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Circulating Non-Esterified Free Fatty Acids in Bangladeshi Patients with Type-2 Diabetes: A Cross-Sectional Analysis

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ABSTRACT

Background and Objectives: Free fatty acids (FFA) significantly influence the relationship between obesity, insulin resistance, and type 2 diabetes. Elevated plasma FFA levels, common in obese individuals, inhibit insulin-stimulated glucose uptake in muscle tissue, indicating peripheral insulin resistance. This study examines the levels of non-esterified free fatty acids (NEFAs) in type 2 diabetes patients in Bangladesh to understand their role in the disease and inform management and treatment strategies.
Materials and Methods: In this cross-sectional study, 134 participants were involved: 100 had previously been diagnosed with type-2 diabetes and 34 served as a non-diabetic control group. Among the diabetic individuals, 60% were newly diagnosed with type-2 diabetes mellitus, 28% had been previously treated but had uncontrolled diabetes, and 11% had been treated and had controlled diabetes and 0.75% had gestational diabetes. The sample included 42.5% males and 57.5% females. Sociodemographic and health data, including BMI, hypertension, cardiovascular history, and lifestyle habits, were collected through questionnaires. Blood pressure was measured per ACC/AHA guidelines. Blood samples were analyzed for glucose, NEFA, and lipid profiles using automated analyzers. NEFA estimation followed specific reagent procedures, while lipid profiling assessed total cholesterol, HDL, LDL, and triglycerides per ADA guidelines. Data were analyzed using Excel and SPSS, with significance set at $p<0.05$.
Results: NEFA levels were significantly higher in diabetic patients (647 µM/L) compared to healthy controls (331.5 µM/L). Significant associations were found between NEFA levels, insulin resistance, and diabetes. Controlled diabetics had lower NEFA levels than uncontrolled ones. Lifestyle factors like work hours and exercise habits correlated with NEFA levels, highlighting their importance in diabetes management.
Conclusion: Elevated NEFA levels are common among diabetic patients in Bangladesh, influenced by disease control status and lifestyle factors. Effective diabetes management

requires comprehensive strategies targeting NEFA levels through medical and lifestyle interventions

Keywords: Non-esterified free fatty acids (NEFAs), type 2 diabetes, insulin resistance, atherosclerosis and cardiovascular disease.

INTRODUCTION

Diabetes mellitus is a long-term metabolic condition that impacts several organs, such as the pancreas, liver, eyes, kidneys, nerves, and blood vessels. A particularly serious complication of diabetes is atherosclerosis, a disease that causes the arteries to harden and narrow, which can result in cardiovascular disease. Individuals with diabetes have a two to four times higher risk of developing cardiovascular disease compared to non-diabetics. Approximately 80% of all diabetes-related fatalities and hospitalizations due to complications are attributed to atherosclerosis. (1)

Non-esterified free fatty acids (NEFAs), also known as free fatty acids (FFAs), circulate in the bloodstream and play vital roles in metabolism. Structurally, NEFAs consist of a hydrophilic carboxylic acid "head" and a hydrophobic hydrocarbon "tail," requiring binding to albumin for transport due to their hydrophobic nature.

NEFAs are major components of triglycerides, which are broken down in adipose tissue by hormone-sensitive lipase, releasing NEFAs and glycerol into the bloodstream. These fatty acids serve as an essential energy source, particularly during fasting or increased energy demand. Insulin regulates NEFA release by suppressing lipolysis, while hormones like glucagon and adrenaline stimulate it. Dietary factors, such as high intake of saturated and trans fats, and physical activity levels also influence NEFA concentrations. Elevated NEFA levels are linked to insulin resistance and inflammation, contributing to metabolic diseases such as Type 2 diabetes and cardiovascular disorders. Understanding the regulation and impact of NEFAs is vital for managing these health issues. (2, 3)

Several genes and their products, combined with environmental factors and lifestyle choices, have been found to play a significant role in the development of diabetes and cardiovascular diseases and their related adverse or deadly outcomes. Among these, Thioredoxin-interacting protein (TXNIP/TBP-2) and the microRNA-34 family have emerged as prominent contributors and essential regulators of diabetes, atherosclerosis, cardiovascular diseases, as well as cancerous and noncancerous cellular proliferation and regeneration.

Thioredoxin-interacting protein (TXNIP), also known as thioredoxin-binding protein-2 (TBP-2), is a key mediator of cellular oxidative stress, glucose, and lipid metabolism and is implicated in various pathological conditions, including dyslipidemia, diabetes, and cardiovascular complications. Elevated TXNIP levels are associated with inflammation, low abundances of glucose transporters on cell membranes, low level of insulin transcription, increased glucagon production, reduced glucagon-like peptide 1 (GLP-1), an incretin hormone, insulin resistance and beta-cell death or dysfunction, changes gut microbiota and trimethylamine N-oxide (TMAO) production, hallmarks of type 2 diabetes.

In 2005, Dutta K K et al. discovered a CpG island within the promoter region of the TXNIP gene. This CpG island, rich in cytosine and guanine dinucleotides, is a common site for epigenetic modifications, particularly DNA methylation. DNA methylation involves the addition of a methyl group to the 5-carbon of cytosine residues in CpG dinucleotides. This epigenetic modification typically suppresses gene expression by interfering with the binding of transcription factors or by recruiting proteins that compact chromatin structure. Aberrant methylation patterns are

associated with various diseases, including diabetes. For the first time, Dutta K K et al. demonstrated that hypermethylation of the CpG island correlates with reduced TXNIP expression in kidney cancers. Conversely, under normal, non-stress conditions, the CpG island tends to be hypomethylated, resulting in higher TXNIP levels. This dynamic regulation is crucial for balancing cellular proliferation in both normal and cancerous kidney tissues. (4, 5, 6)

The methylation status at the cg19693031 site of the TXNIP gene has been found to regulate fasting blood glucose levels in non-diabetic Taiwanese adults. (7) Additionally, the association between TXNIP-cg19693031 DNA methylation (DNAm) and type 2 diabetes (T2D), as well as its correlation with HbA1c, insulin, and fasting glucose levels, has been confirmed. Hypomethylation at TXNIP-cg19693031 is strongly associated with T2D and elevated levels of inflammatory biomarkers, including VCAM-1, ICAM-1, MMP-2, sRAGE, and P-selectin. Notably, the relationship between TXNIP-cg19693031 methylation and T2D is independent of the levels of these inflammatory biomarkers. (8) Another study reported a significant decrease in methylation at all five loci of the TXNIP gene in individuals with T2DM compared to healthy controls. As the methylation levels increased, the risk of developing T2DM significantly decreased. Crucially, interactions among TXNIP methylation, obesity, and hypertriglyceridemia were identified as key factors in the onset of T2DM. (9) At least two recent studies have expanded on this foundational work by exploring the relationship between TXNIP methylation and the risk of developing type 2 diabetes mellitus (T2DM). (10, 11) Two significant studies provide key insights into this connection:

1. **Wu Y et al. (2024):** This nested case-control study investigated the association between TXNIP gene methylation levels and T2DM risk. Participants were classified based on the methylation levels of five loci (CpG1–5) on the TXNIP gene. The study revealed that individuals with high methylation levels at CpG2–5 had a substantial reduction in T2DM risk, with a 61–87% decrease. These findings suggest that maintaining hypermethylation of the TXNIP CpG island in an optimal level may offer a protective effect against T2DM.
2. **Maeda K et al. (2024):** This longitudinal study examined the relationship between TXNIP DNA methylation levels and changes in glycemic traits over four years, focusing on a specific locus (cg19693031) within the TXNIP gene. The study found that individuals with hypomethylation at this locus experienced greater increases in fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) levels compared to those with hypermethylation. These findings indicate that hypomethylation of the TXNIP gene is associated with impaired glucose regulation, potentially serving as an early biomarker for increased diabetes risk.

