NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL BIOLOGY
PROGRAM: MOLECULAR MEDICINE

INVESTIGATION OF METHIONINE METABOLISM IN THE LIVER OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

MASTERS THESIS IN MOLECULAR MEDICINE

JOHNPAUL CHIDILI

NICOSIA
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Supervisor
Prof. Dr Selma YILMAZ
Dr Gökçe AKAN

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APPROVAL

We certify that we have read the thesis submitted by Johnpaul Chidili titled **"THE INVESTIGATION OF METHIONINE METABOLISM IN THE LIVER OF STREPTOZOTOCIN (STZ)- INDUCED DIABETIC RATS"** and that in our combined opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Educational Sciences.

Supervisor: Prof. Dr Selma Yilmaz

Co-supervisor: Dr Gökçe Akan

Examining Committee:	**Name-Surname**	**Signature**
Head of the Committee:	Dr Gülten Tuncel	.....
Committee Member:	Assist. Prof. Emrah Güler	.....
Supervisor:	Prof. Dr Selma Yilmaz	.....

Approved by the Head of the Department

YAKIN DOĞU ÜNİVERSİTESİ TIP FAKÜLTESİ 1988 Prof. Dr. Gonze Mocan

08/02/2024

Prof. Dr Selma Yilmaz
Head of Department

Approved by the Institute of Graduate Studies

/.... /2024

Prof. Dr Kemal Hüsnü Can Başer

Head of the Institute

DECLARATION

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

JOHNPAUL CHIDILI

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JOHNPAUL CHIDILI

ABSTRACT

This study aimed to investigate the impact of streptozotocin (Stz)-induced diabetes on methionine metabolism in the liver and explore potential therapeutic targets for managing diabetic complications.

Using rat models, we extracted total RNA from both the Stz-induced group (10 samples) and the control group (10 samples). The expression levels of methionine adenosyl transferase genes (*Mat1a, Mat2a, and Mat2b*) in rat liver tissues were examined. The findings revealed a significant reduction in mRNA expression levels of *Mat1a* and *Mat2a* ($p=0.029$ and $p=0.007$, respectively) in rats with streptozotocin-induced diabetes compared to the control group. Although the *Mat2b* mRNA expression levels were reduced in the diabetes group, the differences did not reach statistical significance ($p>0.05$).

These results suggest that diabetes disrupts methionine metabolism in the liver, highlighting potential avenues for therapeutic intervention. The observed down-regulation in the gene expression of *Mat1a, Mat2a, and Mat2b* in the livers of rats with diabetes indicates a potential impact on methionine metabolism, supported by the modulation of other genes involved in methyl group metabolism in diabetic contexts. This research provides significant insights into the mechanisms behind diabetes complications and identifies prospective targets for therapeutic interventions.

INVESTIGATION OF METHIONINE METABOLISM IN THE LIVER OF STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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SUMMARY.

The aim of this study is to investigate how streptozotocin-induced diabetes impacts methionine metabolism in the liver of rats, specifically examining the expression levels of critical genes in methionine metabolism, including *Mat1a, Mat2a,* and *Mat2b.* The study seeks to address the gap in understanding how diabetes affects methionine metabolism in the liver, offering valuable insights that may inform future therapeutic strategies and contribute to our broader understanding of metabolic alterations in diabetes.

The hypothesis posits that streptozotocin-induced diabetes will lead to significant alterations in methionine metabolism within the liver. It is expected that the delicate balance of methionine, crucial for energy production and antioxidative processes, will be disrupted under diabetic conditions induced by streptozotocin. This disruption is anticipated to manifest as changes in the activity levels of key enzymes associated with methionine metabolism, including methionine adenosyl transferase and cystathionine beta-synthase. Moreover, notable changes in the expression and functionality of genes associated with methionine metabolism, notably *Mat1a, Mat2a,* and *Mat2b*, are anticipated.

The study utilized a molecular medicine approach to evaluate the expression levels of the specified genes. Liver samples from Wistar rats were induced and collected for analysis. RNA extraction, complementary DNA (cDNA) synthesis, and analysis were performed utilizing gradient PCR and qPCR techniques. The findings indicated a notable reduction in *Mat1a* and *Mat2a* mRNA expression levels in streptozotocin-induced diabetic rats compared to the control group, with statistical significance observed at $p=0.029$ and $p=0.007$, respectively. Although the Mat2b mRNA expression levels showed a decrease in the diabetic group, these variances did not reach statistical significance ($p>0.05$). These findings suggest that diabetes disrupts methionine metabolism in the liver. The findings of this study may uncover potential therapeutic targets for managing diabetic complications and contribute to the broader knowledge of metabolic dysregulation in diabetes.

Keyword: Streptozotocin-induced rat livers, methionine metabolism, gene expression, diabetes, diabetic complications.

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List of Abbreviations

DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
Stz	Streptozotocin
CLD	Chronic Liver Disease
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
HCC	Hepatocellular Carcinoma
cDNA	Complementary DNA
PCR	Polymerase Chain Reaction
Qpcr	Quantitative PCR
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
SAM/SAM Adomet	S-Adenosyl methionine
HDL	High Density Lipoprotein
Alc	Glycated haemoglobin
FFAs	Free Fatty acids
ADA	American Diabetes Association
IDF	International Diabetes Federation
CLD	Chronic Liver Disease
OLEFT Rats	Otsuka Long-Evans Tokushima Fatty rats
TAE	Tris Acetate-EDTA
Etbr	Ethidium bromide
SPSS	Statistical Package for the Social Sciences
ABM	Applied Biology M
dNtp	Deoxynucleotide triphosphate
RT-PCR	Real Time PCR
GC	Guanine-Cytosine
Ct	Cycle Threshold
mRNA	Messenger RNA
AU	Arbitrary Units
AA	Amino Acid

NASH	Non-alcoholic Steatohepatitis
BHMT	Betaine-homocysteine S-methyltransferase
GNMT	Glycine N-methyltransferase
NCBI	National Center for Biotechnology Information

CHAPTER I

1. INTRODUCTION

1.1. AN OVERVIEW OF DIABETES AND ITS IMPACT ON METABOLIC PATHWAYS

Diabetes Mellitus, characterized by impaired glucose regulation is a persistent metabolic condition presents a worldwide health concern due to its increasing prevalence. This condition exerts profound effects on various physiological systems such as deficiency in insulin production, insulin action, or both, particularly impacting intricate metabolic pathways within the body. The liver, a central organ in metabolic regulation, undergoes significant alterations in response to diabetic conditions. As the epicentre of nutrient processing and energy homeostasis, the liver's function becomes intricately entwined with the disruptions caused by diabetes. These changes often lead to disturbances in critical metabolic pathways, contributing to the development of diabetic complications.

One avenue of metabolic investigation gaining prominence is the exploration of methionine metabolism. Methionine, an essential amino acid, plays a pivotal role in cellular processes, serving as a precursor for critical molecules involved in energy production and antioxidative defence mechanisms. Metabolic dysregulation in diabetes extends to diverse biochemical processes, impacting not only glucose metabolism but also lipid and protein metabolism. The liver's intricate involvement in methionine metabolism makes it a focal point for understanding the repercussions of diabetes on these crucial biochemical pathways.

1.1.2. UNDERSTANDING THE SIGNIFICANCE OF METHIONINE METABOLISM IN THE LIVER.

The importance of comprehending methionine metabolism in the context of diabetic pathophysiology lies in its potential to unveil nuanced insights into the intricate interplay between altered metabolic pathways and the progression of diabetic complications. As methionine serves as a precursor for crucial cellular processes, including DNA methylation and protein synthesis, alterations in its metabolism which contributes to the liver's response to diabetic conditions may significantly impact the overall systemic metabolic milieu. Therefore, a detailed investigation into how diabetes influences methionine metabolism in the liver could uncover novel avenues for therapeutic intervention and shed light on the molecular underpinnings of diabetic complications.

1.2. PROBLEM STATEMENT

The prevalence of diabetes is a significant global health concern, with far-reaching implications for various metabolic processes. Methionine metabolism is one of such processes, which plays a crucial role in liver function. Streptozotocin (Stz)-induced diabetes has been shown to impact the liver's metabolic pathways, including methionine metabolism. Understanding the specific alterations in methionine metabolism in the context of Stz-induced diabetes is therefore of great importance.

1.3. PURPOSE OF THE STUDY

For this research, we are aiming to comprehensively examine the impacts of streptozotocin-induced diabetes on methionine metabolism in rat livers. By focusing on the potential alterations in the gene expression of key genes such as *Mat1a, Mat2a* and *Mat2b,* the objective of this study is to gain a thorough understanding of how diabetes affects methionine metabolism and the potential implications for the development of the disease by analysing the above genes.

1.4. RESEARCH QUESTION/HYPOTHESIS

It is hypothesized that the induction of diabetes using Stz will result in significant alterations to methionine metabolism within the liver. The intricate balance of methionine, a key amino acid involved in energy production and antioxidative processes, is expected to be disrupted under diabetic conditions induced by Stz. It is expected that these disturbances will result in alterations in the activity levels of important enzymes linked to methionine metabolism, including methionine adenosyl transferase and cystathionine beta-synthase. The gene expression related to methionine metabolism, especially *Mat1a, Mat2a, and Mat2b* will equally show significant alterations through this induction.

