

Dear Editor:

On behalf of my coauthors, we thank you very much for giving us an opportunity to revise our manuscript (No. 101966). We appreciate the editor and reviewers very much for their positive and constructive comments and suggestions.

We have carefully studied the reviewer's comments and incorporated revisions into the manuscript, highlighted in yellow. We have done our best to address all the feedback provided.

Both the first version of the manuscript and the revised version of the manuscript were edited by American Journal Experts (<http://www.aje.com>), and a certificate is included along with the revised manuscript.

We would like to express our appreciation to you and the reviewers for your feedback. We look forward to hearing from you.

Thank you and best regards.

Yours sincerely,

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Response to comments

Reviewer #1: (Number ID 06209780)

Scientific Quality: Grade C (Good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors:

The article is very well written.

1 Title: The title contains important information about the study. The type of study to be included in the title/abstract as per STROBE guidelines.

-----Response:

We appreciate the reviewer's suggestions very much.

The type of study was included in the abstract: "This observational study involved the systematic recruitment of 585 patients with T2D.....".

2 Authors: The author's details and affiliations are mentioned.

3 Abstract: The abstract reflects the importance, purpose and significant of the study.

4 Key Words: Keywords reflect the content of the manuscript.

5 Introduction: Introduction is written concisely with relevance to the study topic. The purpose of the study is clearly mentioned.

Kindly don't brand the participants as diabetic patients. It can be modified as patients with diabetes.

-----Response:

We appreciate the reviewer's positive comments and the latter suggestions for modification.

In the revised manuscript, "diabetic patients" was revised to "patients with

diabetes".

6 Materials and Methods/Experimental Procedure: Well written. The methods and procedures and sample analysis and statistical analysis are mentioned.

How did the authors divide the patients into 4 groups? What is the cut off value BUN for each group?

-----Response:

We appreciate the reviewer's comments very much.

Patients with diabetes were categorized by quartile levels of BUN (Q1, Q2, Q3, and Q4 groups). The 25th percentile value of BUN was 4.56 mmol/L, the 50th percentile value was 5.65 mmol/L, and the 75th percentile value was 6.86 mmol/L. Therefore, the BUN levels were 2.03–4.56 mmol/L in the Q1 group (147 patients), 4.57–5.65 mmol/L in the Q2 group (147 patients), 5.66–6.86 mmol/L in the Q3 group (146 patients), and 6.87–16.28 mmol/L in the Q4 group (145 patients) (**Table 1**).

7 Results: The results are understandable, and data are represented clearly. Statistics and interpretation of data are very well explained. The Table is represented as the format.

All the correlation values are weak, kindly mention as weak correlation in the results. The authors have done the multivariable linear regression using BUN. My suggestion would be to do the multivariable linear regression using individual groups BUN with nerve latency, amplitude, and NCV; it will define the risk of BUN with DPN appropriately.

-----Response:

We appreciate the reviewer's positive comments and the latter suggestions for modification.

In the **Results** section, the description was revised to "Significant correlations were identified between BUN levels and functional indices, specifically nerve latency, amplitude, and nerve conduction velocity (NCV), for each peripheral nerve. Despite being relatively weak, these correlations reached statistical significance. BUN levels were consistently correlated with the latency, amplitude, and NCV of each peripheral nerve".

In the present study, we aimed primarily to examine the relationship between BUN levels and peripheral nerve function in patients with T2D rather than the association of BUN with the risk of DPN. In future studies, we will investigate the association of BUN with the risk of DPN, and during follow-up, we can explore the relationship between BUN and the incidence of DPN. We have also added this information to the **Limitations** section.

The design of our present study is fundamentally based on the work of Li F et al., as published in the esteemed journal "**Diabetes Research and Clinical Practice**".

The pertinent reference is as follows: Li F et al., [TIR generated by continuous glucose monitoring is associated with peripheral nerve function in type 2 diabetes](#). *Diabetes Res Clin Pract* 2020; **166**: 108289.

8 Discussion: Very well discussed. The results are discussed properly in comparison to other relevant studies. The limitations of the study are undoubtedly mentioned. Future perspective of the study is also mentioned.

The authors need not to mention as their previous study in reference 16; instead they can mention as Xu F et al. found that lower levels of 1,5-anhydro-D-glucitol, a marker for short-term glyceamic fluctuation, were significantly associated with compromised peripheral nerve function.

-----Response:

We appreciate the reviewer's suggestions very much.

In the revised manuscript, the description was revised to "Xu et al. reported that lower levels of 1,5-anhydro-D-glucitol, a marker for short-term glyceamic fluctuation, were significantly associated with compromised peripheral nerve function."