Circulating non-esterified free fatty acids (NEFAs), also known as free fatty acids (FFAs), are released into the bloodstream when triglycerides are hydrolyzed in adipose tissue. High NEFA levels are commonly observed in individuals with type 2 diabetes and are linked to insulin resistance and chronic inflammation. (12) NEFAs can activate TXNIP, contributing to oxidative stress and inflammatory responses, which exacerbate the metabolic disturbances seen in diabetes. Consequently, targeting TXNIP and managing NEFA levels could be potential therapeutic strategies to mitigate insulin resistance and beta-cell dysfunction in type 2 diabetes. TXNIP has been implicated in the pathogenesis of cardiovascular complications associated with diabetes. It has been shown to promote vascular smooth muscle cell proliferation and migration, endothelial dysfunction, and inflammation,

all of which contribute to the development of atherosclerosis and other cardiovascular complications. (13, 14, 15) Therefore, understanding the role of TXNIP in dyslipidemia, diabetes, and cardiovascular complications may provide new insights into the pathophysiology of these diseases and may lead to the development of new therapeutic strategies. (16)

MicroRNA-34a (miR-34a) is a small, non-coding RNA that plays a significant role in the regulation of gene expression related to various cellular processes, including apoptosis, cell cycle control, and metabolism. In the context of type 2 diabetes, miR-34a has gained attention due to its involvement in pancreatic beta-cell function and insulin resistance. Elevated levels of miR-34a have been associated with beta-cell apoptosis and impaired insulin secretion, contributing to the pathogenesis of type 2 diabetes. (17, 18, 19) In addition, miRNA-34a has been implicated in the pathogenesis of cardiovascular disease, including dyslipidemia, atherosclerosis, and myocardial infarction. Therefore, targeting miRNA-34a may represent a potential therapeutic strategy for preventing and treating both diabetes and cardiovascular disease. (20) Palmitate leads microRNA-34a mediated suppression of sirtuin 1 and induce beta cell (INS-1 cells) death. Glucagon-like peptide-1 (GLP-1) reduces fatty acid – induced beta-cell lipotoxicity in diabetes by the inhibition of microRNA-34a. (21)

In our cross-sectional study, we investigated and compared non-esterified free fatty acids (NEFAs) among newly diagnosed and uncontrolled type-2 diabetes mellitus patients, as well as healthy individuals. The aim was to gain insights that could improve the management and treatment of diabetes. Our findings suggest that addressing NEFAs, alongside treating hyperglycemia and insulin resistance, may provide significant benefits for diabetes mellitus patients. Given that NEFA levels in diabetes patients vary based on disease control status and lifestyle factors such as work hours and exercise habits, diabetes care should be tailored to meet the specific needs of different patient groups.

MATERIALS & METHODS

Study Population

This cross-sectional study received approval from the ethics committee of the Gopalganj Diabetic Hospital, located in Gopalganj, Dhaka, Bangladesh. Blood samples were collected from 134 individuals at this hospital between December 2023 and March 2024. The study participants included:

- 60 newly diagnosed diabetes mellitus patients
- 28 patients with treated but uncontrolled diabetes mellitus
- 11 patients with treated and controlled diabetes mellitus
- 34 non-diabetic individuals (control group) with fasting glycemia below 5.9 mmol/L, not treated with glucose-lowering drugs, and without a history of diabetes, metabolic disease, or metabolic syndrome.

Due to inadequate samples or storage issues, NEFA values for some individuals and lipid profiles for others could not be calculated. The data for all 134 participants were accurately recorded in Excel and SPSS spreadsheets, with missing data noted where applicable.

Collection of Participant Data

Medical personnel collected sociodemographic information using a standardized approach. All participants provided informed consent. Data on age, gender, body mass index (BMI), hypertension, history of cardiovascular disease or stroke, family history of diabetes, work stress, physical activity, smoking habits (yes/no), alcohol consumption (yes/no), history of major health issues (yes/no), and pregnancy frequency were obtained through well-structured questionnaires. BMI was calculated as weight (kg) divided by height squared (m^2) (Centers for Disease Control and Prevention, 2022). Blood pressure (BP) was

measured using automated sphygmomanometers on the upper right arm after 10 minutes of rest in a seated position, with the average of two readings recorded. BP was classified according to the American College of Cardiology (ACC)/American Heart Association (AHA) guidelines. (22)

Blood Sample Collection

Blood samples were collected at Gopalganj Diabetic Hospital. Approximately 2 ml of blood was drawn from each participant's peripheral vein under fasting conditions and two hours after ingesting 75 grams of glucose in 250 ml of water. Blood glucose levels were measured using an automatic blood glucose analyzer GA series (Yokohama, Kanagawa, Japan) in the Biochemistry lab of Gopalganj Diabetic Hospital.

Blood Sample Transportation

Collected blood samples were transported to the lab. of the Biochemistry and Molecular Biology Department at Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, using vacuum blood collection EDTA (anticoagulant) tubes in a cooler box (Winner cooler box, Pran RFL Company, Bangladesh).

Centrifugation

Upon arrival at the lab of the Department of Biochemistry and Molecular Biology at Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, 2 ml of each blood sample was transferred from the vacuum blood collection EDTA tube to a 2 ml round bottom closed microcentrifuge tube (Interlab Limited, New Zealand). Plasma was obtained by centrifuging the samples at 3000 rpm for 10 minutes using a TC-SPINPLUS-8 (Topscien Instrument Company Limited, Italy).

NEFA Estimation

The standard was prepared with palmitic acid from DAEJUNG CHEMICALS & METALS CO. LTD, KOREA. Different concentration of palmitic acid solution made with ethanol with different concentration. 100, 200, 400, 600, 1000 µmol/L were run in an assay. The absorbance was measured at 450 nm by using Bio-TeK Instrument Elisa Reader Machine, Inc 100 Tigan street Highland Park, PO Box 998 Winooski, made in USA. The readings for absorbance were taken within 1min after adding sample and freshly made color reagent was used on the 96well ELISA plate. The results expressed as µmol NEFA/L and corresponding absorbances were plotted for standard curve. The trend equation obtained from standard curve was used the calculation of non-esterified free fatty acids in participants' plasma. Sample preparation involved using 0.7N HCl, a copper reagent mixture, and a color reagent freshly prepared daily. The solvent mixture (chloroform, heptane, and methanol in a 49:49:2 ratio) was designated as CHM. The procedure included mixing plasma/serum with HCl, copper reagent, and CHM, followed by oscillation, centrifugation, and reading the sample in an ELISA reader at 450 nm. The NEFA estimation was conducted following Mondal and Baruah (2015), (23) with approximately 100 µL samples analyzed.