Furthermore, the hypothesized alterations in methionine metabolism are speculated to contribute to the development of diabetic complications. Given the central role of the liver in maintaining metabolic homeostasis, disturbances in methionine metabolism may cascade into broader metabolic imbalances, exacerbating the progression of diabetes-related complications. The hypothesis posits that understanding the specific changes in methionine metabolism induced by streptozotocin will provide valuable insights into the intricate molecular mechanisms linking altered hepatic metabolism to the manifestation and progression of diabetic complications.

1.4. SIGNIFICANCE OF THE STUDY IN ADVANCING KNOWLEDGE OF DIABETES-RELATED METABOLIC ALTERATIONS

This study is expected to provide valuable insights into the alterations in methionine metabolism in the context of diabetes, potentially identifying novel targets for therapeutic intervention. Centering on the liver, where methionine metabolism primarily occurs, the study seeks to reveal particular mechanisms that could be involved in the onset of diabetes and its associated complications.

The study will build upon existing research that has demonstrated the impact of diabetes on amino acid metabolism, including methionine (Si Q. *et al.,* 2023 and Ndlovu IS*, et al.*, 2023). Moreover, it will add to the expanding understanding of the metabolic consequences of diabetes in animal models. (Monjani et al., 2014 and Welche et al., 1991).

This thesis presents a thorough examination of changes in the metabolism of liver methionine in diabetic rats induced with Stz, aiming to enhance our understanding of diabetes-related metabolic shifts and pinpointing potential therapeutic targets.

1.5. LIMITATIONS

One potential limitation of this study is the focus on a specific animal model (streptozotocin-induced diabetic rats), which may not fully represent the complexity of methionine metabolism alterations in human diabetes. Additionally, the study's scope may be limited to the specific genes and pathways under investigation, and further research may be needed to explore additional factors influencing methionine metabolism in the context of diabetes.

This research aims to address the gap in understanding the impact of Stz-induced diabetes on methionine metabolism in the liver, providing valuable insights that may inform future therapeutic strategies and contribute to the broader knowledge of metabolic alterations in diabetes.

The genes associated with methionine metabolism, such as *Mat1a, Mat2a, and Mat2b*, are essential for regulating methionine and its related pathways. (Rouillard AD. et al., 2016). By analysing the expression of these genes, the study aims to shed light on the intricate interplay between diabetes and methionine metabolism in the liver.

1.6. OBJECTIVES

- To Investigate the impact of streptozotocin-induced diabetes on methionine metabolism in livers of rats.

- To assess the correlation between altered methionine metabolism genes in streptozotocin-induced diabetes rat.

CHAPTER II

2.0. LITERATURE REVIEW:

2.1. DIABETES

The World Health Organization (WHO) anticipates that the number of individuals with diabetes will reach 366 million worldwide by 2030. (S. Wild, et al., 2004). Diabetes has gradually become one of the most silent and deadly pandemics. As outlined by M. van der Graaf et al. (2004), diabetes mellitus, a multifaceted metabolic condition, is distinguished by persistently elevated levels of blood sugar, insulin, and ketone bodies. This hyperglycaemia, hyperinsulinemia, and ketosis are progressive but also can be solved. The progression of these situations is usually very catastrophic in the body because it is responsible for the majority of the diabetic complications such as cardiomyopathy, encephalopathy, and nephropathy (Zhao L. et at., 2010). There are two main types of diabetes mellitus: Type 1 and Type 2. Type 1 diabetes (T1D) is defined by Pinkse. G.G. et al., 2005 as a T cell-mediated autoimmune disorder and Type 2 diabetes (T2D) has been known to be a metabolic disease for many years (De Candia P, et al., 2019). Diabetes attacks multiple organs and tissues in the body which makes it a systemic metabolic disease (P. Chen, et al., 2007). The metabolic challenges faced by individuals with diabetes, particularly Type 1 diabetes (T1D), present significant management difficulties, with insulin therapy being the primary approach to maintaining metabolic stability (Chai, J. et al., 2022). Additionally, abnormalities in circulatory metabolism due to T1D can influence the metabolic condition of multiple organs. Reduced levels of choline, betaine, and methionine detected in the livers of T1D patients indicate a possible diminishment in their protective capacity against liver injury, potentially attributable to hyperglycaemia. (Chen M. et al., 2019). Various metabolites, including γ-aminobutyric acid, glutamine, myoinositol, and lactate, have been detected in various brain regions, suggesting their potential utility as markers for evaluating health status. Additionally, Type 1 Diabetes (T1D) has been shown to impact lipid metabolism in the meibomian gland and induce aberrant metabolic alterations in the kidney, characterized by oxidative stress and perturbations in the purine metabolic pathway. (Liu. J. et al., 2015). Given the widespread impact of T1D on multiple organs, close monitoring of metabolic status, especially in high-risk individuals prior to seroconversion, is crucial for implementing effective interventions, particularly in paediatric patients (Chen M. et al., 2019).

Diabetes, particularly T2D, is equally associated with various metabolic alterations that can impact different systems in the body. These key metabolic changes and their implications include: brain metabolism alterations, role of adipose tissues, insulin resistance and obesity, blood glucose and hyperglycaemia. Research has shown that T2D can lead to alterations in brain energy metabolism, which may underlie memory impairment and dysfunction. These alterations are linked to factors such as chronic hyperglycaemia, insulin resistance, dyslipidaemia, and hypertension (Garcia-Serrano AM, et al., 2020). The adipose tissue's role in diabetes-related metabolic complications is significant. Chronic hyperglycaemia in diabetes can induce the generation of advanced glycation end products, markers of inflammation, and oxidative stress, all associated with microvascular complications like nephropathy, neuropathy, and retinopathy (Dilworth L, et al., 2021). Insulin resistance is a pivotal factor in the metabolic changes seen in both obesity and diabetes, often co-existing and exerting their effects through insulin resistance, leading to various metabolic alterations and complications (Oyewande AA, et al., 2020). Dysregulated blood glucose and prolonged hyperglycaemia in Type 2 Diabetes (T2D) are linked to metabolic changes, affecting numerous physiological processes and contributing to the development of diabetic microvascular complications (J.M. Sanches et al., 2021 & Miras, A.D., et al., 2019).

2.1.2. INSULIN RESISTANCE AND DIABETICS

According to Cleaveland Clinic (2021), the condition of insulin resistance in the body is a complex one that results to the irresponsiveness of the body's cells to insulin, which leads to elevated blood sugar levels. The dysfunction of this very important response can be a precursor to T2D and amongst numerous other complications in the body. In 2018, the National Institutes of Diabetes and Digestive and Kidney Diseases characterized insulin as a hormone that assists glucose to enter the cells to be used as a source of energy. When cells in the body develop resistance to insulin, i.e., they can no longer recognise it or acknowledge it for its functions, the pancreas which produces this insulin becomes overwhelmed with producing more insulin to compensate. Over time, this can lead to prediabetes and type 2 diabetes and numerous other health concerns such as obesity, hypertension, elevated cholesterol, and triglyceride levels (WebMD, 2024).

According to WebMD, 2024, Insulin resistance could also be called metabolic syndrome which could be seen in as many as 1in 3 Americans. It is caused by excess body fat, especially around the abdomen, lack of physical activity, wrong or unhealthy nutrition,

certain medications, hormonal disorders, and sleep problems. It's important to note insulin resistance, it could be a pointer to some other health conditions that is not only T2D. It could be seen even in T1D and at any age. In addition, not everyone with insulin resistance could be an indication of other health conditions, and not only T2D (Diabetics UK, 2022). Most importantly, all levels of insulin resistance are not diagnosed as diabetes and not all types of diabetes are majorly a result of insulin resistance.

2.1.3. PREDIABETES

This is a less complicated condition that exists as a middle ground between being metabolically heathy and diabetes. You are diagnosed with prediabetes if you already have insulin resistance which means your blood glucose levels are already overshooting and your body's cells are not responding properly to insulin but not enough to be called diabetes. Diabetes is the extreme levels of prediabetes. Symptoms of insulin resistance are not always apparent, and it is typically diagnosed through blood tests to check blood sugar levels (WebMD, 2024). With recent discoveries and innovations, insulin levels can now be measured which even makes it more efficient for diagnosis. Therefore, anyone can develop insulin resistance at different levels either temporarily or chronically. Diabetes doesn't just occur; it usually is a progressive insulin resistance that becomes prediabetes and then over time becomes T2D diabetes if not taken care of. (Cleaveland Clinic, 2021). According to the Centres for Disease Control and Prevention (CDC) in 2022, common indicators of insulin resistance include increased blood glucose levels, elevated triglycerides, and reduced levels of HDL (beneficial) cholesterol, as noted by Diabetes UK (2022). Maintaining a healthy lifestyle, which encompasses consistent physical activity and a well-balanced diet, is essential for effectively managing insulin resistance. These lifestyle changes can enhance insulin sensitivity, potentially preventing or delaying the development of T2D, as highlighted by The National Institutes of Diabetes and Digestive and Kidney Diseases (2018) and the Centres for Disease Control and Prevention (2022).

