International Journal of Medical and Applied Health Science (IJMAHS)

Contents lists available at IJMAHS
International Journal of Medical and Applied Health Science
journal homepage: IJMAHS
Volume 1, No. 2, 2025

Single Nucleotide Polymorphisms Detection of INS gene in Patients with Type 2 Diabetes Mellitus and their Association to some Clinical Features

Mahdi Saber Al-Deresawi [a]

[a] *Department of Biology, College of Science, Wasit University, Iraq.*

ARTICLE INFO

Received: 2025/08/20

Revised: 2025/09/15

Accept: 2025/11/19

Keywords:

T2DM, BMI, HbA1c, INS gene, SNPs.

ABSTRACT

About 90–95% of all occurrences of diabetes are type 2 diabetes mellitus (T2DM), making it the most prevalent kind of the disease. Independent of body mass index, T2DM is a major predictor of incident hypertension. The objective of this study was to detection of some single nucleotide polymorphism in INS gene and their effect in other factors. This study includes (100) patients T2DM collected from Al-Zahraa Teaching Hospital in Wasit, Iraq and individuals used as control group. The results recorded a significant increase in HbA1C levels (8.72±1.22) compared to control (4.44±0.51) also significant increase in male patients T2DM with Hypertension compared to female patients T2DM with Hypertension (19%) and (8%) receptively. According to BMI the results showed the Obese: female (12%) and male (15%), Overweight female (23%) and male (12), Normal weight: female (15%) and male (14%) depending on the Statical analysis there is non-significant between them. The results of matching the sequence showed the T2DM with Hypertension two SNPs in upstream of INS gene 12% (-106C>T), 11% (-106C>T), and two in coding region 8% (244G>T) and 8% silent mutation (353C>T). Obese T2DM two SNPs in upstream of INS gene: 7% (-106C>T) and 7% (-234A>G). Overweight T2DM SNPs in upstream 8% (-106C>T) and 8% (-234A>G). Three SNPs in Normal weight with T2DM: 13%(-106C>T) and 12%(-106C>T) in upstream of INS gene and silent mutation 8% 353C>T in coding region. In conclusion this study reported the SNPs in promoter and coding region of INS gene effecting in its function in T2DM patients and influence in displayed higher BMI value and insulin levels and confirm the link between T2DM and hypertension.

1. Introduction:

About 10% of people worldwide suffer from T2DM, a metabolic disease marked by insulin resistance and increasing pancreatic β-cell dysfunction that eventually results in insulin

[a] Corresponding author email address: malderesawi@uowasit.edu.iq (Mahdi Saber Al-Deresawi).
DOI: https://doi.org/10.22034/ijmahs.v1i2.186
Available online 11/20/2025

3115-7378/BGSA Ltd.

insufficiency (Cerf, 2013). Numerous genetic and metabolic variables contribute to the diverse etiology of type 2 diabetes (Kahn et al., 2014). Approximately 90% of all instances of diabetes are T2DM. A decreased responsiveness to insulin in individuals with T2DM is known as insulin resistance. Because insulin is inefficient in this state, glucose homeostasis is maintained by increasing insulin synthesis. However, insulin production decreases, leading to type 2 diabetes. According to Picke et al. (2019), T2DM is the most prevalent kind of diabetes. Age, obesity, inactivity, and a family history of diabetes are the main risk factors for T2DM (You and Henneberg, 2016). The risk of developing T2DM is 2-4 times higher in people over 45 (Alva et al., 2017). Over 70% of T2DM patients are overweight or obese, indicating a strong correlation between obesity and a higher prevalence of T2DM (Jihua and Riobo, 2019). The chance of having T2DM is increased by 2-4 times if one parent or sibling has the disease (Trikkalinou et al., 2017). Diabetes and hypertension are both part of the metabolic syndrome, and they coexist and affect each other's development.Fifty to eighty percent of patients with type 2 diabetes have consistently high blood pressure (Cheung and Le, 2012). According to one epidemiological investigation, incident hypertension was significantly predicted by type 2 diabetes at baseline, regardless of BMI, sex, age, or T2DM (Tsimihodimos et al., 2018). The objective of this study was to detection some SNPs in promoter and coding region of INS gene and trying to find out whether these SNPs have an effect on some clinical features of T2DM patients.