9 Conclusion: Conclusion is concise and clear. The study reports support the conclusion.

-----Response:

We appreciate the reviewer's positive comments.

10 Acknowledgments: Acknowledgement is not mentioned.

-----Response:

The acknowledgments are not applicable.

11 References: references are relevant. The references are mentioned in order. Few of the references are more than 10 years old. Authors are requested to update the references.

-----Response:

We appreciate the reviewer's suggestions very much.

In the revised manuscript, we have updated several of the older references. However, we have retained certain seminal references pertaining to fundamental research on blood urea nitrogen (BUN) and metabolic disturbances because they continue to provide essential insights.

12 Abbreviations: Abbreviations are mentioned.

13 Language: language quality is good.

14 Manuscript type: Observational study.

15 Scientific research ethics: Authors have mentioned the ethical approval details, study duration in the Methods.

-----Response:

We appreciate the reviewer's positive comments.

Reviewer #2: (Number ID 08339407)

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors:

For abstract

Background: The statement "Increased blood urea nitrogen (BUN) levels have been shown to be associated with the incidence of type 2 diabetes (T2D)" lacks specificity and appears overly general. It should clarify whether BUN is an independent factor or merely associated with broader metabolic disturbances.

-----Response:

We appreciate the reviewer's suggestions very much.

When renal function is relatively normal, elevated blood urea nitrogen (BUN) levels may indicate disruptions in protein metabolism and glycemic variability, reflecting broader metabolic disturbances. Moreover, increased BUN levels have been linked to the incidence of type 2 diabetes (T2D), potentially playing a role in the progression of diabetic complications.

In the **Background** section, the statement was revised to "Increased blood urea nitrogen (BUN) levels have been demonstrated to be associated with broader metabolic disturbances and the incidence of type 2 diabetes (T2D), potentially playing a role in the development of diabetic complications".

Results: The trends for Z scores (latency, amplitude, and NCV) are mentioned but lack specific statistical values to support the claims. Including key p values or effect sizes would make the results more compelling.

-----Response:

Across the quartiles of BUN levels, the overall composite Z-score for latency (F=38.996, p for trend<0.001) showed a significant increasing trend, whereas the overall composite Z-scores for amplitude (F=50.972, p for trend<0.001) and NCV (F=30.636, p for trend<0.001) exhibited a significant decreasing trend.

Conclusion: The statement "Monitoring BUN levels can help proactively identify the risk of peripheral nerve dysfunction early" is overly optimistic without explicitly linking it to actionable intervention

-----Response:

We appreciate the reviewer's suggestions very much.

The statement was not in the **Conclusion** section but in the **Core Tip** section.

In the Core Tip section of the revised manuscript, the statement has been revised to "In the present study, our findings indicate that increased BUN levels are independently associated with compromised peripheral nerve function in patients with type 2 diabetes (T2D) and may serve as a potential risk factor for peripheral nerve dysfunction in these patients. Future interventions to lower BUN levels by improving nutritional status and balancing protein metabolism may alleviate peripheral nerve dysfunction in patients with T2D."

For Introduction

The introduction extensively discusses DPN but repeats information unnecessarily about its complications and prevalence. This redundancy detracts from the focus on

BUN as a central hypothesis. The hypothesis that BUN contributes to peripheral nerve dysfunction in T2D is implied but not explicitly stated. This weakens the rationale for the study.

-----**Response:**

We appreciate the reviewer's suggestions very much, and we have revised the **Introduction** section according to the reviewer's suggestions.

In the revised **Introduction** section:

While the etiology and pathogenesis of DPN in T2D remain inadequately understood, its development is recognized as a multifactorial process resulting from an interplay of metabolic disturbances, oxidative stress, and inflammatory responses. Consequently, identifying potential risk factors and devising appropriate interventions for DPN in clinical practice are of paramount importance.

BUN is increasingly acknowledged as a crucial marker for a series of metabolic disturbances, including insulin resistance, oxidative stress, and inflammation, which can lead to endothelial dysfunction and vascular damage and negatively impact peripheral nerve function. This condition is particularly pertinent in the context of diabetes, where insulin resistance is a major concern. On the basis of these findings, we hypothesized that increased BUN levels may play a role in the development of DPN in patients with T2D and could contribute to compromised peripheral nerve function in these patients.

Methods

Patient Recruitment: While inclusion and exclusion criteria are detailed, the rationale for excluding conditions like anemia or vitamin B12 deficiency is not provided. This omission could raise questions about the generalizability of the findings.

