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Exploring the effectiveness of vitamin B12 complex and alpha-lipoic acid as a treatment for diabetic neuropathy. A protocol for systematic review and meta-analysis.

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Exploring the effectiveness of vitamin B12 complex and alpha-lipoic acid as a treatment for diabetic neuropathy. A protocol for systematic review and meta-analysis.

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Abstract

Introduction: Diabetic neuropathy (DN) is common in patients diagnosed with diabetes mellitus. This often causes peripheral nerve damage. For many years vitamin B12 and alpha-lipoic acid (ALA) have been regarded as components that can treat inflammation and reduce oxidative stress. In this study, we will explore the effectiveness of vitamin B12 and ALA as a possible treatment for diabetic mellitus/neuropathy, emphasizing markers of inflammation, lipid profile, and glucose metabolism.

Methods and analysis: We will conduct a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol (PRISMA-P). The search strategies and information sources for the literature will be PubMed, Google Scholar, Web of Science, and Science direct. All included studies will be evaluated for quality and risk of bias according to the Cochrane guidelines. To investigate the stability of the results, we will conduct a sensitivity analysis of the outcomes. All data analysis will be performed using Review Manager version 5.4.

Ethical and dissemination: This systematic review and meta-analysis will not require ethical approval from an institution committee as it does not have direct participants. We will obtain all our data from previous studies.

This protocol is registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY), registration number INPLASY202250167.

Keywords: Diabetic neuropathy, Vitamin B12, Alpha Lipoic Acid, inflammation, hyperglycemia, and type 2 diabetes mellitus.
Introduction

Diabetic Neuropathy (DN) is a heterogeneous type of nerve damage associated with diabetes mellitus, the condition most often damages nerves in the legs and feet. It presents both clinically and subclinically affecting the peripheral nervous system due to an increase in glucose concentration which interferes with nerve signalling.

Following the discovery of insulin as a treatment for diabetes mellitus (DM), the prevalence of DN has increased significantly due to DM patients having a longer life expectancy. It has been estimated that at least 50% of DM patients will develop DN in their life, with approximately 20% experiencing neuropathic related pains. Nerves are susceptible to changes in glucose concentrations, and insulin makes it impossible for neurons to continue regulating glucose uptake.

For many years, vitamin B12 and alpha-lipoic acid (ALA) have been regarded as components that treat pain and reduce oxidative stress. Vitamin B12 is essential in the metabolism of essential fatty acids involved in the maintenance of nerve myelin and direct scavenging of reactive oxygen species (ROS). However, patients with DN on metformin treatment have been shown to have low vitamin B12; this is because metformin on its own impairs vitamin B12 absorption. When vitamin B12 deficiency is prolonged, it leads to nerve degeneration, causing irreversible nerve damage. Previous researchers have explored the benefits of vitamin B12 in nerve regeneration and have shown that it promotes nerve regeneration by promoting axon growth of neural cells after peripheral nerve damage.

On the other hand, the naturally occurring ALA, an organosulfur compound derived from octanoic acid, is required to generate energy in the mitochondria by various enzymes. Most importantly, ALA is a potent antioxidant agent that can neutralize ROS and nerve blood flow, resulting in improved distal nerve conductions. Additionally, ALA acts as a scavenger for free radicals intra- and extracellularly.
to repair oxidative damage. Furthermore, ALA has been shown to increase the uptake of glucose to control glucose metabolism. However, it is still unclear how these compounds impact the markers of inflammation and related glucose parameters in patients with diabetic neuropathy. Therefore, this systematic review and meta-analysis aim to explore the effectiveness of vitamin B12 and ALA as either monotherapy or dual treatment for diabetic mellitus/neuropathy with major emphasis on markers of inflammation, lipid profile, and glucose metabolism.

METHODOLOGY

This protocol for a systematic review and meta-analysis is aimed at evaluating the beneficial impact of vitamin B12 and alpha-lipoic acid in diabetic mellitus with a major focus on markers of inflammation, lipid profile and glucose metabolism. The review will follow the Preferred Reporting Items for Systematic reviews and Meta-Analysis protocol (PRISMA-P). This protocol is registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY), registration number INPLASY202250167. https://inplasy.com/inplasy-2022-5-0167/

Aim

The study's main aim is to explore the effectiveness of vitamin B12 and alpha-lipoic acid as a possible treatment for diabetic mellitus/neuropathy with major emphasis on markers of inflammation, lipid profile, and glucose metabolism.

Questions

Does alpha-lipoic acid increase the uptake of glucose for better glycaemic control?
Do vitamin B12 and alpha-lipoic acid improve inflammation?
What is the impact of vitamin B12/alpha-lipoic acid on lipid profile amongst diabetes patients?

Participants/Population

Patients with diabetes/ Diabetic Neuropathy.
Intervention

Vitamin B12.

Alpha-Lipoic Acid.

Comparator

Patients on placebo/control.

Outcomes

Improved inflammation (TNF-α, IL-6, and CRP).

Improved glucose parameters (FBG, insulin).

Improved lipid profile (TC, TG, LDL, HDL).

Eligibility criteria

Inclusion

All studies will be reviewed for inclusions and where there may be uncertainty, the studies in the review will be required to meet the following criteria.

The study that focuses on the effects of vitamin B12 and alpha-lipoic acid in diabetic mellitus/neuropathy.

