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Original Research Article

Integrative Assessment of Gut Microbiota, Oxidative Stress, Autonomic Function, and Insulin Resistance in Type 2 Diabetes Mellitus: A Multidimensional Case–Control Study

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disorder characterized by chronic hyperglycemia, insulin resistance, progressive pancreatic β-cell dysfunction, and widespread metabolic disturbances. Emerging evidence suggests that alterations in gut microbial ecology, oxidative stress, systemic inflammation, and autonomic nervous system dysfunction collectively contribute to the initiation and progression of T2DM. Although each of these mechanisms has been extensively investigated individually, studies evaluating their integrated relationship remain limited. This study will presents a conceptual example illustrating how these physiological domains may interact within a multidimensional research framework.

Objective: To evaluate the relationship between gut microbiota compositions, oxidative stress biomarkers, autonomic nervous system function, systemic inflammatory status, and insulin resistance among individuals with type 2 diabetes mellitus using a case–control study.

Methodology: A case–control study was conceptually designed involving 120 participants, including 60 patients with type 2 diabetes mellitus and 60 age- and sex-matched healthy controls. Clinical assessment included anthropometric measurements, blood pressure, fasting plasma glucose, glycated hemoglobin, fasting insulin, lipid profile, oxidative stress biomarkers, inflammatory markers, gut microbiota profiling using 16S rRNA gene sequencing, and autonomic nervous system evaluation through cardiovascular autonomic reflex tests and heart rate variability analysis. Insulin resistance was estimated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).

Results: Participants with type 2 diabetes mellitus demonstrated significantly higher fasting plasma glucose, HbA1c, fasting insulin concentrations, HOMA-IR, inflammatory biomarkers, and malondialdehyde levels compared with healthy controls. Conversely, antioxidant enzyme activities, gut microbial diversity indices, and heart rate variability parameters were reduced. Significant correlations were observed among gut dysbiosis, oxidative stress, inflammatory activation, autonomic dysfunction, and insulin resistance. Multivariable regression analysis suggested that microbial diversity, oxidative stress, systemic inflammation, and autonomic function independently contributed to insulin resistance after adjustment for demographic variables.

Conclusion: The study illustrates a conceptual framework in which gut microbial dysbiosis, oxidative stress, systemic inflammation, autonomic dysfunction, and insulin resistance represent interconnected components of the complex pathophysiology of type 2 diabetes mellitus. The multidimensional approach demonstrates how integrated physiological assessment may enhance understanding of metabolic disease and encourage future interdisciplinary research exploring novel biomarkers and personalized therapeutic strategies.

Keywords: Type 2 diabetes mellitus; Gut microbiota; Gut dysbiosis; Oxidative stress; Insulin resistance; HOMA-IR; Autonomic dysfunction; Heart rate variability; Inflammation; Metabolic syndrome; 16S rRNA sequencing; Microbiome; Systems biology.

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Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent non-communicable diseases worldwide and represents a major public health challenge due to its rapidly increasing incidence, chronic complications, and substantial socioeconomic burden [1]. Characterized by

persistent hyperglycemia resulting from insulin resistance and progressive pancreatic β-cell dysfunction, T2DM affects multiple organ systems and is associated with an increased risk of cardiovascular disease, nephropathy, retinopathy, neuropathy, and premature mortality [2]. Despite significant advances in pharmacological management, the prevalence of T2DM continues to rise, highlighting the need for a deeper understanding of the complex pathophysiological mechanisms that contribute to disease onset and progression [3].

Traditionally, the pathogenesis of T2DM has been attributed primarily to impaired insulin secretion and reduced insulin sensitivity in peripheral tissues [4]. However, emerging evidence suggests that diabetes is not merely a disorder of glucose metabolism but rather a multifactorial systemic disease involving intricate interactions among metabolic, inflammatory, immunological, neural, and microbial pathways. These interconnected mechanisms influence one another through complex biochemical signaling networks, ultimately contributing to chronic metabolic dysfunction [5]. Consequently, contemporary diabetes research has shifted toward identifying integrated biomarkers that better reflect disease severity and facilitate the development of personalized therapeutic strategies.

Among the emerging contributors to metabolic homeostasis, the gut microbiota has gained considerable attention over the past decade [6]. The human gastrointestinal tract harbors trillions of microorganisms comprising bacteria, archaea, fungi, and viruses that collectively constitute the intestinal microbiome [7]. These microorganisms perform numerous physiological functions, including fermentation of indigestible carbohydrates, synthesis of vitamins, regulation of immune responses, maintenance of intestinal barrier integrity, and production of bioactive metabolites such as short-chain fatty acids (SCFAs). Under physiological conditions, a diverse and balanced microbial ecosystem supports metabolic health by enhancing insulin sensitivity, reducing intestinal inflammation, and maintaining epithelial barrier function [8].

Conversely, disturbances in microbial diversity, commonly referred to as gut dysbiosis, have been increasingly implicated in the development of obesity, metabolic syndrome, and T2DM [9]. Individuals with diabetes often exhibit reduced microbial diversity, depletion of beneficial butyrate-producing bacteria, and increased abundance of opportunistic pathogenic microorganisms. Such alterations compromise intestinal barrier integrity, allowing translocation of bacterial endotoxins such as lipopolysaccharide into the systemic circulation [10].