High blood glucose levels associated with diabetes can lead to various long-term complications by damaging organs and blood vessels. Certain indicators are commonly used to speculate or identify insulin resistance in the body. According to a report by WebMD (2024), men should have a waist circumference of 40 inches, while women should aim for 35 inches. Men should have increased levels of high-density lipoprotein (HDL) cholesterol surpassing 40 mg/dL in men and 50 mg/dL in women, and blood pressure readings

surpassing 130/80 mmHg. fasting triglyceride levels exceeding 150 mg/dL, and fasting glucose levels over 100 mg/dL. Some other visible signs are skin tags, dark patches and acanthosis nigricans (velvet skin). Nevertheless, blood testing has been an efficient way of detecting diabetes or maintaining health. This test is called glycated haemoglobin (A1c) and it generally evaluates the average blood sugar for the past 3 months. A1c is preferably normal at a level below 5.7%, 5.7-6.4% is considered prediabetes while above 6.5% on two different tests show T2D.(Cleaveland Clinic, 2021).

Studies have demonstrated that diabetes can have enduring consequences, including harm to both large and small blood vessels. This damage can lead to heart attacks, strokes, and a range of complications impacting the kidneys, eyes, gums, feet, and nerves. Additionally, diabetes can lead to nerve damage contributing to conditions such as erectile dysfunction and chronic kidney disease, potentially progressing to end-stage kidney failure necessitating dialysis or transplantation. Other complications associated with diabetes encompass significant eye ailments like cataracts and glaucoma, as well as skin problems such as bacterial and fungal infections. Moreover, diabetes heightens the likelihood of bone disorders like osteoporosis and cognitive impairments like dementia. Effective management of blood sugar levels, blood pressure, and cholesterol, coupled with adopting a healthy lifestyle, can mitigate the risk of enduring complications from diabetes.

2.1.4. METABOLIC CONSEQUENCES OF DIABETICS

There are several metabolic consequences on the body which can result to various health complications. Firstly, the body becomes resistant to insulin which makes the body incapable of effectively using insulin to regulate blood sugar levels (Watts, M. et al., 2019). Then the body loses its ability to process glucose translating to high blood sugar levels (Martin, L. 2021). This could equally result in an altered brain energy metabolism, a function of memory impairment and dysfunction. (Garcia-Serrano AM et al., 2020). Many organs can be affected as well as from the presence of excessive free fatty acids (FFAs) in the blood. (Jellinger PS. 2007). Also, metabolic alterations arise when the body is producing very low levels of insulin (T1D) according to Martin, L. 2021. Other complications associated to insulin resistance are inflammation and impaired fibrinolysis. (Jellinger PS. 2007). Insulin resistant could be very complex on its own and at different levels. The American Diabetes Association (ADA) classification of "complete" and "partial" remission in T2D was solely determined by glycaemic indicators. These glycaemic markers together with medication usage were

introduced further as a more comprehensive set of criteria that emphasizes optimizing and enhancing metabolic health by the International Diabetes Federation (IDF). They equally included the usage of weight loss, plasma lipids and blood pressure. (Buse JB, et al., 2009 and Dixon JB, et al, 2021).

2.1.5. METABOLIC COMPLICATIONS IN DIABETES INDUCED RAT MODELS

Experiments employ animal models to enhance understanding of pathogenesis, discover novel treatments, and explore rehabilitation methods. The careful selection of a diabetes induction method is pivotal to ensure more dependable outcomes. Various techniques have been employed to induce diabetes mellitus in rodent models, each with its own set of advantages and limitations (Mojani, MS. et al., 2014). Several researches have equally been done using these induced models especially related to diabetes Si Q. et al. (2023) observed that there is limited research exploring the complete metabolic profile of diabetic animals and patients. Previous reports predominantly focused on analysing serum and urine specimens obtained from individuals with diabetes. This research aims at analysing the metabolic consequences of diabetes on the methionine pathway in the liver of this rat models. Typically, the liver, being among the body's vital organs, is frequently targeted by chronic conditions such as diabetes. In rats, when diabetes gets to the liver, it triggers oxidative stress and other biochemical alterations. Studies indicate that rats with diabetes display elevated levels of free radicals and diminished antioxidant activity in their liver tissue, resulting in oxidative stress (Lucchesi AN. et al., 2013, Bilal H M. et al., 2016 and Yazdi HB et al., 2019). The dysregulation of oxidative processes in liver cells could play a significant role in the onset of chronic liver diseases associated with diabetes, including non-alcoholic fatty liver disease, and its potential progression to steatohepatitis (Lucchesi AN. et al., 2013). Additionally, liver damage induced by diabetes is linked to oxidative stress resulting from hyperglycaemia, disruptions in protein and lipid metabolisms, and pathophysiological alterations in the liver (Yazdi HB et al., 2019).

Nevertheless, we are more focused on the alterations of the methionine cycle in the liver of rats when treated with Streptozotocin (Stz). Stz is frequently employed as an antibiotic for various cancer treatments. Moreover, because of its high cytotoxicity toward pancreatic beta-cells, it is frequently employed to induce experimental type 1 diabetes in rodent investigations. (Raza, H. et al., 2012). Stz-induced rats have been shown over time to have organ-specific metabolic alterations but little has been done on the precision of these effects

on the organs. It is equally pertinent to pay attention to the effect of Stz in specific organs as well.

2.1.6. METHIONINE METABOLISM

Methionine, a vital amino acid essential for optimal growth and development (Parkhitko, A. A. et al., 2019), undergoes breakdown in the small intestine, leading to the generation of free methionine. This free methionine is subsequently absorbed and utilized for protein synthesis or converted into S-adenosylmethionine (SAM/AdoMet), as outlined by Anstee, Q. M. et al. (2012). SAM functions as a primary donor of methyl and sulphate groups in various biochemical reactions and is proposed as a treatment for specific diseases (Wunsch, E. et al., 2018). In Anstee, Q. M. et al., (2012) report, chronic liver diseases (CLD) inhibit SAM synthesis, sparking significant interest in leveraging SAM to mitigate disease severity. Furthermore, according to Moon MK. et al., (2010), SAM showed direct inhibition of IKK-β kinase activity was observed in vitro. These findings imply that SAM could potentially mitigate TNFα-induced insulin resistance by impeding the IKK-β/NF-κB pathway, potentially offering a beneficial treatment approach for T2D. However, research on methionine supplementation in clinical settings is still limited and its outcomes remain controversial (Morgan, T. R. et al. 2015). With the above information, it's important to observe that the amount of methionine in the body matters. Interestingly, the production and consumption of most SAM is done in the liver and it is produced from ATP and Methionine (Cantoni, GL. 1952). This study investigates the correlation of the altered genes and the consequences of Stz in the methionine metabolism of the liver of Stz-induced rats.

Methionine metabolism plays a central role, serving as a crucial link between the pathway of folate metabolism and the transsulfuration process within one-carbon metabolism. In addition to its function as a precursor for glutathione synthesis and a fundamental supplier of methyl groups for various biological processes, methionine metabolites also contribute to polyamine synthesis. Disruptions in methionine metabolism can exacerbate pathological damage in diseases. In the context of Chronic Liver Diseases (CLDs), alterations in methionine metabolism components can influence disease pathology through various mechanisms during onset and progression (Li Z, et al., 2020). However, methionine metabolism is vital for improving Type 2 Diabetes (T2D) and for synthesizing polyamines, glutathione, and choline through treatment with S-adenosylmethionine (SAM) (H. Izu et al., 2019). The

administration of SAM was documented to enhance insulin sensitivity and prevention of body weight gain in OLETF rats, a commonly used animal model, are closely linked. for TD2 (Jin CJ. et at., 2007).

2.1.7. THE MECHANISM OF METHIONINE METABOLISM

Methionine metabolism encompasses the methionine or the folate pathway, transsulfuration route, and salvage
pathway.