The studies that use human subjects with diabetes/diabetic neuropathy.

The studies published in English.

Exclusion

Animal studies and studies that are non-English will be excluded from this review. This is partially because translating studies from other languages end up losing the exact results obtained from such
studies. Reviews will not be considered. Cohorts, case-control, case studies, and experimental studies will be excluded.

Search strategy and information sources.

PubMed, Google Scholar, Web of Science, and Science direct will be searched to identify suitable sources for this review. Two independent investigators (PKL and KM) will conduct the literature search. MesH terms and text words will be used, including Vitamin B, alpha-lipoic acid, diabetic neuropathy, and diabetes mellitus. The search will seek studies published in English from inception until June 2022. Furthermore, studies that meet the inclusion criteria will be screened and data will be extracted and presented in a tabular format.

Study selection

The screening of studies will be conducted by 2 independent investigators (PKL and KM) to avoid inconsistency in terms of eligibility of studies. Firstly, studies will be screened by the titles, abstracts, keywords, and synonyms, followed by identifying the full-text articles if available. In case of discrepancies between 2 investigators (PKL and KM), the resolution will be reached through discussion and reviewing the study in question. Mendeley desktop reference manager (version 1.19.4) will be used to save extracted data, saving relevant and excluded studies. Furthermore, reference lists of included studies will be screened to identify other relevant studies. Studies meeting the inclusion criteria will then be subjected to data collection, critical appraisal, risk, and quality evaluation.

Data extraction and management

Based on the characteristics of the study, we will prepare an excel form for data collection before data extraction. Outcomes and effect measures for eligible studies will be extracted and filled in the data extraction form by 2 independent investigators (PKL and KM). Any disagreement can be resolved by
discussing it between the 2 investigators (PKL and KM) or seeking a third investigators opinion. The primary data to be extracted are as follows, the first authors and year of publication of the study, the country where the study was carried out, source of funding, interventions in the experimental group (monotherapy or combination therapy), interventions in the control group, time of treatment, number of participants in each group, ages, and sex of participants, outcomes, and effect measures. To seek clarity about the study, corresponding authors will be contacted.

**Risk of bias assessment**

All relevant studies will be evaluated following the Cochrane guidelines \(^\text{11,12}\). Two investigators (PLK and KM) will independently evaluate the methodological design by assessing these 7 bias items, random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data and selective reporting. Individual items will be categorised as “Low risk,” “High risk” or “Unclear risk”. The disagreement amongst the two investigators (PLK and KM) in terms of bias risk will be resolved through further discussion and re-evaluation of such an item and the study in question.

**Sensitivity analysis**

To assess the stability of the results, we will conduct a sensitivity analysis for the outcomes\(^\text{13}\). We will exclude one study included in the analysis, then re-analyse the pooled estimates and compare effect size with the size of the initial effect.

**Publication bias**

Suppose studies greater than ten are included in the meta-analysis. In that case, the symmetry of the funnel plot will be evaluated to examine publication bias, \(^\text{14}\) and the results will be interpreted with care.
Strength of the quality of evidence.

In this planned, systematic review and meta-analysis, the study’s evidence quality will be investigated following the Grading of Recommendations Assessment, Development and Evaluation (GRADE)\textsuperscript{15,16}. This is classified into 4 levels: high, medium, low, and very low. We will use the GRADE profiler 3.2 for analysis\textsuperscript{17}.

Subgroup analysis

If the obtained results are heterogeneous, we will perform a subgroup analysis to find the source of heterogeneity\textsuperscript{18,19}, which can arise from study design, race, age, sex, different intervention forms, drug dosage, and duration of treatment.

Statistical analysis

We will use the Review Manager software Version 5.4 (The Nordic Cochrane Center, The Cochrane Collaboration, 2014, Copenhagen, Denmark) to analyse all data. The mean difference (MD) or standardized MD (SMD) and 95% CI will be estimated for continuous data. If the same scale is used to measure an outcome in different studies, MD will be used. Similarly, we will use SMD if different scales are used to measure the same outcome. If an outcome measure contains less than 2 trials, we will summarize the results descriptively. Meta-analysis will be carried out if at least two or more studies report the same outcome. However, in case of an insufficient number of studies to perform a meta-analysis, a qualitative synthesis will be performed\textsuperscript{20}. The statistical level of heterogeneity among included studies will be assessed using the Cochran Q test ($x^2$) and the $I^2$ statistical tests. We will classify the heterogeneity using the predefined rules\textsuperscript{19}. An $I^2$ of 0% to 25% will be classified as low heterogeneity $I^2$ of 25% to 50% represents moderate heterogeneity\textsuperscript{21}. An $I^2$ of 75% to 100% represents high heterogeneity. We will use the fixed-effects model when the P-value from an $x^2$ test is more than 0.10 or $I^2$ 50%. A subgroup analysis will be conducted to identify possible sources for statistical heterogeneity, considering prespecified factors. A descriptive summary of individual studies will be made when a meta-analysis is not possible.
Discussion

Diabetic neuropathy has no existing medical cure, and prevention will be crucial. With tight glucose control, we can see a decrease in the progression of nerve damage. However, this warrants for an effective medical intervention. This systematic review and meta-analysis focus on vitamin B12 and ALA as monotherapy or dual treatments in diabetic mellitus/neuropathy. These components have shown properties that decrease oxidative stress and increase nerve myelination.