Lipid Profiling

Lipid profile analysis was conducted to measure total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c) using plasma samples. The analysis was performed in the Biochemistry Lab at Sheikh Sayera Khatun Medical College Hospital, Gopalganj, using the Dimension® Xpand® Plus Integrated Chemistry System (Siemens Healthineers, Erlangen, Germany). Around 300 µL of plasma was used for each sample. Dyslipidemia was

defined based on the American Diabetes Association's guidelines (2007).

Preservation

The blood, Plasma and blood cells in the closed microcentrifuge tubes were preserved at -86 °C in a FROILABO 340L Vertical Deep Freezer (Collégien, France) at the lab of the Department of Biochemistry and Molecular Biology at Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj.

Data Processing and Statistical Analysis

Collected data were compiled, examined, and documented. Data entry and processing were conducted using Microsoft Excel 2016 and SPSS version 25.0 (IBM Corporation). The missing data noted where applicable. Categorical variables were analyzed using the Chi-square test, with statistical significance set at $p < 0.05$.

RESULT

A total of 134 participants were included in this study for lipidemic and non-esterified free fatty acids (NEFAs) analysis, of which 100 had previously been diagnosed with type-2 diabetes and 34 served as a non-diabetic control group. The proportion of diabetic patients in the study was 74.63%, with the remaining 25.37% comprising non-diabetic participants. Among the diabetic individuals, 60% were newly diagnosed with type-2 diabetes mellitus, 28% had been previously treated but had uncontrolled diabetes, and 11% had been treated and had controlled diabetes. The study population included 57 (42.54%) male and 77 (57.46%) female participants, representing a male-to-female ratio of 1:1.35. Only one of the patients had gestational diabetic which represented 1% of the participants.

Table 1. Baseline characteristics of Diabetic participants

Characteristics	Frequency(n=100)	Percentage (%)
Age		
15-40	33	33
41-59	54	54
≥ 60	13	13
Marital status		
Married	94	94
single	6	6
Family History of Diabetes		
Parents	28	28
Spouse	15	15
Others	13	13
No one	44	44
Physical exercise		
No	39	39
Yes	61	61
Eye condition		
Severe	28	28
Blur	36	36
Moderate	7	7
Little	17	17
No	12	12
GI problem		
Yes	67	67
Little	18	18
No	15	15
Allergic problem		
Yes	49	49
No	51	51
Teeth problem		
Yes	55	55

Little	11	11
No	34	34
Nerve problem		
Yes	60	60
Little	12	12
No	28	28
Smoking		
Yes	15	15
No	85	85
Alcohol		
Yes	3	3
No	97	97
Appetite		
Less	7	7
Normal	29	29
High	64	64
Anxiety		
No	0	0
Yes	100	100
Chest pain		
Yes	43	53.75
No	37	46.25
Regular checkup		
Yes	34	34
No	66	66

Table 2. Palmitic Acid Concentration (µM/L) and Corresponding Absorbance at 450 nm

Palmitic acid Conc. µM/L	Absorbance at 450nm
0	0
100	0.0234
200	0.0584
400	0.0914
600	0.1484
1000	0.1814
1500	0.4664

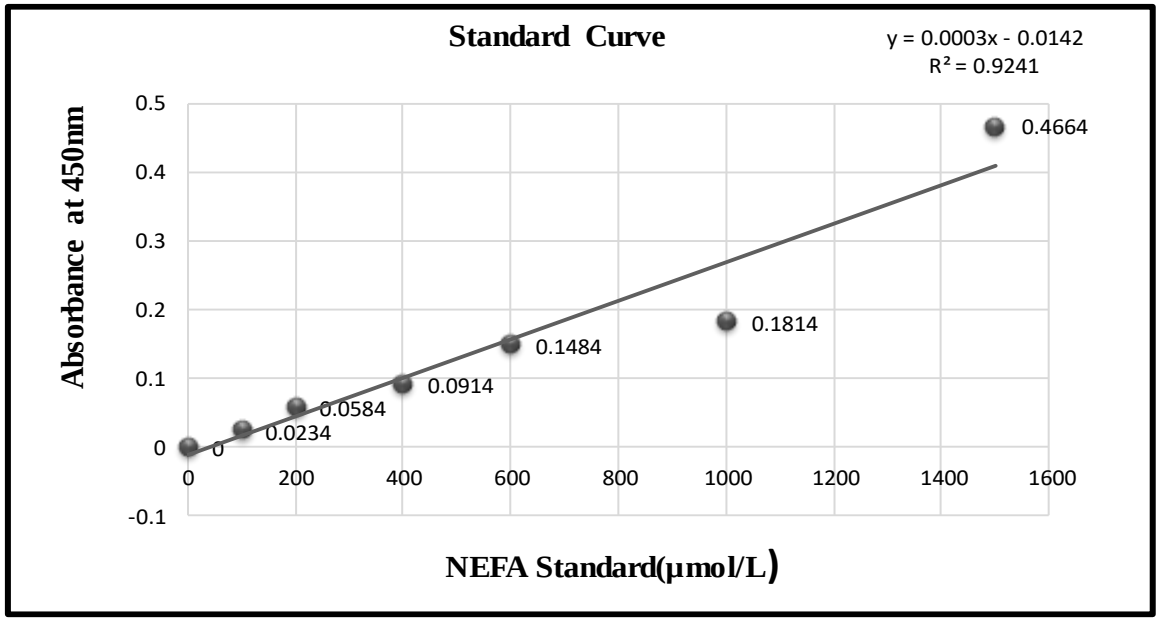

Figure 1. Standard curve with correlation trend for the measurement of NEFAs in the human blood/plasma