2. Materials and methods:

2.1. Subjects and Samples: The present study was performed during the period between from July 2024 to September 2024. Blood samples of (100) T2DM patients with different age were collected from Al-Zahraa Teaching Hospital in Wasit/Iraq. (50) individuals apparently healthy as control group. 3 ml of blood was divided into two parts: 1 ml for HbA1C value and 2 ml in EDTA tube for molecular analysis.

2.2.DNA Extraction: The Wizard genomic DNA purification kit (Promega, USA) was used to separate the genomic DNA from peripheral blood. The kit's instructions were followed in order to extract the DNA.

2.3. Estimation of DNA Concentration: To assess the quality of the samples, the concentration and purity of extracted DNA were measured using the NanoVue Nanodrop spectrophotometer (England). To ascertain the purity of the DNA, the absorbance of the samples was measured at two

different wavelengths (260 and 280 nm). The A260/A280 ratio of approximately 2.0 indicated the purity of the DNA sample.

2.4. Polymerase Chain Reaction: Primers were designed depends on nucleotide sequence of INS gene had been done by using (Primer 3 programme). Bioneer Company/Korea provided the primers as a lyophilized product. Lyophilized primer was dissolved in a DNase/RNase free water to give a final concentration of (100 pmol/µl) as stock solution. The sequences of this primers were showed in table (1): Using a 50µl reaction mixture including 30 µl of Go Taq® Green Master Mix (Promega/USA), 2 µl of F-primer, 2 µl of R-primer, 4 µl of DNA sample, and 12 µl D.W., the polymerase chain reaction (PCR) was conducted in an Applied Biosystem Veriti™ heat cycle. A 7-minute initial denaturation at 95°C was followed by 35 cycles of 1 minute denaturation at 95°C, 1 minute annealing at 60°C, 1 minute extension at 72°C, and 7 minutes final extension at 72°C. This was the usual cycle approach.

Table 1: Primer sequence and its properties.

Sequence (5'->3')	Length	Tm °C	CG%	Product
F- GAAGAGGCCATCAAGCAGATCACT	24	62.73	50.00	319 bp
R- ATTGTTCCACAATGCCACGCT	21	61.43	47.62	

2.5. Gel Electrophoresis: Electrophoresis was used to confirm the target size. PCR products were separated on 2.5% agarose gel electrophoresis and observed by exposure to ultraviolet light (302 nm) after staining with Ethidium Bromide.

2.6. Gene Sequencing: The amplified PCR fragments were subjected to Sanger sequencing using an ABI3730XL automated DNA sequences, and 20 µl of PCR products and primers were sent to Macrogen Corporation in Korea for analysis. The queries were aligned to reference sequences in the Gene Bank using the Basic Local Alignment Search Tool (BLAST): https://blast.ncbi.nlm.nih.gov/

2.7. Sequences Analysis: NCBI's Basic Local Alignment Search Tool Bio ID tool is used to analyze nucleotide databases in order to identify samples that are then uploaded to GenBank (ID). The NCBI nucleotide database (www.ncbi.nlm.gov/nucleotide) provided the sample sequences, which were then used in a multiple alignment with the BLAST.

2.8. Statistical Analysis: The distribution of the sample research based on different factors was found using the Statistical Packages of Social Sciences (SPSS) (2019) application. In this study, a

significant comparison between percentages (0.05 and 0.01 probability) was made using the chi-square test.

3. Results and Discussion:

3.1 Distribution of Patients according to different factors:

The different factors were included in this experiment, the results showed a significant increase in HbA1C levels (8.72±1.22) compared to control (4.44±0.51) also significant increase in male patients T2DM with Hypertension compared to female patients T2DM with Hypertension (19%) and (8%) receptively. According to BMI the results showed in table (2).

