervals

The representative band patterns of PCR-RFLP genotyping of the *PGM1* gene variant p. Tyr420His SNP are shown in **Figure 5.5.**

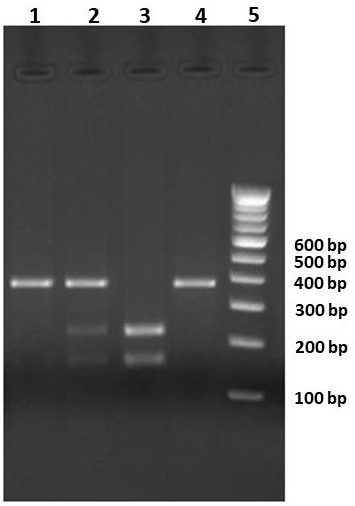

Figure 5.5: Representative PCR-RFLP band pattern of the *PGM1* rs11208257 SNP

Lane 1 represents the TT genotype (375 bp). **Lane 2** represents the TC genotype (375 bp, 226 bp, and 149 bp). **Lane 3** represents the CC genotype (226 bp and 149 bp). **Lane 4** represents an undigested PCR amplicon (375 bp). **Lane 5** represents 100 bp ladder

The association of the genotypic frequency of SNP rs11208257 with PGM enzyme activity was carried out between the study groups, i.e., irrespective of the study groups (n = 126), and the analysis was also carried out for each study group separately (n = 63). The average PGM enzyme activity showed a normal distribution. The data are presented as the mean ± SD. The SNP rs11208257 shows TT, TC, and CC genotypes with PGM enzyme activities of 0.71, 0.54, and 0.15 mU/mg of protein lysate, respectively. The difference between the groups was statistically significant (p = 0.023; Multiple logistic regression). The difference between the groups was statistically significant (p = 0.016; Multiple logistic regression). The data are presented in **Table 5.4.** The effect of SNP rs11208257 on PGM enzyme activity is graphically represented in **Figure 5.6.**

Table 5.4: The association of SNP rs11208257 with PGM enzyme activity

Genotype	**Irrespective of study groups (n = 126)**	**PGM enzyme activity (mU/mg of protein lysate) (n = 126)**	***p* value***
TT	83	0.71 ± 0.21	0.023
TC	39	0.52 ± 0.09	
CC	4	0.15 ± 0.05	

* Multiple logistic regression

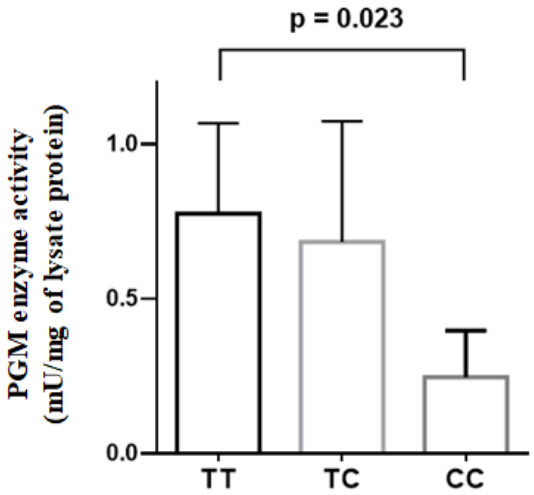

Figure 5.6: Effect of SNP rs11208257 on PGM enzyme activity

Furthermore, the association of SNP rs11208257 and PGM enzyme activity showed a statistically significant difference in the T2DM group (p = 0.017; Multiple logistic regression) and the control group (p = 0.001; Multiple logistic regression). The data are presented in **Table 5.5.** The effect of SNP rs11208257 on enzyme activity in the T2DM and control groups is separately and graphically represented in **Figure 5.7 (a) and (b).**

Table 5.5: The association of SNP rs11208257 with PGM enzyme activity in the study groups

Genotype	T2DM (n = 63)	Enzyme activity (T2DM)	p value*	Controls (n = 63)	Enzyme activity (Controls)	p value*
TT	43	0.74 ± 0.21	0.017	31	0.68 ± 0.20	0.001
TC	17	0.55 ± 0.20		31	0.66 ± 0.17	
CC	3	0.17 ± 0.04		01	0.46 ± 0	

* Multiple logistic regression

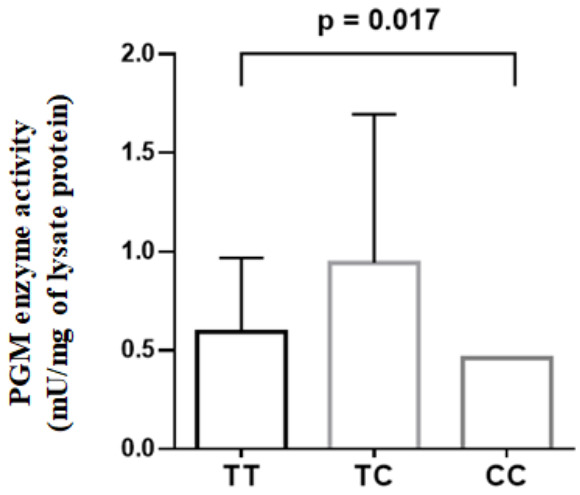

Figure 5.7 (a): Effect of the *PGM1* SNP rs11208257 on PGM enzyme activity in the T2DM group

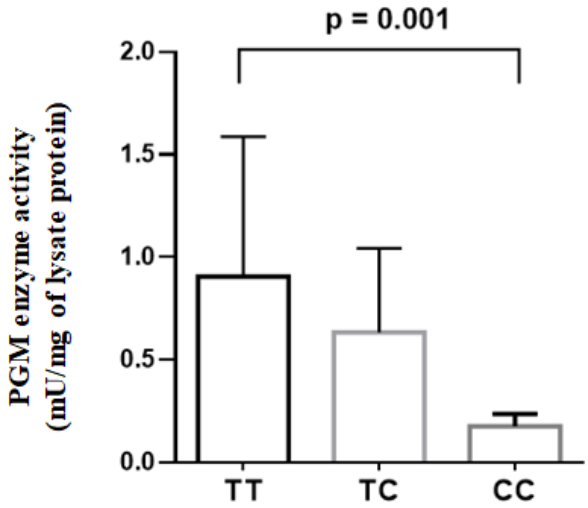

Figure 5.7 (b): Effect SNP rs11208257 on PGM enzyme activity in the control group

5.6. Relationship between PGM enzyme activity, gene expression, and SNP rs11208257:

Stepwise regression was used to analyze the combinatorial impact of *PGM1* gene expression and SNP rs11208257 on PGM enzyme activity. The R-square value was used to check the predictive power of independent variables (*PGM1* gene expression and SNP rs11208257) in determining the dependent variable (PGM enzyme activity).

The results are presented in **Table 5.6.** *PGM1* gene expression was found to determine PGM enzyme activity with a power of 6%, and the power was statistically significant (p = 0.007). Furthermore, SNP rs11208257 was found to determine PGM enzyme activity with a power of 11%, and the power was statistically significant (p = 0.012). Together, *PGM1* gene expression and SNP rs11208257 were capable of determining PGM enzyme activity with a power of 21%, and the determining power was statistically significant (p = 0.001).

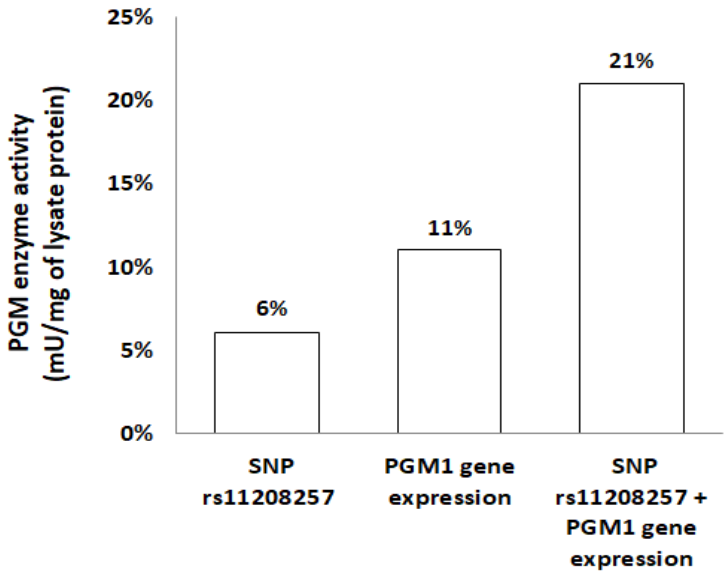

Figure 5.8: Stepwise regression analysis for the determinant of PGM enzyme activity

5.4. PBMC viability under glucose-limiting conditions was reduced in T2DM:

The PBMC viability was measured under glucose-limiting conditions. The first measurement was carried out before glucose deprivation (i.e., under glucose-proficient conditions), and the second measurement was carried out under glucose-limiting conditions.

The percentage of viable cells was used as the measure of cell viability. Cell viability levels showed a normal distribution. Therefore, the mean and standard deviation were

calculated for both groups. Before the induction of glucose deprivation, the mean cell viability levels were 81% in the T2DM group and 83% in the healthy control group. There was no statistically significant difference between the two groups (p = 0.144; Student's t test). This indicates that the baseline levels of cell viability in the two groups were comparable before the induction of glucose deprivation. After inducing glucose deprivation, the mean cell viability levels were 72.3 ± 5.3 in the T2DM group and 87.1 ± 5.7 in the healthy control group. The difference in the cell viability levels of the two groups was statistically significant (p = 0.001; Student's t test). The results are graphically represented in **Figure 5.9.**

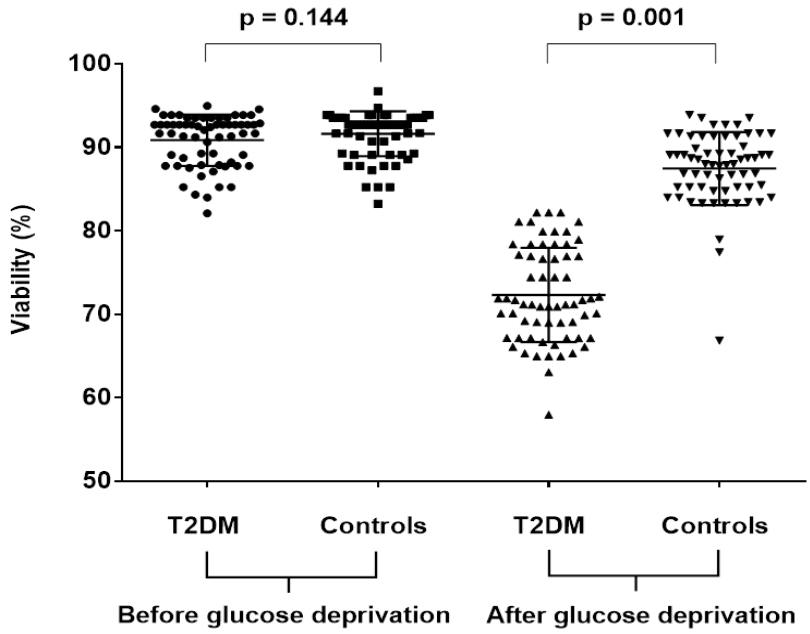

Figure 5.9: PBMC viability under glucose-limiting conditions

The percentage of cell viability in the T2DM group was 70.6 ± 4.7 and 86.8 ± 5.2 in the healthy control group. The percentage of cell viability was 0.81 times lower in the T2DM group than in the healthy control group. The difference in the levels of the two groups was statistically significant (p = 0.001; Student's t test). This indicates that the cell viability levels under glucose limiting conditions were different in the two groups.