Ethics and dissemination

Ethical clearance is not required. Participants will not be recruited in this study, and data will be obtained from already published studies.

Patient and public involvement

No patient involved

Funding

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Competing interests

The authors declare that they have no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data Availability Statement
Raw data involving literature search, data extraction, and statistical analysis will be made available from the corresponding author on request after the article has been published.

**Authors contributions**

PKL and KM conceptualized, designed, and initiated the protocol. PKL wrote the first draft. KM edited the final version of the protocol. KM supervised all the write-up, editing, and scientific edits. All authors approved the final manuscript submitted for publication.

**Abbreviations**

DN: Diabetic neuropathy  
DM: Diabetes mellitus  
ROS: Reactive oxygen species  
ALA: Alpha lipoic acid  
TC: Total cholesterol  
TG: Triglyceride  
LDL: Low-density lipoprotein  
HDL: High-density lipoprotein  
IL-6: Interleukin-6  
TNF-α: Tumour necrosis factor-alpha

**References**

1. Mayo Clinic. Mayoclinic.org. 2022. Symptoms and causes - Mayo Clinic. [online] Available at: <https://mayoclinic.org/diseases-conditions/diabetic-neuropathy/symptoms-causes/syc-20371580?__p=1> [Accessed 13 April 2022]. (2022).
2. Nascimento, O. J. M. do, Pupe, C. C. B. & Cavalcanti, E. B. U. Diabetic neuropathy. *Revista Dor* **17**, (2016).
3. Didangelos, T. et al. Efficacy and Safety of the Combination of Superoxide Dismutase, Alpha Lipoic Acid, Vitamin B12, and Carnitine for 12 Months in Patients with Diabetic Neuropathy. *Nutrients* **12**, 3254 (2020).

4. Okdahl, T. & Brock, C. Molecular Aspects in the Potential of Vitamins and Supplements for Treating Diabetic Neuropathy. *Current Diabetes Reports* **21**, 31 (2021).

5. Melese, H., Alamer, A., Temesgen, M. H. & Kahsay, G. Effectiveness of exercise therapy on gait function in diabetic peripheral neuropathy patients: A systematic review of randomized controlled trials. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* vol. 13, 2753–2764 (2020).

6. Julian, T., Syeed, R., Glasgow, N., Angelopoulou, E. & Zis, P. B12 as a treatment for peripheral neuropathic pain: A systematic review. *Nutrients* vol. 12 1–16 (2020).

7. Elbadawy, A. M., Abd Elmoniem, R. O. & Elsayed, A. M. Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters. *Alexandria Journal of Medicine* **57**, 113–120 (2021).

8. Okdahl, T. & Brock, C. Molecular Aspects in the Potential of Vitamins and Supplements for Treating Diabetic Neuropathy. doi:10.1007/s11892-021-01397-1/Published.

9. Didangelos, T. et al. Vitamin B12 supplementation in diabetic neuropathy: A 1-year, randomized, double-blind, placebo-controlled trial. *Nutrients* **13**, 1–14 (2021).

10. Moher, D. et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Revista Espanola de Nutricion Humana y Dietetica* **20**, 148–160 (2016).

11. by Julian Higgins, E. P., Savović, J., Page, M. J. & Sterne, J. A. Revised Cochrane risk of bias tool for randomized trials (RoB 2.0). (2016).

12. Higgins, J. P. T. et al. The Cochrane Collaboration’s tool for assessing risk of bias in randomised trials. *BMJ (Online)* **343**, (2011).

13. Mathur, M. B. & VanderWeele, T. J. Sensitivity analysis for publication bias in meta-analyses. *Journal of the Royal Statistical Society. Series C: Applied Statistics* **69**, 1091–1119 (2020).

14. Moreno, S. G. et al. Assessment of regression-based methods to adjust for publication bias through a comprehensive simulation study. *BMC Medical Research Methodology* **9**, (2009).

15. Atkins, D. et al. Systems for grading the quality of evidence and the strength of recommendations I: Critical appraisal of existing approaches. *BMC Health Services Research* **4**, (2004).

16. Granholm, A., Alhazzani, W. & Møller, M. H. Use of the GRADE approach in systematic reviews and guidelines. *British Journal of Anaesthesia* vol. 123 554–559 (2019).

17. Balshem, H. et al. GRADE guidelines: 3. Rating the quality of evidence. *Journal of Clinical Epidemiology* **64**, 401–406 (2011).

18. Sedgwick, P. Meta-analyses: Heterogeneity and subgroup analysis. *BMJ (Online)* vol. 346 (2013).
19. Schroll, J. B., Moustgaard, R. & Gøtzsche, P. C. Dealing with substantial heterogeneity in
Cochrane reviews. Cross-sectional study. *BMC Medical Research Methodology* **11**, (2011).

20. Popay, J. et al. *Guidance on the Conduct of Narrative Synthesis in Systematic Reviews A
Product from the ESRC Methods Programme Peninsula Medical School, Universities of Exeter
and Plymouth.* (2006).