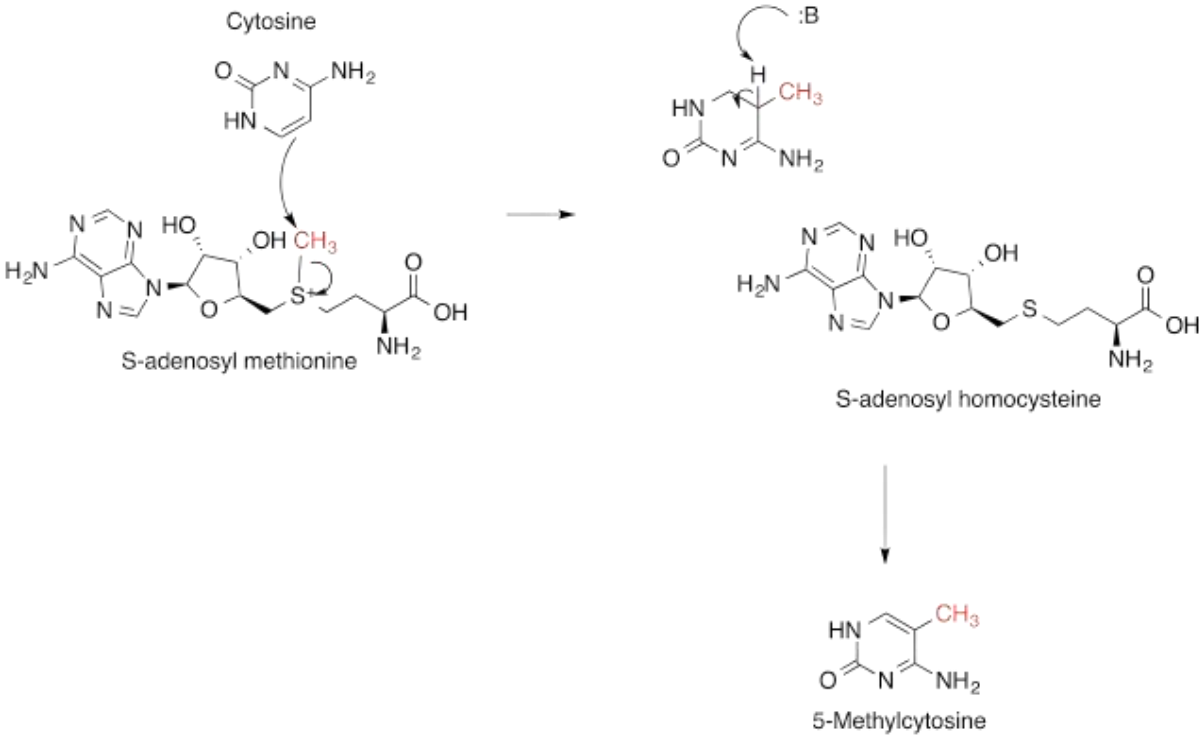


Fig. 1: The methyl transfer reaction resembling SN2. For simplicity, only the SAM cofactor and cytosine base are depicted (Etabs1089., 2014).

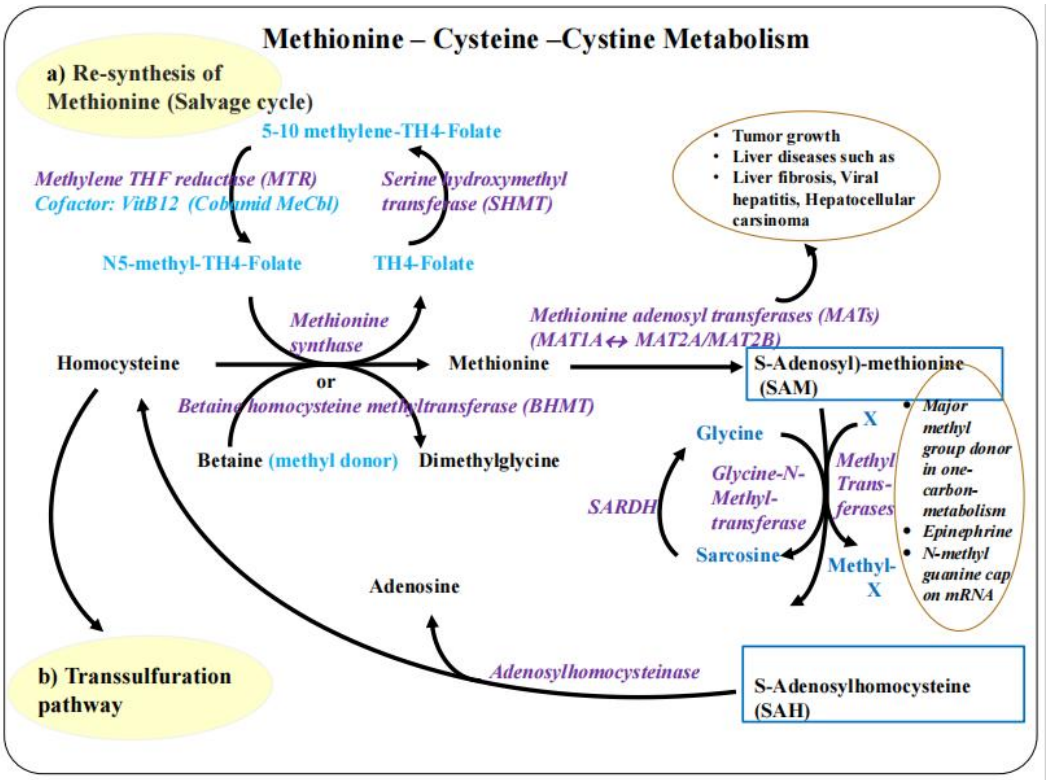


Fig. 2: A. The Methionine – Cysteine – Cystine Metabolism.

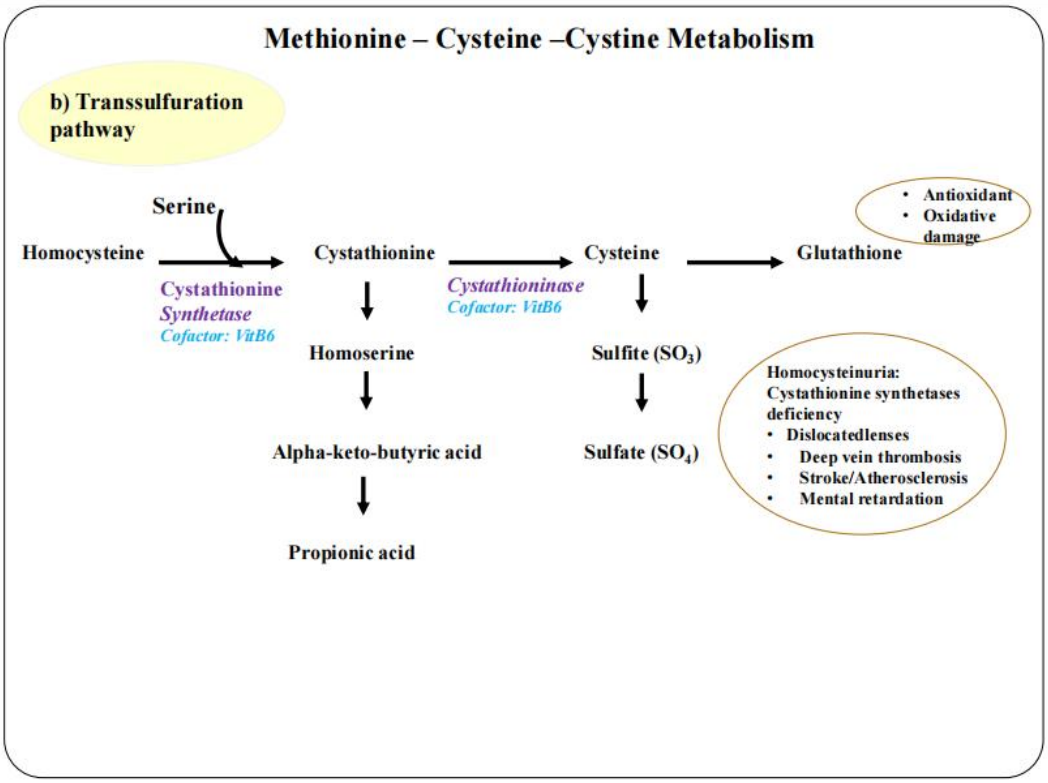


Fig. 3: B. The Methionine – Cysteine – Cystine Metabolism.

2.1.8. GENES OF INTEREST AND THEIR IMPORTANCE

Numerous genes, enzymes, and proteins are involved in the complex pathways, with a particular focus on methionine metabolism. Of interest are the *Mat1a, Mat2a, and Mat2b* genes, pivotal in this cycle. *Mat1a* is primarily expressed in the liver, it serves as an indicator of typical liver maturation and rises during periods of accelerated liver growth and loss of specialization. It plays a critical role in synthesizing S-adenosylmethionine (SAM) from methionine and ATP, crucial for various biological processes. *Mat2a*, expressed in nonhepatic tissues, likewise, it rises during periods of rapid liver growth and dedifferentiation. (Li et al., 2020). *Mat2a,* along with *Mat2b,* forms a dimer with α2 subunits, which is overexpressed in various human epithelial tumours (Li et al., 2020). *Mat2b* (Methionine adenosyl transferase 2b): *Mat2b* codes for a regulatory subunit that forms a complex with the *Mat2a* dimer, creating a heterotetramer (Nordgren et al., 2011). This binding significantly reduces the apparent Km of the complex for methionine, improving the conversion from methionine to SAM (Nordgren et al., 2011). Additionally, *Mat2b* decreases the apparent Km value of *Mat2a* for both methionine and ATP (Nordgren et al., 2011).

Table 1: An Insight of the Mammalian MAT genes.