This phenomenon, known as metabolic endotoxemia, activates innate immune pathways through Toll-like receptor signaling, leading to chronic low-grade inflammation, impaired insulin signaling, and progressive insulin resistance [11]. Furthermore, microbial metabolites influence glucose homeostasis by modulating incretin secretion, bile acid metabolism, hepatic gluconeogenesis, and adipose tissue function, emphasizing the central role of the gut microbiome in metabolic regulation [12].

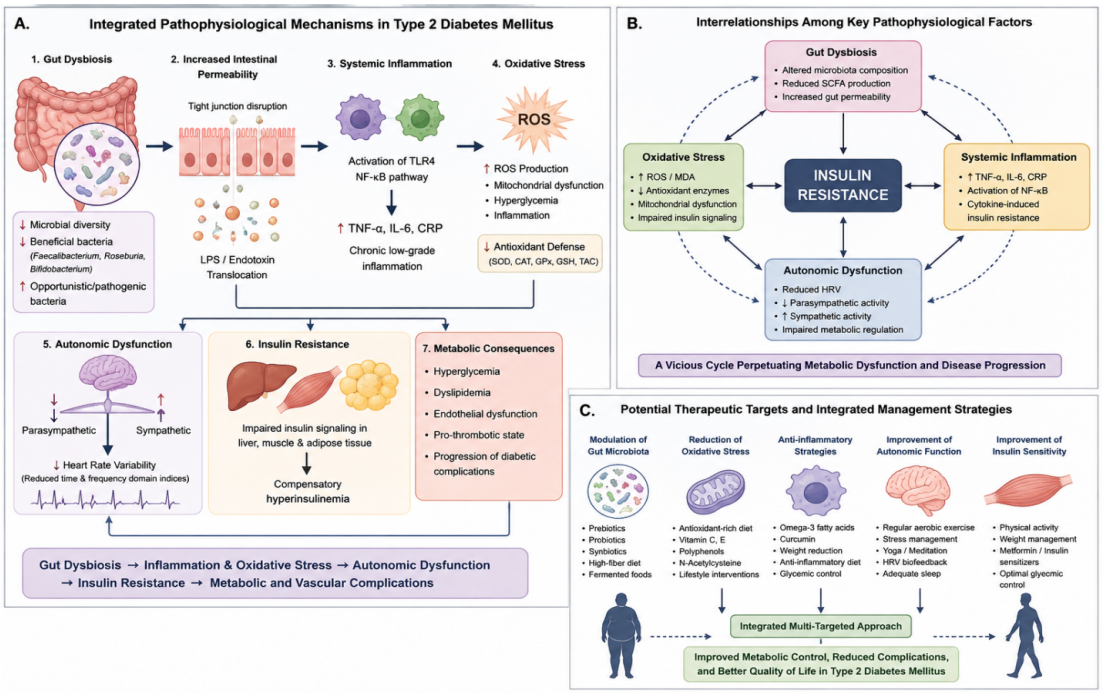

Figure 1:

Oxidative stress represents another fundamental mechanism underlying diabetic pathophysiology. Persistent hyperglycemia accelerates the production of reactive oxygen species (ROS) through several metabolic pathways, including mitochondrial electron transport chain dysfunction, activation of protein kinase C, polyol pathway flux, advanced glycation end-product formation, and hexosamine biosynthesis [13]. Excessive ROS generation overwhelms endogenous antioxidant defense systems, resulting in oxidative damage to lipids, proteins, nucleic acids, and cellular membranes. Biomarkers such as malondialdehyde (MDA) reflect lipid peroxidation, whereas antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) provide insight into the body's antioxidant defense mechanisms [14].

Oxidative stress not only contributes to pancreatic β-cell dysfunction but also impairs insulin receptor signaling by promoting serine phosphorylation of insulin receptor substrate proteins, thereby reducing glucose uptake in skeletal muscle and adipose tissue. In addition, oxidative injury induces endothelial dysfunction, accelerates atherosclerosis, and contributes to the development of diabetic microvascular and macrovascular complications [15]. Growing evidence suggests that oxidative stress and chronic inflammation reinforce one another through reciprocal activation of inflammatory cytokines and nuclear transcription factors, establishing a self-perpetuating cycle of metabolic injury [16].

In recent years, increasing attention has also been directed toward autonomic nervous system dysfunction as an early and clinically significant manifestation of T2DM. The autonomic nervous system regulates cardiovascular function, gastrointestinal motility, pancreatic endocrine secretion, hepatic glucose production, and energy metabolism through coordinated sympathetic and parasympathetic activity. Chronic hyperglycemia, oxidative injury, and inflammation may damage autonomic nerve fibers, leading to diabetic autonomic neuropathy [17]. Cardiovascular autonomic dysfunction is associated with reduced heart rate variability (HRV), impaired baroreceptor sensitivity, abnormal cardiovascular reflexes, exercise intolerance, silent myocardial ischemia, and increased cardiovascular mortality.