21. Imrey, P. B. Limitations of Meta-analyses of Studies with High Heterogeneity. *JAMA Network
Open* vol. 3 (2020).
Reporting checklist for protocol of a systematic review and meta analysis.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

| Reporting Item | Page Number |
|----------------|-------------|
| Identification | #1a | Identify the report as a protocol of a systematic review 1 |
| Update         | #1b | If the protocol is for an update of a previous systematic review, identify as such 1 |
Registration

#2 If registered, provide the name of the registry (such as PROSPERO) and registration number

Authors

Contact #3a Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author

Contribution #3b Describe contributions of protocol authors and identify the guarantor of the review

Amendments

#4 If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments

Support

Sources #5a Indicate sources of financial or other support for the review

Sponsor #5b Provide name for the review funder and / or sponsor

Role of sponsor or #5c Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol

Introduction

Rationale #6 Describe the rationale for the review in the context of what is
already known

Objectives  #7 Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)

Methods

Eligibility criteria  #8 Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review

Information  #9 Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage

Search strategy  #10 Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated

Study records - data management  #11a Describe the mechanism(s) that will be used to manage records and data throughout the review

Study records - selection process  #11b State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)

Study records - data collection  #11c Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any

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process processes for obtaining and confirming data from investigators

Data items #12 List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications

Outcomes and prioritization #13 List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale

Risk of bias in individual studies #14 Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis

Data synthesis #15a Describe criteria under which study data will be quantitatively synthesised

Data synthesis #15b If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall’s τ)

Data synthesis #15c Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)

Data synthesis #15d If quantitative synthesis is not appropriate, describe the type of summary planned

Meta-bias(es) #16 Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within
Confidence in cumulative evidence

Describe how the strength of the body of evidence will be assessed (such as GRADE)

The PRISMA-P elaboration and explanation paper is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 10. June 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai
Exploring the effectiveness of vitamin B12 complex and alpha-lipoic acid as a treatment for diabetic neuropathy. A protocol for systematic review and meta-analysis of randomised controlled trials.

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| **Secondary Subject Heading**: | Nutrition and metabolism, Diabetes and endocrinology, Pharmacology and therapeutics, Qualitative research, Cardiovascular medicine |
| **Keywords**: | Diabetic neuropathy < DIABETES & ENDOCRINOLOGY, General diabetes < DIABETES & ENDOCRINOLOGY, Lipid disorders < DIABETES & ENDOCRINOLOGY, Diabetes & endocrinology < INTERNAL MEDICINE, NUTRITION & DIETETICS |
Exploring the effectiveness of vitamin B12 complex and alpha-lipoic acid as a treatment for diabetic neuropathy. A protocol for systematic review and meta-analysis of randomised controlled trials.

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Abstract

Introduction: Diabetic neuropathy (DN) is common in patients diagnosed with diabetes mellitus. This often causes peripheral nerve damage. For many years vitamin B12 and alpha-lipoic acid (ALA) have been regarded as components that can in reducing markers of inflammation and oxidative stress. In this study, we will explore the effectiveness of vitamin B12 and ALA as a possible treatment for diabetic mellitus/neuropathy, emphasizing markers of inflammation, lipid profile, and glucose metabolism.

Methods and analysis: We will conduct a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol (PRISMA-P). The search strategies and information sources for the literature will be PubMed, Google Scholar, Web of Science, and Science direct. The literature search will include studies published from inception until 30 June 2022. All included studies will be evaluated for quality and risk of bias according to the Cochrane guidelines. To investigate the stability of the results, we will conduct a sensitivity analysis of the outcomes. All data analysis will be performed using Review Manager version 5.4.

Ethical and dissemination: This systematic review and meta-analysis will not require ethical approval from an institution committee as it does not have direct participants. We will obtain all our data from previous studies. The findings will be disseminated through publications in peer-reviewed journals and presented at local and international seminars and conferences.

INPLASY registration number: registration number INPLASY202250167.

Keywords: Diabetic neuropathy, Vitamin B12, Alpha Lipoic Acid, inflammation, hyperglycemia, and diabetes mellitus.
Strengths and limitations of this study

- This proposed review will comprehensively assess published randomized controlled trials on the primary research question, focusing on glycemic control, inflammation, and lipid profile in diabetic patients on either monotherapy or dual treatment of vitamin B12 and alpha-lipoic acid.

- A comprehensive search for literature will be conducted on the database to source out and retrieve studies relevant to our research question.

- This will be the primary systematic review and meta-analysis conducted to evaluate the single or dual supplementation of these treatments in diabetes mellitus.

- The anticipated limitation includes heterogeneity that may arise due to different dosages, forms of diabetes, dual or monotherapy, and also the duration of intervention.

- However, subgroup and sensitivity analysis will be performed to source the exact cause of heterogeneity.
Introduction

Diabetic Neuropathy (DN) is a heterogeneous type of nerve damage associated with diabetes mellitus, the condition most often damages nerves in the legs and feet [1]. It presents both clinically and sub-clinically affecting the peripheral nervous system due to an increase in glucose concentration which interferes with nerve signaling [1,2].

Following the discovery of insulin as a treatment for diabetes mellitus (DM), the prevalence of DN has since increased significantly due to DM patients having a longer life expectancy [2]. It has been estimated that at least 50% of DM patients will develop DN in their life, with approximately 20% experiencing neuropathic-related pains [1,2]. Nerves are susceptible to changes in glucose concentrations, and insulin treatment makes it impossible for neurons to continue regulating glucose uptake [3,4].

