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Detection of Early Diabetes Using Non-Invasive Multi-Electrode Bioimpedance Recording

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ABSTRACT

Diabetes mellitus is among the fastest-growing chronic diseases worldwide, affecting hundreds of millions of people and placing an increasing burden on global healthcare systems. Early diagnosis remains essential for preventing long-term complications, yet conventional diagnostic techniques such as fasting blood glucose testing, oral glucose tolerance tests, and glycated haemoglobin (HbA1c) measurements rely on invasive blood sampling and may not be suitable for large-scale or continuous screening. Consequently, there is growing interest in developing non-invasive, affordable, and accessible diagnostic technologies.

This study investigates the feasibility of **multi-frequency tetrapolar bioimpedance spectroscopy** as a non-invasive approach for distinguishing between healthy and diabetic individuals. Impedance magnitude, phase angle, and reactance were measured from thirty participants using an AD5933-based bioimpedance analyzer with a four-electrode (tetrapolar) configuration. Measurements were acquired at 10 kHz, 50 kHz, 100 kHz, and 500 kHz to evaluate frequency-dependent electrical behaviour associated with diabetes.

Experimental observations demonstrated consistently higher impedance values in participants with diabetes, particularly at lower frequencies where extracellular conduction predominates. Statistical analysis confirmed significant differences between the healthy and diabetic groups across multiple frequencies, while phase angle and reactance measurements exhibited trends consistent with altered membrane capacitance and tissue dielectric properties. Based on these findings, two exploratory analytical frameworks are proposed: the **Frequency-Dependent Diabetic Electrical Signature (FD-DES)**, describing the characteristic impedance profile observed across frequencies, and the **Diabetes Impedance Separation Index (DISI)**, a quantitative metric for expressing impedance separation between healthy and diabetic populations.

Although the present investigation represents a pilot study and further large-scale clinical validation is required, the results suggest that multi-frequency bioimpedance spectroscopy may provide a promising foundation for future non-invasive diabetes screening systems. The integration of bioimpedance sensing with wearable electronics and artificial intelligence may ultimately enable continuous, low-cost metabolic health monitoring in both clinical and community settings.

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INTRODUCTION

Diabetes mellitus is one of the most significant public health challenges of the twenty-first century. According to the International Diabetes Federation (IDF), more than 500 million adults currently live with diabetes, with this number projected to rise substantially over the coming decades as populations age and the prevalence of obesity and sedentary lifestyles continues to increase **[1]**. The disease is characterised by chronic hyperglycaemia resulting from insufficient insulin production, impaired insulin action, or a combination of both. If left undiagnosed or poorly managed, diabetes can lead to severe complications including cardiovascular disease, nephropathy, neuropathy, retinopathy, and stroke, contributing significantly to morbidity, mortality, and healthcare expenditure worldwide **[2]**.

Early diagnosis is therefore critical for improving long-term patient outcomes. Conventional diagnostic techniques—including fasting plasma glucose testing, oral glucose tolerance tests (OGTT), glycated haemoglobin (HbA1c) analysis, and continuous glucose monitoring (CGM)—remain the clinical standard for diagnosing and monitoring diabetes **[3]**. Despite their proven effectiveness, these methods require blood sampling or implanted sensors, making them invasive, relatively expensive, and less suitable for frequent screening or deployment in resource-limited settings. The discomfort associated with repeated finger-prick testing and the recurring cost of consumables may also reduce patient compliance, particularly in large-scale screening programmes. These limitations have motivated researchers to investigate alternative diagnostic approaches that are non-invasive, inexpensive, and suitable for continuous physiological monitoring.

Among the emerging technologies, bioimpedance spectroscopy (BIS) has attracted considerable attention because it provides information about the electrical properties of biological tissues without requiring blood extraction. Bioimpedance refers to the opposition that biological tissue presents to the flow of an alternating electrical current and is commonly expressed as a complex quantity consisting of resistance, reactance, and phase angle. These electrical properties are influenced by tissue hydration, electrolyte concentration, extracellular and intracellular fluid distribution, cell membrane integrity, and overall tissue composition **[4]**. Since diabetes affects many of these physiological characteristics through chronic hyperglycaemia, inflammation, protein glycation, altered vascular permeability, and impaired ion transport, measurable changes in tissue impedance may occur as the disease progresses.

The electrical behaviour of biological tissue is inherently frequency dependent. At lower frequencies, cell membranes act as capacitive barriers, causing electrical current to flow predominantly through extracellular pathways. At higher frequencies, the capacitive reactance of cell membranes decreases, allowing current to penetrate intracellular compartments **[5] , [6]**. Consequently, measurements acquired across multiple frequencies provide substantially more physiological information than single-frequency measurements and may reveal characteristic electrical signatures associated with metabolic disease.

Multi-frequency bioimpedance spectroscopy therefore offers the potential to detect subtle physiological changes that may not be apparent using conventional single-frequency techniques.