The percentage of cell viability in the two groups is graphically represented in **Figure 5.10.**

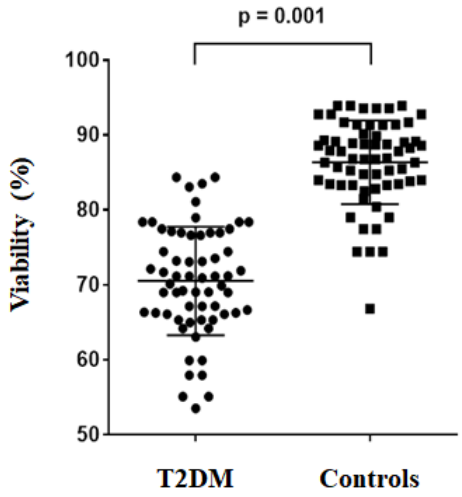

Figure 5.10: Glucose deprivation-induced viability of PBMCs from T2DM and healthy subjects

5.5 Glycogen degradation under glucose-limiting conditions is reduced in T2DM:

The glycogen levels were measured in PBMCs at two time intervals in T2DM and healthy subjects. The first measurement was carried out before glucose deprivation (i.e., under glucose-proficient conditions), and the second measurement was carried out under glucose-deprived conditions.

The glycogen levels showed a normal distribution. Therefore, the mean and standard deviation were calculated for both study groups. Before the induction of glucose deprivation, the mean glycogen levels were 81% in the T2DM group and 83% in the healthy control group. There was no statistically significant difference between the two groups (p = 0.432; Student's t test). This indicates that the baseline levels of glycogen in the two groups were comparable before the induction of glucose deprivation. After inducing glucose deprivation, the mean glycogen levels were 27.5 ± 7.2 in the T2DM group and 14.8 ± 5.6 in the healthy control group. There was a statistically significant

difference in the levels between the two groups (p = 0.001; Student's t test). The results are graphically represented in **Figure 5.11.**

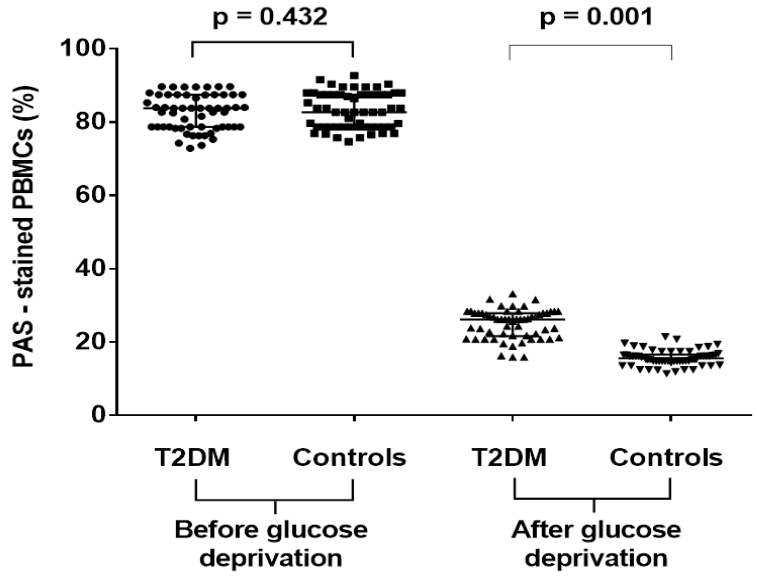

Figure 5.11: Glycogen levels in PBMCs under glucose limiting conditions

The glycogen degradation levels were 55.4 ± 3.5 in the T2DM group and 69.5 ± 4.2 in the healthy control group. The levels of glycogen degradation were 0.8 times lower in the T2DM patients than in the healthy control group. The difference between the two groups was found to be statistically significant (p = 0.001; Student's t test). This indicates that the levels of glycogen degradation under glucose limiting conditions were different in the two groups. The levels of glycogen degradation in the two groups are graphically represented in **Figure 5.12.**

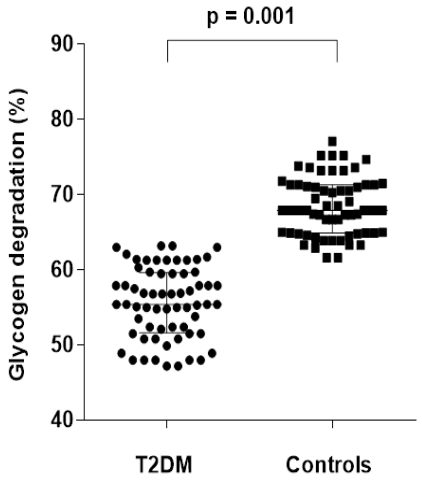

Figure 5.12: Glycogen degradation in PBMCs from T2DM and healthy controls

Correlation analysis was carried out between PBMC viability and glycogen degradation after combining the data from both study groups. Pearson's correlation test was used, as the data showed a normal distribution. The graphical representation of the results is given in **Figure 5.13.** The two parameters showed statistically significant correlation (r = - 0.46; p = 0.012; Pearson's correlation test). The correlation was negative, i.e., reciprocal in the relationship and the magnitude of the correlation was moderate. Furthermore, correlation analysis was also carried out for each study group separately. The correlation was statistically significant in both the T2DM (r = - 0.41; p = 0.008; Pearson's correlation test) and control (r = - 0.36; p = 0.013; Pearson's correlation test) groups. However, the correlation between the two groups was statistically significant, and the magnitude of the correlation was moderate.

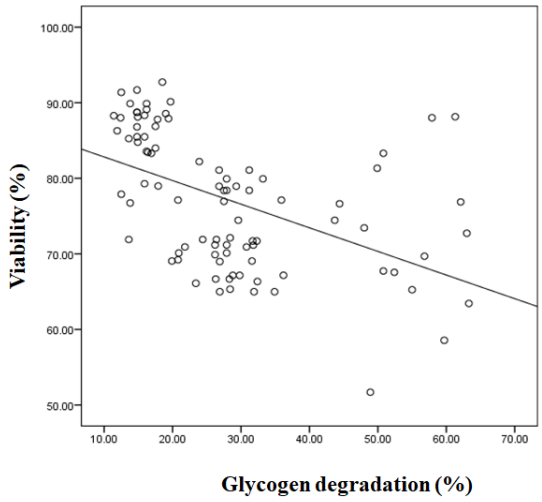

Figure 5.13: Correlation between glycogen degradation and viability in PBMCs under glucose limiting conditions

Correlation analysis was carried out between PBMC viability and PGM enzyme activity after combining the data from both study groups. Pearson's correlation test was used, as the levels of both study parameters showed a normal distribution. The graphical representation of the results is given in **Figure 5.14.** The two parameters showed statistically significant moderate positive correlations (r = 0.37; p = 0.019; Pearson's correlation test).

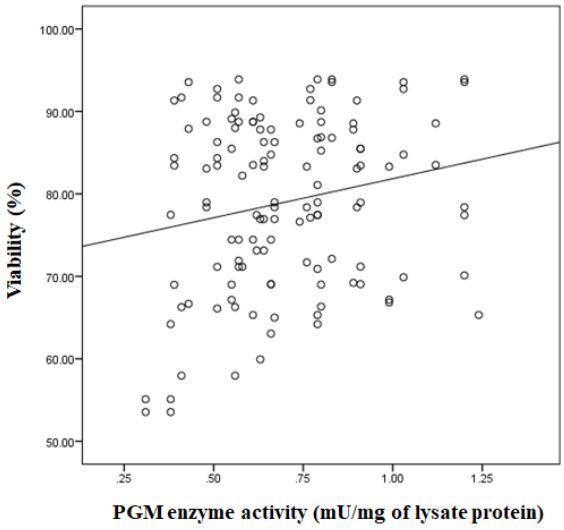

Figure 5.14: Correlation between PBMC viability and PGM enzyme activity under glucose-limiting conditions

5.6. Relationship between PGM enzyme activity, PBMC viability, and glycogen degradation:

Stepwise regression was used to analyze the combinatorial effect of PGM enzyme activity and glycogen degradation on PBMC viability. The results are presented in **Figure 5.15**. The R-square value was used to check the power of the independent variables (PGM enzyme activity and glycogen degradation) in determining the dependent variable (PBMC viability). PGM enzyme activity could determine PBMC viability with a power of 12%, and the power was statistically significant (p = 0.015). Furthermore, glycogen degradation could determine PBMC viability with a power of 16%, and the power was statistically significant (p = 0.001). Together, PGM enzyme activity and glycogen degradation were capable of determining PBMC viability with a power of 23%, and the power was statistically significant (p = 0.001).

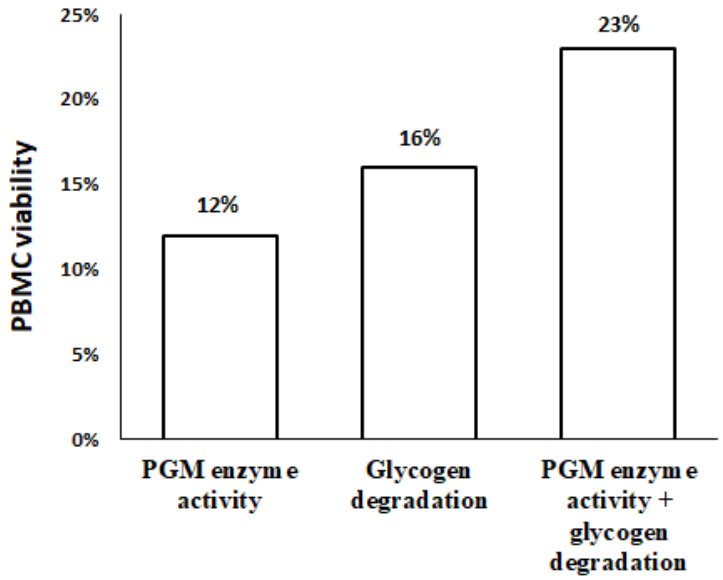

Figure 5.15: Stepwise regression analysis for the determinant of PBMC viability.

