Official Study Title

Evaluation of Serum Irisin Levels as a Potential Biomarker in Patients With Diabetic Polyneuropathy: A Single-center Prospective Cross-sectional Observational Study

NCT Number

"NCT not yet assigned"

Document Type

Study Protocol with Statistical Analysis Plan

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Version 1.0

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30 june 2026

STUDY PROTOCOL

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Evaluation of Serum Irisin Levels as a Potential Biomarker in Patients with Diabetic Polyneuropathy: A Single-Center Prospective Cross-Sectional Observational Study

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Version 1.0

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30 June 2026

PROTOCOL SYNOPSIS

Item	Description
Study Title	Evaluation of Serum Irisin Levels as a Potential Biomarker in Patients with Diabetic Polyneuropathy
Study Design	Single-center prospective observational case-control study
Study Center	Kanuni Sultan Süleyman Training and Research Hospital, Istanbul, Türkiye

Item	Description
Study Population	Adults with Type 2 Diabetes Mellitus and healthy volunteers
Sample Size	81 participants
Study Groups	DPN+, DPN−, Healthy Controls
Primary Endpoint	Serum irisin concentration
Secondary Endpoints	HbA1c, fasting plasma glucose, BMI, lipid profile, correlation analyses
Biospecimen	Venous blood
Laboratory Method	Commercially available ELISA kit
Study Duration	june 2026 – September 2026

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1. BACKGROUND

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. The prevalence of Type 2 Diabetes Mellitus (T2DM) continues to increase worldwide, representing one of the major global public health challenges. Chronic hyperglycemia contributes to the development of microvascular and macrovascular complications that substantially increase morbidity and mortality.

Diabetic peripheral neuropathy (DPN) is among the most common chronic complications of T2DM, affecting approximately 30–50% of individuals during the course of the disease. It is characterized by progressive degeneration of peripheral nerves leading to sensory loss, pain, impaired proprioception, muscle weakness, gait disturbances, balance impairment, foot ulceration, and increased risk of falls. These complications negatively affect functional capacity, independence, and quality of life.

Although chronic hyperglycemia is considered the principal pathogenic factor, multiple mechanisms contribute to the development of diabetic neuropathy, including oxidative stress, mitochondrial dysfunction, endothelial injury, chronic low-grade inflammation, impaired neurovascular blood flow, and metabolic dysregulation.

Identification of novel biomarkers reflecting these pathophysiological mechanisms may facilitate earlier diagnosis and improve understanding of disease progression.

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2. SCIENTIFIC RATIONALE

Irisin is a peptide hormone (myokine) generated through cleavage of fibronectin type III domain-containing protein 5 (FNDC5) and released primarily from skeletal muscle during physical activity. Since its discovery, irisin has attracted considerable scientific interest because of its effects on glucose metabolism, insulin sensitivity, energy expenditure, lipid metabolism, and inflammatory pathways.

Experimental evidence suggests that irisin promotes glucose uptake, enhances insulin sensitivity, reduces oxidative stress, suppresses inflammatory cytokine production, and improves mitochondrial function. These biological effects suggest that irisin may exert protective effects against metabolic disorders and diabetic complications.

Several clinical studies have demonstrated altered circulating irisin concentrations in obesity, metabolic syndrome, and Type 2 Diabetes Mellitus. However, evidence regarding the relationship between serum irisin concentrations and diabetic peripheral neuropathy remains limited and inconsistent. Some investigators have reported significantly decreased serum irisin levels among patients with diabetic neuropathy, whereas others have observed no significant differences.

Because diabetic peripheral neuropathy is closely associated with chronic inflammation, oxidative stress, endothelial dysfunction, and impaired glucose metabolism, serum irisin may represent a promising biomarker reflecting disease severity and metabolic impairment.

The present study has therefore been designed to evaluate serum irisin concentrations in patients with diabetic peripheral neuropathy and to investigate possible associations between irisin levels and metabolic characteristics.

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3. STUDY OBJECTIVES

Primary Objective

To compare serum irisin concentrations among patients with Type 2 Diabetes Mellitus and diabetic peripheral neuropathy, patients with Type 2 Diabetes Mellitus without neuropathy, and healthy control participants.

Secondary Objectives

- To evaluate the relationship between serum irisin concentrations and HbA1c levels.
- To investigate the association between serum irisin concentrations and fasting plasma glucose.
- To determine the relationship between serum irisin concentration and body mass index.
- To evaluate the association between serum irisin concentration and serum lipid profile.
- To investigate correlations between serum irisin concentration and duration of diabetes.
- To determine whether serum irisin concentration may serve as a potential biomarker for diabetic peripheral neuropathy.