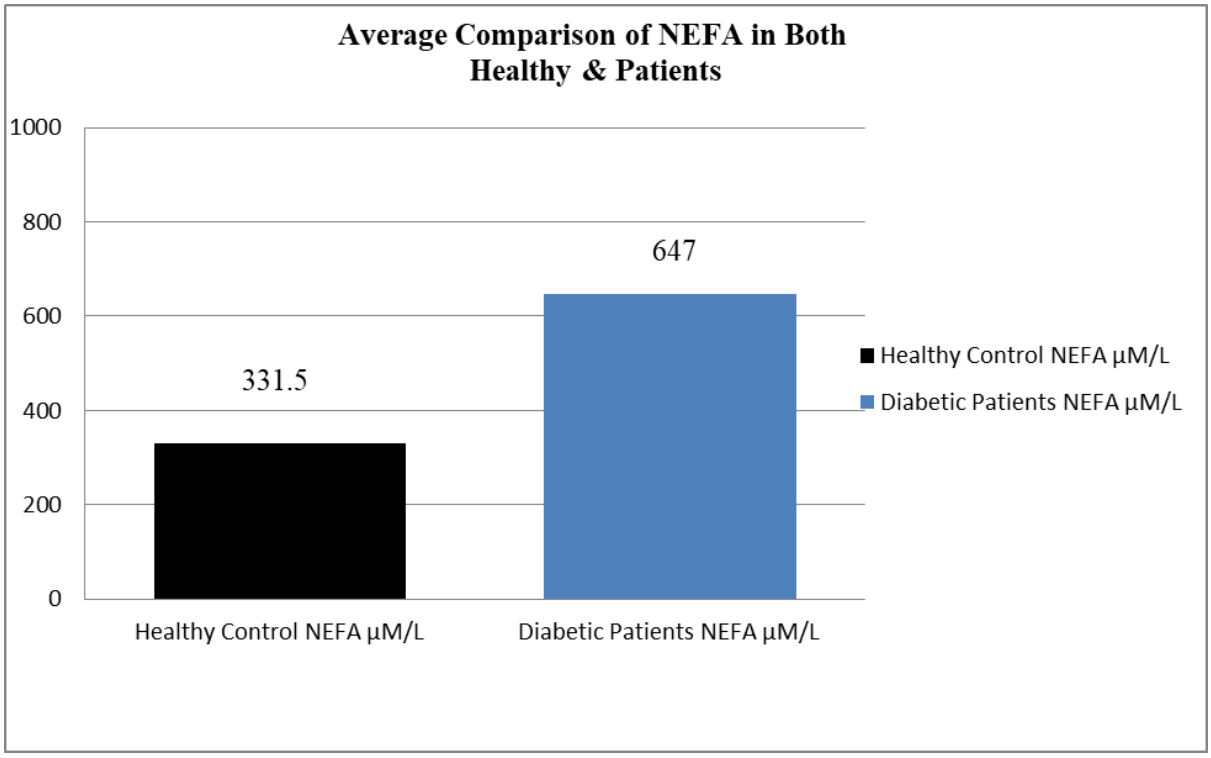

Figure 2. Bar chart for the average values of NEFAs in the plasma of healthy peoples and diabetes patients.

Table 3. Frequency and Percentage Distribution of NEFA Values Among Participants Categorized by Normal, Insulin-Resistant, and Diabetic Conditions

		Frequency	Percent	Valid Percent
Valid	normal	63	47.0	57.3
	Insulin resistant	19	14.2	17.3
	diabetic	28	20.9	25.5
	Total	110	82.1	100.0
Missing	System	24	17.9	
Total		134	100.0	

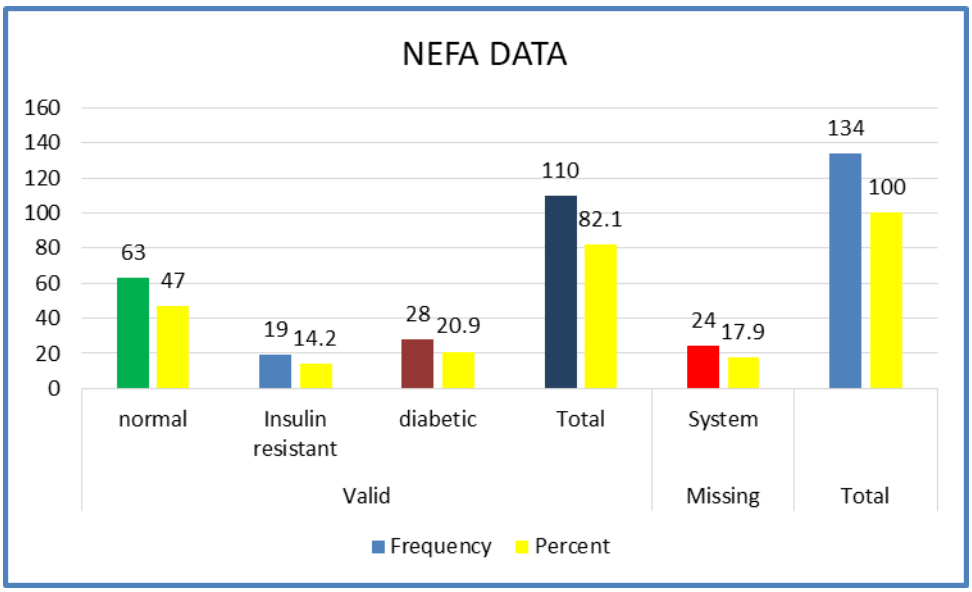

Figure 3. Comparison of NEFA Levels between Diabetic Patients and Healthy Controls

NEFA levels were significantly higher in diabetic patients compared to healthy controls. The average NEFA level in diabetic patients was 647 µM/L (SD = 108), while in healthy controls it was 331.5 µM/L (SD = 75). The difference was statistically significant ($p < 0.01$, t-test). Among the 134 patients, 24 had missing NEFA data due to inadequate samples, leaving 110 patients with available NEFA data. A chi-square test revealed a significant correlation between NEFA levels and the type of diabetic condition ($\chi^2 = 25.64$, $p < 0.01$). In terms of gender:

- Males: 45 patients (24 normal, 9 insulin-resistant, 12 diabetic)
- Females: 65 patients (39 normal, 10 insulin-resistant, 16 diabetic)

There was no significant difference between genders in terms of NEFA levels ($p > 0.05$).
A chi-square test revealed a significant correlation between NEFA levels and the type of diabetic condition ($p < 0.01$). Among the 110 patients:

- Normal NEFA: 8 old diabetic, 28 newly diagnosed, 26 healthy control, 1 gestational diabetic.
- Insulin-resistant: 7 old diabetic, 10 newly diagnosed, 2 healthy control.
- Diabetic NEFA: 10 old diabetic, 16 newly diagnosed, 2 healthy control.

Table 4. Cross-Tabulation of NEFA Levels with BMI Categories

		NEFA Category			Total
		Normal	Insulin-resistance	Diabetic	
Condition	Normal	37	12	16	65
	Overweight	23	5	9	37
	Obese	3	2	3	8
Total		63	19	28	110

Among the patients, 65 had normal BMI. Where 37 with normal NEFA level,12 were in insulin-resistance category & 16 had high NEFA and fitted in diabetic category.
37 of the patients were overweight where 23 had normal NEFA level, 5 had Insulin-resistance & 9 fitted in diabetic category.
In this study 8 patients were obese where 3 of them had normal NEFA, 2 of them were insulin resistance & rest 3 were in diabetic category. The percentage of obese patients having high NEFA was 37.5% which was higher than the other 2 category.
So people who are in obese condition had higher possibility to have high NEFA as well as the risk of having diabetics.