DISCUSSION

The purpose of this study was to evaluate the role of PGM in T2DM by a combination of biochemical, genetic, and gene expression studies. The main findings of this study are as follows:

a) PGM enzyme activity is reduced in T2DM
b) *PGM1* gene expression is downregulated in T2DM
c) *PGM1* rs11208257 SNP is associated with T2DM
d) Cell viability and glycogen degradation under glucose-limiting conditions are reduced in T2DM

The inference and significance of each finding are discussed individually below:

Inference 1: PGM enzyme activity is suboptimal in T2DM

PGM enzyme activity was found to be lower in T2DM patients than in healthy subjects. This indicates that suboptimal PGM enzyme activity is associated with T2DM. Furthermore, PGM enzyme activity also showed a correlation with the indices of glycemic control, viz., fasting blood glucose, random blood glucose, postprandial blood glucose, and glycated hemoglobin (figure 5.2). The correlation was negative or reciprocal in nature. This relationship indicates that the PGM enzyme may be involved in regulating blood glucose levels. PGM enzymes are involved in regulating blood glucose levels by facilitating glycogen synthesis. Blood glucose can be lowered only when it is absorbed by the cells. Furthermore, the cells can absorb glucose only if they can metabolize the excess glucose or store it in the form of glycogen. PGM is an important enzyme involved in the storage of excess blood sugar in the form of glycogen inside cells. The observation of the negative relationship between parameters of blood sugar regulation and the PGM enzyme is in conformity with the mechanism

by which the PGM enzyme contributes to its regulation. However, the effect size of the correlation between PGM enzyme activity and indices of glycemic control was moderate. The limited effect size indicates that the PGM enzyme may not be the principal factor but one of the several factors involved in regulating blood glucose levels.

The current understanding is that the impairment of the glycogen pathway in T2DM arises mainly due to defects in enzymes such as glycogen synthase and glycogen phosphorylase [Hojlund et al., 2009; Damsbo et al., 1991]. The results of this study support the inclusion of PGM in the list of enzymes responsible for the impairment of the glycogen pathway in T2DM.

Inference 2: Suboptimal PGM enzyme activity linked to reduced gene expression

PGM1 gene expression was found to be lower in the PBMCs of T2DM patients than in those of healthy controls. This indicates that the *PGM1* gene is downregulated in T2DM (figure 5.3). Furthermore, a positive correlation was found between PGM1 enzyme activity and *PGM1* gene expression. This relationship indicates that suboptimal PGM enzyme activity may arise due to the downregulation of the corresponding *PGM1* gene. However, the effect size of the correlation was moderate. The limited effect size indicates that downregulated gene expression may not be the major factor but one of the several factors responsible for the reduction in PGM enzyme activity.

Inference 3: Suboptimal PGM enzyme activity linked to gene polymorphism

The minor allele frequency of SNP rs11208257 was significantly higher among T2DM patients than among healthy volunteers (Table 5.2). This indicates that SNP

rs11208257 is associated with the risk of T2DM. This relationship is expected since this SNP has been shown to reduce the structural stability of the PGM enzyme.

Further analysis was carried out to determine whether this SNP may be responsible for the reduction in PGM enzyme activity in T2DM. This SNP results in c.1258 T>C substitution in the cDNA, which then leads to p. Y420H substitution in the protein chain. The protein chain with histidine at position 420 in the protein chain is thermodynamically less stable than the protein chain with tryptophan [Cheng et al., 2006]. Therefore, the C allele of this SNP is the risk allele. The average PGM enzyme activity was highest in the PBMCs of individuals who were homozygous for the major allele (TT) and lowest in individuals who were homozygous for the minor allele (CC). Intermediate levels were found in the individuals who were heterozygous (TC). The difference in the average PGM enzyme activity among the three groups was statistically significant. This relationship indicates that SNP rs11208257 may contribute to the reduction of PGM enzyme activity.

The association between SNP rs11208257 and T2DM was evaluated by using various genetic models (Table 5.3). Statistically, a significant association was seen in the case of dominant and additive genetic models but not with recessive models. This indicates that a single copy of the risk allele is sufficient to predispose the individual to develop T2DM. Furthermore, the additive model agrees with the progressive reduction of the PGM enzyme activity with increasing copy number of the risk alleles.

The role of genetic variation in the *PGM1* gene in the pathogenesis indicated by this study also agrees with a previous report. Inshaw and coworkers showed that T2DM is associated with SNP rs2269247 located in the intergenic region 0.5 Mb from the

PGM1 gene [Inshaw et al., 2021]. The implications of this report were limited since a tag SNP was discovered. In contrast, this study provides evidence for a functional SNP in the *PGM1* gene.

Reduction in PGM enzyme activity was linked to both downregulation of *PGM1* gene expression and SNP rs11208257. Stepwise regression analysis showed that *PGM1* gene expression and SNP rs11208257 had an additive effect in determining PGM enzyme activity (figure 5.8).

Inference 4: Suboptimal PGM enzyme activity reduces cell viability under glucose-limiting conditions

Experiments were carried out to determine whether PGM enzyme activity affects cell physiology. Cytoplasmic glycogen becomes the main source of glucose when the extracellular supply is limited. The PGM enzyme plays a key role in mobilizing glucose from glycogen. Therefore, a reduction in PGM enzyme activity may compromise glucose mobilization from glycogen and reduce cell viability under glucose-limiting conditions. In agreement with this hypothesis, a positive correlation was observed between PGM enzyme activity and cell viability (figure 5.14). However, the effect size was moderate. This indicates that PGM enzyme activity may not be the only factor responsible for cell viability under glucose-limiting conditions. The role of the PGM enzyme in cell viability indicated by this study agrees with similar observations in tumor cells [Jin et al., 2018]. Under low nutritional conditions, knockdown of the *PGM1* gene in HEK293K, MCF-7, and HeLa cell lines was found to reduce cell proliferation [Bae et al., 2014].

Next, the role of glycogen degradation as an additional factor determining cell viability was checked. Glycogen degradation was reduced in the PBMCs of T2DM patients (figure 5.13). Additionally, glycogen degradation showed a negative correlation with PBMC viability. However, the effect size was moderate, indicating that glycogen degradation may be one of the several factors responsible for cell viability under glucose-limiting conditions. The reduction in PBMC viability was linked to both reduced PGM enzyme activity and glycogen degradation. Stepwise regression analysis showed that PGM enzyme activity and glycogen degradation have an additive effect in determining PBMC viability (figure 5.15). This study shows that PGM enzyme activity is suboptimal in T2DM, probably due to downregulated gene expression and genetic variation. Furthermore, suboptimal PGM enzyme activity has been shown to impair cell viability under glucose-limiting conditions.

SUMMARY AND CONCLUSION

T2DM is a common metabolic disorder that arises due to the disruption of glucose homeostasis. In addition to other mechanisms, the glycogen pathway regulates glucose homeostasis by converting excess glucose into glycogen. The phosphoglucomutase (PGM) enzyme catalyzes the key reaction that links the glycogen pathway and glucose metabolism. Studies have shown that the glycogen pathway is abnormal in diabetes. The purpose of this study was to determine the role of PGM in T2DM by a combination of biochemical, genetic, and gene expression studies.

This study was carried out by following the case–control design. The case group (n = 63) comprised T2DM patients, while the control group (n = 63) comprised healthy individuals. Experimental studies were carried out using PBMCs since the transcriptional profile of PBMCs in T2DM patients has been shown to correlate with the pathophysiology of the disease.

PGM enzyme activity was measured in the PBMC lysate. PGM enzyme activity was found to be comparatively reduced in T2DM patients compared to healthy controls (p = 0.043; Student's t test). Furthermore, PGM enzyme activity showed a reciprocal relationship with the indices of glycemic controls. Then, *PGM1* gene expression and a common functional variation in the *PGM1* gene (SNP rs11208257; p. Tyr420His) were explored as the likely sources for the reduced PGM enzyme activity. *PGM1* gene expression was downregulated in T2DM patients compared to healthy subjects (fold difference = 0.59; p = 0.032; unpaired t test). A positive correlation was observed between PGM enzyme activity and *PGM1* gene expression (r = 0.35; p = 0.016; Pearson's correlation test). Furthermore, SNP rs11208257 was found to be associated with T2DM (p = 0.001; Student's t test). The PGM enzyme activity was relatively

lower in samples with the TC and CC genotypes (0.54 mU/mg of protein lysate) than in samples with the TT genotype (0.69 mU/mg of protein lysate) (p = 0.018; Multiple logistic regression).

The physiological impact of reduced PGM enzyme activity on cell viability was evaluated under glucose-limiting conditions. It was found that PBMC viability was reduced in T2DM patients compared to healthy subjects (p = 0.001; Student's t test). The glycogen degradation showed a reciprocal relationship with PBMC viability, and the effect was moderate (r = -0.46; p = 0.012; Pearson's correlation test). This relationship indicates that glycogen degradation is one of the several factors responsible for PBMC viability under glucose-limiting conditions. There was a positive correlation between PGM enzyme activity and PBMC viability under glucose-limiting conditions (p = 0.001, r = 0.35; Pearson's correlation test). This indicates that reduced PGM enzyme activity affects PBMC viability under glucose-limiting conditions.

This study shows that PGM enzyme activity is suboptimal in T2DM, probably due to downregulated gene expression and genetic variation. Furthermore, suboptimal PGM enzyme activity is shown to impair cell viability under glucose-limiting conditions. This study adds the PGM enzyme to the list of defects that are responsible for impairing the glycogen pathway in T2DM. This is the first attempt to link the PGM enzyme with the pathogenesis of T2DM.

LIMITATIONS OF THE STUDY

The glycogen pathway is directional. It involves both synthesis and degradation. However, only the degradation component was measured. The PGM enzyme does not have a direct role in glycogen degradation. Instead, it is necessary for channelling glucose produced from glycogen degradation to glycolysis. However, the PGM enzyme is necessary for glycogen synthesis. Excess glucose can be converted into glycogen only if the PGM enzyme can channel it into the glycogen pathway. Future studies should check for the relationship between PGM enzyme activity and glycogen synthesis. This aspect will confirm the role of PGM enzyme activity in impairing the glycogen pathway in T2DM.