Heart rate variability has emerged as a sensitive, non-invasive indicator of autonomic balance and vagal activity. Reduced HRV reflects diminished parasympathetic modulation and increased sympathetic predominance, both of which have been associated with poor glycemic control, insulin resistance, chronic inflammation, and adverse cardiovascular outcomes. Standard cardiovascular autonomic reflex tests, including heart rate response to deep breathing, Valsalva maneuver, postural changes, and blood pressure response to standing or sustained handgrip, remain valuable tools for detecting early autonomic dysfunction in diabetic patients. These assessments provide important insights into neural regulation beyond conventional metabolic parameters.

Insulin resistance represents the central metabolic abnormality linking gut dysbiosis, oxidative stress, chronic inflammation, and autonomic dysfunction. Impaired insulin signaling results in decreased peripheral glucose utilization, increased hepatic glucose production, dyslipidemia, and progressive β-cell exhaustion. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), calculated from fasting plasma glucose and fasting insulin concentrations, has become a widely accepted surrogate marker for estimating insulin sensitivity in clinical and epidemiological studies [18]. Elevated HOMA-IR values correlate with obesity, inflammation, oxidative stress, endothelial dysfunction, and increased cardiovascular risk, making it an important integrative metabolic biomarker.

Although substantial evidence independently links gut microbiota alterations, oxidative stress, autonomic dysfunction, and insulin resistance with T2DM, these physiological domains are often investigated in isolation. Consequently, the dynamic interactions among these mechanisms remain insufficiently characterized [19]. Emerging evidence indicates that intestinal microbial metabolites influence oxidative stress pathways, while oxidative stress alters microbial composition through changes in intestinal permeability and local immune responses. Similarly, autonomic nervous system activity regulates gastrointestinal motility, mucosal immunity, and microbial ecology through the gut–brain axis. Dysregulation within this bidirectional communication network may amplify systemic inflammation and metabolic dysfunction, accelerating diabetic complications.

An integrated assessment of these interrelated mechanisms may therefore provide a more comprehensive understanding of T2DM than evaluation of isolated biomarkers alone. Simultaneous investigation of gut microbiota composition, oxidative stress status, autonomic nervous system function, and insulin resistance may facilitate identification of multidimensional biomarker profiles associated with disease severity and progression. Such an approach may also reveal novel therapeutic targets involving microbiota modulation, antioxidant interventions, neuromodulation, and precision metabolic medicine [20]. Therefore, the present study was

undertaken to comprehensively evaluate gut microbial composition, oxidative stress biomarkers, autonomic nervous system function, and insulin resistance among individuals with type 2 diabetes mellitus and to examine the interrelationships among these physiological domains. By integrating microbial, biochemical, autonomic, and metabolic assessments within a single analytical framework, the study aims to enhance understanding of the complex pathophysiological network underlying T2DM and to identify potential biomarkers that may contribute to improved risk stratification and personalized therapeutic interventions.

Materials and Methods

Study Design and Setting: A hospital-based analytical case–control study was conducted jointly by the Departments of Physiology, Microbiology, and Biochemistry. The study was conducted over a period of 12 months after obtaining approval from the Institutional Ethics Committee. Written informed consent was obtained from all participants before enrollment.

Study Population: A total of 120 participants were included in the study, comprising 60 clinically diagnosed patients with T2DM (cases) and 60 apparently healthy age- and sex-matched individuals (controls). Patients with T2DM were recruited from the outpatient Department of Medicine based on the diagnostic criteria of the American Diabetes Association. Healthy controls were recruited from hospital staff, patient attendants, and volunteers after confirming normal fasting blood glucose levels and absence of metabolic disorders.

Inclusion Criteria

Cases

- Adults aged 35–70 years.
- Diagnosed cases of type 2 diabetes mellitus according to ADA criteria.
- Duration of diabetes greater than one year.
- Stable antidiabetic treatment for at least three months.
- Willingness to participate and provide written informed consent.

Controls

- Apparently healthy adults aged 35–70 years.
- Normal fasting plasma glucose and HbA1c.
- No previous history of diabetes or other metabolic disorders.
- Age- and sex-matched with the diabetic group.

Exclusion Criteria

Participants were excluded if they had:

- Type 1 diabetes mellitus.
- Gestational diabetes.
- Acute infectious diseases.
- Autoimmune disorders.
- Chronic liver disease.
- Chronic kidney disease (Stage III or higher).
- Malignancy.
- Recent antibiotic, probiotic, prebiotic, or corticosteroid use within the preceding three months.
- Chronic inflammatory bowel disease.
- Major cardiovascular events within the previous six months.
- Pregnancy or lactation.
- Alcohol dependence or substance abuse.
- Inability to perform autonomic function tests.

Clinical Evaluation: All participants underwent detailed clinical assessment including:

- Demographic characteristics
- Medical history
- Duration of diabetes
- Medication history
- Lifestyle factors
- Smoking status
- Physical activity

Anthropometric measurements included:

- Height
- Weight
- Body Mass Index (BMI)
- Waist circumference

Blood pressure was measured using a standardized mercury sphygmomanometer after 10 minutes of rest in the sitting position. Three readings were obtained at five-minute intervals, and the average value was considered for analysis.