Table 5. Cross-Tabulation of NEFA Levels with Chest Pain Incidence

NEFA Category		Chest-Pain		Total
		yes	no	
	normal	14	49	63
	Insulin resistant	12	7	19
	diabetic	26	2	28
Total		52	58	110

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	41.127[a]	2	.000
Likelihood Ratio	46.004	2	.000
Linear-by-Linear Association	40.561	1	.000
N of Valid Cases	110		
a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.98.			

There was a significant association between high NEFA levels and the presence of chest pain, a marker for cardiovascular disease. Chi-square tests gave p value <0.01 which is

highly significant. Most of the patients with high level of NEFA have chest pain. Chest pain is related to CVD. So NEFA level increase can be a parameter of CVD related problem in diabetes patients.

Table 6. Cross-Tabulation of NEFA Levels with Physical Exercise Frequency

Count				
		Exercise		Total
		yes	no	
NEFA Category	normal	34	29	63
	Insulin-resistance	5	14	19
	diabetic	6	22	28
Total		45	65	110

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	10.514[a]	2	.005
Likelihood Ratio	10.899	2	.004
Linear-by-Linear Association	9.608	1	.002
N of Valid Cases	110		
a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.77.			

The relationship between physical exercise and NEFA levels was significant ($p < 0.05$). Patients with normal NEFA levels were more likely to engage in regular physical exercise compared to those with higher NEFA levels.

The association of NEFA with HDL, LDL, TG, and TC resulted in a p-value greater than 0.05, indicating that the relationship is not statistically significant.

In summary, the study concludes that NEFA levels are higher in diabetic patients compared to healthy controls, with a strong association between high NEFA levels and increased risk of cardiovascular issues. Regular physical exercise was correlated with lower NEFA levels, indicating its potential role in managing NEFA and reducing diabetes-related complications. Gender did not significantly affect NEFA levels, but BMI and physical activity did show significant correlations with NEFA.

DISCUSSION

The present study aimed to investigate the non-esterified free fatty acids (NEFAs) analysis of diabetic and non-diabetic individuals. The results showed that the majority of the study population had type-2 diabetes, with 44.78% of participants newly diagnosed, 20.89% previously treated but uncontrolled, and 8.21% previously treated and controlled. In contrast, only 34 participants (25.37%) served as the non diabetic control group. The high proportion of diabetic patients in the study highlights the importance of understanding the relationship between diabetes and NEFA level. The study population included slightly more females than males, with a ratio of 1:1.35. The majority of participants were over the age of 40, and nearly all reported being married. A high percentage of the diabetic patients had a family history of diabetes.

The key findings of the study are summarized as follows:

1. NEFA Levels in Diabetic vs. Healthy Individuals: The study observed that diabetic patients had significantly higher NEFA levels compared to healthy controls. The average NEFA level in diabetic patients was 647 µM/L, while in healthy controls, it was 331.5 µM/L.
2. Gender Differences in NEFA Levels: The analysis revealed that NEFA levels were slightly higher in females compared to males within the diabetic group. This difference, however, was not statistically significant.

3. Association with Diabetic Complications: The study found a significant association between elevated NEFA levels and the presence of chest pain, which is a common complication in diabetic patients. Those with higher NEFA levels reported more frequent occurrences of chest pain.
4. Impact of Physical Exercise: There was a significant inverse relationship between regular physical exercise and NEFA levels. Patients who engaged in regular physical activity had lower NEFA levels, suggesting a protective effect of exercise against the elevation of NEFAs.
5. Other Factors: No significant correlation was found between NEFA levels and work hours or family history of diabetes. However, NEFA levels were significantly associated with BMI, indicating that higher BMI was correlated with higher NEFA levels.

Some studies conducted with Bangladeshi, Indian, and Pakistani populations have also reported a similar prevalence of dysregulated NEFA levels among individuals with type-2 diabetes. For example, Paul BR et al. (2010) found significantly higher fasting NEFA levels in impaired glucose tolerance (IGT) and T2DM groups compared to controls after adjusting for age, sex, WHR, TG, and fasting insulin. (24) Hossain IA et al. (2018) observed a significant negative association between NEFA levels and insulin sensitivity in prediabetic subjects with NAFLD. (25) Patel JV et al. (2005) and Abate N et al. (2004) found variations in NEFA levels correlated with insulin sensitivity in Asian Indian populations. (26, 27) Shelgikar KM et al. and Wium C et al. (2013) reported higher NEFA levels and insulin resistance in Indian and Pakistani immigrants compared to native populations. (28, 29) Elevated NEFA levels are known to exacerbate insulin resistance by interfering with insulin signaling pathways and impairing glucose uptake in muscle tissues. Bonden & Shulman reported high NEFA levels to insulin resistance and poor glycemic control in T2DM patients. (30) Elevated NEFA levels are known to exacerbate insulin resistance by interfering with insulin signaling pathways and impairing glucose uptake in muscle tissues. (31) This reinforces the role of NEFA as both a marker and a contributor to the pathophysiology of T2DM.

Our study also highlighted a significant correlation between NEFA levels and BMI. Obese patients demonstrated a higher prevalence of elevated NEFA levels compared to those with normal or overweight BMI categories. This is consistent with evidence suggesting that adipose tissue in obese individuals releases more free fatty acids, contributing to higher circulating NEFA levels and increased metabolic risk. (32) This finding emphasizes the need for weight management strategies in diabetic patients to mitigate elevated NEFA levels and associated risks.

Interestingly, no significant gender differences were observed in NEFA levels, suggesting that the metabolic impact of elevated NEFA is similar across genders in the studied population. This aligns with some studies indicating that gender differences in NEFA levels are minimal when controlling for other factors like BMI and physical activity. (33)

The strong association between high NEFA levels and the presence of chest pain, a surrogate marker for cardiovascular disease (CVD), is noteworthy. Elevated NEFA levels have been implicated in the development of atherosclerosis and other cardiovascular conditions due to their role in promoting inflammation, endothelial dysfunction, and lipid deposition. (34) The significant correlation found in our study ($p < 0.01$) which underscores the importance of monitoring NEFA levels as part of cardiovascular risk assessment in diabetic patients.

Our findings also indicate a significant relationship between physical exercise and lower NEFA levels. Patients engaging in

regular physical activity had lower NEFA levels, which is consistent with the role of exercise in improving lipid metabolism and enhancing insulin sensitivity. (35) This supports the recommendation of incorporating regular physical activity into the management plans for diabetic patients to help control NEFA levels and improve overall metabolic health.

Overall, these studies highlighted the high prevalence of elevated NEFA levels among individuals with diabetes in South Asia and underscore the need for effective strategies to keep NEFA level normal and prevent its associated complications.

Several genes and their corresponding products are critically linked with diabetes and cardiovascular diseases and with their detrimental or fatal consequences. Based on the very current understanding, TXNIP (TBP-2) and MicroRNA-34a are the two major players among them. The following discussions may account a significant part of our findings of elevated NEFA level in type-2 diabetes mellitus and related complications specially microvascular and macrovascular complications including the diabetic cardiovascular pathologies.