NEW KNOWLEDGE GENERATED

a) This study provides the first evidence to link PGM with T2DM through a combination of biochemical, gene expression, genetic and cellular studies.

b) This is the first study to explore the status of glycogen degradation in PBMCs. The results of this study establish the use of PBMCs as surrogate cells for studying glycogen metabolism in T2DM.

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LIST OF PRESENTATIONS AND PUBLICATIONS

PRESENTATIONS:

1. Praveen Kumar KS, Prabhakar K, Sharath B. Novel association of *PGM1* gene variant with type 2 diabetes mellitus. Poster session presented at 88th Annual meeting of the Society of biological chemists, India (SBCI-2019) and conference on Advances at the Interface of Biology & Chemistry; 2019 Oct 31 to Nov 3; DAE Convention Centre, Anushaktinagar, Mumbai

2. Praveen Kumar KS, Prabhakar K, Sharath B. Enzyme activity of phosphoglucomutase1 in type 2 diabetes mellitus. Oral session presented at ABGCON 2021, National virtual conference and workshop; 2021 Aug 27 to Aug 28; Sri Balaji Medical College and Hospital, Chennai

PUBLICATIONS:

1. Praveen Kumar K S, Kamarthy P, Balakrishna S, Manu M S, Ramaswamy S. Association between phosphoglucomutase-1 gene Y420H polymorphism and type 2 diabetes mellitus: A Case-control study. Arch Med Health Sci. 2021; 9: 225-228

2. Praveen Kumar K S, Prabhakar K, Balakrishna S. Glucose deprivation-induced glycogen degradation and viability are altered in peripheral blood mononuclear cells of type 2 diabetes patients. Ukrainian Biochemical Journal. (Manuscript accepted)

3. Praveen Kumar K S, Prabhakar K, Balakrishna S. Reduced phosphoglucomutase1 activity impairs the survival of peripheral blood mononuclear cells under glucose-limiting conditions in diabetic patients. Manuscript submitted.

MASTER CHART

Master chart: T2DM patients

Sl. No	code	(M/F)	FBS	PPBS	HbA_{1C}	Genotype	Cell Viability	Enzyme activity	*PGM1* gene expression	Gly deg T2DM
1	DC1	F	131	185	9.1	TT	71.9	0.57	0.95	61.7
2	DC2	M	125	173	9.1	TT	70.1	1.20	7.22	55.4
3	DC3	M	124	210	10.1	TT	70.9	0.79	3.25	51.5
4	DC4	F	169	181	11.2	TT	76.6	0.74	3.15	47.2
5	DC5	M	135	198	7.8	TT	66.1	0.51	4.25	56.8
6	DC6	M	186	172	9.1	TC	69.0	0.39	7.04	61.3
7	DC7	F	142	168	10.1	TC	74.4	0.57	3.25	51.5
8	DC8	F	122	198	9.3	TC	65.0	0.67	2.96	47.2
9	DC 9	M	133	148	9.1	TT	63.1	0.66	0.54	56.8
10	DC10	F	128	128	8.1	TC	67.2	0.99	6.74	61.3
11	DC11	M	136	138	9.3	TT	78.9	0.91	5.42	61.4
12	DC12	F	186	138	10.1	TT	69.2	0.89	8.68	63.2
13	DC13	F	206	126	8.4	TT	78.4	1.20	5.34	59.5
14	DC14	M	156	138	10.1	TC	66.7	0.43	6.02	56.9
15	DC15	F	130	138	8.6	CC	77.1	0.77	5.34	55.1
16	DC16	M	154	210	9.2	TT	66.3	0.80	5.16	57.9
17	DC17	M	169	181	10.1	TC	69.9	1.03	4.09	55.3
18	DC18	M	135	198	8.7	TT	72.1	0.83	8.06	55.4
19	DC19	F	162	198	9.3	TT	69.0	0.66	6.22	48.9
20	DC20	F	169	181	10.1	TC	71.2	0.91	8.19	50.8
21	DC21	M	135	198	7.8	TT	71.7	0.76	5.18	48.0
22	DC22	F	186	172	9.1	TC	82.2	0.58	8.78	59.7
23	DC23	M	142	168	10.1	TT	81.1	0.79	4.88	55.0
24	DC24	M	122	198	9.3	TT	59.9	0.63	2.90	52.4
25	DC25	F	186	172	9.1	TC	67.1	0.55	6.63	63.0
26	DC26	F	142	168	10.1	TT	77.0	0.64	7.06	52.4
27	DC27	M	148	188	9.9	TT	65.3	0.61	2.31	52.1
28	DC28	M	184	178	9.3	TT	71.2	0.51	3.22	50.8
29	DC29	F	132	179	8.6	TC	78.4	0.48	3.67	48.0
30	DC30	M	185	203	9.2	TT	58.0	0.56	5.98	49.9
31	DC31	F	136	138	9.3	TT	64.2	0.38	0.11	61.3
32	DC32	M	133	148	7.3	TC	55.1	0.31	4.29	53.8
33	DC33	F	169	181	10.1	TC	53.5	0.38	6.88	57.9
34	DC34	M	135	198	6.9	TC	77.5	0.79	5.20	59.5
35	DC35	M	186	172	7.5	TC	66.3	0.41	3.05	55.0
36	DC36	F	142	168	10.1	CC	83.1	0.90	1.16	56.8
37	DC37	F	122	198	6.8	TT	84.3	0.39	4.47	53.5
38	DC38	M	133	148	9.3	CC	83.5	1.12	6.45	57.9
39	DC39	F	186	172	7.4	TT	73.1	0.62	2.09	48.0

40	DC40	M	142	168	10.1	TT	69.0	0.80	5.69	52.4
41	DC41	F	122	198	9.1	TT	77.0	0.8	3.09	54.8
42	DC42	M	133	148	6.7	TT	65.3	0.7	3.05	57.9
43	DC43	F	186	172	9.3	TC	71.2	1.0	1.16	48.0
44	DC44	F	162	198	10.1	TC	78.4	1.7	4.47	60.3
45	DC45	M	133	148	7.8	TT	69.0	1.4	5.20	55.4
46	DC46	M	133	169	7.6	TT	76.6	0.99	6.02	51.5
47	DC47	F	128	178	7.6	TT	66.1	0.91	5.34	57.2
48	DC48	F	136	163	7.3	TT	69.0	0.89	5.16	54.8
49	DC49	M	152	198	6.8	TT	74.4	1.20	4.09	61.3
50	DC50	F	142	176	8.1	TT	65.0	0.43	8.06	61.4
51	DC51	F	132	184	8.1	TT	63.1	0.77	6.22	63.2
52	DC52	F	130	195	8.3	TC	67.2	0.80	8.19	59.5
53	DC53	M	132	176	7.8	TC	78.9	1.03	5.18	56.9
54	DC54	M	138	184	7.9	TT	69.2	0.83	2.31	62.1
55	DC55	M	126	176	7.3	TT	78.4	0.79	3.22	57.9
56	DC56	M	128	162	7.9	TT	66.7	0.63	3.67	61.3
57	DC57	M	132	183	7.2	TT	71.2	0.55	5.98	55.4
58	DC58	F	120	194	8.1	TC	78.4	0.64	0.11	48.9
59	DC59	F	128	178	7.9	TC	58.0	0.61	7.29	50.8
60	DC60	F	128	168	8	TT	64.2	0.51	6.88	48.0
61	DC61	F	136	187	7.7	TC	55.1	0.48	5.2	59.7
62	DC62	F	138	198	8	TT	53.5	0.56	3.05	55.0
63	DC63	F	142	173	6.8	TT	78.9	0.80	5.42	57.5

Master chart: Healthy controls

Sl. No	patient code	(M/F)	FBS	PPBS	HbA_{1C}	Genotype	Cell Viability	Enzyme activity	*PGM1* gene expression	Gly deg Ctrl
1	NDC1	F	120	134	4.9	TT	66.8	0.80	3.62	61.7
2	NDC2	F	110	153	4.8	TT	83.4	0.59	4.34	55.4
3	NDC3	F	120	143	2.8	TT	88.6	0.32	4.95	51.5
4	NDC4	M	100	154	2.8	TC	77.4	0.51	3.79	47.2
5	NDC5	M	140	130	4.8	TT	87.9	1.24	4.18	56.8
6	NDC6	M	110	132	5	TT	91.4	0.74	6.86	61.3
7	NDC7	M	110	138	4.9	TT	85.3	0.88	3.04	51.5
8	NDC8	M	130	124	4.9	TC	92.7	0.69	3.98	47.2
9	NDC9	M	120	128	4.9	TC	93.6	1.51	1.42	56.8
10	NDC10	F	115	184	4.9	TT	93.9	1.39	2.16	61.3
11	NDC11	F	120	143	5.2	TT	87.8	0.49	2.98	61.4
12	NDC12	F	110	138	4.9	TT	85.5	0.91	4.56	63.2
13	NDC13	M	126	138	4.9	TC	83.3	0.79	5.85	59.5
14	NDC14	M	130	129	3.8	TT	74.4	0.63	3.89	56.9
15	NDC15	M	136	154	5.1	TT	86.3	0.54	5.16	55.1
16	NDC16	M	138	154	4.9	TT	88.8	1.05	4.02	57.9
17	NDC17	F	130	124	4.9	TC	89.9	0.64	5.54	55.3
18	NDC18	F	120	128	4.9	TT	90.1	0.32	1.41	55.4
19	NDC19	F	120	132	3.8	TC	86.9	0.80	4.05	48.9
20	NDC20	M	110	120	5.1	TT	88.0	0.81	4.41	50.8
21	NDC21	M	130	128	4.8	TT	79.0	1.24	2.73	48.0
22	NDC22	M	110	128	5.1	TT	91.7	0.83	1.57	59.7
23	NDC23	M	120	134	3.8	TC	91.3	0.69	4.78	55.0
24	NDC24	F	110	138	4.9	TT	84.0	0.60	3.56	52.4
25	NDC25	M	120	148	5.1	TT	89.1	1.15	2.77	63.0
26	NDC26	F	110	134	4.6	TC	89.3	0.46	3.78	52.4
27	NDC27	F	130	148	5.2	TC	86.7	0.80	3.27	52.1
28	NDC28	F	140	148	8	TT	86.8	1.25	3.51	50.8
29	NDC29	F	126	145	5	TT	84.8	0.76	-3.06	48.0
30	NDC30	F	115	184	4.8	TT	88.7	0.98	5.31	49.9
31	NDC31	M	130	124	5.2	TT	92.7	0.70	1.68	61.3
32	NDC32	F	130	129	4.9	TT	93.6	0.43	2.65	53.8
33	NDC33	F	130	128	5.1	TT	93.9	0.55	2.12	57.9
34	NDC34	M	126	138	3.8	CC	87.8	0.87	3.04	59.5
35	NDC35	F	130	129	4.9	TT	85.5	1.19	4.06	55.0
36	NDC36	F	130	124	5.2	TC	83.3	0.50	5.06	56.8
37	NDC37	F	120	128	4.9	TC	74.4	1.27	1.60	53.5