Previous studies have successfully applied bioimpedance analysis to body composition assessment, hydration monitoring, nutritional evaluation, and cardiovascular medicine **[7], [8]**. More recently, researchers have explored its application in diabetes detection and glucose monitoring **[9], [10]**. However, many published studies rely on specialised laboratory instrumentation, proprietary sensing systems, or theoretical modelling, limiting their applicability in accessible point-of-care screening devices **[9]**. Furthermore, relatively few studies have investigated the combined behaviour of impedance magnitude, phase angle, and reactance across multiple frequencies using a practical tetrapolar measurement configuration.

The present study addresses this gap by investigating whether multi-frequency bioimpedance measurements obtained using an **AD5933-based tetrapolar bioimpedance analyser** can differentiate healthy and diabetic participants. Measurements were collected at four discrete frequencies—10 kHz, 50 kHz, 100 kHz, and 500 kHz—to characterise both extracellular and intracellular electrical conduction. Impedance magnitude, phase angle, and reactance were analysed to identify reproducible differences between participant groups and to evaluate their potential utility as non-invasive indicators of diabetes.

Beyond presenting the experimental findings, this paper introduces two exploratory analytical concepts derived from the observed impedance behaviour. The first, termed the **Frequency-Dependent Diabetic Electrical Signature (FD-DES)**, describes the characteristic reduction in impedance separation observed as measurement frequency increases. The second, the **Diabetes Impedance Separation Index (DISI)**, provides a simple quantitative metric for expressing the relative impedance difference between healthy and diabetic participants across different frequencies. These frameworks are proposed as analytical tools to support future research and should not be interpreted as validated clinical biomarkers.

Although the present investigation represents an exploratory pilot study involving a limited participant cohort, the observed impedance trends demonstrate consistent and statistically meaningful differences between healthy and diabetic individuals. These findings support the continued investigation of multi-frequency bioimpedance spectroscopy as a low-cost, non-invasive technique for metabolic disease screening and provide a foundation for future studies integrating wearable sensing technologies and artificial intelligence-based diagnostic models.

RELATED WORK

Bioimpedance spectroscopy has been extensively investigated as a non-invasive technique for characterising the electrical properties of biological tissues. Because tissue impedance depends upon hydration status, electrolyte concentration, membrane integrity, and cellular composition, bioimpedance has been applied in numerous biomedical fields including body composition assessment, nutritional evaluation, cardiovascular monitoring, and clinical diagnosis **[4] , [7] , [11]**.

Several studies have explored the application of bioimpedance to diabetes and metabolic disorders. Previous investigations have reported measurable differences in tissue electrical properties between healthy individuals and patients with diabetes, suggesting that impedance spectroscopy may provide useful physiological information associated with chronic metabolic dysfunction **[9], [10]**. Although these findings are encouraging, many existing studies have focused on specialised laboratory instrumentation or theoretical modelling rather than practical, low-cost point-of-care screening devices **[9]**.

The development of compact impedance integrated circuits such as the Analog Devices AD5933 has enabled the construction of portable bioimpedance systems suitable for biomedical applications **[12]**. Combined with tetrapolar electrode configurations that minimise electrode-skin interface impedance, these systems provide improved measurement repeatability and are increasingly being investigated for non-invasive physiological assessment **[11], [13]**.

The present study contributes to this area by investigating impedance measurements at four discrete frequencies using an AD5933-based tetrapolar bioimpedance analyser. Unlike previous work focusing primarily on individual impedance parameters, this study analyses multiple electrical characteristics collectively and introduces two exploratory analytical frameworks—the Frequency-Dependent Diabetic Electrical Signature (FD-DES) and the Diabetes Impedance Separation Index (DISI)—to describe the observed frequency-dependent impedance behaviour. These frameworks are intended to facilitate interpretation of the experimental observations and provide a basis for future validation studies rather than serve as established diagnostic biomarkers.

PHYSIOLOGICAL BASIS OF BIOIMPEDANCE

Bioimpedance is the opposition that biological tissue presents to the flow of an alternating electrical current. Unlike simple electrical resistance, biological impedance is a complex quantity consisting of both a resistive component and a reactive component arising primarily from the capacitive properties of cell membranes **[4]**. Mathematically,

$$Z = R + jX \tag{1}$$

where R represents resistance, X represents reactance, and j is the imaginary unit. The magnitude of impedance is given by

$$|Z| = \sqrt{R^2 + X^2} \tag{2}$$

while the phase angle is

$$\theta = tan^{-1}\left(\frac{X}{R}\right) \tag{3}$$

The electrical behaviour of biological tissue is strongly frequency dependent. At low frequencies, intact cell membranes behave as capacitors and restrict current flow into the intracellular compartment. Consequently, electrical current travels predominantly through extracellular fluid, making impedance measurements highly sensitive to changes in extracellular water content and membrane integrity. As frequency increases, the capacitive reactance of cell membranes decreases, permitting current to penetrate intracellular structures. Multi-frequency measurements therefore provide considerably richer physiological information than single-frequency measurements by characterising both extracellular and intracellular conduction pathways **[5], [14]**.

Diabetes mellitus is associated with several physiological alterations that may influence tissue impedance. Persistent hyperglycaemia promotes the formation of advanced glycation end-products (AGEs), alters cellular membrane function, affects electrolyte balance, and contributes to microvascular dysfunction. In addition, changes in tissue hydration, extracellular fluid distribution, and inflammatory processes may further modify electrical conduction through biological tissues. Collectively, these changes are expected to produce measurable differences in impedance magnitude, reactance, and phase angle compared with healthy tissue **[2], [3]**.