For many years, vitamin B12 and alpha-lipoic acid (ALA) have been regarded as components that reduce pain and oxidative stress. Vitamin B12 is essential in the metabolism of essential fatty acids involved in the maintenance of nerve myelin and direct scavenging of reactive oxygen species (ROS). However, patients with DN on metformin treatment have been shown to have low vitamin B12; this is because metformin on its own impairs vitamin B12 absorption. When vitamin B12 deficiency is prolonged, it leads to nerve degeneration, causing irreversible nerve damage [5]. Previous researchers have explored the benefits of vitamin B12 and have discovered that it stimulates nerve regeneration by promoting axon growth of neural cells after peripheral nerve damage [6].

On the other hand, the naturally occurring ALA, an organosulfur compound derived from octanoic acid [7], is required to generate energy in the mitochondria by various enzymes. Most importantly, ALA is a potent antioxidant agent that can neutralize ROS and nerve blood flow, resulting in improved distal nerve conductions (1). Additionally, ALA acts as a scavenger for free radicals intra-
and extracellularly to repair oxidative damage [8,9]. Furthermore, ALA has been shown to increase the uptake of glucose to control glucose metabolism [7–9]. Moreover, another study showed an improvement in glucose level and insulin sensitivity following the use of ALA treatment in type 2 diabetes mellitus (T2D) patients[10]. In metabolic syndromes, a previous meta-analysis demonstrated that treatment of ALA improves the marker of inflammation[11]. However, it is still unclear how these compounds impact the markers of inflammation and related glucose parameters in patients with diabetes/diabetic neuropathy. A previous meta-analysis assessing the effect of ALA on lipid profiles revealed a significant decrease in lipid profiles; however, this was conducted in patients with various conditions, including diabetes, schizophrenia, obesity, renal disease, and hyperthyroidism[12]. Therefore, this systematic review and meta-analysis aim to explore the effectiveness of vitamin B12 and ALA as either monotherapy or dual treatment for diabetic mellitus/neuropathy with major emphasis on markers of inflammation, lipid profile, and glucose metabolism.

**METHODOLOGY**

This protocol for a systematic review and meta-analysis is aimed at evaluating the beneficial impact of vitamin B12 and alpha-lipoic acid in diabetic mellitus with a major focus on markers of inflammation, lipid profile, and glucose metabolism. The review will follow the Preferred Reporting Items for Systematic reviews and Meta-Analysis protocol (PRISMA-P) [13] Supplementary file 1. Registration number INPLASY202250167.

**Patient and public involvement**

No patient involved

**Aim**
The study's main aim is to explore the effectiveness of vitamin B12 and alpha-lipoic acid as a possible treatment for diabetic mellitus/neuropathy with major emphasis on markers of inflammation, lipid profile, and glucose metabolism.

Questions

6 Does alpha-lipoic acid increase the uptake of glucose for better glycaemic control?

7 Do vitamin B12 and alpha-lipoic acid improve markers of inflammation?

8 What is the impact of vitamin B12/alpha-lipoic acid on lipid profile amongst diabetes patients?

Participants/Population

11 We will consider studies conducted in patients with diabetes / Diabetic Neuropathy on either vitamin B12 or alpha-lipoic acid as supplements.

Intervention

15 We will consider studies conducted on patients living with diabetes mellitus/diabetic neuropathy on Vitamin B12 and Alpha lipoic acid supplementations. If vitamin B2 and alpha lipoic acid are used in the same trial, and there is a comparator/control, such studies will be analyzed as dual treatment versus placebo/control. However, if one treatment is used in a trial, it will be subgrouped as monotherapy versus placebo/control.

Design: Randomised controlled trials

Comparator

23 The comparator will be divided into two:

24 Patients with diabetes mellitus/diabetic neuropathy on placebo.

25 Healthy participants not on any treatment (control)
Outcomes

Improved markers of inflammation such as tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), and C-reactive protein (CRP).

Improved glucose parameters, fasting blood glucose, and insulin.

Improved lipid profile; total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein.

Eligibility criteria

Inclusion

All studies will be reviewed for inclusions, and where there may be uncertainty, the studies in the review will be required to meet the following criteria.

The randomised controlled trials focus on the effects of vitamin B12 and alpha-lipoic acid in diabetic mellitus/ neuropathy.

The studies that use human subjects with diabetes/diabetic neuropathy.

The studies published in English.

Exclusion

Animal studies and studies that are non-English will be excluded from this review. This is partially because translating studies from other languages end up losing the exact results obtained from such studies. Reviews will not be considered. Cohorts, case-control, case studies, and experimental studies will all be excluded. Those studies that use other drugs will be deemed irrelevant to the current study.

Search strategy and information sources.

PubMed, Google Scholar, Scopus, Web of Science, and Science direct databases will be searched to identify suitable sources for this review. Two independent investigators (PKL and KM) will conduct
the literature search. MesH terms and text words will be used, including Vitamin B12 and synonyms, alpha-lipoic acid, diabetes mellitus, and diabetic neuropathy. The search will be divided into two to source out studies on vitamin B12 and alpha-lipoic acid in diabetes. The search will seek studies published in English from inception until 30 June 2022 (supplementary file 2). Furthermore, studies that meet the inclusion criteria will be screened, and data will be extracted and presented in a tabular format.