38	NDC38	M	120	132	4.9	TC	86.3	0.80	3.94	57.9
39	NDC39	M	100	154	5	TT	88.8	1.10	4.93	48.0
40	NDC40	F	140	130	4.3	TT	79.0	0.58	-1.71	52.4
41	NDC41	F	110	132	4.6	TT	91.7	0.76	0.65	54.8
42	NDC42	F	115	184	4.9	TT	91.3	0.70	2.12	57.9
43	NDC43	F	130	124	5.1	TT	83.4	0.98	3.04	48.0
44	NDC44	M	130	129	3.8	TT	88.6	1.70	1.06	60.3
45	NDC45	F	120	134	4.9	TC	77.4	1.43	3.94	55.4
46	NDC46	M	100	148	3.8	TT	88.8	1.15	2.77	51.5
47	NDC47	F	150	138	4.3	TT	89.9	0.96	3.78	57.2
48	NDC48	F	120	132	4.9	TT	90.1	0.80	3.27	54.8
49	NDC49	M	115	132	4.8	TT	86.9	1.25	4.41	61.3
50	NDC50	F	121	158	4.8	TT	88.0	0.76	2.73	61.4
51	NDC51	F	110	141	4.6	TT	79.0	0.98	1.57	63.2
52	NDC52	F	115	148	5	TC	91.7	1.12	4.78	59.5
53	NDC53	M	120	151	4.9	TC	91.3	1.03	3.56	56.9
54	NDC54	M	115	138	3.8	TT	84.8	1.55	2.77	62.1
55	NDC55	M	110	158	4.9	TT	88.7	1.51	3.78	57.9
56	NDC56	M	120	132	5.1	TT	92.7	1.39	3.27	61.3
57	NDC57	M	120	138	4.9	TT	93.6	1.14	3.51	55.4
58	NDC58	F	110	124	5.1	TC	93.9	0.91	2.16	48.9
59	NDC59	F	120	128	4.9	TC	87.8	0.79	0.98	50.8
60	NDC60	F	100	132	4.9	TT	85.5	1.10	4.56	48.0
61	NDC61	F	140	120	4.8	TC	77.4	0.94	5.85	59.7
62	NDC62	F	110	128	3.8	TT	87.9	0.91	3.89	55.0
63	NDC63	F	110	128	4.9	TT	87.8	1.49	0.98	57.5

APPENDICES

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SDUAHER

SRI DEVARAJ URS MEDICAL COLLEGE

Tamaka, Kolar

INSTITUTIONAL ETHICS COMMITTEE

Members

1. **Dr. D.E.Gangadhar Rao, (Chairman)** Prof. & HOD of Zoology, Govt. Women's College, Kolar,
2. **Dr. Sujatha.M.P, (Member Secretary),** Assoc. Prof. of Anesthesia, SDUMC,
3. Dr. C.S.Babu Rajendra Prasad, Prof. of Pathology, SDUMC
4. Dr. Srinivasa Reddy.P, Prof. & HoD of Forensic Medicine, SDUMC
5. Dr. Prasad.K.C, Professor of ENT, SDUMC
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10. Dr. Pavan.K, Asst. Prof of Surgery, SDUMC
11. Dr. Talasila Sruthi, Assoc. Prof. of OBG, SDUMC
12. Dr. Mahendra.M , Asst. Prof. of Community Medicine, SDUMC
13. Dr. Mamata Kale, Asst. Professor of Microbiology, SDUMC

No. SDUMC/KLR/IEC/30/2019-20 **Date:06-06-2019**

PRIOR PERMISSION TO START OF STUDY

The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Ph.D study entitled **"Role of phosphoglucomutase 1 in type 2 diabetes mellitus an integrated biochemical, genetic and gene expression study"** being investigated by **Mr. Praveen Kumar.K.S,** Dr. Sharath.B & Dr. Prabhakar.K[1] in the Departments of Cell Biology and Molecular Genetics, & Medicine[1] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

Sujatha .M.P

Member Secretary

Member Secretary
Institutional Ethics Committee
Sri Devaraj Urs Medical College
Tamaka, Kolar.

Chairman

CHAIRMAN
Institutional Ethics Committee
Sri Devaraj Urs Medical College.
Tamaka, Kolar

PATIENT PROFORMA

Topic: "Role of phosphoglucomutase 1 in Type 2 Diabetes Mellitus: An Integrated Biochemical, Genetic and Gene Expression study"

Date:

Patient Data			
Patient's Name			
IP/OP Number			
Age			
Gender		□M □F	
Contact number			
Address			
Clinical Signs And Symptoms			
Hypertension		□Yes □ No □ Unknown	
Hyperglycemia/ Euglycemia		□Yes □ No □ Unknown	
Weight loss/ Fatigue		□Yes □ No □ Unknown	
Polyuria		□Yes □ No □ Unknown	
Polyphagia		□Yes □ No □ Unknown	
Polydipsia		□Yes □ No □ Unknown	
Systolic		Diastolic	
Laboratory Investigations			
RBS (mg/dL)		HDL(mg/dL)	
FBS (mg/dL)		LDL(mg/dL)	
PPBS (mg/dL)		HbA1c	
Serum Creatinine (mg/dL)		Urine sugar	
Other complications/ Remarks			

PART I: PATIENT INFORMATION SHEET

Name of the project :	Role of phosphoglucomutase 1 in Type 2 Diabetes Mellitus: An Integrated Biochemical, Genetic and Gene Expression study
Name of the Research Scholar :	Mr. Praveen Kumar K.S.
Name of Organization :	Sri Devaraj Urs Academy of Higher Education and Research. Tamaka, Kolar.

Purpose of the study: Diabetes is a disease involving an abnormal increase in blood sugar levels. One of the complications of high blood sugar levels is impaired glucose, malfunctioning of glycogenesis, and gluconeogenesis in the liver, which is a major storage organ, where the entire process takes place. The enzyme involved in the process is Phosphoglucomutase1, which is the catalytic enzyme that plays a key regulatory role in maintaining glucose homeostasis and glycogen metabolism in the cell. Abnormal functioning of the PGM enzyme leads to the disease condition. One such complication of glucose metabolism in Type 2 Diabetes Mellitus. The purpose of this study is to evaluate the role of PGM1 enzyme activity in the development of T2DM. The results obtained from this study will contribute towards the development of therapies that can halt the development of T2DM.

P**articipant selection:** Individuals aged ≥18 years, who are not suffering from diabetes and also do not have a history of any known chronic diseases will be included in this study.

Voluntary Participation: Your participation in this study is entirely voluntary. There is no compulsion to participate in this study. You will be in no way affected if you do not wish to participate in the study. You are required to sign only if you voluntarily agree to participate in this study. Further, you are at liberty to withdraw from the study at any time. We assure you that your withdrawal will not affect your treatment by the concerned physician in any way.

Procedure: We will be collecting a small volume of blood (3 ml) from your arm. The sample will be used for genomic analysis. Clinical and family history is also necessary.

Duration: The research will take place for about 3 years.

Risks: No drug will be tested on you. 3 ml of blood will be collected using a sterile and disposable needle and syringe. Standard of care for the treatment of Type 2 diabetes will be given to you irrespective of your decision to enroll in the project.

Benefits: This study will be useful to understand the role of PGM1 in the development of Type 2 diabetic patients. The results gathered from this study will be beneficial in the management of

diseases. Participation in this study does not involve any cost for you. Also, no monetary compensation will be paid for your participation in this study.

Storage of samples: The samples collected from you may be stored at -80^0 C for future research projects on molecular studies on PGM1. In such an event, ethics clearance will be obtained.

Confidentiality: All information collected from you will be strictly confidential & will not be disclosed to anyone except if it is required by the law. This information collected will be used only for research. This information will not reveal your identity.

Sharing of Results: The results obtained from this study will be published in scientific/Medical Journals/Medical conferences.

For any information, you are free to contact the investigator. This study has been approved by the Institutional Ethics Committee & has been started only after their formal approval. The sample collected will be stored in the institute and I request you to permit us to store and use this sample for any future study. This document will be stored in the safe locker & a copy given to you for information. Person in charge of providing a standard of care to the patients:

Dr. Prabhakar K. Professor,
Dept. of General Medicine
Sri Devaraj Urs Medical College, Tamaka, Kolar.
Mob: 9845209858Mob: 9845209858

HEALTHY CONTROL INFORMATION SHEET

Name of the project :	Role of phosphoglucomutase 1 in Type 2 Diabetes Mellitus: An Integrated Biochemical, Genetic and Gene Expression study
Name of the Research Scholar :	Mr. Praveen Kumar K.S.
Name of Organization :	Sri Devaraj Urs Academy of Higher Education and Research. Tamaka, Kolar.

Purpose of the study: Diabetes is a disease involving an abnormal increase in blood sugar levels. One of the complications of high blood sugar levels is impaired glucose, malfunctioning of glycogenesis and gluconeogenesis in the liver results in causing disease. The enzyme involved in the process is Phosphoglucomutase 1, which is the catalytic enzyme that plays a key regulatory role in maintaining glucose homeostasis and glycogen metabolism in the cell. Abnormal functioning of the PGM1 enzyme leads to the disease condition. One such complication of glucose metabolism in Type 2 Diabetes mellitus. The purpose of this study is to evaluate the role of PGM1 enzyme activity in the development of T2DM. The results obtained from this study will contribute towards the development of therapies that can halt the development of T2DM.

P**articipant selection:** Individuals aged ≥18 years, who are not suffering from diabetes and also do not have a history of any known chronic diseases will be included in this study.

Voluntary Participation: Your participation in this study is entirely voluntary. There is no compulsion to participate in this study. You will be in no way affected if you do not wish to participate in the study. You are required to sign only if you voluntarily agree to participate in this study. Further, you are at liberty to withdraw from the study at any time. We assure you that your withdrawal will not affect your treatment by the concerned physician in any way.

Procedure: We will be collecting a small volume of blood (3 ml) from your arm. The sample will be used for immunological analysis. Clinical and family history is also necessary.