Table 2: Distribution of Patients study according to difference factors

Factors		No.	Percentage 100%	P- value
Gender	Female	50	50%	1.00 NS
	Male	50	50%	
HbA1C	Patients	100	8.72 ± 1.22	0.001**
	Control	50	4.44 ± 0.51	
Hypertension	Female	8	8%	0.0343 *
	Male	19	19%	
Obese	Female	12	12%	0.5637 NS
	Male	15	15%	
Overweight	Female	23	23%	0.7630 NS
	Male	21	12%	
Normal weigh	Female	15	15%	0.8527 NS
	Male	14	14%	

** (P≤0.05), NS: Non-Significant.

The results showed the association of T2DM with hypertension and agreed with (Petrie et al.,2018). Risk factors such as endothelial dysfunction (Meigs et al., 2004), vascular inflammation, arterial remodeling, atherosclerosis, dyslipidemia, and obesity are comparable and closely related to those of hypertension and T2DM. In terms of cardiovascular problems, these risk factors also significantly overlap (Petrie et al., 2018). In the past, HbA1c was regarded as the gold standard test for identifying diabetic patients and assessing blood glucose management. A laboratory test called HbA1c measures the amount of normal blood glucose levels during the preceding three months in patients with diabetes. These three months assess the total HbA1c value, taking into

account any changes in HbA1c over the previous six weeks as a result of dietary changes or glucose control during the time (Evans et al., 2020). According to one epidemiological investigation, incident hypertension was significantly predicted by T2DM at baseline, regardless of sex, age, body mass index, or T2DM (Tsimihodimos et al., 2018). With more than 70% of T2DM patients being overweight or obese, obesity is strongly associated with a higher prevalence of T2DM (Riobo, 2013). Increased BMI has been shown in multiple studies to be a risk factor for inadequate glycemic control in patients T2DM (Haber et al., 2024). Obesity exacerbates insulin resistance, leading to elevated blood glucose levels and higher HbA1c (Boutari et al.,2023). Additional research has supported our findings, demonstrating that the detrimental metabolic effects of extra adipose tissue, especially visceral fat, are linked to higher HbA1c and increased BMI (Emont et al., 2022). In several cohorts, Kodama et al. (2014) found a strong correlation between increased HbA1c levels and elevated BMI, indicating that obesity worsens insulin resistance and, as a result, impairs glycemic management.

3.2. Molecular Detection:

To determine the SNPs there are steps should be carrying out:

3.2.1. DNA Extraction:

Within 24 to 72 hours of aspiration, blood samples were obtained for the DNA isolation process; figure (1) illustrates how the amount of genomic DNA and its purity were determined to affect the band integrity and DNA concentration.

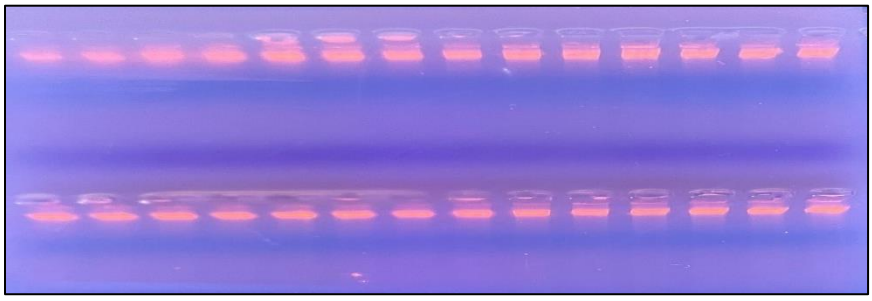


Figure 1: Gel electrophoresis of Total DNA extraction .1% Agarose gel at 100 volts for 40 min. then visualized under U.V after staining with EtBr.

3.2.2. Polymerase Chain Reaction Products:

The advantage of PCR in which a given segments of DNA can be amplified millions of times invitro, this technique doesn't require highly purified DNA preparations as it works well with partially purified DNA samples and the present study, we have described the genetic analysis for

the INS gene and the pattern of PCR products presented in figures (2). The PCR products relied in differences of intensity but identical the amplified bands.