4. PROTOCOL SYNOPSIS

Item	Description
Official Title	Evaluation of Serum Irisin Levels as a Potential Biomarker in Patients with Diabetic Polyneuropathy: A Single-Center Prospective Cross-Sectional Observational Study
Protocol ID	KAEK/2026.05.190
Study Type	Observational

Item	Description
Study Design	Prospective, analytical, cross-sectional, case-control study
Study Center	Kanuni Sultan Süleyman Training and Research Hospital, Istanbul, Türkiye
Study Duration	June 2026 – September 2026
Estimated Enrollment	81 participants
Study Groups	27 DPN+, 27 DPN−, 27 Healthy Controls
Primary Objective	To evaluate serum irisin concentrations in patients with diabetic peripheral neuropathy.
Primary Outcome	Serum irisin concentration (ng/mL)
Secondary Outcomes	HbA1c, fasting plasma glucose, BMI, lipid profile, correlation analyses
Biospecimen	Venous blood
Laboratory Method	Commercially available validated ELISA kit
Statistical Software	IBM SPSS Statistics Version 29.0
Ethics Approval	University of Health Sciences Türkiye Ethics Committee

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5. STUDY DESIGN

This investigation is designed as a **single-center, prospective, analytical, observational, cross-sectional case-control study** conducted at the Internal Medicine Outpatient Clinic of Kanuni Sultan Süleyman Training and Research Hospital.

Eligible participants will be recruited consecutively during the study period and allocated into one of three predefined study groups based on clinical history and electrophysiological evaluation.

Group 1 – Diabetic Peripheral Neuropathy (DPN+)

Patients with Type 2 Diabetes Mellitus diagnosed with diabetic peripheral neuropathy based on clinical examination and nerve conduction studies.

Group 2 – Diabetes Without Neuropathy (DPN−)

Patients with Type 2 Diabetes Mellitus without evidence of diabetic peripheral neuropathy.

Group 3 – Healthy Controls

Age- and sex-matched healthy volunteers without diabetes mellitus or peripheral neuropathy.

All laboratory measurements and clinical evaluations will be performed at baseline.

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6. STUDY POPULATION

The study population will consist of adult individuals attending the Internal Medicine Outpatient Clinic.

Eligible participants will be recruited consecutively until the required sample size has been achieved.

A total of **81 participants** will be enrolled.

- 27 participants with diabetic peripheral neuropathy
- 27 diabetic participants without neuropathy
- 27 healthy controls

Participants of both sexes will be included.

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7. ELIGIBILITY CRITERIA

Inclusion Criteria

Participants must fulfill all of the following criteria:

- Age between 30 and 75 years.
- Diagnosis of Type 2 Diabetes Mellitus for at least one year (diabetic groups only).
- Ability to understand the study procedures.
- Ability to provide written informed consent.
- Willingness to participate voluntarily.

Healthy Controls

Healthy volunteers must additionally fulfill the following:

- No history of diabetes mellitus.
- Normal fasting plasma glucose.
- No history of peripheral neuropathy.

Exclusion Criteria

Participants meeting any of the following criteria will be excluded:

- Vitamin B12 deficiency.
- Chronic alcohol abuse.
- Chemotherapy-induced neuropathy.

- Peripheral neuropathy of non-diabetic origin.
- Chronic kidney disease (eGFR <60 mL/min/1.73 m^2).
- Acute or chronic infection.
- Active inflammatory disease.
- Autoimmune disease.
- Malignancy.
- Pregnancy.
- Myopathies.
- Neuromuscular disorders.
- Parkinson disease.
- Stroke.
- Multiple sclerosis.
- Severe orthopedic disorders affecting mobility.
- Inability to provide informed consent.

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8. RECRUITMENT PROCEDURES

Potential participants will be identified during routine outpatient visits.

Patients meeting the preliminary eligibility criteria will receive verbal and written information regarding the study.

After obtaining written informed consent, eligibility will be confirmed through review of medical history, laboratory findings, and neurological examination.

Healthy volunteers will be recruited from hospital staff and accompanying relatives after screening for eligibility.

Recruitment will continue until the planned sample size has been achieved.

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9. SAMPLE SIZE CALCULATION

Sample size estimation was performed using **G*Power Version 3.1**.

The following assumptions were used:

- Effect size (f): 0.40
- Alpha error: 0.05
- Statistical power: 80%
- Number of study groups: 3

The minimum required sample size was calculated as **66 participants**.