Duration: The research will take place for about 3 years.

Risks: No drug will be tested on you. 3 ml of blood will be collected using a sterile and disposable needle and syringe. Standard of care for the treatment of Type 2 diabetes will be given to you irrespective of your decision to enroll in the project.

Benefits: This study will be useful to understand the role of PGM1 in the development of Type 2 diabetic patients. The results gathered from this study will be beneficial in the management of diseases. Participation in this study does not involve any cost for you. Also, no monetary compensation will be paid for your participation in this study.

Storage of samples: The samples collected from you may be stored at -80^0 C for future research projects on molecular studies on PGM1. In such an event, ethics clearance will be obtained.

Confidentiality: All information collected from you will be strictly confidential & will not be disclosed to anyone except if it is required by the law. This information collected will be used only for research. This information will not reveal your identity.

Sharing the Results: The results obtained from this study will be published in scientific/Medical Journals/Medical conferences

For any information, you are free to contact the investigator. This study has been approved by the Institutional Ethics Committee & has been started only after their formal approval. The sample collected will be stored in the institute and I request you to permit us to store and use this sample for any future study.

This document will be stored in the safe locker & a copy given to you for information. For any further clarification you are free to contact:

Dr. Prabhakar K. Professor,
Dept. of General Medicine
Sri Devaraj Urs Medical College, Tamaka, Kolar.
Mob: 9845209858Mob: 9845209858

PART II:

INFORMED CONSENT FORM (Patients)

Note: Consent for the case group (patients with Type 2 Diabetics) should be obtained from the participant.

Name of Participant (Patient/Volunteer): _____

- This research study has been explained to me; I have been given the chance to discuss it and ask questions. All of my questions have been answered to my satisfaction.
- I have read each page of the Patient Information Sheet or it has been read to me.
- I agree to allow access to my health information as explained in the patient information sheet. (In case of the patient only)
- I agree to allow the collection of 3ml blood samples and health data for the research purposes explained in the Patient Information Sheet.
- I voluntarily consent to the storage of my sample for future research projects.
- I understand that all the information collected will be kept confidential.
- I voluntarily consent to take part in this research study.

Participant's signature or thumb impression

	Name	**Signature**	**Date**
Participant			
Witness 1			
Witness 2			
The person taking consent*			

Date: _____

If illiterate: Two literate witnesses must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should include their thumb-print as well.

PART II:

INFORMED CONSENT FORM (Healthy controls)

Note: Consent for the control group (Healthy individuals without Type 2 Diabetics) should be obtained from the participant.

Name of Participant (Healthy control /Volunteer): _____

- This research study has been explained to me; I have been given the chance to discuss it and ask questions. All of my questions have been answered to my satisfaction.
- I have read each page of the Patient Information Sheet or it has been read to me.
- I agree to allow access to my health information as explained in the patient information sheet. (In case of the patient only)
- I agree to allow the collection of 3ml blood samples and health data for the research purposes explained in the Patient Information Sheet.
- I voluntarily consent to the storage of my sample for future research projects.
- I understand that all the information collected will be kept confidential.
- I voluntarily consent to take part in this research study.

Participant's signature or thumb impression

	Name	**Signature**	**Date**
Participant			
Witness 1			
Witness 2			
The person taking consent*			

Date: _____

If illiterate: Two literate witnesses must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Illiterate participants should include their thumb-print as well.

ಭಾಗ 1: ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿ ಪತ್ರ
ರೋಗಿಯ ಮಾಹಿತಿ ಪತ್ರ

ಯೋಜನೆಯ ಹೆಸರು	ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್‌ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್‌ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ
ಸಂಶೋಧಕಹೆಸರು	ಪ್ರವೀಣ್ ಕುಮಾರ್ ಕೆ.ಎಸ್.
ಸಂಸ್ಥೆಯ ಹೆಸರು	ಶ್ರೀ ದೇವರಾಜ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಸಂಸ್ಥೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಟಮಕ, ಕೋಲಾರ.

ಭಾಗವಹಿಸುವವರು ಆಯ್ಕೆ : ≥18 ವರ್ಷಕ್ಕಿಂತ ಮೇಲ್ಪಟ್ಟ ಟೈಪ್ 2 ಮಧುಮೇಹದಿಂದ ಬಳಲುತ್ತಿರುವ ರೋಗಿಗಳು. ಎಲ್ಲಾ ಡಯಾಬಿಟಿಕ್ ರೋಗಿಗಳಿಗೆ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವ ತಮ್ಮ ನಿರ್ಧಾರವನ್ನು ಮಾತ್ರ ಪರಿಗಣಿಸದೆ ಆರ್. ಎಲ್. ಜಾಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರಕ್ಕೆ ಬರುವ ಮಧುಮೇಹ ಕ್ಲಿನಿಕ್ ಮೂಲಕ ಚಿಕಿತ್ಸೆಯ ಪ್ರಮಾಣಿತ ಆರೈಕೆ ನೀಡಲಾಗುವುದು. ಆಸ್ಪತ್ರೆಯ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರದಲ್ಲಿನ ಮಧುಮೇಹ ಕ್ಲಿನಿಕ್ನ ಉಸ್ತುವಾರಿ ವೈದ್ಯರನ್ನು ಅಧ್ಯಯನದಲ್ಲಿ ಸಹ-ಮಾರ್ಗದರ್ಶಿಯಾಗಿ ಸೇರಿಸಲಾಗಿದೆ. ಅವರು ತಮ್ಮ ತೊಡಕುಗಳನ್ನು ಲೆಕ್ಕಿಸದೆಯೇ ಎಲ್ಲ ಮಧುಮೇಹ ರೋಗಿಗಳ ಎಲ್ಲಾ ಪರೀಕ್ಷೆಗಳು ಮತ್ತು ಕ್ಲಿನಿಕಲ್ ನಿರ್ವಹಣೆಯನ್ನು ಸಹಕರಿಸುತ್ತಾರೆ.

ಸ್ವಯಂ ಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ: ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂ ಪ್ರೇರಿತವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ನಿರ್ಬಂಧವಿಲ್ಲ. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ, ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನೀವು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದರೆ ಮಾತ್ರ ನೀವು ಸಹಿ ಮಾಡಬೇಕಾಗುತ್ತದೆ. ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಸ್ವಾತಂತ್ರ್ಯವಾಗಿರುತ್ತೀರಿ. ನಿಮ್ಮ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆಯು ವೈದ್ಯರನ್ನು ಯಾವುದೇ ರೀತಿಯಲ್ಲಿ ಯಾವುದೇ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ ಎಂದು ನಾವು ಭರವಸೆನೀಡುತ್ತೇವೆ.

ವಿಧಾನ: ನಾವು ನಿಮ್ಮ ಕೈಯಿಂದ ಸಣ್ಣ ಪ್ರಮಾಣದ ರಕ್ತವನ್ನು (5 ಮಿ.ಲೀ) ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. ಈ ಮಾದರಿಯನ್ನು ರೋಗ ನಿರೋಧಕ ವಿಶ್ಲೇಷಣೆಗಾಗಿ ಬಳಸಲಾಗುತ್ತದೆ. ಕುಟುಂಬದ ಕ್ಲಿನಿಕಲ್ ಇತಿಹಾಸವೂ ಅವಶ್ಯಕವಾಗಿರುತ್ತದೆ.

ಅವಧಿ : ಸಂಶೋಧನೆ ಸುಮಾರು 3 ವರ್ಷಗಳ ಕಾಲ ನಡೆಯುತ್ತದೆ.

ಅಪಾಯಗಳು: ನಿಮ್ಮ ಮೇಲೆ ಯಾವುದೇ ಔಷಧಿ ಪರೀಕ್ಷಿಸಲಾಗುವುದಿಲ್ಲ. 3 ಮಿ. ಲೀ ರಕ್ತವನ್ನು ಸೂಜಿ ಮತ್ತು ಸಿರಿಂಜ್ ಬಳಸಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಯೋಜನೆಯಲ್ಲಿ ತೊಡಗಿಸಿಕೊಳ್ಳುವ ನಿಮ್ಮನಿರ್ಧಾರವನ್ನುಹೊರತುಪಡಿಸಿ ಟೈಪ್ 2 ಮಧುಮೇಹ ಹೊಂದಿರುವ ರೋಗಿಗಳಿಗೆ ಗುಣಮಟ್ಟದ ಚಿಕಿತ್ಸೆಯನ್ನು ನೀಡಲಾಗುತ್ತದೆ.

ಪ್ರಯೋಜನಗಳು: ಡಯಾಬಿಟಿಕ್ ರೋಗಿಗಳಲ್ಲಿ ಫಾಸ್ಫೊಗ್ಲೂಕೊಮ್ಯುಟೇಸ್ ಬೆಳವಣಿಗೆಯನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ಈ ಅಧ್ಯಯನವು ಉಪಯುಕ್ತವಾಗಿದೆ. ಈ ಅಧ್ಯಯನದಿಂದ ಸಂಗ್ರಹಿಸಿದ ಫಲಿತಾಂಶಗಳು ರೋಗಗಳ ನಿರ್ವಹಣೆಗೆ ಅನುಕೂಲಕರವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ

ಭಾಗವಹಿಸುವಿಕೆಯು ನಿಮಗೆ ಯಾವುದೇ ವೆಚ್ಚವನ್ನುಒಳಗೊಂಡಿರುವುದಿಲ್ಲ. ಅಲ್ಲದೆ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗೆ ಯಾವುದೇ ವಿತ್ತೀಯ ಪರಿಹಾರವನ್ನು ಪಾವತಿಸಲಾಗುವುದಿಲ್ಲ.

ಮಾದರಿಗಳಶೇಖರಣೆ: ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗಾಗಿ ನಿಮ್ಮ ರಕ್ತದ ಮಾದರಿಯಿಂದ ವಂಶವಾಹಿ ವಸ್ತುಗಳನ್ನು ಸಂಗ್ರಹಿಸಬಹುದು. ಅಂತಹ ಸಂದರ್ಭದಲ್ಲಿ, ಎಥಿಕ್ಸ್ಕಮಿಟಿಯಿಂದ ಅನುಮತಿ ಪಡೆಯುವ ಮೊದಲು ಅದನ್ನು ಪಡೆಯಬಹುದು.