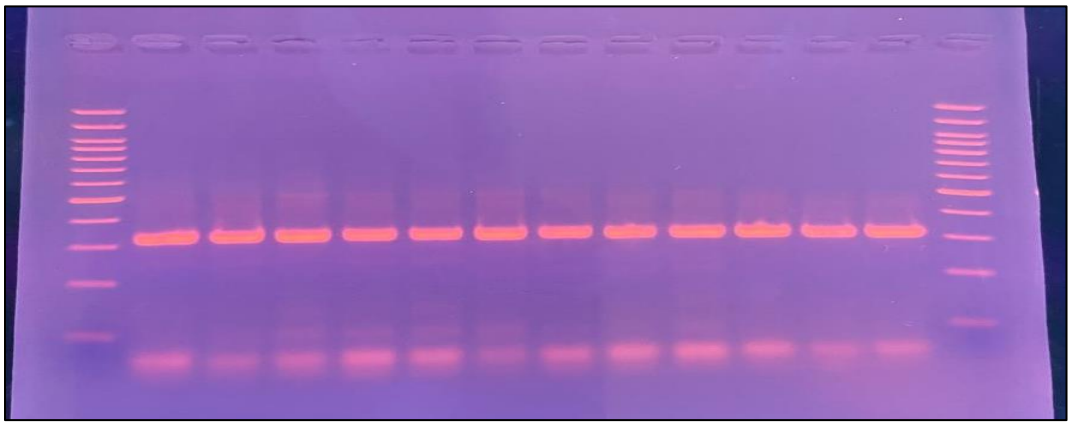
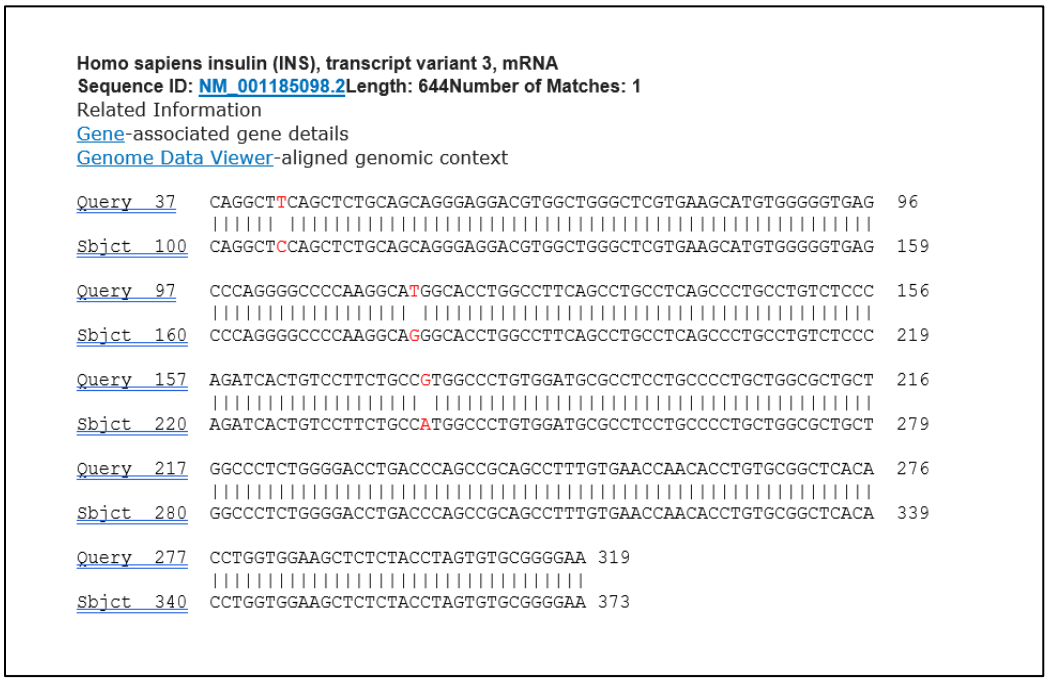


Figure 2: Electrophoresis of PCR of INS gene on agarose gel for 90 minutes, electrophoresis was done on a 2% agarose gel with an 80 volt. Lane M is a (100 bp) ladder, Lane 2,3,4,5,6,7,8,9,10 ,11,12,13 was a positive (319 bp)

3.2.3. PCR Products Purification and Sequencing:

The EZ-10 spin column DNA cleanup minipreps Kit (Bio Basic Inc., Canada) was used to purify the PCR product for sequencing. According to https://blast.ncbi.nlm.nih.gov/Blast.cgi#1843419891, the sequencing results were aligned to the reference subject. Figure (3) illustrated the alignment process for a single sample.