Several specific and non-specific inhibitors of TXNIP, including SRI-37330 (substituted quinazoline sulfonamide), verapamil (U.S. FDA approved antihypertensive drug), W2476 [9- ((1-(4-acetyl-phenyloxy)-ethyl)-2-)adenine], and quinazolin-4(3H)-one derivatives have shown potential in improving diabetes. SRI-37330 is an orally bioavailable, non-toxic small molecule that has effectively rescued mice from streptozotocin- and obesity-induced diabetes. (36) Verapamil has also shown promising results in improving lipid metabolism, with a significant decrease in total cholesterol, triglycerides, and LDL cholesterol observed in type 2 diabetes patients after 12 weeks of treatment. (37) Similarly W2476 has been shown to have a significant effect on lipid metabolism and glycemic control, with the potential to reduce the risk of cardiovascular disease in patients with diabetes. (38) Quinazolin-4(3H)-one derivatives have also been evaluated for their inhibitory effects on TXNIP and have shown potential as α-glucosidase inhibitors, which could improve the metabolic status of diabetic patients by modulating lipid metabolism. (39, 40)

Preclinical studies have investigated the use of various miRNA-34a inhibitors, such as antagomirs, locked nucleic acids (LNAs), and small molecule inhibitors, to target miRNA-34a in CVDs, with promising results. (41, 42) However, further studies are needed to investigate the safety and efficacy of miRNA-34a inhibition in human clinical trials.

Thioredoxin-interacting protein (TXNIP), also known as thioredoxin-binding protein-2 (TBP-2), is a key mediator of cellular oxidative stress and is implicated in various pathological conditions, including dyslipidemia, diabetes, and cardiovascular complications. (43, 44) TXNIP has been shown to play a critical role in glucose and lipid metabolism, and its dysregulation has been linked to abnormal NEFA homeostasis.

Studies have shown that the knockdown of TXNIP can significantly reduce the levels of non-esterified free fatty acids (NEFAs) in the bloodstream. This reduction is believed to be due to TXNIP's influence on glucose and lipid metabolism (45, 46). TXNIP knockdown in adipocytes has been observed to improve insulin sensitivity, which in turn enhances glucose uptake and reduces lipolysis—the process through which triglycerides are broken down into NEFAs and glycerol. This effect has been demonstrated in various experimental models, where the inhibition of TXNIP leads to a decrease in the release of NEFAs from adipose tissue. (47)

Moreover, TXNIP is known to be a negative regulator of glucose uptake by interacting with glucose transporter type 1 (GLUT1) and impairing its function. By reducing TXNIP expression, the activity of GLUT1 is enhanced, promoting better glucose utilization and reducing the substrate availability for NEFA production. (48)

Thioredoxin-interacting protein (TXNIP) significantly influences fatty acid uptake by cells. This regulatory role is critical in understanding how TXNIP contributes to metabolic homeostasis and the pathophysiology of metabolic disorders.

TXNIP is known to modulate the expression and activity of various transporters and enzymes involved in lipid metabolism. Specifically, TXNIP has been shown to affect the uptake of fatty acids by influencing the expression of CD36, a major fatty acid translocase. CD36 facilitates the transport of long-chain fatty acids into cells, and its expression is upregulated in conditions of increased TXNIP activity. Studies have indicated that TXNIP promotes the transcription of CD36, thereby enhancing fatty acid uptake and contributing to lipid accumulation in cells. (49)

Moreover, TXNIP's role in oxidative stress and inflammation further exacerbates its impact on fatty acid uptake. By inducing oxidative stress, TXNIP can alter cellular signaling pathways that regulate lipid metabolism, leading to increased fatty acid uptake and storage. This process is particularly detrimental in the context of insulin resistance and type 2 diabetes, where excess fatty acid uptake contributes to ectopic lipid deposition and metabolic dysfunction. (47, 48) Reducing TXNIP expression or activity could help modulate fatty acid uptake, thereby preventing lipid overload and improving metabolic health. (46)

Thioredoxin-interacting protein (TXNIP) has been implicated in inducing incomplete fatty acid oxidation in mitochondria and contributing to insulin resistance. This connection is particularly evident in the context of metabolic disorders where mitochondrial dysfunction and impaired fatty acid metabolism play crucial roles.

Incomplete fatty acid oxidation in the mitochondria results in the accumulation of fatty acid intermediates, which can disrupt cellular functions and contribute to metabolic stress. TXNIP has been shown to interfere with the normal process of fatty acid oxidation by affecting key regulatory enzymes and mitochondrial function. Studies have demonstrated that TXNIP overexpression leads to impaired fatty acid oxidation, resulting in the buildup of lipid intermediates and subsequent metabolic complications. (49)

In addition to its role in fatty acid metabolism, TXNIP is also a significant player in the development of insulin resistance. By promoting oxidative stress and inflammation, TXNIP disrupts insulin signaling pathways, leading to decreased insulin sensitivity in peripheral tissues. This impairment in insulin action is a hallmark of type 2 diabetes and other related metabolic disorders. (47, 48)

Therefore, targeting TXNIP not only aids in controlling oxidative stress but also plays a pivotal role in managing lipid metabolism, thereby reducing the levels of NEFAs, improving mitochondrial fatty acid metabolism, alleviating mitochondrial dysfunction and finally enhancing insulin sensitivity. (46) This mechanism underscores the therapeutic potential of TXNIP inhibition in metabolic disorders such as diabetes and obesity, where elevated NEFA levels are a common pathological feature. (45, 46, 47, 48)

MicroRNA-34a (miR-34a) plays a significant role in the regulation of fatty acid uptake and metabolism in cells. As a part of the microRNA family, miR-34a exerts its effects by post-transcriptionally regulating gene expression, impacting various metabolic pathways.

MiR-34a has been shown to influence the expression of genes involved in lipid metabolism, including those that regulate fatty acid uptake. One key target of miR-34a is the gene encoding for the fatty acid translocase CD36. CD36 is a critical transporter that facilitates the uptake of long-chain fatty acids into cells. Studies have demonstrated that miR-34a downregulates CD36 expression, thereby reducing fatty acid uptake. For example, a study by Fu et al. (2012) (50) revealed that overexpression of miR-34a in hepatocytes

and adipocytes led to decreased CD36 levels and subsequently reduced fatty acid uptake.

In addition to its effects on fatty acid uptake, miR-34a also impacts broader aspects of lipid metabolism. MiR-34a has been found to target other metabolic genes, including those involved in fatty acid synthesis and oxidation. For instance, miR-34a represses the expression of SIRT1, a key regulator of lipid metabolism and mitochondrial function. The inhibition of SIRT1 by miR-34a leads to altered fatty acid oxidation and storage, contributing to metabolic imbalances and conditions such as obesity and type 2 diabetes. (51)

Furthermore, miR-34a has been linked to the regulation of hepatic lipid metabolism. A study by Lee et al. (2010) (52) showed that miR-34a expression is elevated in the liver of obese and diabetic mice, leading to increased lipid accumulation and hepatic steatosis. This study highlighted the role of miR-34a in modulating genes involved in lipid synthesis and breakdown, underscoring its contribution to metabolic disorders.