Mammalian MAT (Methionine adenosyltransferase) genes	**MAT protein encoded (subunits)**	**Isoenzymes of MAT genes**	**Expressed in**
Mat1a High amino acid sequence similarity to *Mat2a,* different enzyme activity Kenza	MATα1 Catalytic subunit	*Mati* (a homotetramer) MATIII (a homodimer)	Liver (Mostly in hepatocytes), Pancreas (acinar cells)
Mat2a	Catalytic subunit MATα2	MATII encoded by *Mat2a* gene (gamma; α2 and β interaction)	Extrahepatic tissues, Also in the hepatic stellate cells (HSCs) and Kupffer

		(predominant in fetal liver; after birth, *Mat1a* replaces with MATII) MAT II (gamma)	cells of liver
Mat2b	Regulatory subunit MATβ Multiple transcript variants encoding different isoforms are resulted by Alternative splicing. Two major splicing variants: MATβ (same as V1) and V2 (differ from V1 for 20 amino acids at the beginning of N-terminal	MATII (gamma; α2 and β interaction) MATII is regulated by both β and V2 (lower the Km for methionine, β also lowers Ki for S-adenosyl-methionine-SAM).	Extrahepatic tissues, Also, in the hepatic stellate cells (HSCs) and Kupffer cells of liver Ubiquitous expression in lymph node, thyroid and 25 other tissues (See NIH Gene bank for the references)

CHAPTER III

MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. CHEMICAL REAGENTS AND KITS

Trizol reagent (Hibrizol, Hibrigen, Istanbul, Turkey) was utilized for RNA isolation procedures. Subsequently, cDNA synthesis was performed using the ABM OneScript Plus cDNA Synthesis Kit from Applied Biological Materials Inc. (ABM), located in Richmond, Canada. Following qPCR experiments were conducted using 2X Sybr Green qPCR Mix sourced from Hibrigen, Istanbul, Turkey., Catalog MG-SSGD-01-1000), using a 50 bp DNA ladder (Thermo Scientific™, Pittsburg, USA, catalog number: MG-LDR-50-5) as a molecular weight marker.

3.1.2. EQUIPMENT

MetiSafe® Laminar Air Flow Cabinet (Ankara, Turkey), MetiSafe® PCR Cabinet (Ankara, Turkey), Nano-drop™ 2000/2000c Spectrophotometer (Thermo-scientific, Pittsburg, USA), RotorGene Real-Time PCR (Qiagen, Hilden, Germany), Spin (Qiagen, Hilden, Germany). DNR Bio Imaging Systems MiniBIS Pro (DNR Bio Imaging Systems, Neve Yamın, Israel), the gel electrophoresis apparatus and power source are sourced from Cleaver Scientific, based in Rugby, UK.

3.1.3. OLIGONUCLEOTIDES

In this project, the primers used were sourced from Bioligo Company (Turkey).

3.1.4. STANDARD SOLUTIONS

50x stock solution of Tris-Acetate/EDTA (TAE) electrophoresis buffer (Sambrook J. et al., 1989) was prepared by dissolving it in 1 ml of water. This 50x TAE buffer was then diluted to 1x concentration using a mixture of 20 ml from 50x stock solution with 980 ml of distilled water. This dilution aimed to decrease the TAE concentration, potentially minimizing any delay in band movement during electrophoresis.

3.1.5. OTHER CHEMICAL AGENTS

Gel electrophoresis was performed using an agarose powder obtained from Sigma-Aldrich with catalogue number 11388983001 to visualize the PCR product samples. A fluorescent

dye, ethidium bromide (EtBr), obtained from Sigma-Aldrich (catalogue number E1385), was used to render the PCR products visible.

3.1.6. COMPUTER SOFTWARES

The GelCapture Software facilitated the viewing, analysis, and storage of gel images and imaging data. RNA quantification was performed using the Nano-drop™ 2000/2000c software. Data statistics were conducted using the Statistical Package for the Social Sciences (SPSS).

3.1.7. ETHICAL CLEARANCE

The Ethical Committee for Scientific Research at Near East University approved the collection of samples (project no. 2024/171/ 171).

3.1.8. DIABETES MODEL

Twenty (20) male experimental rats called Wistar albino rats, each aged 6 months and weighing 250 g, were used in this study. These rats were randomly divided into two equal groups: Group 1, identified as the Diabetic group, and Group 2, assigned as the Control group.

3.2. METHODS

3.2.1. INJECTION OF STZ AND PREPARATION OF SAMPLE

The rats were rendered diabetic using a modification of the method suggested by Saad and colleagues in 1992, wherein a solitary administration of Streptozotocin (Stz) at a dosage of 50 mg/kg was suspended in a citrate buffer with a concentration of 0.07 M at pH 6 and given via intraperitoneal injection (Sigma, St. Louis, MO, USA).

3.2.2. SAMPLE COLLECTION

After the follow-up period concluded, a ketamine and xylazine mixture at doses of 75 mg/kg and 10 mg/kg, respectively) was given to anesthetize and euthanize all animals. Liver tissues from the rats were promptly dissected (approximately 30 mg tissue) and then stored in 500 µl Trizol reagent at -80°C after extraction.

3.2.3. RNA ISOLATION

Liver tissue RNA isolation from both the diabetes and control groups was conducted using the Trizol method, employing the Trizol reagent from Hibrizol, Hibrigen, Istanbul, Turkey. Initially, 500 µl of Trizol reagent was included to disrupt the cellular membrane, accompanied by adding 100 µl of chloroform then vortex mixing for 15-20 seconds. The solution underwent centrifugation at 14,000 × g for 15 minutes at 4°C, leading to the formation of three separate phases: organic, interphase, and aqueous. The RNA-containing upper aqueous phase was meticulously transferred to a fresh tube, and RNA precipitation was induced by the addition of 250 µl isopropyl alcohol. After incubation at 15-30°C, the RNA was pelleted by centrifugation at 14,000 x g for 10 minutes at 4°C after incubating at room temperature for 10 minutes. Following this, the supernatant was removed, and an initial homogenization was achieved by adding a 1:1 ratio of 75% ethanol to Trizol reagent. After vortexing, the solution underwent centrifugation at 10,000 x g for 5 minutes at 4°C, with the washing step repeated. Subsequently, ethanol was removed, and the sample was dried with dry air for 5-10 minutes to achieve high purity. Finally, 20 µl of distilled water or nuclease-free water was used to elute RNA.

3.2.4. RNA QUANTIFICATION

The quantification and purity of RNA were assessed using the NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific, Pittsburgh, USA). These spectrophotometers offer full spectrum UV-vis analysis, enabling assessment not only of RNA quality and purity but also of DNA, protein, and various other compounds. Key advantages of this device include the capacity to quantify sample volumes as small as 0–5 µl. Optimal ratios for RNA range between 1.8ng and 2ng, while for DNA, the maximum ratio is 2.1. Results obtained from the NanoDrop analysis are presented in the results section, following which our experiment proceeded upon confirming sufficient RNA quantity and quality.

3.2.5. SYNTHESIS OF COMPLEMENTARY DNA (cDNA)

In performing the cDNA synthesis, the ABM One Script Plus cDNA synthesis kit was commercially sourced from ABM, headquartered in Richmond, Canada. The kit includes the dNTPs mix, One Script Plus reverse transcriptase, Oligo (dT) primer, One Script Plus RT reaction buffer and anchored oligo(dT) primer are all stored within the temperature range of -

15°C to -25°C. In the experimental protocol, 500ng of RNA was mixed with 1µl of oligo(dt) primers (for eukaryotes), 1µl of reverse transcriptase enzyme, 1µl of deoxyribonucleotides triphosphate (dNTPs), 4µl of buffer solution, and nuclease-free water to make a total volume of 20 µl. The experiment was conducted within a laminar flow hood classified as category II. After adding all components to the reaction tubes, conventional PCR was used for cDNA synthesis via reverse transcription, followed by incubation at 55°C for 15 minutes.

3.2.6. PRIMER DESIGN

The primers targeting the genes *Mat1a, Mat2a, and Mat2b* were designed utilizing Primer-BLAST from the National Centre for Biotechnology Information (NCBI).

Table 1: List containing gene-specific primer sequences along with their respective melting temperatures, product sizes and GC content.