Homo sapiens insulin (INS), transcript variant 3, mRNA
Sequence ID: NM_001185098.2 Length: 644 Number of Matches: 1
Related Information
Gene-associated gene details
Genome Data Viewer-aligned genomic context

```
Query  37   CAGGCTTCAGCTCTGCAGCAGGGAGGACGTGGCTGGGCTCGTGAAGCATGTGGGGGTGAG  96
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  100  CAGGCTCCAGCTCTGCAGCAGGGAGGACGTGGCTGGGCTCGTGAAGCATGTGGGGGTGAG  159

Query  97   CCCAGGGGCCCCAAGGCATGGCACCTGGCCTTCAGCCTGCCTCAGCCCTGCCTGTCTCCC  156
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  160  CCCAGGGGCCCCAAGGCAGGGCACCTGGCCTTCAGCCTGCCTCAGCCCTGCCTGTCTCCC  219

Query  157  AGATCACTGTCCTTCTGCCGTGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCT  216
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  220  AGATCACTGTCCTTCTGCCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCT  279

Query  217  GGCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACA  276
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  280  GGCCCTCTGGGGACCTGACCCAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACA  339

Query  277  CCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAA 319
            |||||||||||||||||||||||||||||||||
Sbjct  340  CCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAA 373
```

Figure 3: Method of alignment for one sample to detection SNPs

3.2.4. INS cDNA Downloading:

The INS cDNA is important to localization of codons in gene and this necessary for fixation the site of SNPs and their effecting in amino acids alteration. Figure (4) showed the complete cDNA according to:

https://asia.ensembl.org/Homo_sapiens/Transcript/Sequence_cDNA?db=core;g=ENSG00000254647;r=11:2159779-2161221;t=ENST00000397262

3.2.5. DNA Sequence Results

After examining the sequence, it was found that all the examined samples had altered genotypes in terms of the variants in the promoter and coding region of INS gene. Table (3) showed the type of mutations and their percentage.