-----**Response:**

We appreciate the reviewer's comments very much.

In the **Study Design and Patient Recruitment** section, we specified exclusion criteria such as "(7) history of anemia or folic acid or vitamin B12 deficiency" because anemia or vitamin B12 deficiency may lead to peripheral neuropathy.

Z-Score Standardization: The justification for using Z-scores to analyze nerve function is not clearly explained. This may confuse readers unfamiliar with the method's advantages.

-----**Response:**

In the **Peripheral Nerve Function Assessment** section, we have provided a methodology to calculate overall composite Z-scores for latency, amplitude, and NCV, detailed as follows: "After standardization of the functional data related to motor and sensory nerves using Z-scores, overall composite Z-scores for latency, amplitude, and nerve conduction velocity (NCV) were calculated. This calculation was achieved by averaging the respective functional parameters across all motor and sensory nerves. Specifically, the composite Z-score for latency was derived by calculating the mean of the latency Z-scores across all peripheral nerves."

Discussion

The discussion heavily relies on speculation about mechanisms linking BUN to peripheral nerve dysfunction, such as oxidative stress and endothelial dysfunction, without direct evidence from this study.

The statement that "Elevated BUN levels may increase the risk of compromised peripheral nerve function" is overly definitive for an observational study. The discussion should emphasize the need for future research to establish causality. The clinical implications are underdeveloped.

Although BUN is identified as a potential biomarker, there is no clear explanation of how monitoring or reducing BUN would translate into improved outcomes for DPN patients.

-----Response:

In the **Discussion** section, we discuss the potential mechanisms connecting BUN to peripheral nerve dysfunction. On the one hand, we elucidate this relationship via foundational evidence; on the other hand, we establish a basis for future basic research aimed at investigating the specific mechanisms by which BUN contributes to nerve damage in T2D patients.

Reviewer #3 also suggested adding potential mechanisms that link BUN to compromised peripheral nerve function in the **Discussion**.

In the revised **Discussion** section, the statement was revised to "Elevated BUN levels were found to be independently associated with compromised peripheral nerve function in patients with T2D." The discussion also emphasized that "Future longitudinal studies are necessary to establish a causal relationship between increased BUN levels and impaired peripheral nerve function in this population, which may further elucidate the clinical implications of our findings."

In the revised **Discussion** section, we also added "These findings suggest that interventions aimed at reducing BUN levels through the enhancement of nutritional status and the regulation of protein metabolism may mitigate peripheral nerve dysfunction in this patient population."

Results

The trends presented in the tables and text often lack interpretation. For example, the increase in latency with higher BUN quartiles is statistically significant, but the clinical importance is not discussed.

The correlation analyses (Table 2) show strong statistical results but do not address whether these correlations remain robust after adjusting for potential confounders like hydration status or dietary intake.

Some trends, such as the association of BUN with NCV, appear weak despite being statistically significant (e.g., low r-values). This could be misleading without context.

-----Response:

The trends illustrated in the tables are also elucidated in the **Results** section. Statistical analysis revealed a statistically significant increase or decrease in a variable across higher BUN quartiles, indicating a significant correlation between the variable and the BUN levels.

Table 2 presents the results of Pearson's correlation analysis examining the associations between BUN levels and the functional indices of each peripheral nerve as well as overall peripheral nerve function. **Tables 3, 4, and 5** further illustrate the relationships between BUN levels and overall peripheral nerve function, adjusted for potential clinical confounders using multivariate linear regression analyses. These relationships remain robust after adjustment.

Concerning the potential confounding factor of hydration status, patients experiencing dehydration-related issues typically present with clinical symptoms such as fatigue, xerostomia, and hypotension, all of which have been previously ruled out in this study. Additionally, dietary protein intake, which could influence BUN levels,

was not assessed in our analysis. This drawback has been noted in the **limitations** section.

Certain trends, such as the correlations between BUN and NCVs, exhibited weak associations, as indicated by low correlation coefficients, despite achieving statistical significance (**Table 2**). Nevertheless, these associations remained significant even after controlling for other potential clinical confounders in multivariate linear regression analyses (**Tables 3, 4, and 5**).

Reviewer #3: (Number ID 07918561)

Scientific Quality: Grade D (Fair)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments to Authors:

The letter provides constructive feedback on the manuscript exploring the role of BUN (blood urea nitrogen) as a risk factor for diabetic peripheral neuropathy (DPN) in type 2 diabetes (T2D). The main points of the feedback are:

Introduction:

It would help to briefly define DPN and explain how it develops, linking it to the study's exploration of risk factors like BUN.