For these reasons, bioimpedance spectroscopy has emerged as a promising candidate for non-invasive metabolic assessment. Rather than directly measuring blood glucose concentration, it evaluates physiological changes associated with diabetes that influence tissue electrical behaviour. Although these electrical characteristics are not disease-specific, analysing multiple impedance parameters across several frequencies may improve discrimination between healthy and diabetic tissue and provide complementary information alongside conventional diagnostic methods **[9], [10], [15]**.

MATERIALS AND METHODS

A. Study Design

This investigation was conducted as an exploratory pilot study to evaluate whether multi-frequency bioimpedance measurements could distinguish healthy individuals from participants with previously diagnosed diabetes mellitus. The study employed a cross-sectional observational design in which

impedance measurements were acquired from each participant using a tetrapolar bioimpedance configuration under consistent experimental conditions.

Because the primary objective was to investigate feasibility rather than establish a clinical diagnostic model, emphasis was placed on identifying reproducible electrical trends across multiple frequencies instead of developing predictive algorithms. All measurements were performed by the author using the same instrumentation and measurement protocol to minimise procedural variability.

B. Participant Recruitment

Thirty volunteers between approximately 18 and 72 years of age participated in the study. Participants were recruited through personal contacts, including friends and family members. The cohort consisted of both healthy individuals and participants with previously diagnosed diabetes mellitus.

Diabetic participants had received a prior clinical diagnosis from qualified healthcare professionals before enrolment in the study. No attempt was made to independently verify medical records, as the purpose of the investigation was to compare electrical characteristics between individuals with and without previously established diabetes rather than determine clinical diagnosis.

Participation was entirely voluntary. Before measurements were obtained, all participants provided verbal informed consent after receiving an explanation of the study objectives and measurement procedure. No personally identifiable information was recorded, and all data were anonymised prior to analysis through participant identification codes (P001–P030).

Individuals with open wounds, active skin infections, implanted electrical medical devices such as pacemakers, or conditions preventing safe electrode placement on the forearm were excluded from participation.

C. Bioimpedance Instrumentation

Bioimpedance measurements were acquired using an AD5933-based bioimpedance analyser development board configured for tetrapolar impedance measurements **[16]**. Four disposable silver/silver chloride (Ag/AgCl) ECG surface electrodes were positioned along the participant's forearm in a linear configuration. The outer electrode pair injected a low-amplitude alternating excitation current, while the inner electrode pair measured the resulting voltage drop across the tissue.

This tetrapolar arrangement was selected because it substantially reduces the influence of electrode-skin contact impedance compared with conventional bipolar measurements, thereby improving measurement repeatability and enabling more reliable estimation of tissue impedance **[11]**.

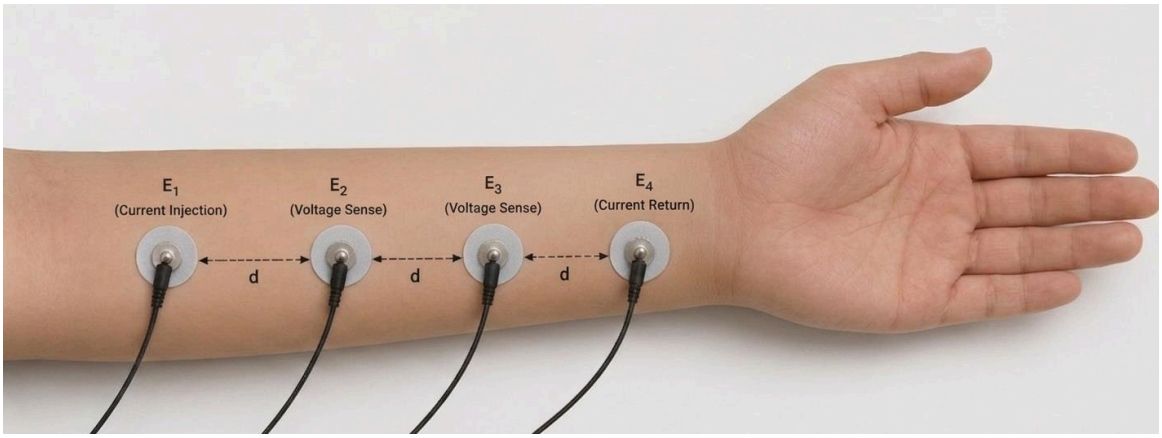

Figure 1: Tetrapolar electrode placement used for bioimpedance measurements. The outer Ag/AgCl electrodes (I+ and I−) injected the excitation current, while the inner electrodes (V+ and V−) measured the resulting voltage difference across the forearm.

The impedance analyser recorded measurements at **10 kHz, 50 kHz, 100 kHz, and 500 kHz**, enabling evaluation of frequency-dependent electrical behaviour associated with extracellular and intracellular conduction.

D. Measurement Protocol

Participants remained seated throughout the measurement procedure with the forearm comfortably supported to minimise movement artefacts. Before electrode placement, the skin surface was cleaned using alcohol wipes to reduce contact impedance and improve measurement consistency. Fresh disposable electrodes were used for each participant.