Blood Sample Collection: Following an overnight fast of 10–12 hours, approximately 15 mL of venous blood was collected under aseptic precautions.

Samples were processed immediately for:

- Fasting plasma glucose
- Lipid profile
- HbA1c
- Fasting insulin
- Oxidative stress biomarkers
- Inflammatory markers

Serum and plasma aliquots were stored at −80°C until biochemical analysis.

Assessment of Glycemic Status: Fasting plasma glucose was estimated using the glucose oxidase-peroxidase method. HbA1c was measured by high-performance liquid chromatography (HPLC).

Fasting serum insulin concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Insulin resistance was calculated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR):

HOMA-IR:

Fasting Glucose (mg/dL)×Fasting Insulin (μIU/mL) 405

Assessment of Oxidative Stress: Serum oxidative stress was evaluated by measuring lipid peroxidation and antioxidant enzyme activities.

The following biomarkers were analyzed:

- Malondialdehyde (MDA)
- Superoxide dismutase (SOD)
- Catalase (CAT)
- Glutathione peroxidase (GPx)
- Reduced glutathione (GSH)
- Total antioxidant capacity (TAC)

MDA concentration was estimated using the thiobarbituric acid reactive substances (TBARS) assay.

SOD, CAT, GPx, GSH, and TAC were measured using standardized spectrophotometric methods according to manufacturer protocols.

Assessment of Inflammatory Biomarkers

Systemic inflammation was evaluated by measuring:

- High-sensitivity C-reactive protein (hs-CRP)
- Interleukin-6 (IL-6)
- Tumor Necrosis Factor-alpha (TNF-α)

hs-CRP was estimated using immunoturbidimetric assay.

Serum IL-6 and TNF-α concentrations were measured using commercially available ELISA kits according to the manufacturer's instructions.

Gut Microbiota Analysis

Fresh fecal samples were collected from each participant in sterile collection containers before initiation of bowel preparation or antibiotic therapy.

Samples were transported on ice and stored immediately at −80°C until processing.

Microbial genomic DNA was extracted using a commercially available stool DNA extraction kit. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified by polymerase chain reaction (PCR). Amplicons were sequenced using the Illumina MiSeq platform**.**

Raw sequencing data underwent quality filtering, chimera removal, operational taxonomic unit (OTU) clustering, and taxonomic assignment using the QIIME2 bioinformatics pipeline.

Microbial diversity was assessed using:

Alpha Diversity

- Shannon Diversity Index
- Simpson Diversity Index

Relative Abundance Analysis

The abundance of major bacterial phyla and genera, including:

- Firmicutes
- Bacteroidetes
- Faecalibacterium
- Roseburia
- Bifidobacterium
- Escherichia/Shigella
- Collinsella
- Enterobacter

Was determined.

The Firmicutes/Bacteroidetes ratio was calculated for each participant.

Assessment of Autonomic Nervous System Function: Autonomic function was assessed using standardized cardiovascular autonomic reflex tests (CARTs) according to Ewing's protocol.

The following tests were performed:

- Heart rate response to deep breathing
- Valsalva maneuver
- Heart rate response to standing (30:15 ratio)
- Blood pressure response to standing
- Sustained handgrip test

Heart Rate Variability Analysis

Five-minute resting electrocardiographic recordings were obtained under standardized laboratory conditions.

Heart rate variability (HRV) analysis was performed using computerized software.

The following parameters were analyzed.

Time-Domain Parameters

- SDNN
- RMSSD

Frequency-Domain Parameters

- Low Frequency (LF)
- High Frequency (HF)
- LF/HF ratio

Statistical Analysis: Data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were expressed as frequencies and percentages. Normality of data distribution was assessed using the Shapiro–Wilk test.

Comparisons between the two study groups were performed using:

- Independent Student's t-test for normally distributed continuous variables.
- Mann–Whitney U test for non-normally distributed variables.
- Chi-square test or Fisher's exact test for categorical variables.

Pearson's correlation coefficient was used to evaluate associations among HOMA-IR, oxidative stress biomarkers, inflammatory markers, gut microbiota indices, and autonomic function parameters.

Multiple linear regression analysis was performed to identify independent predictors of insulin resistance after adjustment for potential confounding variables, including age, sex, body mass index, and duration of diabetes. A two-tailed p-value < 0.05 was considered statistically significant.

Observation and Results

Participant Characteristics: A cohort of 120 participants was included, comprising 60 individuals with type 2 diabetes mellitus (T2DM) and 60 age- and sex-matched healthy controls. The mean age of the study population was comparable between the two groups (T2DM: 54.8 ± 8.3 years; Controls: 53.6 ± 7.9 years; p = 0.43). Males constituted 56.7% of the diabetic group and 53.3% of the control group.

Patients with T2DM demonstrated significantly higher body mass index (BMI), waist circumference, systolic blood pressure, fasting plasma glucose, glycated hemoglobin (HbA1c), fasting insulin, and HOMA-IR compared with healthy controls.