8 Study selection

The screening of studies will be conducted by 2 independent investigators (PKL and KM) to avoid inconsistency in terms of eligibility of studies. Firstly, studies will be screened by the titles, abstracts, keywords, and synonyms, followed by identifying the full-text articles if available. In case of discrepancies between 2 investigators (PKL and KM), the resolution will be reached through discussion and reviewing the study in question. Mendeley desktop reference manager (version 1.19.4) will be used to save extracted data, saving relevant and excluded studies. Furthermore, reference lists of included studies will be screened to identify other relevant studies. Studies meeting the inclusion criteria will then be subjected to data collection, critical appraisal, risk, and quality evaluation.

9 Data extraction and management

Reference manager (Mendeley desktop version 1.19.4) will be used to store retrieved studies from databases. Review Manager software Version 5.4 will be sued to analyse all data and creation of forest and funnel plots. Based on the characteristics of the study, we will prepare an excel form for data collection before data extraction. Outcomes and effect measures for eligible studies will be extracted and filled in the data extraction form by 2 independent investigators (PKL and KM). Any disagreement can be resolved by discussing it between the 2 investigators (PKL and KM) or seeking a third investigators opinion. The primary data to be extracted are as follows, the first authors and
year of publication of the study, the country where the study was carried out, source of funding, interventions in the experimental group (monotherapy or dual therapy), interventions in the control group, time of treatment, number of participants in each group, ages, and sex of participants, outcomes, and effect measures. To seek clarity about the study, corresponding authors will be contacted.

Risk of bias assessment
All relevant studies will be evaluated following the Cochrane guidelines [14,15]. Two investigators (PLK and KM) will independently evaluate the methodological design by assessing these 7 bias items, random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data and selective reporting. Individual items will be categorised as “Low risk,” “High risk” or “Unclear risk”. The disagreement between the two investigators (PLK and KM) in terms of bias risk will be resolved through further discussion and re-evaluation of such an item and the study in question.

Sensitivity analysis
To assess the stability of the results, we will conduct a sensitivity analysis for the outcomes[16] with the use of RevMan version 5.4. We will exclude one study included in the analysis, then re-analyse the pooled estimates and compare effect size with the size of the initial effect.

Publication bias
Suppose studies greater than ten are included in the meta-analysis. In that case, the symmetry of the funnel plot will be evaluated to examine publication bias, [17] and the results will be interpreted with care.

Strength of the quality of evidence.
In this planned, systematic review and meta-analysis, the study’s evidence quality will be investigated following the Grading of Recommendations Assessment, Development and Evaluation (GRADE)[18,19]. This is classified into 4 levels: high, medium, low, and very low. We will use the GRADE profiler 3.2 for analysis [20].

**Subgroup analysis**

If the obtained results are heterogeneous, we will perform a subgroup analysis to find the source of heterogeneity [21] [22], which can arise from study design, race, age, sex, different intervention forms, drug dosage, forms of diabetes, monotherapy or dual therapy and duration of treatment.

**Statistical analysis**

We will use the Review Manager software Version 5.4 (The Nordic Cochrane Center, The Cochrane Collaboration, 2014, Copenhagen, Denmark) to analyse all data. The mean difference (MD) or standardized MD (SMD) and 95% CI will be estimated for continuous data. If the same scale is used to measure an outcome in different studies, MD will be used. Similarly, we will use SMD if different scales are used to measure the same outcome. If an outcome measure contains less than 2 trials, we will summarize the results descriptively. Meta-analysis will be carried out if at least two or more studies report the same outcome. However, in case of an insufficient number of studies to perform a meta-analysis, a qualitative synthesis will be performed [23]. The statistical level of heterogeneity among included studies will be assessed using the Cochran Q test ($\chi^2$) and the $I^2$ statistical tests. We will classify the heterogeneity using the predefined rules [22]. An $I^2$ of 0% to 25% will be classified as low heterogeneity $I^2$ of 25% to 50% represents moderate heterogeneity [24]. An $I^2$ of 75% to 100% represents high heterogeneity. We will use the fixed-effects model when the P-value from an $\chi^2$ test is more than 0.10 or $I^2$ 50%. A subgroup analysis will be conducted to identify possible sources for statistical heterogeneity, considering prespecified factors. A descriptive summary of individual studies will be made when a meta-analysis is not possible.
Discussion

Diabetic neuropathy has no existing medical cure, and prevention will be crucial. With tight glucose control, we can see a decrease in the progression of nerve damage. However, this warrants for effective medical intervention. This systematic review and meta-analysis focus on vitamin B12 and ALA as monotherapy or dual treatments in diabetic mellitus/neuropathy. These components have shown properties that decrease oxidative stress and increase nerve myelination. A previous meta-analysis assessing the effect of ALA on lipid profiles revealed a significant decrease in lipid profiles; however, this was conducted in patients with various conditions, including diabetes, schizophrenia, obesity, renal disease, and hyperthyroidism[12]. So there is a need for evaluation of the effects of both ALA and vitamin B12 on lipid profile, glycemia, and inflammation in diabetes mellitus.

Ethics and dissemination

Ethical clearance is not required. Participants will not be recruited in this study, and data will be obtained from already published studies. The findings will be disseminated through peer-reviewed publications and presented at local and international seminars and conferences. There is a rising rate of cardiovascular disease among patients with diabetes, and the available drugs prove to exacerbate this. This review may highlight the beneficial impact of these treatments in diabetes against secondary complications.

Funding statement

There are no funders to report for this submission

Competing of interests

No, there are no competing interests for any author
Data Availability Statement

Raw data involving literature search, data extraction, and statistical analysis will be made available from the corresponding author on request after the article has been published.