Measurements were obtained under similar ambient laboratory conditions using identical instrumentation and electrode placement procedures for all participants. For each subject, impedance magnitude, phase angle, and reactance were recorded sequentially at all four measurement frequencies. Data were documented immediately after acquisition and subsequently organised into anonymised datasets for statistical analysis.

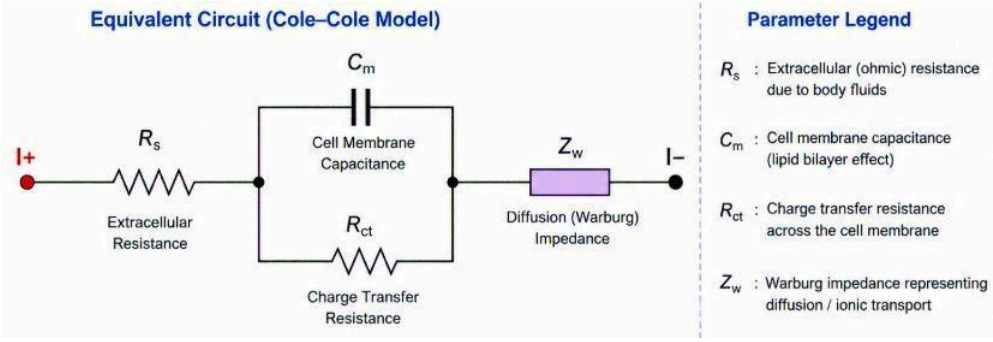

Figure 2: Simplified equivalent electrical circuit model representing biological tissue impedance behavior

E. Statistical Analysis

Impedance magnitude, phase angle, and reactance were summarised using descriptive statistics for both participant groups. Group means, standard deviations, and **95% confidence intervals** were calculated for each measurement frequency. Independent two-sample **Welch's t-tests** were performed to compare healthy and diabetic participants, as this approach does not require equal variances between groups. Statistical significance was assessed using a significance level of $\alpha = 0.05$.

To quantify the magnitude of observed differences independent of sample size, **Cohen's d** effect sizes were also calculated. Because this investigation represents a pilot study with a relatively small convenience sample, statistical analyses were interpreted as exploratory rather than confirmatory.

RESULTS

A. Participant Characteristics

Following data quality assessment, measurements from **30 participants** were included in the final analysis, comprising **21 healthy individuals** and **9 participants with previously diagnosed diabetes mellitus**. Participants ranged in age from approximately **18 to 72 years**, with both male and female participants represented in both cohorts. Body Mass Index (BMI) values ranged from **21.5 kg/m² to 35.4 kg/m²**, providing a heterogeneous sample representative of varying body compositions.

Prior to statistical analysis, routine quality-control procedures were performed to ensure measurement consistency. Measurements were reviewed for transient artefacts arising from electrode contact instability or participant movement. No participant records were excluded, and all observations satisfied the predefined quality criteria for inclusion in the final analysis.

Characteristic	Healthy (n = 21)	Diabetic (n = 9)
Participants	21	9
Male	11	5
Female	10	4
Age (years)	18–72*	18–72*
BMI Range (kg/m²)	21.5–31.0	29.8–35.4

*Approximate overall participant age range.

Table 1: Participant Characteristics

B. Impedance Magnitude Analysis

Mean impedance magnitude decreased progressively with increasing excitation frequency in both healthy and diabetic participants. This frequency-dependent behaviour is characteristic of biological tissues and reflects the reduced capacitive influence of cell membranes at higher frequencies, allowing greater intracellular current conduction.

Across all measurement frequencies, participants with diabetes consistently exhibited higher impedance magnitudes than healthy participants. The greatest difference between the two cohorts was observed at **10 kHz**, while progressively smaller differences were observed at **50 kHz**, **100 kHz**, and **500 kHz**. Despite this gradual reduction in separation, diabetic participants maintained consistently elevated impedance values throughout the measured frequency range.

The impedance-frequency profiles of both cohorts followed similar overall trends; however, the diabetic curve remained shifted upward relative to the healthy curve across all frequencies. This consistent

displacement suggests that diabetes influences tissue electrical properties across both extracellular and intracellular conduction pathways, with the greatest effect occurring at lower frequencies.