The introduction should include a brief mention of known mechanisms behind DPN (e.g., oxidative stress, inflammation) to provide context for why studying BUN as a risk factor is important.

The mechanistic link between elevated BUN levels and DPN is underdeveloped. The introduction could elaborate on how BUN might contribute to vascular damage, inflammation, or renal dysfunction, which could affect nerve function.

-----Response:

We appreciate the reviewer's suggestions very much, and we have revised the **Introduction** section according to the reviewer's suggestions.

In the revised **Introduction** section:

While the etiology and pathogenesis of DPN in T2D remain inadequately understood, its development is recognized as a multifactorial process resulting from an interplay of metabolic disturbances, oxidative stress, and inflammatory responses. Consequently, identifying potential risk factors and devising appropriate interventions for DPN in clinical practice are of paramount importance.

BUN is increasingly acknowledged as a crucial marker for a series of metabolic disturbances, including insulin resistance, oxidative stress, and inflammation, which can lead to endothelial dysfunction and vascular damage and negatively impact peripheral nerve function.

The introduction should emphasize how identifying BUN as a risk factor could lead to earlier diagnosis or targeted treatments for DPN, potentially improving patient outcomes and reducing long-term complications.

-----Response:

If a relationship between increased BUN levels and compromised peripheral nerve function is identified, interventions to lower BUN levels may improve nutritional status and balance protein metabolism, which may alleviate peripheral nerve dysfunction in patients with T2D. We have added this information in the **Discussion** section.

The study's objective should be more clearly stated and specific research questions should be outlined to guide the reader's understanding of the study's aims.

The hypothesis could be made more specific, such as examining whether elevated BUN levels are associated with both the presence and severity of DPN, to provide clearer focus.

-----Response:

We appreciate the reviewer's suggestions very much.

In the revised Introduction section: ".....On the basis of these findings, we hypothesized that increased BUN levels may play a role in the development of DPN in patients with T2D and could contribute to compromised peripheral nerve function in these patients." "Therefore, we..... analyze the associations between BUN levels and peripheral nerve function in these patients."

In the present study, we aimed primarily to examine the relationship between BUN levels and peripheral nerve function in patients with T2D rather than the association of BUN with the risk of DPN. In future studies, we will investigate the associations of BUN with the risk of DPN and the severity of DPN. We have also added this information to the **Limitations** section.

The design of our present study is fundamentally based on the work of Li F et al., as published in the esteemed journal "**Diabetes Research and Clinical Practice**". The pertinent reference is as follows: TIR generated by continuous glucose monitoring is associated with peripheral nerve function in type 2 diabetes, *Diabetes Res Clin Pract*, 2020, **166**: 108289.

Methods:

Clarify participant recruitment and include a control group to differentiate diabetes-specific effects.

-----Response:

We appreciate the reviewer's suggestions very much.

In the **Study Design and Patient Recruitment** section:

According to the inclusion and exclusion criteria, 585 eligible T2D patients with complete data were enrolled in the study.

BUN may be a potential risk factor for compromised peripheral nerve function in T2D patients, but BUN is not a specific biomarker for peripheral nerve injury. Therefore, we compared indices of peripheral nerve function among the four subgroups categorized by quartile levels of BUN. We did not establish a control group.

Address how confounding variables, such as medications, are controlled for.

-----Response:

The confounding variables, including **medications**, were all adjusted in the multivariable linear regression analysis (**Tables 3, 4, and 5**).

Medications included statins and hypoglycemic drugs; the latter contained insulin, secretagogues, metformin, thiazolidinediones (TZDs), α -glucosidase inhibitors (AGIs), dipeptidyl peptidase-4 inhibitors (DPP-4Is), sodium–glucose cotransporter-2 inhibitors (SGLT-2Is), and glucagon–like peptide–1 receptor agonists (GLP-1RAs).

Provide more details on standardization of nerve conduction measurements, handling of missing data, and statistical power analysis.

-----Response:

In the **Peripheral Nerve Function Assessment** section, we have provided a methodology to calculate overall composite Z-scores for latency, amplitude, and NCV, detailed as follows: "After standardization of the functional data related to motor and sensory nerves using Z-scores, overall composite Z-scores for latency, amplitude, and nerve conduction velocity (NCV) were calculated. This calculation was achieved by averaging the respective functional parameters across all motor and sensory nerves. Specifically, the composite Z-score for latency was derived by calculating the mean of the latency Z-scores across all peripheral nerves."