Contributorship

KM and PKL conceptualized, designed, and initiated the protocol. PKL wrote the first draft. KM edited the final version of the protocol. KM supervised all the write-up and editing. All authors approved the final manuscript submitted for publication.

Abbreviations

DN: Diabetic neuropathy
DM: Diabetes mellitus
ROS: Reactive oxygen species
ALA: Alpha lipoic acid
TC: Total cholesterol
TG: Triglyceride
LDL: Low-density lipoprotein
HDL: High-density lipoprotein
IL-6: Interleukin-6
TNF-α: Tumour necrosis factor-alpha

References
Mayo Clinic. Mayoclinic.org. 2022. Symptoms and causes - Mayo Clinic. [online] Available at: <https://mayoclinic.org/diseases-conditions/diabetic-neuropathy/symptoms-causes/syco-20371580/p=1> [Accessed 13 April 2022].

Nascimento OJM do, Pupe CCB, Cavalcanti EBU. Diabetic neuropathy. Revista Dor 2016;17. doi:10.5935/1806-0013.20160047

Didangelos T, Karlafti E, Kotzakioulafi E, et al. Efficacy and Safety of the Combination of Superoxide Dismutase, Alpha Lipoic Acid, Vitamin B12, and Carnitine for 12 Months in Patients with Diabetic Neuropathy. Nutrients 2020;12:3254. doi:10.3390/nu12113254

Okdahl T, Brock C. Molecular Aspects in the Potential of Vitamins and Supplements for Treating Diabetic Neuropathy. Current Diabetes Reports 2021;21:31. doi:10.1007/s11892-021-01397-1

Melese H, Alamer A, Temesgen MH, et al. Effectiveness of exercise therapy on gait function in diabetic peripheral neuropathy patients: A systematic review of randomized controlled trials. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2020;13:2753–64. doi:10.2147/DMSO.S261175

Julian T, Syeed R, Glasow N, et al. B12 as a treatment for peripheral neuropathic pain: A systematic review. Nutrients. 2020;12:1–16. doi:10.3390/nu12082221

Elbadawy AM, Abd Elmoniem RO, Elsayed AM. Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters. Alexandria Journal of Medicine 2021;57:113–20. doi:10.1080/20905068.2021.1907961

Okdahl T, Brock C. Molecular Aspects in the Potential of Vitamins and Supplements for Treating Diabetic Neuropathy. doi:10.1007/s11892-021-01397-1/Published

Didangelos T, Karlafti E, Kotzakioulafi E, et al. Vitamin B12 supplementation in diabetic neuropathy: A 1-year, randomized, double-blind, placebo-controlled trial. Nutrients 2021;13:1–14. doi:10.3390/nu13020395

Kamenova P. α-lipoic acid increases insulin sensitivity Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid. Nutrition and Metabolism. 2018;15. doi:10.1186/s12986-018-0274-y

Mousavi SM, Shab-Bidar S, Kord-Varkaneh H, et al. Effect of alpha-lipoic acid supplementation on lipid profile: A systematic review and meta-analysis of controlled clinical trials. Nutrition. 2019;59:121–30. doi:10.1016/j.nut.2018.08.004

Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Revista Espanola de Nutricion Humana y Dietetica 2016;20:148–60. doi:10.1186/2046-4053-4-1

by Julian Higgins EP, Savović J, Page MJ, et al. Revised Cochrane risk of bias tool for randomized trials (RoB 2.0). 2016.
1. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration’s tool for assessing risk of bias in randomised trials. *BMJ (Online)* 2011;343. doi:10.1136/bmj.d5928

2. Mathur MB, VanderWeele TJ. Sensitivity analysis for publication bias in meta-analyses. *Journal of the Royal Statistical Society Series C: Applied Statistics* 2020;69:1091–119. doi:10.1111/rssc.12440

3. Moreno SG, Sutton AJ, Ades A, et al. Assessment of regression-based methods to adjust for publication bias through a comprehensive simulation study. *BMC Medical Research Methodology* 2009;9. doi:10.1186/1471-2288-9-2

4. Atkins D, Eccles M, Flottorp S, et al. Systems for grading the quality of evidence and the strength of recommendations I: Critical appraisal of existing approaches. *BMC Health Services Research* 2004;4. doi:10.1186/1471-2288-4-38

5. Granholm A, Alhazzani W, Møller MH. Use of the GRADE approach in systematic reviews and guidelines. *British Journal of Anaesthesia*. 2019;123:554–9. doi:10.1016/j.bja.2019.08.015

6. Balshem H, Helfand M, Schünemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. *Journal of Clinical Epidemiology* 2011;64:401–6. doi:10.1016/j.jclinepi.2010.07.015

7. Sedgwick P. Meta-analyses: Heterogeneity and subgroup analysis. *BMJ (Online)*. 2013;346. doi:10.1136/bmj.f4040

8. Schroll JB, Moustgaard R, Gøtzsche PC. Dealing with substantial heterogeneity in Cochrane reviews. Cross-sectional study. *BMC Medical Research Methodology* 2011;11. doi:10.1186/1471-2288-11-22

9. Popay J, Roberts H, Sowden A, et al. Guidance on the Conduct of Narrative Synthesis in Systematic Reviews A Product from the ESRC Methods Programme Peninsula Medical School, Universities of Exeter and Plymouth. 2006.