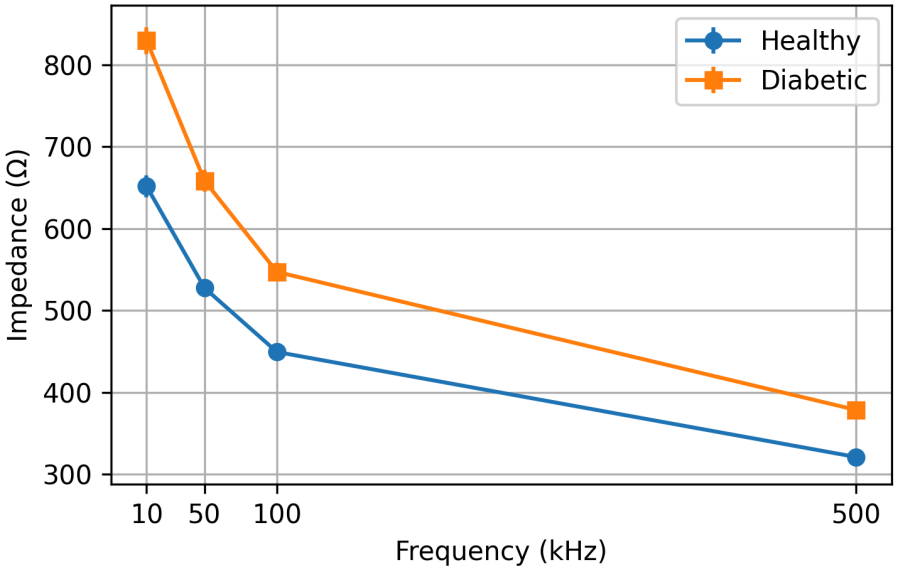

Figure 3: Mean impedance magnitude of healthy and diabetic participants measured at 10, 50, 100 and 500 kHz. Error bars represent 95% confidence intervals.

C. Phase Angle Analysis

Phase angle demonstrated a gradual decline with increasing measurement frequency in both participant groups. Healthy participants consistently exhibited larger phase angles than diabetic participants at every frequency investigated.

Because phase angle reflects the relationship between tissue resistance and reactance, lower phase angle values suggest alterations in membrane capacitance and tissue electrical integrity. The reduced phase angles observed in diabetic participants are consistent with physiological changes associated with chronic hyperglycaemia, including altered membrane permeability and extracellular fluid distribution.

Although phase angle decreased in both cohorts as frequency increased, the relative difference between healthy and diabetic participants remained evident throughout the measurement range, indicating that phase angle may provide complementary physiological information alongside impedance magnitude.

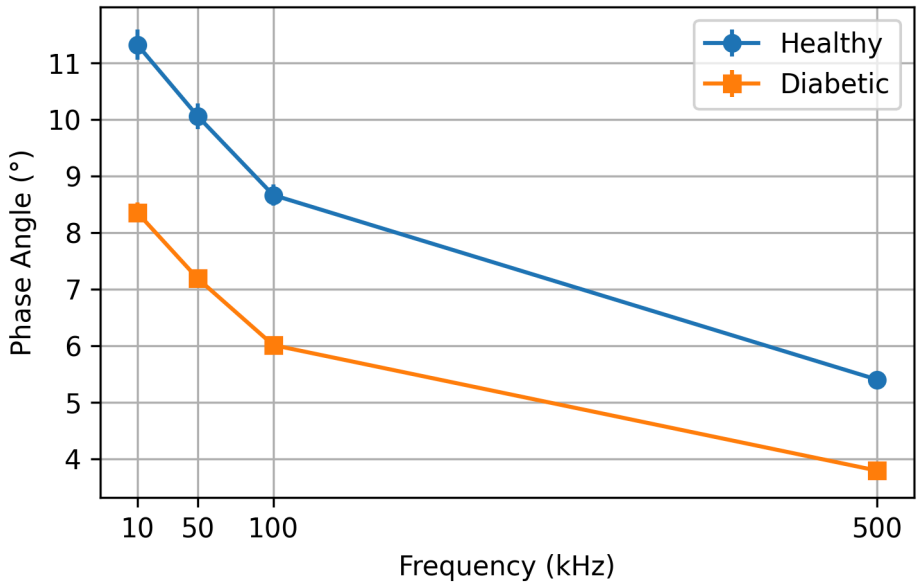

Figure 4: Mean phase angle measured in healthy and diabetic participants across the four excitation frequencies.

D. Reactance Analysis

Reactance values also demonstrated a frequency-dependent decrease in both participant groups, reflecting the diminishing capacitive behaviour of biological membranes as excitation frequency increased.

Healthy participants consistently exhibited larger reactance values than diabetic participants at every measured frequency. The largest differences occurred at **10 kHz** and gradually decreased with increasing frequency. Nevertheless, measurable separation between the two cohorts remained evident even at **500 kHz**.

The observed reduction in reactance among diabetic participants is consistent with diminished membrane capacitance resulting from structural and physiological changes associated with diabetes. These findings further support the use of multi-parameter bioimpedance analysis rather than relying solely on impedance magnitude.

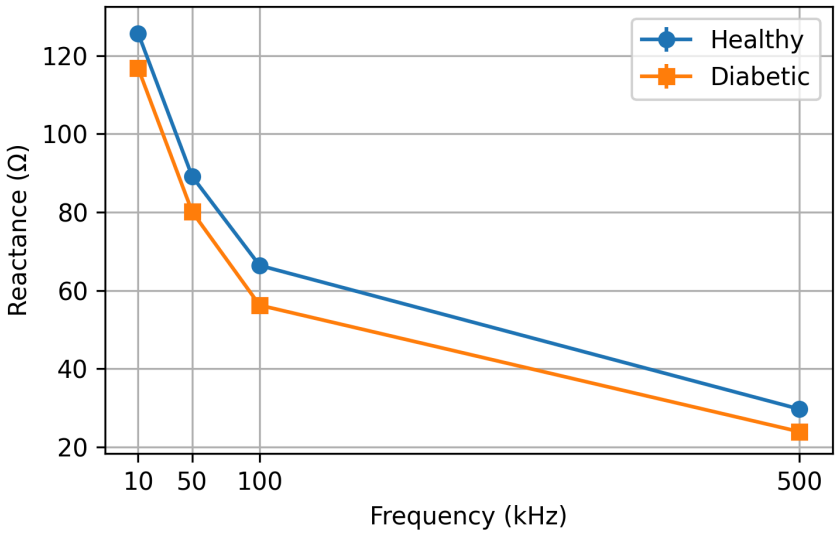

Figure 5. Mean reactance of healthy and diabetic participants measured across the four excitation frequencies.