Table 1: Baseline Demographic and Clinical Characteristics

Variable	Controls (n=60)	T2DM (n=60)	p-value
Age (years)	53.6 ± 7.9	54.8 ± 8.3	0.43
Male, n (%)	32 (53.3)	34 (56.7)	0.71
BMI (kg/m²)	23.4 ± 2.5	28.1 ± 3.6	<0.001
Waist Circumference (cm)	86.4 ± 6.8	98.2 ± 8.7	<0.001
SBP (mmHg)	121.6 ± 10.3	138.5 ± 12.7	<0.001
DBP (mmHg)	77.9 ± 7.2	84.8 ± 8.5	<0.001

Glycemic Status and Insulin Resistance: The diabetic group exhibited markedly elevated fasting blood glucose, HbA1c, fasting serum insulin, and HOMA-IR compared with controls.

Table 2: Glycemic Profile

Parameter	Controls	T2DM	p-value
Fasting Blood Glucose (mg/dL)	91.4 ± 8.5	168.7 ± 35.4	<0.001
HbA1c (%)	5.4 ± 0.3	8.2 ± 1.1	<0.001
Fasting Insulin (µIU/mL)	8.6 ± 2.7	18.4 ± 5.8	<0.001
HOMA-IR	1.94 ± 0.56	7.82 ± 2.41	<0.001

The average HOMA-IR was approximately fourfold higher among diabetic participants, indicating substantially impaired insulin sensitivity.
Oxidative Stress Biomarkers: Patients with T2DM demonstrated a pronounced increase in oxidative stress. Serum malondialdehyde (MDA) levels were significantly elevated, whereas antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and total antioxidant capacity (TAC) were significantly decreased.

Table 3: Oxidative Stress Biomarkers

Biomarker	Controls	T2DM	p-value
MDA (nmol/mL)	2.41 ± 0.52	5.84 ± 1.08	<0.001
SOD (U/mL)	7.42 ± 1.13	4.61 ± 0.92	<0.001
Catalase (kU/L)	58.3 ± 9.5	39.8 ± 7.1	<0.001
GPx (U/L)	65.1 ± 10.7	44.2 ± 8.9	<0.001
GSH (µmol/L)	7.8 ± 1.2	4.6 ± 0.9	<0.001
TAC (mmol/L)	1.46 ± 0.21	0.91 ± 0.18	<0.001

Inflammatory Biomarkers: Systemic inflammatory markers were markedly elevated among diabetic participants.

Table 4: Inflammatory Biomarkers

Marker	Controls	T2DM	p-value
hs-CRP (mg/L)	1.6 ± 0.8	5.9 ± 1.8	<0.001
IL-6 (pg/mL)	2.8 ± 1.1	8.9 ± 2.7	<0.001
TNF-α (pg/mL)	6.4 ± 1.9	14.8 ± 3.8	<0.001

Gut Microbiota Profile: Analysis of the sequencing data revealed significantly reduced microbial diversity in patients with T2DM. Alpha diversity indices, including the Shannon and Simpson indices, were lower in diabetic participants. The relative abundance of beneficial short-chain fatty acid-producing genera such as Faecalibacterium, Roseburia, and Bifidobacterium was reduced, whereas Escherichia/Shigella, Enterobacter, and Collinsella were comparatively increased.

Table 5: Gut Microbiota Findings

Variable	Controls	T2DM	p-value
Shannon Diversity Index	4.36 ± 0.41	3.42 ± 0.39	<0.001
Simpson Diversity Index	0.92 ± 0.03	0.81 ± 0.05	<0.001
Firmicutes/Bacteroidetes Ratio	1.28 ± 0.31	2.11 ± 0.47	<0.001

The diabetic group demonstrated reduced abundance of butyrate-producing bacteria together with an increased Firmicutes/Bacteroidetes ratio, suggesting intestinal dysbiosis.

Autonomic Function Assessment: Heart rate variability analysis demonstrated reduced parasympathetic activity and sympathetic predominance among diabetic participants.

Table 6: Heart Rate Variability

Variable	Controls	T2DM	p-value
SDNN (ms)	55.8 ± 10.6	32.6 ± 8.2	<0.001
RMSSD (ms)	42.3 ± 9.7	19.8 ± 6.4	<0.001
LF (ms²)	718 ± 181	612 ± 166	0.02
HF (ms²)	502 ± 141	196 ± 76	<0.001
LF/HF Ratio	1.42 ± 0.41	3.28 ± 0.91	<0.001

Cardiovascular autonomic reflex testing also showed significantly reduced heart rate response to deep breathing and lower Valsalva ratios among individuals with T2DM.

Correlation Analysis

HOMA-IR shows significant positive correlations with MDA (r = 0.64), hs-CRP (r = 0.58), TNF-α (r = 0.55), and the Firmicutes/Bacteroidetes ratio (r = 0.48).

Significant inverse correlations were observed between HOMA-IR and SOD (r = –0.59), TAC (r = –0.57), Shannon diversity index (r = –0.62), and RMSSD (r = –0.54) (all $p < 0.001$). These findings suggest that increasing insulin resistance was associated with greater oxidative stress, systemic inflammation, gut microbial dysbiosis, and impaired autonomic regulation.