GENE NAME AND ASCERTION NUMBER	PRIMER NAME	SEQUENCES (5' – 3')	GC%	SELF COMPLEMETARITY (5'-3')	PRODUCT SIZE
β-ACTIN (NM_031144.3)	Forward	AGAGGGAAATCGTGCGTGACA	52.38		138bp
	Reverse	CGATAGTGATGACCTGACCGTCA	52.17		
MAT1A (NM_012860.2	Forward	CCGGGAAGCTTAGCTCTGTC	60.00	7.00/3.00	140
	Reverse	GATGTTTGCAGATTGCCGGG	55.00	4.00/3.00	
MAT2A (NM_134351)	Forward	AGAAAGTGGTTCGTGAAGCCATA	43.48	6.00/2.00	169
	Reverse	CCCTGATCTCCTGCACCAATG	57.14	4.00/3.00	
MAT2B NM_001044282.1	Forward	TGCACTGTGAAAAGCTGCAC	50.00	*6:00/2:00*	94
	Reverse	GACGCCAAGAAACGTGAGGT	55.00	*4.00/3.00*	

3.2.6. OPTIMIZATION OF PRIMERS USING GRADIENT PCR

In the optimization phase, we prepared a 100 µM stock solution of oligomers for the primers of each of the three genes. To create a 10 µM concentration of the working solution for this stock primer, it was diluted by combining 90 µl of deionized water with 10 µl of the stock primer. The primers employed in the gradient PCR included *Mat1a, Mat2a, and Mat2b*. Gradient PCR was conducted using the SimpliAmpTM Thermal Cycler (appliedbiosystems

by Thermo Fisher Scientific) to determine the ideal temperature conditions for RT-PCR. Each of the specified genes underwent gradient PCR at temperatures ranging from 58°C to 64°C. The findings of calculations conducted on three samples and one negative control are depicted in Table 1.

Table 2: *The preparation of the gradient PCR mixture.*

Component	**1X**	**5X**
Taq	10µl	50µl
Forward Primer	1µl	5µl
Reverse Primer	1µl	5µl
dH_2O	6µl	30µl
cDNA pool	2µl	-

During the experimental procedure, 20µl was dispensed into the Eppendorf PCR tubes, comprising 18µl from the final mix and an extra 2µl of cDNA pool. A cDNA pool was created by diluting 1µl of rat cDNA (at a concentration of 10 µl) with 90 µl of dH2O to achieve a 1:10 pool dilution. This procedure was repeated for three specimens and one negative control. Each gene was subjected to the experimental protocol separately using gene-specific primers. The gradient PCR in Table 1 employed annealing temperatures ranging from 56°C to 62°C.

Table 3: *Optimal Parameters used for gradient PCR.*

	Stage	**Temperature**	**Time**	**Cycles**
Steps	Initial denaturation	94°C	3 mins	
	Denaturation	94°C	30 sec	35 cycles
	Annealing	56°C - 62°C	30 sec	

Extension	72°C	40 sec
Final extension	72°C	7 mins

3.2.7. AGAROSE GEL ELECTROPHORESIS

The PCR product underwent gel electrophoresis employing Agarose (Sigma-Aldrich, catalogue no. 11388983001). A 2% gel concentration was prepared by dissolving 5 grams of agarose in 250 ml TAE buffer and heating the solution in a microwave until clear. After cooling in a laminar flow hood, the solution was poured into a 20cm x 20cm tray treated with Ethidium Bromide (EtBr) sourced from Sigma-Aldrich with catalogue number E1385). PCR products (10µl) were loaded into wells, along with a Thermo Scientific™ 50 base pair DNA ladder sourced from Pittsburg, USA. The gel was run at 105 volts for 30 minutes, and the bands were detected using an ultraviolet transilluminator of the DNR Bioimaging system sourced from Neve Yamin, Israel.

3.2.8. QUANTITATIVE-PCR (qPCR) FOR THE HOUSE KEEPING GENE (*B-ACTIN*)

The category II laminar flow hood was utilized to provide a sterile environment in performing the qPCR reactions. Prior to use, reagents and plasticware underwent sterilization. The table presented below provides details of 20 samples comprising the qPCR master mix employed for analysing the housekeeping gene β-actin.

Table 4: *Preparation of the qPCR mixture for β-actin analysis.*

Component	**1X**	**25X**
SYBR Green	5 µl	125 µl
Forward Primers	0.5 µl	12.5 µl
Reverse Primers	0.5 µl	12.5 µl
dH_20	1 µl	25 µl

Throughout the experimental protocol, 10µl was pipetted into the Eppendorf PCR tubes, comprising 7µl from the resultant mixture and an extra 3µl of cDNA.

Table 5: *The ideal parameters for qPCR analysis of β-actin.*

	Stage	Temperature	Time	Cycle
Steps	Initial denaturation	95°C	3 mins	
	Denaturation	95°C	15 seconds	40 cycles
	Annealing	60°C	30 seconds	
	Extension	72°C	30 seconds	

3.2.9. QUANTITATIVE-PCR (QPCR)

The category II laminar flow hood was utilized to provide a sterile environment in performing the qPCR reactions. Before utilization, all reagents and plasticware underwent sterilization. The table provided below displays 20 samples of the qPCR master mix.

Table 6: *Preparation for qPCR mixture for Mat1a, Mat2a and Mat2b.*

Component	1X	25X
SYBR green	5 µl	125 µl
Forward Primers	0.5 µl	12.5 µl
Reverse Primers	0.5 µl	12.5 µl
dH_20	1 µl	25 µl

For this PCR procedure, a total volume of 10 µl was utilized, transferred into eppendorf PCR tubes. This comprised 7 µl from the resultant mixture and an extra 3µl of cDNA.

Table 7: *The optimal qPCR conditions for Mat1a and Mat2a were determined and documented at various stages.*

	Stage	Temperature	Time	Cycle
Steps	Initial denaturation	95°C	2 mins	
	Denaturation	95°C	15 secs	40 cycles
	Annealing	62°C	30 secs	
	Extension	72°C	30 secs	

Table 8: *The optimal qPCR conditions for Mat2b were documented and presented at various stages.*

	Stage	Temperature	Time	Cycle
Steps	Initial denaturation	95°C	3 mins	
	Denaturation	95°C	15 secs	40 cycles
	Annealing	60°C	45 secs	
	Extension	72°C	30 secs	

3.3.1. INVESTIGATION OF THE GENE EXPRESSION LEVELS

The expression levels of *Mat1a, Mat2a, Mat2b*, and the housekeeping gene β-Actin were measured using SYBR Green and gene-specific primers via qPCR in the Insta Q96™ Plus Real-time PCR Detection System. Total RNA was converted to cDNA and diluted fivefold

for use as templates, with each sample analysed in triplicate and mean values calculated for further analysis.

3.3.2. STATISTICAL ANALYSIS

The statistical analysis utilized the statistical software SPSS (Statistical Package for the Social Sciences 25.0, SPSS Inc., Chicago, IL, USA) and the data is presented as the mean value ± standard error (SE). Gene expression information was acquired in Cycle Threshold (Ct) values. Ct represents the cycle number where logarithmic PC plots intersect a calculated threshold line. The gene expression between depots were compared using the 2ΔΔCt method. The ΔΔCt was calculated by subtracting the Ct value from the target gene compared to the housekeeping gene. Statistical analyses were conducted to compare normally distributed continuous variables using Student's t-test, and abnormally distributed variables were compared using the Mann-Whitney U test. The statistical significance level between the control and patient group was taken as $p < 0.05$ (p less than 0.05).

CHAPTER 1V

4.1. RESULTS

This chapter presents the outcomes of the experiment conducted, which primarily relied on real-time PCR analysis. Additionally, statistical analysis through one-way ANOVA student's T-test was employed to interpret these results. This approach facilitated the generation of both numerical and graphical representations showcased in this chapter.

4.2. DIABATES MODEL

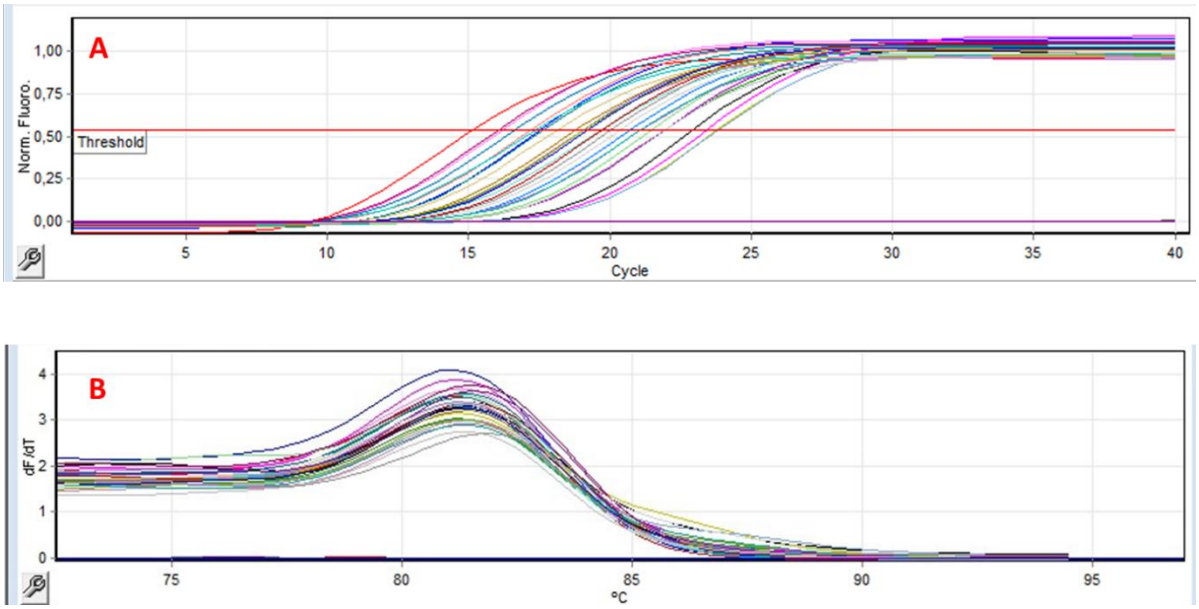
The streptozotocin-induced rat group underwent a 12-hour fast before and a 4-hour fast after streptozotocin administration. Blood samples of 0.1 ml were obtained from the tail vein of diabetic rats on days 1, 2, and 14, and their glucose levels were measured using a digital glucose meter (Gluco Leader; HMD BioMedical Inc., Hsinchu, Taiwan). On the first day, all subjects were given drinking water containing 15% dextrose, and after streptozotocin injection, rats exhibiting blood glucose levels exceeding 200 mg/dl (11.1 mmol/l) were classified as diabetic. Glucose levels remained normal in the control group, which did not receive streptozotocin.