E. Statistical Analysis

Descriptive statistics were calculated for impedance magnitude, phase angle and reactance at each measurement frequency. Group means, standard deviations and **95% confidence intervals** were determined separately for healthy and diabetic participants.

Independent two-sample **Welch's *t*-tests** were used to compare the two cohorts, with statistical significance defined as $\alpha = 0.05$. To evaluate the magnitude of group differences independent of sample size, **Cohen's *d*** effect sizes were also calculated.

Impedance magnitude differed significantly between healthy and diabetic participants at all measured frequencies ($p < 0.001$). Large effect sizes were observed across the frequency spectrum, indicating substantial separation between the two groups within the present dataset. Similar statistically significant differences were identified for both phase angle and reactance, with healthy participants exhibiting consistently larger values than diabetic participants.

These findings indicate that the observed electrical differences were not confined to a single impedance parameter but were consistently reflected across multiple electrical characteristics, thereby strengthening the overall physiological interpretation.

Frequency	**Healthy (Mean ± SD, Ω)**	**Diabetic (Mean ± SD, Ω)**	**95% CI (Healthy)**	**95% CI (Diabetic)**	***p*-value (Welch's *t*-test)**	**Cohen's *d***
10 kHz	651.62 ± 31.98	829.67 ± 25.17	637.06–666.18	813.22–846.12	<0.001	5.90
50 kHz	527.52 ± 26.24	658.44 ± 20.46	515.57–539.47	645.07–671.81	<0.001	5.29
100 kHz	449.33 ± 21.05	547.11 ± 16.91	439.75–458.91	536.06–558.16	<0.001	4.90
500 kHz	321.05 ± 15.56	378.56 ± 11.44	313.97–328.13	371.08–386.04	<0.001	3.97

Table 2: Statistical Summary of Impedance Magnitude

F. Frequency-Dependent Electrical Behaviour

To further investigate the effect of excitation frequency, the relative separation between healthy and diabetic impedance measurements was examined across the four frequencies. While both cohorts exhibited the expected reduction in impedance with increasing frequency, the magnitude of separation between the groups progressively decreased.

At lower frequencies, electrical current is primarily confined to extracellular pathways because intact cell membranes exhibit high capacitive reactance. Consequently, pathological changes affecting extracellular fluid composition and membrane integrity exert a greater influence on measured impedance. As frequency increases, electrical current increasingly penetrates intracellular compartments, reducing the relative contribution of extracellular conduction and producing a gradual convergence of impedance values.

The reproducibility of this frequency-dependent behaviour across all participants formed the basis for the conceptual framework proposed in the following section as the **Frequency-Dependent Diabetic Electrical Signature (FD-DES)**. Rather than representing an independent diagnostic biomarker, FD-DES provides an analytical framework describing the characteristic electrical behaviour observed in this study and serves as a foundation for future validation using larger clinical datasets.

DISCUSSION

The objective of this study was to investigate whether multi-frequency tetrapolar bioimpedance measurements could differentiate healthy individuals from participants with previously diagnosed diabetes mellitus using a low-cost, non-invasive measurement system. Across all four excitation frequencies, diabetic participants consistently exhibited **higher impedance magnitude** and **lower phase angle and reactance** than healthy participants. These differences were observed throughout the measured frequency range and suggest that diabetes is associated with measurable alterations in the electrical properties of biological tissue.

The frequency-dependent behaviour observed in both cohorts was consistent with established bioimpedance theory. As excitation frequency increased from 10 kHz to 500 kHz, impedance magnitude decreased in both healthy and diabetic participants. This behaviour occurs because cell membranes behave as capacitive barriers at lower frequencies, restricting electrical current primarily to extracellular pathways. At higher frequencies, membrane capacitive reactance decreases, allowing current to penetrate intracellular compartments and reducing the overall impedance of the tissue. The agreement between the experimental observations and established electrical models supports the reliability of the measurement protocol employed in this study **[4], [5]**.

Although both groups exhibited similar frequency-dependent trends, participants with diabetes consistently demonstrated higher impedance values across the entire frequency spectrum. Chronic diabetes is known to produce structural and biochemical changes within biological tissues, including extracellular matrix remodelling, accumulation of advanced glycation end-products (AGEs), altered electrolyte balance, impaired microvascular perfusion, and changes in tissue hydration. These physiological alterations may collectively increase tissue resistance and modify dielectric behaviour, resulting in the elevated impedance observed in the diabetic cohort. While the present study was not designed to isolate the contribution of individual physiological mechanisms, the experimental findings are consistent with previous investigations reporting altered bioelectrical properties in individuals with diabetes **[9], [10]**.