The regulatory effects of miR-34a on fatty acid uptake and metabolism underscore its potential as a therapeutic target for metabolic diseases. Modulating miR-34a levels could help restore normal lipid metabolism and improve metabolic health.

However, there were some limitations in our study which should be taken into consideration. M Mondal and K K Baruah (23) used light of 440nm wave length to quantitate the final colored product in NEFA estimation. But we used the light of 450nm wave length which may have some effect on the obtained NEFA concentration in our experiments and may generate dissimilarities to some extent between the scenarios reported by other groups and ours. We only included 134 participants, which may not be representative of the entire population of patients with type-2 diabetes mellitus in this district of Bangladesh. This may limit the generalizability of the study's findings to other populations. The dietary habits of the participants were not considered in the study. The study used a cross-sectional design, which can only establish a correlation between elevated NEFA level in the fasting plasma and diabetes but cannot determine causality or the temporal sequence of events. Additionally, the sample size, particularly in subgroups like gestational diabetes, was small. Longitudinal studies are needed to better understand the relationship between diabetes and dysregulated NEFA homeostasis over time.

CONCLUSION

In conclusion, elevated NEFA levels are prevalent among Bangladeshi patients with T2DM and are associated with increased BMI, physical inactivity, and higher cardiovascular risk. These findings highlight the importance of NEFA as a biomarker for metabolic health and the potential benefits of weight management and physical activity in mitigating elevated NEFA levels. Integrating NEFA level monitoring into routine clinical practice could enhance the management of T2DM and associated complications.

Declaration by Authors

Esita Halder conducted this research work (thesis) as a partial requirement for the completion of her M.Sc. degree in Biochemistry and Molecular Biology from Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, under the guidance and supervision of Dr. Khokon Kumar Dutta from the same department. The project also received significant contributions from all other co-authors, including project design, facilities, data analysis, and guidance. The authors' guidelines, experimental design, and laboratory data were used to generate the manuscript with the assistance of ChatGPT, an artificial intelligence program developed by OpenAI.

Ethical Approval: Approved by the Gopalganj Diabetic Hospital, Gopalganj, Dhaka, Bangladesh.

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REFERENCES

1. American Diabetes Association. (2019). Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes-2019. Diabetes care, 42(Supplement 1), S103-S123.
2. Sobczak, A. I. S., Blindauer, C. A., & Stewart, A. J. Changes in plasma free fatty acids associated with type-2 diabetes. *Nutrients*. 2019; 11(9):2022.
3. Itoh, Y., Kawamata, Y., Harada, M. et al. Free fatty acids regulate insulin secretion from pancreatic β cells through GPR40. *Nature*. 2003; 422(6928):173-176.
4. Dutta, K. K., Nishinaka, Y., Masutani, H. et al. Two distinct mechanisms for loss of thioredoxin-binding protein-2 in oxidative stress-induced renal carcinogenesis. Lab Invest; 2005 Jun: 85(6):798-807.
5. Kim, M. J., Lee, H. J., Choi, M. Y. et al. UHRF1 Induces Methylation of the *TXNIP* Promoter and Down-Regulates Gene Expression in Cervical Cancer. Mol Cells; 2021 Mar 31: 44(3):146-159.
6. Zhang, P., Gao, J., Wang, X. et al. A novel indication of thioredoxin-interacting protein as a tumor suppressor gene in malignant glioma. Oncol Lett; 2017 Aug: 14(2):2053-2058.
7. Tsai, H. H., Shen, C. Y., Ho, C. C. et al. Interaction between a diabetes-related methylation site (TXNIP cg19693031) and variant (GLUT1 rs841853) on fasting blood glucose levels among non-diabetics. J Transl Med. 2022 Feb 14; 20(1):87.
8. Xiang, Y., Wang, Z., Hui, Q. et al. DNA Methylation of TXNIP Independently Associated with Inflammation and Diabetes Mellitus in Twins. Twin Res Hum Genet. 2021 Oct; 24(5):273-280.
9. Zhang, D., Cheng, C., Cao, M. et al. TXNIP hypomethylation and its interaction with obesity and hypertriglyceridemia increase type 2 diabetes mellitus risk: A nested case-control study. J Diabetes. 2020 Jul; 12(7):512-520.
10. Wu, Y., Chen, W., Zhao, Y. *et al.* Visit to visit transition in *TXNIP* gene methylation and the risk of type 2 diabetes mellitus: a nested case-control study. *J Hum Genet.* 2024 Jul; 69(7):311-319.
11. Maeda, K, Fujii, R., Yamada, H. *et al.* Association between DNA methylation levels of thioredoxin-interacting protein (TXNIP) and changes in glycemic traits: a longitudinal population-based study. *Endocrine Journal*. 2024 Jun 18; 71 (6): 593-601.
12. Alhawiti, N. M., Al Mahri, S., Aziz, M. A. et al. TXNIP in Metabolic Regulation: Physiological Role and Therapeutic Outlook. *Current Drug Targets*. 2017; 18(9):1095-1103.
13. Boden, G. Obesity, insulin resistance and free fatty acids. *Current Opinion in Endocrinology, Diabetes and Obesity*. 2008; 15(2):119-125.
14. Shah, M. S., Brownlee, M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. Circ Res. 2016; 118(11):1808-1829.
15. Wang, Y., De Keulenaer, G. W., Lee, R. T. Vitamin D(3)-up-regulated protein-1 is a stress-responsive gene that regulates cardiomyocyte viability through interaction with Bcl-2 and Bcl-xl. *Journal of Biological Chemistry*. 2002; *277*(30):26496-26500.
16. Shao, M., Zhou, H., Wu, Y., et al. Thioredoxin-interacting protein (TXNIP) in metabolic and cardiovascular diseases. J Diabetes. 2020; 12(3):186-197.
17. Wei, R., Yang, J., Liu, G. Q., et al. Dynamic expression of microRNAs during the differentiation of human embryonic stem cells into insulin-producing cells. Gene. 2013; 518(2):246-55.