To compensate for possible exclusions and incomplete laboratory data, the final planned enrollment was increased to **81 participants**, corresponding to **27 participants per study group**.

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10. STUDY PROCEDURES

Each participant will attend a single study visit.

The following assessments will be performed.

Clinical Assessment

- Medical history
- Duration of diabetes
- Medication use
- Smoking status
- Blood pressure
- Height
- Weight
- Body Mass Index
- Waist circumference

Neurological Evaluation

Participants will undergo neurological examination by an experienced physician.

The diagnosis of diabetic peripheral neuropathy will be confirmed using:

- Clinical neurological examination
- Nerve conduction studies (Electromyography)

Blood Sampling

Following an overnight fast of at least eight hours, approximately **10 mL** of venous blood will be collected.

Blood samples will be processed within one hour after collection.

Serum will be separated by centrifugation at **3000 rpm for 10 minutes**.

Aliquots will be stored at **–80°C** until analysis.

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11. LABORATORY ANALYSIS

Serum irisin concentrations will be measured using a commercially available, validated enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions.

Routine biochemical analyses will include:

- HbA1c
- Fasting plasma glucose
- Total cholesterol
- HDL cholesterol
- LDL cholesterol
- Triglycerides
- Creatinine
- Vitamin B12 (when clinically indicated)

Laboratory personnel performing ELISA measurements will be blinded to participants' clinical group assignments.

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12. OUTCOME MEASURES

Primary Outcome

Serum Irisin Concentration

Serum irisin concentration (ng/mL) measured by ELISA at baseline.

Secondary Outcomes

- Glycated hemoglobin (HbA1c)
- Fasting plasma glucose
- Body Mass Index
- Serum lipid profile
- Correlation between serum irisin concentration and:
    - HbA1c
    - Fasting plasma glucose
    - BMI
    - Duration of diabetes
    - Lipid profile
    - Presence of diabetic peripheral neuropathy

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13. STATISTICAL ANALYSIS PLAN (SAP)

Statistical Software

All analyses will be performed using **IBM SPSS Statistics Version 29.0**.

Significance Level

Two-sided p values less than **0.05** will be considered statistically significant.

Descriptive Statistics

Continuous variables will be expressed as:

- Mean ± Standard Deviation (SD), or
- Median (Interquartile Range)

Categorical variables will be presented as:

- Frequency (n)
- Percentage (%)

Normality Assessment

Distribution of continuous variables will be evaluated using:

- Shapiro–Wilk test
- Histogram
- Q-Q plot

Group Comparisons

Normally distributed variables:

- Independent Samples t-test
- One-Way ANOVA

Non-normally distributed variables:

- Mann–Whitney U test
- Kruskal–Wallis test

Categorical variables:

- Chi-square test
- Fisher's Exact test (when appropriate)

Correlation Analysis

- Pearson correlation
- Spearman rank correlation

Multivariable Analysis

Voluntary Participation

Participation is entirely voluntary. You may withdraw from the study at any time without affecting your medical care or legal rights.

Contact Information

If you have questions regarding the study, you may contact the Principal Investigator using the contact details provided by the study center.

Consent Statement

I have read and understood the information provided above. I have had the opportunity to ask questions and have received satisfactory answers. I voluntarily agree to participate in this study.

Participant Name: _____

Signature: _____

Date: _____

Researcher Name: _____

Signature: _____

Date: _____

15. DATA MANAGEMENT

15.1 Data Collection

All study data will be collected prospectively using standardized Case Report Forms (CRFs) specifically developed for this study. Data will be obtained from participant interviews, medical records, physical examinations, neurological assessments, and laboratory analyses.

Each participant will be assigned a unique study identification number to ensure confidentiality. Personal identifiers will not be entered into the study database.

Collected data will include:

- Demographic characteristics
- Medical history
- Duration of diabetes
- Current medications
- Anthropometric measurements
- Neurological examination findings
- Electrophysiological examination results
- Laboratory measurements
- Serum irisin concentration
- HbA1c

- Fasting plasma glucose
- Lipid profile
- Body Mass Index

15.2 Data Entry

Data will be entered into a secure electronic database by trained research personnel.

Double-data entry procedures will be implemented for critical variables to minimize transcription errors.

Data accuracy will be verified by periodic comparison with the original source documents.

15.3 Data Storage

Electronic data will be stored on password-protected institutional computers with restricted access.

Paper documents will be stored in locked filing cabinets within the Department of Internal Medicine.

Biological samples will be labeled using only the participant study identification number.

Serum samples will be stored at **−80°C** until laboratory analyses have been completed.