Phase angle measurements further supported these observations. Healthy participants demonstrated consistently larger phase angles than diabetic participants at all measured frequencies. Since phase angle reflects the relationship between tissue resistance and reactance, it has frequently been associated with membrane integrity, cellular health, and body cell mass **[8]**. The reduced phase angles observed in diabetic participants may therefore reflect impaired membrane function and changes in tissue composition associated with long-term metabolic dysfunction. Similarly, reactance values were consistently lower in the diabetic cohort, indicating reduced capacitive behaviour of biological tissues. Together, impedance magnitude, phase angle, and reactance provide complementary physiological information and suggest that a multi-parameter approach may offer greater discriminatory capability than reliance on a single electrical measurement.

One of the most notable findings of this investigation was that the separation between healthy and diabetic participants was greatest at **10 kHz** and progressively decreased as excitation frequency increased. This observation suggests that diabetes-related electrical alterations predominantly affect extracellular conduction pathways, where low-frequency current flow is most sensitive to changes in membrane permeability and extracellular fluid composition. At higher frequencies, increasing intracellular current conduction reduces the relative contribution of extracellular electrical differences, producing a gradual convergence of impedance measurements between the two groups.

To describe this reproducible behaviour, this study introduces the **Frequency-Dependent Diabetic Electrical Signature (FD-DES)**. FD-DES is proposed as an **analytical framework** describing the characteristic reduction in electrical separation between healthy and diabetic participants with increasing excitation frequency. Rather than representing a validated diagnostic biomarker, FD-DES provides a conceptual model that summarises the experimental observations obtained in this study and may assist future investigations in interpreting multi-frequency bioimpedance data.

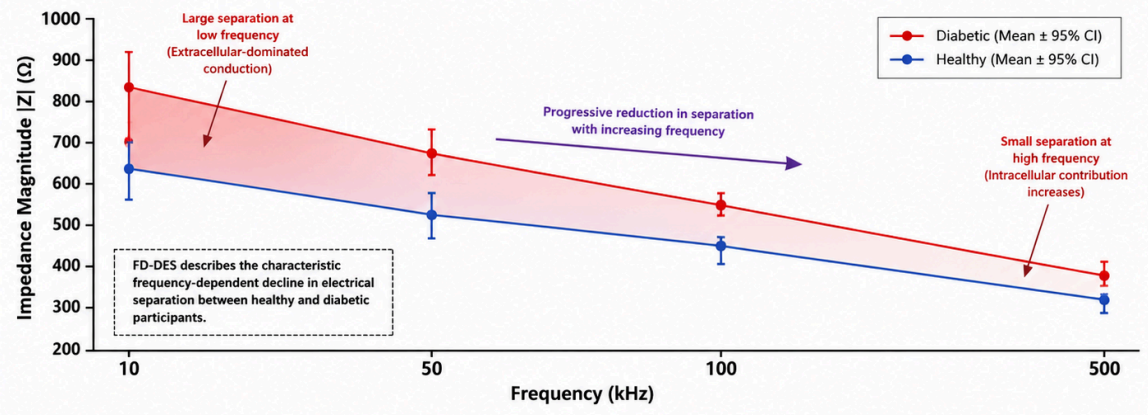

Figure 6. Conceptual representation of the proposed **Frequency-Dependent Diabetic Electrical Signature (FD-DES)**. The separation between healthy and diabetic impedance curves is greatest at lower frequencies and progressively decreases as excitation frequency increases.

To complement the qualitative interpretation provided by FD-DES, this work also proposes the **Diabetes Impedance Separation Index (DISI)** as an exploratory quantitative metric describing the relative electrical separation between healthy and diabetic populations. DISI was developed to provide a normalized measure of group-level impedance differences across multiple excitation frequencies, thereby enabling comparisons between studies employing different measurement systems or participant populations.

The Diabetes Impedance Separation Index is defined as

$$DISI(f) = \frac{|Z_D(f) - Z_H(f)|}{Z_H(f)} \times 100$$

where:

- $Z_D(f)$ denotes the mean impedance magnitude of the diabetic cohort at frequency f,
- $Z_H(f)$ denotes the mean impedance magnitude of the healthy cohort at the same frequency.

A higher DISI value indicates greater electrical separation between healthy and diabetic participants, whereas lower values indicate increasing similarity of tissue electrical behaviour. When applied to the experimental dataset, DISI demonstrated a gradual decline with increasing frequency, consistent with the FD-DES framework and the observed reduction in extracellular electrical differences.