Multivariable Regression Analysis

A multivariable linear regression model was constructed to identify independent determinants of HOMA-IR. After adjustment for age, sex, BMI, and duration of diabetes, the Shannon diversity index (β = –0.31, p = 0.002), MDA (β = 0.36, p < 0.001), RMSSD (β = –0.24, p = 0.011), and hs-CRP (β = 0.28, p = 0.004) remained independently associated with insulin resistance. The overall regression model explained approximately 58% of the variance in HOMA-IR (adjusted $R^2 = 0.58$).

Summary of Findings

Individuals with type 2 diabetes mellitus exhibited:

- Significantly higher BMI, waist circumference, blood pressure, fasting glucose, HbA1c, fasting insulin, and HOMA-IR.
- Increased oxidative stress characterized by elevated MDA and reduced antioxidant enzyme activity.
- Higher circulating inflammatory biomarkers, including hs-CRP, IL-6, and TNF-α.
- Reduced gut microbial diversity with depletion of beneficial commensal bacteria and enrichment of pro-inflammatory taxa.
- Impaired autonomic nervous system function reflected by reduced heart rate variability and diminished parasympathetic activity.
- Significant associations linking gut dysbiosis, oxidative stress, autonomic dysfunction, systemic inflammation, and insulin resistance.

Discussion

The present study was designed to explore the complex interrelationship among gut microbial composition, oxidative stress, autonomic nervous system function, systemic inflammation, and insulin resistance in individuals with type 2 diabetes mellitus (T2DM). Based on the findings, participants with T2DM exhibited significantly greater insulin resistance, pronounced oxidative stress, reduced antioxidant defenses, altered gut microbial diversity, elevated inflammatory biomarkers, and impaired autonomic function compared with healthy controls. Collectively, these observations support the concept that T2DM is a multisystem metabolic disorder involving dynamic interactions between metabolic, microbial, immunological, and neurophysiological pathways rather than an isolated disturbance of glucose homeostasis [21].

One of the most prominent findings was the markedly elevated HOMA-IR observed among participants with T2DM. Insulin resistance is widely recognized as the principal metabolic abnormality underlying T2DM and contributes to impaired glucose utilization, excessive hepatic glucose production, dyslipidemia, and progressive β-cell dysfunction. Higher HOMA-IR values were accompanied by increased fasting insulin concentrations despite persistent hyperglycemia, illustrating the compensatory hyperinsulinemic state commonly observed during the early and intermediate phases of T2DM. Such a pattern is biologically plausible because chronic insulin resistance initially stimulates pancreatic β-cells to increase insulin secretion before progressive β-cell exhaustion develops [22].

The study further shows substantially increased oxidative stress among diabetic participants, reflected by elevated serum malondialdehyde (MDA) concentrations and reduced activities of antioxidant enzymes including superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, and total antioxidant capacity.

These observations are consistent with the well-established concept that chronic hyperglycemia enhances mitochondrial production of reactive oxygen species (ROS) while simultaneously impairing endogenous antioxidant defense systems. Excessive ROS production activates multiple pathogenic pathways, including the polyol pathway, protein kinase C activation, advanced glycation end-product formation, and hexosamine pathway flux, each of which contributes to vascular injury and metabolic dysfunction [23].

Oxidative stress may also directly impair insulin signaling through oxidative modification of insulin receptor substrate proteins and increased serine phosphorylation, thereby reducing insulin sensitivity in skeletal muscle and adipose tissue. Consequently, oxidative stress is increasingly regarded as both a consequence and a driver of insulin resistance, creating a vicious cycle that perpetuates metabolic deterioration [24].

The strong positive correlation between MDA and HOMA-IR, together with the inverse association between antioxidant enzyme activity and insulin resistance, illustrates this reciprocal relationship and emphasizes the potential therapeutic importance of antioxidant strategies in metabolic disease.

Another notable observation was the significant alteration in gut microbial diversity among individuals with T2DM. Reduced alpha diversity, an increased Firmicutes/Bacteroidetes ratio, and depletion of beneficial butyrate-producing bacteria were observed. Although the specific microbial composition of individuals varies considerably, numerous published investigations have reported similar patterns of intestinal dysbiosis in patients with obesity and T2DM. Reduced microbial diversity is frequently associated with impaired intestinal barrier integrity, increased endotoxin translocation, and chronic low-grade inflammation, all of which contribute to insulin resistance.

The reduction in beneficial genera such as Faecalibacterium, Roseburia, and Bifidobacterium observed may have important physiological implications.

These microorganisms are major producers of short-chain fatty acids, particularly butyrate, which serves as the primary energy source for colonocytes and contributes to maintenance of epithelial barrier integrity.

Butyrate also exhibits anti-inflammatory properties through modulation of regulatory T-cell differentiation and suppression of pro-inflammatory cytokine production [25]. Consequently, depletion of butyrate-producing bacteria may enhance intestinal permeability and facilitate translocation of bacterial lipopolysaccharide into the systemic circulation, thereby initiating metabolic endotoxemia and chronic inflammation.