15.4 Confidentiality

All participant information will remain strictly confidential.

Only authorized investigators will have access to identifiable study data.

Results will be reported only in aggregate form, ensuring that individual participants cannot be identified.

All study procedures will comply with institutional policies regarding data protection and confidentiality.

15.5 Data Retention

Study documents will be retained for the period required by institutional regulations and applicable national legislation.

Following completion of the required retention period, paper records will be securely destroyed and electronic data permanently deleted according to institutional policies.

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16. QUALITY ASSURANCE

To ensure data quality and protocol compliance, several quality assurance procedures will be implemented throughout the study.

These procedures include:

- Investigator training before study initiation.
- Standardized operating procedures for participant recruitment.
- Standardized neurological examinations.
- Standardized blood collection procedures.
- Standardized laboratory analyses.
- Calibration of laboratory equipment.
- Routine monitoring of data completeness.
- Verification of informed consent documentation.
- Periodic review of Case Report Forms.
- Internal quality control of ELISA measurements.

Laboratory personnel performing serum irisin analyses will be blinded to participant group allocation.

Any protocol deviations will be documented and reviewed by the principal investigator.

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17. ETHICAL CONSIDERATIONS

The study will be conducted in accordance with:

- The Declaration of Helsinki (2013 revision)
- Good Clinical Practice (ICH-GCP) principles applicable to observational studies
- Turkish regulations governing biomedical research involving human participants

Ethical approval has been obtained from the University of Health Sciences Türkiye Clinical Research Ethics Committee before initiation of participant recruitment.

Written informed consent will be obtained from every participant before any study-specific procedure is performed.

Participation is entirely voluntary.

Participants may withdraw from the study at any time without providing a reason and without affecting their future medical care.

No experimental intervention will be administered during the study.

Only one fasting venous blood sample will be collected.

Potential risks are minimal and are limited to temporary discomfort, bruising, or minor bleeding associated with venipuncture.

Participant privacy and confidentiality will be protected throughout the study.

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18. SAFETY CONSIDERATIONS

As this is a non-interventional observational study, no study-related treatment will be administered.

The only study-related procedure associated with minimal risk is venous blood collection.

Any unexpected adverse event occurring during blood sampling will be managed according to standard clinical practice.

Serious adverse events are not anticipated because no investigational medicinal product or medical device is being evaluated.

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19. DISSEMINATION POLICY

Study findings will be disseminated regardless of the direction or magnitude of the results.

Results may be presented at national and international scientific meetings.

The findings may also be submitted for publication in peer-reviewed international journals.

No participant-identifiable information will be disclosed in any publication or presentation.

Authorship will follow the recommendations of the International Committee of Medical Journal Editors (ICMJE).

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20. DATA SHARING STATEMENT

Individual participant data (IPD) will not be made publicly available.

Only anonymized, aggregated study results will be reported in scientific publications and conference presentations.

Requests for additional analyses may be considered by the principal investigator following publication of the primary study results and in accordance with institutional policies.

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21. STUDY LIMITATIONS

Potential limitations of the study include:

- Single-center study design.
- Relatively small sample size.
- Cross-sectional assessment precluding causal inference.
- Single baseline measurement of serum irisin concentration.
- Possible influence of unmeasured confounding variables.

Despite these limitations, the study is expected to provide valuable information regarding the association between serum irisin concentrations and diabetic peripheral neuropathy.

Multiple linear regression analysis will be performed to determine independent predictors of serum irisin concentration after adjustment for potential confounding variables.

Potential covariates include:

- Age
- Sex
- BMI
- HbA1c
- Duration of diabetes

Missing Data

Incomplete datasets will be excluded from analyses requiring complete observations. The frequency and reasons for missing data will be reported.

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14. INFORMED CONSENT FORM (ICF)

Purpose of the Study

You are invited to participate in a research study investigating whether serum irisin levels may serve as a biomarker for diabetic peripheral neuropathy.

Study Procedures

If you agree to participate, you will undergo:

- Clinical assessment
- Neurological examination
- Collection of one fasting blood sample (approximately 10 mL)
- Routine laboratory measurements
- Serum irisin analysis

Participation is expected to require approximately **30–45 minutes**.

Possible Risks

The study involves minimal risk. Blood collection may cause temporary discomfort, bruising, or rarely minor bleeding or infection.

Possible Benefits

Participants may not receive direct medical benefit. However, the results may contribute to improved understanding of diabetic peripheral neuropathy and future patient care.

Confidentiality

Your personal information will remain confidential. Study data will be coded and stored securely. Only authorized research personnel will have access to identifiable information.