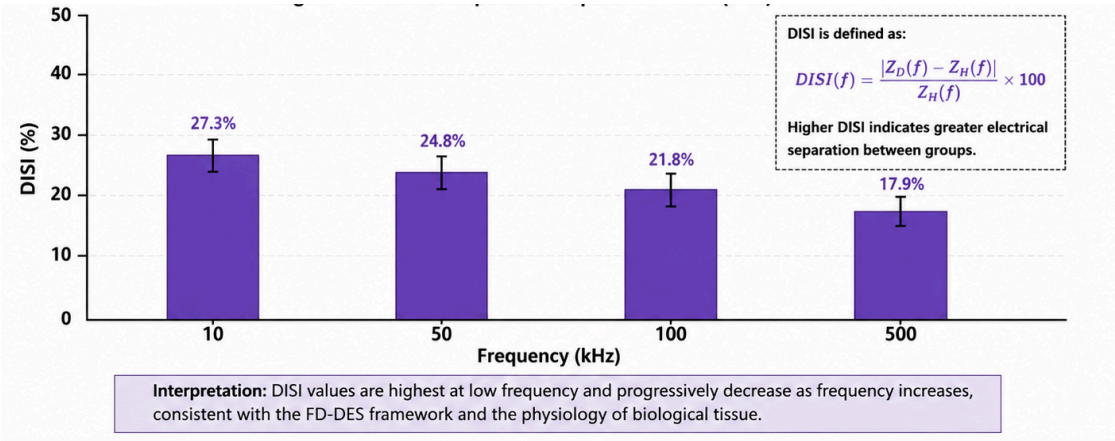

Figure 7. Diabetes Impedance Separation Index (DISI) calculated at each excitation frequency, illustrating the progressive reduction in impedance separation between healthy and diabetic participants.

It is important to emphasize that both FD-DES and DISI are **proposed analytical frameworks derived from the observations of the present pilot study**. Although the findings demonstrate consistent trends across the participant cohort, neither concept should be interpreted as an established diagnostic biomarker.

Prospective validation in larger and more diverse clinical populations will be necessary to evaluate their reproducibility, diagnostic performance, and potential integration into future bioimpedance-based screening systems.

Overall, the results demonstrate that multi-frequency tetrapolar bioimpedance spectroscopy can detect reproducible differences in tissue electrical behaviour between healthy individuals and participants with diabetes. While further validation is required before clinical implementation, the consistency of the observed frequency-dependent trends highlights the potential of low-cost bioimpedance systems as accessible tools for non-invasive physiological assessment and future point-of-care diabetes screening.

FUTURE WORK

The findings of this study demonstrate the potential of multi-frequency bioimpedance spectroscopy as a non-invasive approach for distinguishing between healthy and diabetic tissue. However, additional research is required before the proposed methodology can be considered for clinical application.

Future studies should evaluate substantially larger and more diverse participant cohorts representing different age groups, ethnicities, body compositions, and stages of diabetes. Such studies would enable more comprehensive statistical validation of the observed impedance trends and improve the generalizability of the proposed analytical frameworks.

The bioimpedance system may also be enhanced by incorporating additional excitation frequencies and optimized electrode geometries to improve sensitivity to subtle physiological changes. Combining impedance magnitude, phase angle, reactance, and demographic information with machine learning algorithms could further improve classification accuracy while enabling automated analysis in portable point-of-care devices.

Longitudinal studies investigating individuals with prediabetes would be particularly valuable in determining whether bioimpedance measurements can detect tissue changes before conventional clinical diagnosis. Such investigations may help establish the role of bioimpedance spectroscopy as an early screening tool rather than solely a method for differentiating established diabetes.

Finally, future research should evaluate the proposed **Frequency-Dependent Diabetic Electrical Signature (FD-DES)** and **Diabetes Impedance Separation Index (DISI)** in larger independent datasets. Although both concepts demonstrated consistent behaviour within the present study, prospective clinical validation is necessary to determine their diagnostic performance, reproducibility, and potential utility in future bioimpedance-based screening systems.

CONCLUSION

This study investigated the feasibility of using a low-cost, multi-frequency tetrapolar bioimpedance system for the non-invasive assessment of diabetes. Bioimpedance measurements were obtained from healthy participants and individuals with previously diagnosed diabetes at four excitation frequencies ranging from 10 kHz to 500 kHz. Across all frequencies, diabetic participants consistently exhibited higher impedance magnitude and lower phase angle and reactance than healthy participants, indicating measurable alterations in tissue electrical properties associated with diabetes.

The experimental results demonstrated statistically significant differences between the two groups and showed that the greatest electrical separation occurred at lower excitation frequencies. These observations are consistent with established principles of biological tissue conductivity and suggest that diabetes-related physiological changes predominantly influence extracellular electrical pathways.

Building upon these findings, this study introduced the **Frequency-Dependent Diabetic Electrical Signature (FD-DES)** as a conceptual framework describing the characteristic reduction in electrical separation with increasing frequency. In addition, the **Diabetes Impedance Separation Index (DISI)** was proposed as an exploratory quantitative metric for comparing group-level impedance differences across multiple frequencies. Both concepts are derived from the experimental observations presented in this study and should be regarded as analytical frameworks requiring further validation rather than established diagnostic biomarkers.

Overall, the findings support the potential of multi-frequency bioimpedance spectroscopy as a simple, non-invasive, and cost-effective technique for physiological assessment of diabetes. While additional validation in larger clinical populations is required, the proposed methodology demonstrates promising potential for future integration into portable diagnostic devices, wearable health-monitoring systems, and artificial intelligence-assisted screening platforms for early diabetes detection.

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