ಗೋಪ್ಯತೆ:ವೈಯಕ್ತಿಕ ಗುರುತಿನ ಬಗ್ಗೆ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನೂ ಗೌಪ್ಯವಾಗಿರಿಸಲಾಗುವುದು ಮತ್ತು ಕಾನೂನಿನ ಅಗತ್ಯವನ್ನು ಹೊರತು ಪಡಿಸಿ ಯಾರಿಗೂ ಅದನ್ನು ಬಹಿರಂಗ ಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಯೋಜನೆಯ ಶೋಧಕರು ಮಾತ್ರ ಗುರುತಿನ ವಿವರಗಳಿಗೆ ಪ್ರವೇಶವನ್ನುಹೊಂದಿರುತ್ತಾರೆ.

ಫಲಿತಾಂಶಗಳನ್ನುಹಂಚಿಕೆ: ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಫಲಿತಾಂಶಗಳು ವೈಜ್ಞಾನಿಕ / ವೈದ್ಯಕೀಯ ಜರ್ನಲ್ / ಮೆಡಿಕಲ್ ಸಮ್ಮೇಳನಗಳಲ್ಲಿ ಪ್ರಕಟವಾಗುತ್ತವೆ.

ಯಾವುದೇ ಮಾಹಿತಿಗಾಗಿ ನೀವು ತನಿಖೆದಾರರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ. ಈ ಅಧ್ಯಯನವು ಸಾಂಸ್ಥಿಕ ನೀತಿ ಶಾಸ್ತ್ರ ಸಮಿತಿಯಿಂದ ಅನುಮೋದಿಸಲ್ಪಟ್ಟಿದೆ ಮತ್ತು ಅವರ ಔಪಚಾರಿಕ ಅನುಮೋದನೆಯ ನಂತರ ಮಾತ್ರ ಪ್ರಾರಂಭಿಸಲ್ಪಟ್ಟಿದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾದರಿಯನ್ನು ಇನ್ಸ್ಟಿಟ್ಯೂಟ್ನಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುವುದು ಮತ್ತು ಭವಿಷ್ಯದ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಈ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸಲು ಮತ್ತು ಬಳಸಲು ನಮಗೆ ಅನುಮತಿಸಲು ನಾನು ವಿನಂತಿಸುತ್ತೇನೆ.

ಈ ಡಾಕ್ಯುಮೆಂಟ್ ಅನ್ನುಸುರಕ್ಷಿತ ಲಾಕರ್ನಲ್ಲಿ ಮತ್ತು ಮಾಹಿತಿಗಾಗಿ ನಿಮಗೆ ನೀಡಿದ ಪ್ರತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಈ ಕೆಳಗಿನ ಸಂಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ:

ಡಾ. ಪ್ರಭಾಕರ್ ಕೆ.
ಪ್ರೊಫೆಸರ್ , ಸಾಮಾನ್ಯ ಔಷಧ ವಿಭಾಗ
ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು,
ತಮಕಾ, ಕೋಲಾರ.
ಮೊಬೈಲ್: 9845209858

1: ಸಾಮಾನ್ಯ ಗುಂಪಿನ ಮಾಹಿತಿ ಪತ್ರ

ಯೋಜನೆಯ ಹೆಸರು	ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್‌ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್‌ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ
ಸಂಶೋಧಕಹೆಸರು	ಪ್ರವೀಣ್ ಕುಮಾರ್ ಕೆ.ಎಸ್.
ಸಂಸ್ಥೆಯ ಹೆಸರು	ಶ್ರೀ ದೇವರಾಜ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಸಂಸ್ಥೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ಟಮಕ, ಕೋಲಾರ.

ಭಾಗವಹಿಸುವವರು ಆಯ್ಕೆ : ≥ 18 ವರ್ಷ ವಯಸ್ಸಿನ ವ್ಯಕ್ತಿಗಳು, ಮಧುಮೇಹದಿಂದ ಬಳಲುತ್ತಿರುವವರು ಮತ್ತು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಯಾವುದೇ ದೀರ್ಘಕಾಲೀನ ರೋಗಗಳ ಇತಿಹಾಸವನ್ನು ಹೊಂದಿಲ್ಲದಿರುವವರು.

ಸ್ವಯಂ ಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ: ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂ ಪ್ರೇರಿತವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ನಿರ್ಬಂಧವಿಲ್ಲ. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ, ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ನೀವು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸಿದರೆ ಮಾತ್ರ ನೀವುಸಹಿಮಾಡಬೇಕಾಗುತ್ತದೆ. ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ಸ್ವಾತಂತ್ರ್ಯವಾಗಿರುತ್ತೀರಿ. ನಿಮ್ಮ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆಯು ವೈದ್ಯರನ್ನು ಯಾವುದೇ ರೀತಿಯಲ್ಲಿ ಯಾವುದೇ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ ಎಂದು ನಾವು ಭರವಸೆನೀಡುತ್ತೇವೆ.

ವಿಧಾನ: ನಾವು ನಿಮ್ಮ ಕೈಯಿಂದ ಒಂದು ಸಣ್ಣ ಪ್ರಮಾಣದ ರಕ್ತವನ್ನು (3 ಮಿ.ಲೀ) ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. ಈ ಮಾದರಿಯನ್ನು ರೋಗ ನಿರೋಧಕ ವಿಶ್ಲೇಷಣೆಗಾಗಿ ಬಳಸಲಾಗುತ್ತದೆ. ವೈದ್ಯಕೀಯ ಇತಿಹಾಸವೂ ಅವಶ್ಯಕ.

ಅವಧಿ : ಸಂಶೋಧನೆ ಸುಮಾರು 3 ವರ್ಷಗಳ ಕಾಲ ನಡೆಯುತ್ತದೆ.

ಅಪಾಯಗಳು: ನಿಮ್ಮ ಮೇಲೆ ಯಾವುದೇ ಔಷಧಿ ಪರೀಕ್ಷಿಸಲಾಗುವುದಿಲ್ಲ. 3 ಮಿ.ಲೀ ರಕ್ತವನ್ನು ಸೂಜಿ ಮತ್ತು ಸಿರಿಂಜ್ ಬಳಸಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಯೋಜನೆಯಲ್ಲಿ ತೊಡಗಿಸಿಕೊಳ್ಳುವ ನಿಮ್ಮನಿರ್ಧಾರವನ್ನುಹೊರತುಪಡಿಸಿ ಟೈಪ್ 2 ಮಧುಮೇಹ ಹೊಂದಿರುವ ರೋಗಿಗಳಿಗೆ ಗುಣಮಟ್ಟದ ಚಿಕಿತ್ಸೆಯನ್ನು ನೀಡಲಾಗುತ್ತದೆ.

ಪ್ರಯೋಜನಗಳು: ಡಯಾಬಿಟಿಕ್ ರೋಗಿಗಳಲ್ಲಿ ಫಾಸ್ಫೊಗ್ಲೂಕೊಮ್ಯುಟೇಸ್ ಬೆಳವಣಿಗೆಯನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ಈ ಅಧ್ಯಯನವು ಉಪಯುಕ್ತವಾಗಿದೆ. ಈ ಅಧ್ಯಯನದಿಂದ ಸಂಗ್ರಹಿಸಿದ ಫಲಿತಾಂಶಗಳು ರೋಗಗಳ ನಿರ್ವಹಣೆಗೆ ಅನುಕೂಲಕರವಾಗಿರುತ್ತದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವಿಕೆಯು ನಿಮಗೆ ಯಾವುದೇ ವೆಚ್ಚವನ್ನು ಒಳಗೊಂಡಿರುವುದಿಲ್ಲ. ಅಲ್ಲದೆ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗೆ ಯಾವುದೇ ವಿತ್ತೀಯ ಪರಿಹಾರವನ್ನುಪಾವತಿಸಲಾಗುವುದಿಲ್ಲ.

ಮಾದರಿಗಳ ಶೇಖರಣೆ: ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗಾಗಿ ನಿಮ್ಮ ರಕ್ತದ ಮಾದರಿಯಿಂದ ವಂಶವಾಹಿ ವಸ್ತುಗಳನ್ನು ಸಂಗ್ರಹಿಸಬಹುದು. ಅಂತಹ ಸಂದರ್ಭದಲ್ಲಿ, ನೈತಿಕ ಸಮಿತಿಯಿಂದ ಅನುಮತಿ ಪಡೆಯುವ ಮೊದಲು ಅದನ್ನು ಪಡೆಯಬಹುದು.

ಗೋಪ್ಯತೆ: ವೈಯಕ್ತಿಕ ಗುರುತಿನ ಬಗ್ಗೆ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನೂ ಗೌಪ್ಯವಾಗಿರಿಸಲಾಗುವುದು ಮತ್ತು ಕಾನೂನಿನ ಅಗತ್ಯವನ್ನು ಹೊರತು ಪಡಿಸಿಯಾರಿಗೂ ಅದನ್ನು ಬಹಿರಂಗ ಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಯೋಜನೆಯ ಶೋಧಕರು ಮಾತ್ರ ಗುರುತಿನ ವಿವರಗಳಿಗೆ ಪ್ರವೇಶವನ್ನುಹೊಂದಿರುತ್ತಾರೆ.

ಫಲಿತಾಂಶಗಳನ್ನುಹಂಚಿಕೆ: ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಫಲಿತಾಂಶಗಳು ವೈಜ್ಞಾನಿಕ / ವೈದ್ಯಕೀಯ ಜರ್ನಲ್ / ಮೆಡಿಕಲ್ ಸಮ್ಮೇಳನಗಳಲ್ಲಿ ಪ್ರಕಟಿಸಲಾಗುತ್ತದೆ.

ಯಾವುದೇ ಮಾಹಿತಿಗಾಗಿ ನೀವು ತನಿಖೆದಾರರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ. ಈ ಅಧ್ಯಯನವು ಸಾಂಸ್ಥಿಕ ನೀತಿಶಾಸ್ತ್ರ ಸಮಿತಿಯಿಂದ ಅನುಮೋದಿಸಲ್ಪಟ್ಟಿದೆ ಮತ್ತು ಅವರ ಔಪಚಾರಿಕ ಅನುಮೋದನೆಯ ನಂತರ ಮಾತ್ರ ಪ್ರಾರಂಭಿಸಲ್ಪಟ್ಟಿದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾದರಿಯನ್ನು ಸಂಸ್ಥೆಯಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುವುದು ಮತ್ತು ಭವಿಷ್ಯದ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಈ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸಲು ಮತ್ತು ಬಳಸಲು ನಮಗೆ ಅನುಮತಿಸಲು ನಾನು ವಿನಂತಿಸುತ್ತೇನೆ.