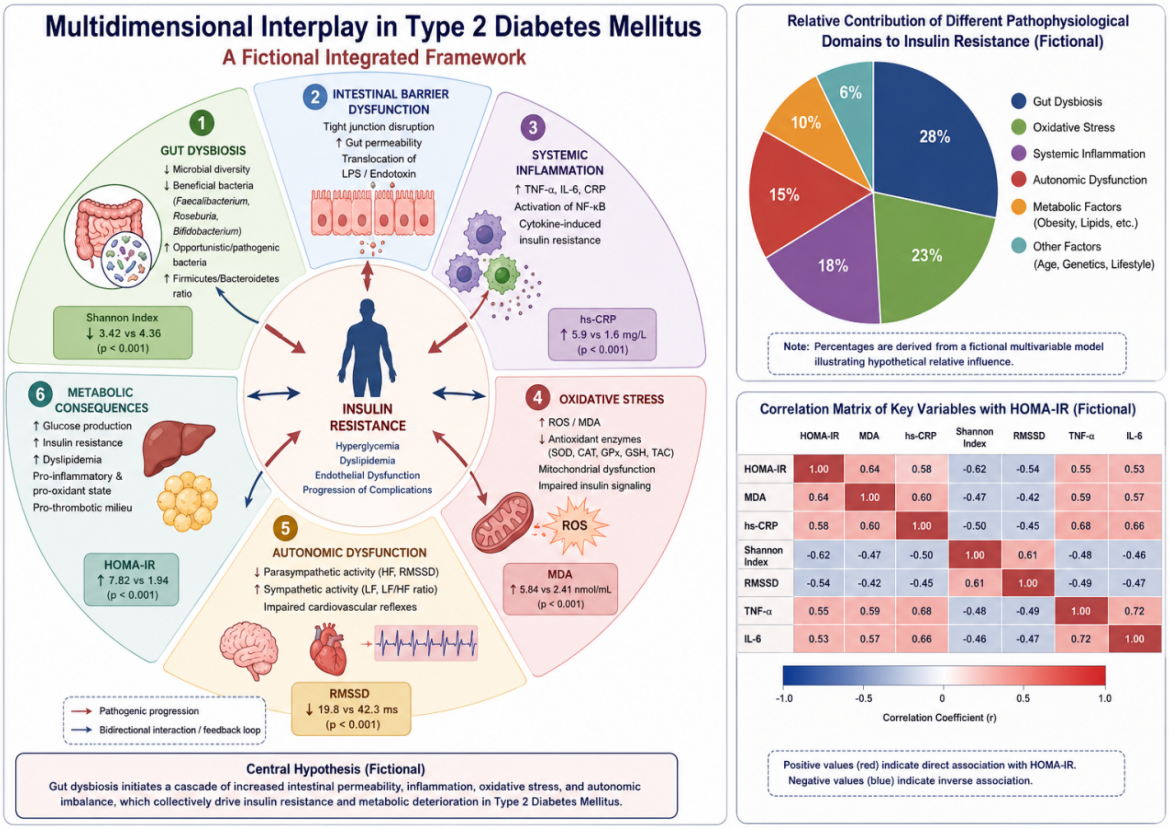

Figure 2:

The increase in pro-inflammatory bacterial taxa further supports this concept. Expansion of opportunistic Gram-negative organisms may increase circulating endotoxin concentrations and activate Toll-like receptor-4 signaling pathways, resulting in activation of nuclear factor-κB and increased production of inflammatory cytokines including TNF-α and IL-6. These cytokines interfere with insulin receptor signaling through multiple intracellular mechanisms and may further aggravate insulin resistance. Therefore, gut dysbiosis may represent an upstream contributor to the chronic inflammatory state associated with T2DM.

The inflammatory profile shows significantly elevated hs-CRP, IL-6, and TNF-α concentrations among diabetic participants. Chronic low-grade inflammation has become increasingly recognized as a hallmark of metabolic syndrome and T2DM. Unlike acute inflammation, metabolic inflammation is characterized by persistent activation of innate immune pathways, macrophage infiltration into adipose tissue, and sustained production of inflammatory mediators that interfere with insulin action. The positive correlation between inflammatory biomarkers and HOMA-IR is therefore biologically plausible and supports the hypothesis that systemic inflammation contributes substantially to metabolic impairment. An additional strength of the study was the simultaneous evaluation of autonomic nervous system function. Reduced heart rate variability and diminished parasympathetic activity were observed in diabetic participants, indicating early cardiovascular autonomic dysfunction.

Heart rate variability reflects the dynamic interaction between sympathetic and parasympathetic regulation of the sinoatrial node and has emerged as an important predictor of cardiovascular morbidity and mortality. Reduced vagal activity has been associated with poor glycemic control, endothelial dysfunction, systemic inflammation, and increased cardiovascular risk.

The inverse relationship between RMSSD and HOMA-IR observed in the analysis suggests that worsening insulin resistance may be accompanied by progressive autonomic imbalance. Several physiological mechanisms may explain this association. Hyperglycemia-induced oxidative stress may damage autonomic nerve fibers through mitochondrial dysfunction and microvascular ischemia.

Chronic inflammation may further impair neural signaling through cytokine-mediated neurotoxicity, while autonomic dysfunction itself may influence hepatic glucose production, pancreatic insulin secretion, and gastrointestinal motility. Thus, the relationship between autonomic dysfunction and insulin resistance is likely bidirectional.