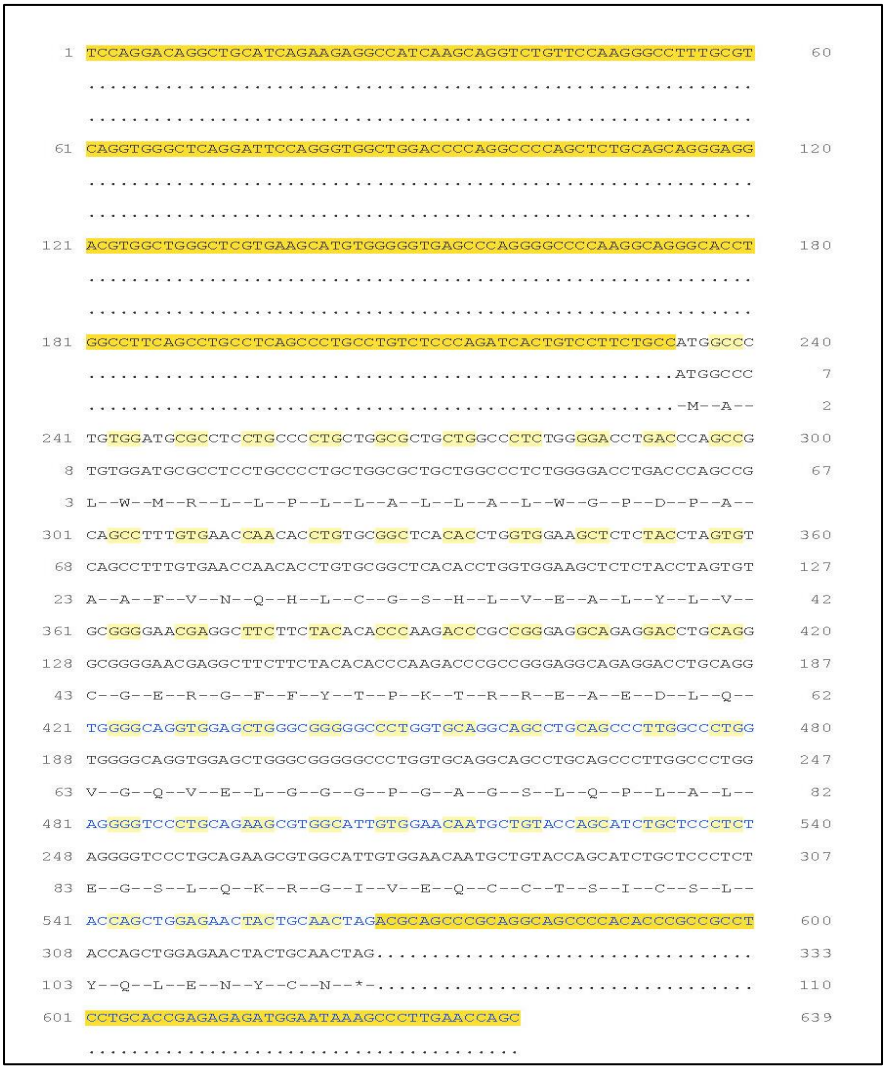


Figure 4: Complete cDNA sequence of insulin gene. The colors indicate to: Alternating codons, Alternating codons, An exon, Another exon, UTR

Table 3: The results of matching the sequence according

T2DM Patients	Wild type	Mutant type	Name of mutation	a.a change	Percentage
T2DM with Hypertension	C	T	-106C>T	UTR	12%
	A	G	-234A>G	UTR	11%
	TGG	TAG	244G>T	W>Stop	8%
	TAC	TAT	353C>T	Y>Y Silent	8%
Obese T2DM	C	T	-106C>T	UTR	7%
	A	G	-234A>G	UTR	7%
Overweight T2DM	C	T	-106C>T	UTR	8%
	A	G	-234A>G	UTR	8%
Normal weight T2DM	C	T	-106C>T	UTR	13%
	TAC	TAT	353C>T	Y>Y Silent	8%
	C	T	-106C>T	UTR	12%

The results of genetic alteration in untranslated region (5′-UTR) upstream and coding region of INS gene were confirmed the work by Fajan et al., (2011) who documented single gene mutations (monogenic or Mendelian), demonstrating that single base pair changes result in changes to protein function and sequence, and are adequate to induce hyperglycemia in type 2 diabetes. This finding indicated that there was a significant association between a protein-coding variant and type 2 diabetes, but not enough to conclusively link a gene to the pathophysiology of the disease. The SNP regions, which contain both protein-coding and noncoding variation, may be a hope for establishing a more direct link between associated signals and biological function. -106C>T and -234A>G are mutations in the 5′-UTR (promoter) of the INS gene that affect gene expression. Many physiological processes depend on the post-transcriptional control of gene expression, which is mostly handled by the untranslated region (UTR). Two SNPs showed significant differences in genotype frequencies between analyzed groups influence in displayed higher BMI value and insulin levels. Bonnefond and Froguel (2015) reported the many loci SNPs in promoter of INS gene that effecting in β cell or islet function in T2DM patients. In other hand; our findings detection the nonsense mutation (244G>T) converts the codon (TGG) coding to tryptophan to stop codon

(TAG) that effecting INS synthesis by shorting the protein leading to non-functional or partially functional protein. The mutation in cording region (353C>T) was silent mutation that unchanged in type of amino acid.

Conclusion:

Single nucleotide polymorphisms, which are genetic variations in a DNA sequence that occur in populations with a specific frequency, are significant. Finding SNPs linked to T2DM could help prevent the disease and forecast an individual's risk. This knowledge could be useful in the development of new medications, and gene therapy is a viable therapeutic option. Given that type 2 diabetes may have a causative effect on hypertension, our findings emphasize the importance of screening for and monitoring hypertension, especially systolic blood pressure, as well as maintaining an optimal glycemic profile in general populations. This study highlights the relationship between BMI and HbA1c levels in individuals with type 2 diabetes, underscoring the critical role that weight management plays in optimizing glycemic control.

References Format:

[1] Alva, M. L., Hoerger, T. J., Zhang, P., and Gregg, E. W. (2017). Identifying risk for type 2 diabetes in different age cohorts: does one size fit all?. BMJ Open Diabetes Research and Care, 5(1), e000447.