18. Chakraborty, C., Doss, C. G., Bandyopadhyay, S., et al.. Influence of miRNA in insulin signaling pathway and insulin resistance: micro-molecules with a major role in type-2 diabetes. Wiley Interdiscip Rev RNA. 2014; 5(5):697-712.
19. García Jacobo, R., Uresti-Rivera, E., Portales-Pérez, D., et al. Circulating miR-146a, miR-34a and miR-375 in type 2 diabetes patients, pre-diabetic and normal glycaemic individuals in relation to β-cell function, insulin resistance and metabolic parameters. Clin Exp Pharmacol. 2019; 46(12):1092-1100.
20. Hua, C. C., Liu, X. M., Liang, L. R. et al. Targeting the microRNA-34a as a Novel Therapeutic Strategy for Cardiovascular Diseases. Front Cardiovasc Med. 2022; 8:784044.
21. Han, Y. B., Wang, M. N., Li, Q. et al. MicroRNA-34a contributes to the protective effects of glucagon-like peptide-1 against lipotoxicity in INS-1 cells. Chin Med J (Engl). 2012 Dec; 125(23):4202-8.
22. Whelton, P. K., Carey, R. M., Aronow, W. S. et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension*. 2018; 71(6):e13-e115.
23. Mondal, M., & Baruah, K. K. Development of A Rapid Microtiterplate Based Colorimetric Method for Estimation of Non-esterified Fatty Acids in Bovine Plasma. 2015; 4(3):29–34.
24. Paul, B. R., Akter, S., Hossain, I. A., et al. A study on the association of circulating nonesterified fatty acid (NEFA) with impaired glucose tolerance and insulin resistance in a Bangladeshi urban population. 17th Diabetes & Endocrine Conference (at Dhaka, Bangladesh). 2010.
25. Hossain, I. A., Shah, M. R., Ali, L. Association of Serum Nonesterified Fatty Acid and Insulin Resistance with Nonalcoholic Fatty Liver Disease-A Study from Bangladeshi Prediabetic Subjects. J Diabetes Metab. 2018; 9:9
26. Patel, J. V., Vyas, A., Prabhakaran, D. Nonesterified fatty acids as mediators of glucose intolerance in Indian Asian populations. Diabetes Care. 2005; 28(6):1505-7.
27. Abate, N., Chandalia, M., Snell, P. G. et al. Adipose tissue metabolites and insulin resistance in nondiabetic Asian Indian men. J Clin Endocrinol Metab. 2004; 89(6):2750-5.
28. Shelgikar, K. M., Naik, S. S., Khopkar, M. et al. Circulating lipids and cardiovascular risk in newly diagnosed non-insulin-dependent diabetic subjects in India. Diabet Med. 1997; 14(9):757-61.
29. Wium, C., Aasheim, E. T., Ueland, T. et al. Differences in insulin sensitivity, lipid metabolism and inflammation between young adult Pakistani and Norwegian patients with type 2 diabetes: a cross sectional study. BMC Endocr Disord. 2013; 13:49.
30. Boden, G., & Shulman, G. I. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *European Journal of Clinical Investigation*. 2002; 32 Suppl 3:14-23.
31. Roden, M. How free fatty acids inhibit glucose utilization in human skeletal muscle. *News in Physiological Sciences*. 2004; 19:92-96.
32. Boden, G. Obesity and free fatty acids. *Endocrinology and Metabolism Clinics of North America*. 2008; 37(3):635-646.
33. Mittendorfer, B. Insulin resistance: sex matters. *Current Opinion in Clinical Nutrition & Metabolic Care*. 2005; 8(4):367-372.
34. Stevanovic, S., Thiebaud, D., Bilezikian, J. P., et al. Effects of free fatty acids on glucose transport and phosphorylation in human skeletal muscle. *Journal of Clinical Investigation*. 1987; 80(5):1309-1318.
35. Perseghin, G., Price, T. B., Petersen, K. F., et al. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *New England Journal of Medicine*. 1996; 335(18):1357-1362.
36. Thielen, L. A., Chen, J., Jing, G., et al. Identification of an anti-diabetic, orally available small molecule that regulates TXNIP expression and glucagon action. Cell Metabolism. 2020; 32(3):353-365.
37. Carnovale C, Dassano A, Mosini G, et al. The β-cell effect of verapamil-based treatment in patients with type 2 diabetes: a

systematic review. Acta Diabetologica. 2020; 57(1):117-131.
38. Gonzalez-Monjal E, Fernandez-Rodriguez E, Perez-Pevida B, et al. W2476, a new selective PPARα modulator, improves lipid metabolism and reduces inflammation in type 2 diabetic rats. Scientific Reports. 2020; 10(1):11675.
39. Moheb SM, Ghanadian M, Sharifzadeh M, et al. Synthesis and biological evaluation of quinazolin-4(3H)-one derivatives as potential inhibitors of thioredoxin-interacting protein and α-glucosidase for the treatment of type 2 diabetes. Eur J Med Chem. 2022; 226:114716.
40. Li X, Yu Y, Li W, et al. Discovery of novel quinazolin-4(3H)-one derivatives as potent thioredoxin-interacting protein inhibitors for the treatment of type 2 diabetes. Bioorg Chem. 2023; 118:105173.
41. He, X., Liang, X., Zhu, Y et al. Advances in research on miRNA-34a and its application prospects in cardiovascular diseases. Life Sciences. 2020; 246:117393.
42. Boon, R. A., Iekushi, K., Lechner, S. et al. MicroRNA-34a regulates cardiac ageing and function. Nature. 2013; 495(7439):107-110.
43. Shao W, Yu Z, Chiang Y, et al. TXNIP regulates peripheral glucose metabolism in humans. PLoS Med. 2013; 10(6):e1001562.
44. Zhou Y, Wang Y, Wang J, et al. Thioredoxin-interacting protein (TXNIP) in cancer and diabetes mellitus. J Cell Physiol. 2018; 233(12):9169-9180
45. Chen, J., Hui, S. T., Couto, F. M. et al. Knockdown of TXNIP alleviates insulin resistance and reduces inflammation in high-fat diet-fed mice. *Diabetes, Obesity & Metabolism.* 2016; *18*(3):313-320.
46. Wang, Y., Lin, Y., Goto, H. et al. TXNIP deficiency ameliorates obesity-associated metabolic dysfunction. *Journal of Clinical Investigation.* 2013; *123*(1):256-265.
47. Minn, A. H., Hafele, C., & Shalev, A. Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. *Endocrinology.* 2005; *146*(5):2397-2405.
48. Parikh, H., Carlsson, E., Chutkow, W. A. et al. TXNIP regulates peripheral glucose metabolism in humans. *PLoS Medicine.* 2007; *4*(5):e158.
49. Shen, N., Huan, Y., Shen, Z. et al. TXNIP knockdown ameliorates mitochondrial dysfunction and insulin resistance by reducing fatty acid oxidation. *Cell Metabolism.* 2018; *28*(5):765-776.e4.
50. Fu, X., Dong, B., Tian, Y. MicroRNA-34a regulates hepatic lipid metabolism by targeting the fatty acid translocase CD36. *American Journal of Physiology-Endocrinology and Metabolism.* 2012; *303*(8):E843-E852.
51. Choi, S. E., Fu, T., Seok, S. et al. MicroRNA-34a inhibits hepatic autophagy by downregulating SIRT1 and inducing lipid accumulation. *Molecular and Cellular Biology.*2013; *33*(11):2535-2545.
52. Lee, J., Padhye, A., Sharma, A. et al. MicroRNA-34a repression in the liver protects against obesity-induced hepatic steatosis and inflammation. *Journal of Biological Chemistry.*2010; *285*(56):41369-41378.

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