4.3. GENE EXPRESSION ANALYSIS

Mat1a, Mat2a, and *Mat2b* expression levels were studied in liver tissues collected after the 14th day of the development of diabetes caused by Stz injection for all rats from study groups (Figure 3).

The mRNA levels of expression of *Mat1a, Mat2a, and Mat2b* were detected to decrease in comparison to the control group, among those with diabetes (Figure 4). Specifically, *Mat1a* mRNA expression levels were significantly down-regulated by 6.6-fold in liver tissues collected on the 14th day of diabetes development induced by Stz injection, compared to control group rats ($p=0.029$). Similarly, *Mat2a* mRNA expression levels were significantly down-regulated by 5.5-fold in diabetic rat's liver tissues compared to control group rats ($p=0.007$). However, the mRNA levels of the *Mat2b* gene expression were 3-fold decreased

in the livers of rats in the diabetes group compared to those in the control group, however, the



variances were not statistically significant ($p>0.05$).

Fig. 3: Gene expression analysis. Panel A represents the fluorescence curves and the threshold line that was used to detect Ct values, Panel B represents the melting curve.

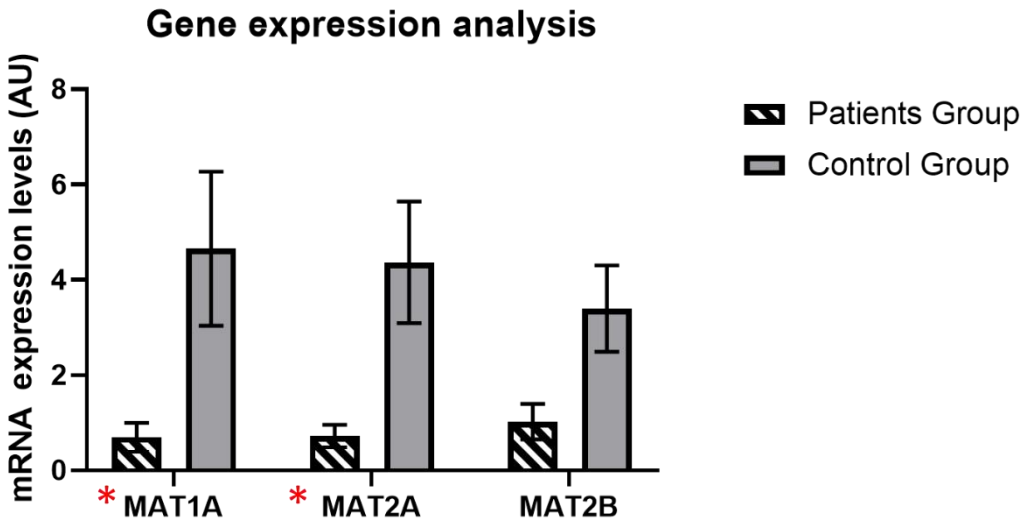


Fig. 4: Presents the relative expression levels of *Mat1a, Mat2a, and Mat2b* in streptozotocin-induced diabetic rats compared to the control group. The calculations were performed utilizing the Mann-Whitney U test, where a p-value of ≤ 0.05 was deemed

statistically significant. The p-values in bold (p=0.029 and p=0.007) retain their significance even after Bonferroni correction. Expression levels are measured in Arbitrary Units (AU).

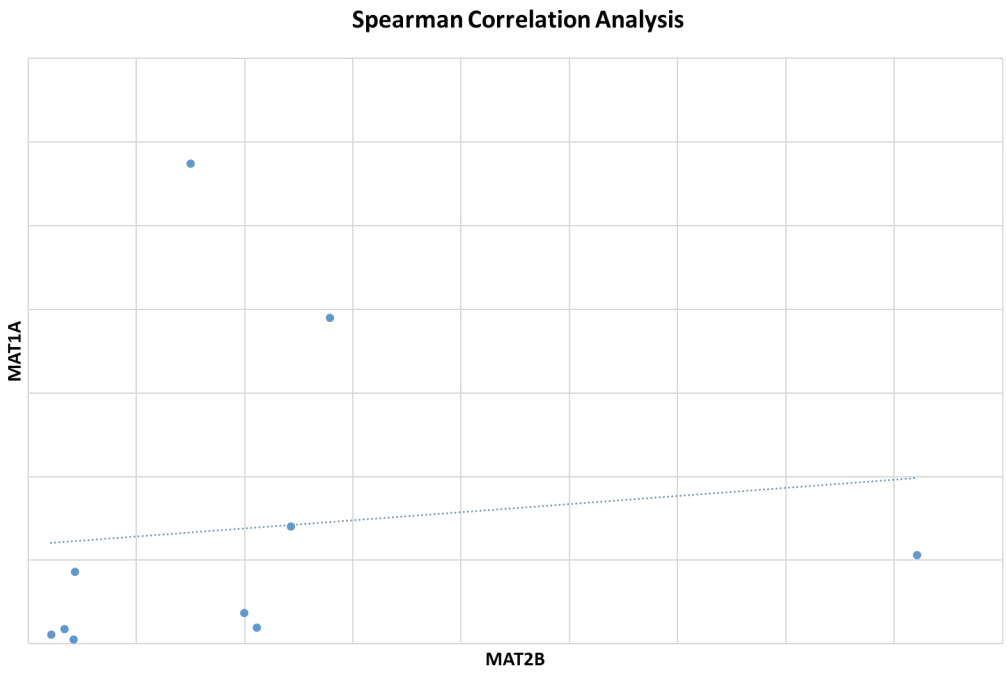


Fig. 5: Graph depicting the correlation of gene expression levels in streptozotocin-induced diabetic rats.

4.4. THE CORRELATION ANALYSIS

Spearman correlation analysis showed a direct correlation between the mRNA expression levels of *Mat1a and Mat2b* genes in the liver tissues of diabetic rats (r = 0.673, p = 0.003). (Fig. 5).

Spearman correlation analysis is performed to investigate partial association between the variables. The result showed a moderate level of relationship between the mRNA expression levels of Mat1a and Mat2b genes in the liver of tissues of diabetic rats. (r = 0.673, p = 0.003)

CHAPTER V

DISCUSSION AND CONCLUSION

5.1. DISCUSSION

Diabetes as a condition that exerts profound effects on various physiological systems particularly impacts intricate metabolic pathways within the body. Since 1963, researchers have extensively utilized streptozotocin to induce diabetes in experimental animals, documenting its biological properties. (Salih, N., et al 1988). It displays considerable efficacy by selectively harming pancreatic β-cells and disrupting cellular metabolic oxidative mechanisms (Papaccio, G. et al., 2000). Diabetes induced by Stz closely resembles inadequately treated human diabetes, and the features observed in human patients begin to manifest within 24 hours after Stz injection. (Shafrir, E. 1996) This study successfully induced diabetes mellitus in mice through intraperitoneal administration of Stz, leading to a swift decrease in glucose levels within 24 hours, exceeding 200 mg/dl (11.1 mmol/l). This decline can be attributed to Stz's diabetogenic effects, which damage β-cells in the pancreatic islets of Langerhans, consistent with previous methods of inducing Stz-induced diabetes in mice. (Riedl et al. 2011, Al-Badri, S. T. et at., 2011 and N D Salih, et al., 2009). However, Glanville N.T. et al. (1984) found that hypermethioninemia in Wistar rats treated with Stz was correlated with the administered dose of the drug. Additionally, scientists have noted that indications of diabetogenic toxicity usually vanish within a 14-day timeframe. Alterations in experimental conditions are most likely the result of insulin insufficiency, as the introduction as exogenous insulin administration reverses these effects (Watkins, J.B. et al., 1995). Salih, N., D. et al. (2014) explored the immediate and prolonged impacts of Stz-induced diabetes on liver functionality, examining post-Stz injection at two, four, and six weeks.