A total of 585 eligible T2D patients with complete data were enrolled in the study. T2D patients with missing data were not included in the study.

Regarding the statistical power analysis, we provided test statistics (F value, Z value and χ^2 value) in the trend analysis and provided t values and R^2 values in the multivariable linear regression analysis.

Results:

The footnotes for figures should clearly explain key aspects of the data, including the methodologies, statistical analyses, and interpretation of results.

They must contextualize the figure within the study, helping readers understand the significance of the findings and their potential impact on clinical practice and future research. The goal is to provide clarity and depth in the explanations, ensuring that the reader fully grasps the relevance and implications of the data presented in each figure.

-----Response:

We have provided detailed footnotes for the figures in the revised manuscript.

Figure 2 Graphical correlations between blood urea nitrogen levels and overall peripheral nerve functional indices.

Pearson's correlation coefficient (r) and the corresponding p value were based on total data ($n=585$).

BUN: blood urea nitrogen; NCV: nerve conduction velocity

A: overall nerve latency; **B:** overall nerve amplitude; **C:** overall nerve NCV

Figure 3 Graphical correlations between blood urea nitrogen levels and functional indices of motor and sensory nerves.

Pearson's correlation coefficient (r) and the corresponding p value were based on total data ($n=585$).

BUN: blood urea nitrogen; NCV: nerve conduction velocity

A: motor nerve latency; **B:** motor nerve amplitude; **C:** motor nerve NCV; **D:** sensory nerve latency; **E:** sensory nerve amplitude; **F:** sensory nerve NCV

Discussion:

Discuss the causal relationship between BUN and nerve dysfunction and the potential mechanisms involved.

-----Response:

We have added this information to the revised **Discussion** section.

Elevated BUN levels were found to be independently associated with compromised peripheral nerve function in patients with T2D. Future longitudinal studies are necessary to establish a causal relationship between increased BUN levels and impaired peripheral nerve function in this population, which may further elucidate the clinical implications of our findings.

There may be potential mechanistic links between increased BUN levels and compromised peripheral nerve function in individuals with T2D.

Explore the clinical implications of using BUN as a diagnostic or prognostic tool for DPN.

-----Response:

BUN may be a potential risk factor for compromised peripheral nerve function in patients with T2D, but it is not a specific biomarker for peripheral nerve injury. Therefore, BUN may not be suitable as a diagnostic tool for DPN.

Elevated BUN levels were independently linked to compromised peripheral nerve function in patients with T2D, which may imply a poor outcome for DPN patients.

Address the generalizability of findings and integrate BUN's relationship with other DPN risk factors like glycemic control and inflammation.

-----Response:

The present study was limited to patients with T2D in a single center in China; therefore, the results may not be generalizable. We have addressed this issue in the **Limitations** section.

Increased BUN levels indicate poor glycemic control. Huang et al. [37] revealed that increased BUN levels were well correlated with short- and long-term glycemic variability. In our present study, BUN levels were positively related to HbA1c. We have addressed these issues in the **Discussion** section.

Suggest future research to investigate the specific mechanisms linking BUN to nerve damage in T2D.

-----Response:

In accordance with the reviewer's comments, we have added this suggestion in the revised **Discussion** section.

There may be potential mechanistic links between increased BUN levels and compromised peripheral nerve function in individuals with T2D. However, as these mechanisms are currently supported by indirect evidence, we propose performing fundamental research to elucidate the specific pathways through which BUN

contributes to nerve damage in patients with T2D.

Comments from the journal Editor-in-Chief

The study may be of interest, although there are several pitfalls, including the lack of a STROBE diagram to ensure transparency, the NCV mentioned as an EMG (which cannot assess sensory function) and procedural details and data availability. These issues should also be assessed in detail during the peer review process to consider the suitability of the paper.

-----Response:

We appreciate the editor's comments very much.

A study diagram has been incorporated into **Figure 1**.

Electromyography (EMG) nerve conduction studies represent the most sensitive, objective, and reliable techniques for quantifying nerve function, including nerve latency, amplitude, and conduction velocity, in both motor and sensory nerves. In terms of sensory function assessment, quantitative sensory testing (QST) is typically employed; however, it was not utilized in the present study.

The design of our present study is fundamentally based on the work of Li F et al., as published in the esteemed journal "**Diabetes Research and Clinical Practice**". The pertinent reference is as follows: TIR generated by continuous glucose monitoring is associated with peripheral nerve function in type 2 diabetes, *Diabetes Res Clin Pract*, 2020, **166**: 108289.