[2] Boutari C, DeMarsilis A, Mantzoros CS. Obesity and diabetes. Diabet Res Clin Pract. 2023; 202:110773. doi: 10.1016/j.diabres.2023.110773.

[3] Cerf M.E. Beta Cell Dysfunction and Insulin Resistance. Front. Endocrinol. 2013; 4:37. doi: 10.3389/fendo.2013.00037.

[4] Cheung B.M., Li C. Diabetes and hypertension: Is there a common metabolic pathway? Curr. Atheroscler. Rep. 2012; 14:160–166. doi: 10.1007/s11883-012-0227-2.

[5] Czech MP. Mechanisms of insulin resistance related to white, beige, and brown adipocytes. mol Metab. 2020; 34:27–42. doi: 10.1016/j.molmet.2019.12.014.

[6] Emont MP, Jacobs C, Essene AL, et al. A single-cell atlas of human and mouse white adipose tissue. Nature. 2022;603(7903):926–933. doi: 10.1038/s41586-022-04518-2.

[7] Evans, M., Welsh, Z., Ells, S., and Seibold, A. (2020). The impact of flash glucose monitoring on glycaemic control as measured by HbA1c: a meta-analysis of clinical trials and real-world observational studies. Diabetes Therapy, 11(1), 83-95.

[8] Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med. 2001;345(13):971-980. doi:10.1056/NEJMra002168.

[9] Haber R, Ghezzawi M, Puzantian H, et al. Mortality risk in patients with obesity and COVID-19 infection: a systematic review and meta-analysis. Metabolism. 2024; 155:155812. doi: 10.1016/j.metabol.2024.155812.

[10] Jihua, S. and Riobo,S.P. (2019). The Impact of Genetic Variation on Type 2 Diabetes: Insights Into Rare Variant Susceptibility. Diss. University of Copenhagen, Faculty of Science, Department of Biology, Obesity and diabetes. Nutr Hosp, 2013. 28 Suppl 5: p. 138-43.

[11] Kahn S.E., Cooper M.E., Del Prato S. Pathophysiology and Treatment of Diabetes: Perspectives on the Past, Present and Future. Lancet. 2014; 383:1068–1083. doi: 10.1016/S0140-6736(13)62154-6.

[12] Kodama S, Horikawa C, Fujihara K, et al. Quantitative relationship between body weight gain in adulthood and incident type 2 diabetes: a meta-analysis. Obes Rev. 2014;15(3):202–214. doi: 10.1111/obr.12129

[13] Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. Jama. 2004; 291:1978–86.

[14] Park C, Guallar E, Linton JA, Lee DC, Jang Y, Son DK, et al. Fasting glucose level and the risk of incident atherosclerotic cardiovascular diseases. Diabetes Care. 2013; 36:1988–93.

[15] Petrie JR, Guzik TJ, Touyz RM. Diabetes, hypertension, and cardiovascular disease: clinical insights and vascular mechanisms. Can J Cardiol. 2018; 34:575–84.

[16] Picke, A. K., Campbell, G., Napoli, N., Hofbauer, L. C., and Rauner, M. (2019). Update on the impact of type 2 diabetes mellitus on bone metabolism and material properties. Endocrine connections, 8(3), R55-R70.

[17] Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. J Clin Invest. 2016;126(1):12–22. doi: 10.1172/JCI77812

[18] SPSS (2019). Statistical Packages of Social Sciences- SPSS/ IBM Statistics 26 step by step. 16th Edition. https://doi.org/10.4324/9780429056765

[19] Trikkalinou, A., Papazafiropoulou, A. K., and Melidonis, A. (2017). Type 2 diabetes and quality of life. World journal of diabetes, 8(4), 120.

[20] Tsimihodimos V, Gonzalez-Villalpando C, Meigs JB, Ferrannini E. Hypertension and Diabetes Mellitus: Coprediction and time trajectories. Hypertension. 2018; 71:422–8.

[21] You, W. P., and Henneberg, M. (2016). Type 1 diabetes prevalence increasing globally and regionally: the role of natural selection and life expectancy at birth. BMJ open diabetes research and care, 4(1), e000161.