One of the most important functions that occur in the liver is the methionine metabolism. Being integral to one-carbon metabolism, it is closely intertwined with various pathophysiological processes (Anstee, Q. M. et al., 2012). An increasing number of

preclinical studies suggest that changes in the methionine cycle contribute to development of CLD (Pascale, R.M. et al., 2019). In this study, we focused on the effects of diabetes as one of the diseases that can causes several dysfunctions in the liver with emphasis to the methionine metabolism. Methionine, recognized as the most toxic AA, undergoes significant metabolism primarily in the liver (Glanville N.T. et al., 1984). The impaired ability to metabolize methionine observed in Stz-induced diabetic rats can be anticipated as a result of hepatocellular damage done when Stz is used, according to Laguens RP, et al., 1980, and Feldman S. et al., 1977. However, Glanville N.T. et al., 1984, also notes in their study that factors other than insulin deficiency contributes to the restricted capability of rats with Stz-induced diabetes in metabolizing methionine. Maturation of hepatic methionine metabolism heavily relies on *Mat1a*, and the deletion of *Mat1α* in the germline of mice fosters the development of Non-alcoholic Steatohepatitis (NASH). (C. Alarcon-Vila et al. 2023). Furthermore, the shift from *Mat1a to Mat2a/Mat2b* leads to a decline in SAM levels, which is pivotal in fibrosis and liver cancer. (Murray, B. et at., 2019). The investigation conducted by C. Alarcon-Vila et al. (2023) employed both dietary interventions and genetic manipulation models to perturb methionine metabolism. A common feature observed in these diverse approaches is the reduction in SAM levels, a vital intermediary metabolite generated from methionine through the action of *Mat1a.*

In addition to exploring methionine metabolism in rat livers with Stz-induced diabetes, the investigation extended to human liver samples, revealing significant alterations in metabolic profiles between diabetic and control livers. Diabetic livers exhibited lower methionine levels, indicating disruptions in methionine metabolism linked to liver diseases. Research emphasized the critical role of methionine in liver health, as its irregular metabolism can cause liver damage and influence various metabolic pathways.

Moreover, within the context of Stz-induced diabetes in human livers, specific modifications were observed in the expression of *MAT1A, MAT2A*, and *MAT2B* genes. Studies have indicated that in cases of hepatocellular carcinoma (HCC), there is a down-regulation of *MAT1A* expression, while *MAT2A* and *MAT2B* exhibit increased expression. These alterations in gene expression are associated with liver diseases and the development of hepatic malignancies. *MAT2A* expression is regulated through transcriptional and post-transcriptional mechanisms, involving regulatory elements like Sp1, c-Myb, NFκB, and AP-1. Additionally, heightened *MAT2B* expression can affect AdoMet levels and confer growth

advantages to hepatoma cells. These differential gene expressions are pivotal in determining liver health and the progression of liver diseases.

In our study, we observed decreased expression of *Mat1a, Mat2a, and Mat2b* genes in the livers of rats induced with streptozotocin-induced diabetes compared to the control group. Particularly, *Mat1a and Mat2a* mRNA levels showed significant down-regulation by 6.6-fold and 5.5-fold, respectively, in the diabetic group compared to the control group. While *Mat2b* gene expression also decreased (3-fold), the difference did not reach statistical significance ($p>0.05$). These findings demonstrates that Stz-induced diabetes affects methionine metabolism in rat livers. Other studies have reported similar impacts of diabetes on methionine metabolism. One study found that streptozotocin-induced diabetes increased the activity and mRNA levels of betaine-homocysteine S-methyltransferase (BHMT) in rat livers (Ratnam S, et al., 2005). Another study reported that diabetes, in combination with retinoic acid, increased glycine N-methyltransferase (GNMT) activity, which is involved in methyl group metabolism (Nieman, K. M. et al., 2004). These results shows that diabetes holds the capacity to impact expression of genes related to methionine metabolism, which could result in changes to methionine metabolism within the liver.

The decrease in mRNA expression levels of *Mat1a, Mat2a, and Mat2b* in the liver tissues of rats with diabetes compared to the control group suggests a potential disruption of methionine adenosyl transferase *(Mat)* genes in diabetes. This finding is consistent with altered expression of other genes in diabetic rat models, such as sialin, hepcidin, and Bcl-2 family genes, as reported in various studies (Yousefzadeh, N. et al., 2023 and Sánchez-González C, et al., 2021). This suggests widespread alterations in gene expression in various tissues of diabetic rats, consistent with the observed dysregulation of *Mat* genes in the liver. Moreover, assessing the expression of Bcl-2 family genes and the activity of Caspase-3 in rats with diabetes revealed alterations in the expression of these genes, suggesting the engagement of apoptotic pathways in diabetes (Jafari A. I, et al., 2008).

Furthermore, a study on streptozotocin-induced diabetic rats reported dysregulation of differentially expressed mRNAs, further supporting the widespread changes in gene expression associated with diabetes (Kim, J. H. et al., 2023). Furthermore, insulin was observed to specifically enhance SREBP-1c mRNA levels in rat livers, emphasizing the intricate regulatory mechanisms governing gene expression in diabetes (Shimomura et al., 1999).

5.2. CONCLUSION

This comprehensive study sought to investigate the effects of streptozotocin-induced diabetes on liver methionine metabolism, offering potential therapeutic avenues for diabetic complications. Our findings revealed a significant decrease in *Mat1a and Mat2a* mRNA expression levels in the liver tissues of streptozotocin-induced diabetic rats compared to controls. Although *Mat2b* mRNA expression levels were also reduced in the diabetic group, the differences were not statistically significant. These results suggest that diabetes disrupts methionine metabolism in the liver, providing insights into potential therapeutic strategies. Moreover, our study aligns with human research, indicating alterations in *MAT1A, MAT2A*, and *MAT2B* gene expression under diabetic conditions, associated with liver diseases and cancer progression. Transcriptional and post-transcriptional regulation of *MAT2A* expression involves regulatory factors such as Sp1, c-Myb, NFκB, and AP-1. While both human and rat studies show down-regulation of *MAT1A* and *MAT2A,* the extent varies between species. In accordance with Shimomura et al., 1999, Kim, J. H. et al., 2023 and Kogot-Levin A. et al., 2023, our rat study provides further insights into the altered activity and mRNA levels of enzymes involved in methyl group metabolism under diabetic conditions, enhancing our understanding of diabetes' effects on liver methionine metabolism.

RECOMMENDATION

Analytical techniques such as mass spectrometry should be employed to quantify key enzymes participating in methionine metabolism, such as methionine adenosyl transferase and cystathionine beta-synthase. Utilizing bioinformatic tools is crucial for analysing the impact of diabetes on methionine metabolism pathways, and further investigation into additional genes associated with methionine metabolism in diabetic conditions is warranted. Understanding the underlying mechanisms driving changes in gene expression, particularly the involvement of regulatory factors like Sp1, c-Myb, NFκB, and AP-1 in mediating *Mat2a* expression, is essential for identifying potential therapeutic targets for managing diabetic liver complications. Exploring interventions targeting methionine metabolism pathways to mitigate diabetic liver damage, including modulation of *Mat1a, Mat2a, and Mat2b* gene expression or manipulation of other enzymes involved in methyl group metabolism, is recommended. Additionally, comprehensive metabolomic analyses are needed to assess the broader metabolic alterations linked to diabetic liver complications, elucidating how changes in methionine metabolism interact with other metabolic pathways and contribute to overall liver dysfunction in diabetes. Investigating the genetic and epigenetic mechanisms underlying the dysregulation of *Mat1a*, *Mat2a*, and *Mat2b* genes in diabetic liver tissues, and assessing the potential contributions of genetic variations or epigenetic modifications to alterations in gene expression, would further enhance our understanding of diabetic liver pathophysiology.

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Appendices

Appendix 1

YAKIN DOĞU ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi **17/01/2024**
Toplantı No **: 2024/171**
Proje Başvuru No **: 171**

Yakın Doğu Üniversitesi, Tıp Fakültesi'nden, sorumlu araştırmacı Dr. Gökçe AKAN tarafından hazırlanan 'Streptozotosin İle Oluşturulan Diyabetik Sıçanların Karaciğerinde Metiyonin Metabolizmasının Araştırılması." Başlıklı araştırma önerisi kurulumuz tarafından uygun bulunmuştur.

1. Prof. Dr. Emine KOÇ (BAŞKAN)
2. Prof. Dr. Tamer YILMAZ (ÜYE)
3. Prof. Dr. Nurettin ABACIOĞLU (ÜYE)
4. Prof. Dr. Dilek ARSOY (ÜYE)
5. Prof. Dr. Aysel KÜKNER (ÜYE)
6. Prof. Dr. Vedat SAĞMANLIGİL (ÜYE)
7. Prof . Dr. Ahmet Özer ŞEHİRLİ (ÜYE)
8. Vet. Hek. Ahmet SERHATOĞLU (ÜYE)
9. Vet. Hek. Meliha TEMİZEL (ÜYE)
10. Avukat Ömür Güneş ÖZTÜRK (ÜYE)

Appendix 2

JOHN PAUL'S FINAL PLAGIARISM

ORİJİNALLİK RAPORU

%12	%11	%9	%4
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

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Bibliyografyayı Çıkart	üzerinde		