ಈ ಪ್ರತಿಯನ್ನು ಸುರಕ್ಷಿತ ಲಾಕರ್ನಲ್ಲಿ ಮತ್ತು ಮಾಹಿತಿಗಾಗಿ ನಿಮಗೆ ನೀಡಿದ ಪ್ರತಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ.ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಈ ಕೆಳಗಿನ ಸಂಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ:

ಡಾ. ಪ್ರಭಾಕರ್ ಕೆ.
ಪ್ರೊಫೆಸರ್ , ಸಾಮಾನ್ಯ ಔಷಧ ವಿಭಾಗ
ಶ್ರೀ ದೇವರಾಜ್ ಅರಸ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು,
ತಮಕಾ, ಕೋಲಾರ.
ಮೊಬೈಲ್: 9845209858

ತಿಳಿವಳಿಕೆಯ ಸಮ್ಮತಿ ನಮೂನೆ (ರೋಗಿಗಳು)

ವಿಷಯ: ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ

ಗಮನಿಸಿ: ರೋಗಿಯ ಗುಂಪು (ಟೈಪ್ 2 ಮಧು ಮೇಹ ರೋಗಿಗಳು) ಒಪ್ಪಿಗೆ ಪಡೆಯುವವರು ಭಾಗವಹಿಸುವವರಿಂದ ಪಡೆಯಬೇಕು.

ಪಾಲ್ಗೊಳ್ಳುವವರ ಹೆಸರು (ರೋಗಿಯ / ಸ್ವಇಚ್ಛೆಯಿಂದ ಪಾಲ್ಗೊಳ್ಳುವವರು) : _____

- ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನವನ್ನು ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ; ಇದನ್ನು ಚರ್ಚಿಸಲು ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶ ನೀಡಲಾಗಿದೆ. ನನ್ನ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನ ತೃಪ್ತಿಗೆ ಉತ್ತರ ನೀಡಲಾಗಿದೆ.
- ನಾನು ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯ ಪ್ರತಿ ಪುಟವನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ನನಗೆ ಓದಲಾಗಿದೆ.
- ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿದಂತೆ ನನ್ನ ಆರೋಗ್ಯ ಮಾಹಿತಿಯನ್ನು ಪಡೆಯಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ. (ರೋಗಿಗೆಮಾತ್ರ)
- ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿರುವ ಸಂಶೋಧನಾ ಉದ್ದೇಶಗಳಿಗಾಗಿ 3 ಮಿ. ರಕ್ತ ಮಾದರಿ ಮತ್ತು ಆರೋಗ್ಯದ ಡೇಟಾ ಸಂಗ್ರಹಣೆಯನ್ನು ಕೂಡಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.
- ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುವುದು ಎಂದು ನಾನು ಅರ್ಥ ಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.
- ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗೆ ನನ್ನ ಮಾದರಿ ಸಂಗ್ರಹಿಸುವುದಕ್ಕೆ ನಾನು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೇನೆ.
- ನಾನು ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸುತ್ತೇನೆ
- ಭಾಗವಹಿಸುವವರ ಸಹಿ ಅಥವಾ ಹೆಬ್ಬೆರಳು ಗುರುತು

ದಿನಾಂಕ: _____

ಅನಕ್ಷರಸ್ಥನಾಗಿದ್ದರೆ: ಎರಡು ಸಾಕ್ಷರತಾ ಸಾಕ್ಷಿಗಳು ಸಹಿಹಾಕಬೇಕು (ಸಾಧ್ಯವಾದರೆ,ಈ ವ್ಯಕ್ತಿಯನ್ನು ಸಹಭಾಗಿಯು ಆರಿಸಬೇಕು ಮತ್ತು ಸಂಶೋಧನಾತಂಡಕ್ಕೆಯಾವುದೇಸಂಪರ್ಕವನ್ನುಹೊಂದಿಲ್). ಅನಕ್ಷರಸ್ಥರು ಭಾಗವಹಿಸುವವರು ತಮ್ಮಹೆಬ್ಬೆರಳು–ಮುದ್ರಣವನ್ನೂ ಸಹ ಒಳಗೊಂಡಿರಬೇಕು. ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಸಮ್ಮತಿರೂಪದ ನಿಖರವಾದ ಓದುವಿಕೆಗೇ ನಾನು ಸಾಕ್ಷಿಯಾಗಿದ್ದೇನೆ ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ವ್ಯಕ್ತಿಗೆ ಅವಕಾಶವಿದೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾನೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

	ಸಾಕ್ಷಿ 1	ಸಾಕ್ಷಿ 2
ಹೆಸರು		
ಸಹಿ		
ದಿನಾಂಕ		

ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ನಾನು ಓದಿದ್ದೇನೆ ಮತ್ತು ವಿವರಿಸಿದ್ದೇನೆ ಮತ್ತು ಈ ಪ್ರತಿಗಳ ಪ್ರತಿಯನ್ನು ಅವರಿಗೆ ನೀಡಲಾಗಿದೆ.

ಸಮ್ಮತಿಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಹೆಸರು _____
ಒಪ್ಪಿಗೆ ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಸಹಿ _____ ದಿನಾಂಕ: _____

ತಿಳಿವಳಿಕೆಯ ಸಮ್ಮತಿ ನಮೂನೆ (ಆರೋಗ್ಯಕರ ವ್ಯಕ್ತಿಗಳು)

ವಿಷಯ: ಟೈಪ್ 2 ಡಯಾಬಿಟಿಸ್ ಮೆಲ್ಲಿಟಸ್ನಲ್ಲಿ ಫಾಸ್ಫೋಗ್ಲುಕೊಮುಟೇಸ್ನ ಪಾತ್ರ: ಸಮಗ್ರ ಜೈವಿಕ ರಾಸಾಯನಿಕ, ಜೆನೆಟಿಕ್ ಮತ್ತು ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿ ಅಧ್ಯಯನ

ಗಮನಿಸಿ: ಆರೋಗ್ಯಕರ ವ್ಯಕ್ತಿಗಳ ಒಪ್ಪಿಗೆ ಪಡೆಯುವವರು ಭಾಗವಹಿಸುವವರಿಂದ ಪಡೆಯಬೇಕು.

ಪಾಲ್ಗೊಳ್ಳುವವರ ಹೆಸರು (ಆರೋಗ್ಯಕರ ವ್ಯಕ್ತಿ): _____

- ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನವನ್ನು ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ; ಇದನ್ನು ಚರ್ಚಿಸಲು ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶ ನೀಡಲಾಗಿದೆ. ನನ್ನ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನ್ನತೃಪ್ತಿಗೆ ಉತ್ತರ ನೀಡಲಾಗಿದೆ.
- ನಾನು ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯ ಪ್ರತಿಪುಟವನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ನನಗೆ ಓದಲಾಗಿದೆ. .
- ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿದಂತೆ ನನ್ನ ಆರೋಗ್ಯ ಮಾಹಿತಿಗೆ ಪ್ರವೇಶವನ್ನು ಅನುಮತಿಸಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.
- ರೋಗಿಯ ಮಾಹಿತಿಹಾಳೆಯಲ್ಲಿ ವಿವರಿಸಿರುವ ಸಂಶೋಧನಾ ಉದ್ದೇಶಗಳಿಗಾಗಿ 3 ಮಿ.ಲೀ ರಕ್ತ ಮಾದರಿ ಮತ್ತು ಆರೋಗ್ಯದ ಡೇಟಾ ಸಂಗ್ರಹಣೆಯನ್ನು ಅನುಮತಿಸಲು ನಾನು ಒಪ್ಪುತ್ತೇನೆ.
- ಭವಿಷ್ಯದ ಸಂಶೋಧನಾ ಯೋಜನೆಗಳಿಗೆ ನನ್ನ ಮಾದರಿ ಸಂಗ್ರಹಿಸುವುದಕ್ಕೆ ನಾನು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೇನೆ.
- ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನುಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುವುದು ಎಂದು ನಾನು ಅರ್ಥ ಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.
- ನಾನು ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಸ್ವಯಂ ಪ್ರೇರಣೆಯಿಂದ ಸಮ್ಮತಿಸುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರ ಸಹಿ ಅಥವಾ ಹೆಬ್ಬೆರಳು ಗುರುತು

ದಿನಾಂಕ: _____

ಅನಕ್ಷರಸ್ಥನಾಗಿದ್ದರೆ: ಎರಡು ಸಾಕ್ಷರತಾ ಸಾಕ್ಷಿಗಳು ಸಹಿ ಹಾಕಬೇಕು (ಸಾಧ್ಯವಾದರೆ, ಈ ವ್ಯಕ್ತಿಯನ್ನು ಸಹಭಾಗಿಯು ಆರಿಸಬೇಕು ಮತ್ತು ಸಂಶೋಧನಾ ತಂಡಕ್ಕೆ ಯಾವುದೇ ಸಂಪರ್ಕವನ್ನು ಹೊಂದಿಲ್ಲ. ಅನಕ್ಷರಸ್ಥರು ಭಾಗವಹಿಸುವವರು ತಮ್ಮ ಹೆಬ್ಬೆರಳು-ಮುದ್ರಣವನ್ನೂ ಸಹ ಒಳಗೊಂಡಿರಬೇಕು. ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಸಮ್ಮತಿ ರೂಪದ ನಿಖರವಾದ ಓದುವಿಕೆಗೇ ನಾನು ಸಾಕ್ಷಿಯಾಗಿದ್ದೇನೆ ಮತ್ತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ವ್ಯಕ್ತಿಗೆ ಅವಕಾಶವಿದೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾನೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

	ಸಾಕ್ಷಿ 1	ಸಾಕ್ಷಿ 2
ಹೆಸರು		
ಸಹಿ		
ದಿನಾಂಕ		

ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ನಾನು ಓದಿದ್ದೇನೆ ಮತ್ತು ವಿವರಿಸಿದ್ದೇನೆ ಮತ್ತು ಈ ಒಂದು ಪ್ರತಿಯನ್ನು ಅವರಿಗೆ ನೀಡಲಾಗಿದೆ.

ಸಮ್ಮತಿಯನ್ನುತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಹೆಸರು _____
ಒಪ್ಪಿಗೆ ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿಯ ಸಹಿ _____ ದಿನಾಂಕ: _____

RECOMMENDATIONS

Overall, the results of this study support the conclusion that phosphoglucomutase is suboptimal in type 2 diabetes mellitus, probably due to downregulated gene expression and destabilizing genetic variation. Furthermore, the suboptimal activity of phosphoglucomutase appears to impair PBMC viability under glucose-limiting conditions.

- This study highlights the importance of phosphoglucomutase in the pathogenesis of type 2 diabetes mellitus.
- Phosphoglucomutase may be considered a target for developing anti-diabetic drugs.