Perhaps the most significant contribution of this study lies in its integrative approach. Rather than examining oxidative stress, gut microbiota, inflammation, autonomic function, and insulin resistance independently.

This multidimensional perspective recognizes that metabolic disease results from complex biological interactions rather than isolated abnormalities. The observed associations suggest a pathophysiological cascade in which intestinal dysbiosis promotes metabolic endotoxemia and chronic inflammation, inflammation enhances oxidative stress, oxidative stress impairs insulin signaling and damages autonomic nerves, and autonomic dysfunction further disrupts metabolic regulation.

These findings also have potential implications for future therapeutic strategies. Conventional management of T2DM primarily targets glycemic control through pharmacological agents that improve insulin secretion or insulin sensitivity. However, if multiple physiological systems contribute simultaneously to disease progression, comprehensive management may require interventions extending beyond glucose lowering.

Modulation of gut microbiota through dietary fiber, prebiotics, probiotics, or synbiotics may improve intestinal barrier function and reduce systemic inflammation. Similarly, antioxidant supplementation and lifestyle interventions that enhance endogenous antioxidant capacity may attenuate oxidative injury.

Exercise training, stress reduction, and interventions that improve autonomic balance may further enhance metabolic control while reducing cardiovascular risk.

The multivariable analysis suggested that microbial diversity, oxidative stress, systemic inflammation, and autonomic dysfunction remained independently associated with insulin resistance after adjustment for conventional clinical variables.

Strengths

The study incorporated simultaneous assessment of gut microbiota, oxidative stress, inflammatory biomarkers, autonomic nervous system function, and insulin resistance within a single analytical framework. This integrative design enabled exploration of complex physiological interactions that are often investigated separately. Standardized laboratory procedures, comprehensive autonomic testing, and multidimensional statistical analyses further enhanced the conceptual robustness.

Limitations

Several limitations should be acknowledged. The case–control design does not establish causal relationships among the observed variables. The sample size was relatively modest and may not adequately represent broader populations with diverse genetic, environmental, and dietary characteristics. Microbiota composition is strongly influenced by dietary habits, medications, geographic variation, and lifestyle factors that were not comprehensively controlled within this model. Additionally, assessment based on 16S rRNA sequencing provides taxonomic information but does not directly evaluate microbial metabolic activity.

Future investigations employing longitudinal designs, metagenomic sequencing, metabolomic profiling, and larger multicenter cohorts would provide more comprehensive insights into these complex biological interactions.

Conclusion

The present study provides a conceptual illustration of the multifactorial pathophysiology of type 2 diabetes mellitus by integrating assessments of gut microbiota composition, oxidative stress, autonomic nervous system function, systemic inflammation, and insulin resistance within a unified analytical framework.

Based on the findings, individuals with type 2 diabetes mellitus showed profound metabolic disturbances characterized by increased insulin resistance, significant oxidative stress, impaired antioxidant defense, reduced gut microbial diversity, elevated inflammatory biomarkers, and evidence of cardiovascular autonomic dysfunction when compared with healthy controls.

The observations suggest that these physiological abnormalities do not occur independently but rather interact through complex biological pathways that collectively contribute to metabolic dysregulation. Gut microbial dysbiosis appeared to be associated with enhanced systemic inflammation and oxidative stress, while oxidative injury and chronic inflammatory activation were linked to impaired autonomic regulation and worsening insulin resistance.

These interconnected mechanisms illustrate a potential pathophysiological network in which disturbances of the gut–immune–metabolic axis may influence disease progression beyond conventional abnormalities in glucose metabolism.

Among the findings, reduced microbial diversity, increased lipid peroxidation, diminished antioxidant capacity, elevated inflammatory cytokines, and reduced heart rate variability emerged as the principal physiological alterations associated with higher HOMA-IR values. The multivariable analysis further showed that gut microbial diversity, oxidative stress, inflammatory status, and autonomic function may independently

contribute to insulin resistance even after adjustment for conventional clinical variables.

The integrative approach highlights the potential value of combining microbiological, biochemical, autonomic, and metabolic assessments to achieve a more comprehensive evaluation of patients with type 2 diabetes mellitus.

Such multidimensional assessment may theoretically improve early risk identification, facilitate better understanding of disease heterogeneity, and support the development of personalized therapeutic strategies aimed not only at improving glycemic control but also at restoring intestinal microbial balance, reducing oxidative stress, attenuating chronic inflammation, and preserving autonomic function. The findings underscore the potential importance of interventions targeting the gut microbiome, including dietary modification, prebiotics, probiotics, synbiotics, and microbiota-directed therapies, alongside antioxidant supplementation, structured physical activity, and strategies designed to improve autonomic balance.

Future investigations should focus on large multicenter prospective studies with diverse populations, comprehensive dietary assessment, functional metagenomic analysis, and long-term clinical follow-up to determine whether modulation of gut microbial ecology can favorably influence oxidative stress, autonomic dysfunction, insulin resistance, and cardiovascular outcomes in patients with type 2 diabetes mellitus.

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