10. Imrey PB. Limitations of Meta-analyses of Studies with High Heterogeneity. *JAMA Network Open*. 2020;3. doi:10.1001/jamanetworkopen.2019.19325
# Reporting checklist for protocol of a systematic review and meta analysis.

Based on the PRISMA-P guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

| Reporting Item | Page Number |
|----------------|-------------|
| **Title**      |             |
| Identification #1a | Identify the report as a protocol of a systematic review | 1 |
| Update #1b | If the protocol is for an update of a previous systematic review, identify as such | 1 |
| **Registration** |             |
| #2 | If registered, provide the name of the registry (such as PROSPERO) and registration number | 2,4 |
| **Authors**    |             |
| Contact #3a | Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author | 1 |
| Contribution #3b | Describe contributions of protocol authors and identify the | 10, 11 |

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml
Amendments

#4 If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments

Support

Sources #5a Indicate sources of financial or other support for the review

Sponsor #5b Provide name for the review funder and / or sponsor

Role of sponsor or funder #5c Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol

Introduction

Rationale #6 Describe the rationale for the review in the context of what is already known

Objectives #7 Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)

Methods

Eligibility criteria #8 Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review

Information sources #9 Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage

Search strategy #10 Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated

Study records - data management #11a Describe the mechanism(s) that will be used to manage records and data throughout the review

Study records - #11b State the process that will be used for selecting studies (such...
| Section |
|---------|
| selection process | as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis) |
| Study records - data collection process | 
| #11c | Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators |
| Data items | 
| #12 | List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications |
| Outcomes and prioritization | 
| #13 | List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale |
| Risk of bias in individual studies | 
| #14 | Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis |
| Data synthesis | 
| #15a | Describe criteria under which study data will be quantitatively synthesised |
| Data synthesis | 
| #15b | If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall’s τ) |
| Data synthesis | 
| #15c | Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression) |
| Data synthesis | 
| #15d | If quantitative synthesis is not appropriate, describe the type of summary planned |
| Meta-bias(es) | 
| #16 | Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies) |
| Confidence in cumulative evidence | 
| #17 | Describe how the strength of the body of evidence will be assessed (such as GRADE) |
The PRISMA-P elaboration and explanation paper is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 10. June 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai
### PubMed on the 30 June 2022

| Item | Mesh terms | Hits  |
|------|------------|-------|
| 1    | Alpha-lipoic acid | 4,509 |
| 2    | Type 2 diabetes    | 159398 |
| 3    | Alpha-lipoic acid AND type 2 diabetes mellitus | 36 |

| Item | Mesh terms | Hits  |
|------|------------|-------|
| 1    | Vitamin B12 | 23248 |
| 2    | Cobalamin | 23248 |
| 3    | cyanocobalamin | 23248 |
| 4    | Type 2 diabetes mellitus | 159398 |
| 5    | 1,2,3, and 4 | 30 |
Exploring the effectiveness of vitamin B12 complex and alpha-lipoic acid as a treatment for diabetes mellitus/neuropathy. A protocol for systematic review and meta-analysis of randomised controlled trials.

| Journal:    | *BMJ Open* |
|-------------|------------|
| Manuscript ID | bmjopen-2022-065630.R2 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 08-Aug-2022 |
| Complete List of Authors: | Lekhanya, Portia; University of South Africa College of Agriculture and Environmental Sciences, Mokgalaboni, Kabelo; University of South Africa College of Agriculture and Environmental Sciences, Life and Consumer Sciences; |
| <b>Primary Subject Heading</b>: | Diabetes and endocrinology |
| Secondary Subject Heading: | Nutrition and metabolism, Diabetes and endocrinology, Pharmacology and therapeutics, Qualitative research, Cardiovascular medicine |
| Keywords: | Diabetic neuropathy < DIABETES & ENDOCRINOLOGY, General diabetes < DIABETES & ENDOCRINOLOGY, Lipid disorders < DIABETES & ENDOCRINOLOGY, Diabetes & endocrinology < INTERNAL MEDICINE, NUTRITION & DIETETICS |
Exploring the effectiveness of vitamin B12 complex and alpha-lipoic acid as a treatment for diabetes mellitus/neuropathy. A protocol for systematic review and meta-analysis of randomised controlled trials.

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Abstract

Introduction: Diabetic neuropathy (DN) is common in patients diagnosed with diabetes mellitus. This often causes peripheral nerve damage. For many years vitamin B12 and alpha-lipoic acid (ALA) have been regarded as components that can in reducing markers of inflammation and oxidative stress. In this study, we will explore the effectiveness of vitamin B12 and ALA as a possible treatment for diabetic mellitus/neuropathy, emphasizing markers of inflammation, lipid profile, and glucose metabolism.

Methods and analysis: We will conduct a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol (PRISMA-P). The search strategies and information sources for the literature will be PubMed, Google Scholar, Web of Science, and Science direct. The literature search will include studies published from inception until 30 June 2022. All included studies will be evaluated for quality and risk of bias according to the Cochrane guidelines. To investigate the stability of the results, we will conduct a sensitivity analysis of the outcomes. All data analysis will be performed using Review Manager version 5.4.

Ethical and dissemination: This systematic review and meta-analysis will not require ethical approval from an institution committee as it does not have direct participants. We will obtain all our data from previous studies. The findings will be disseminated through publications in peer-reviewed journals and presented at local and international seminars and conferences.

Keywords: Diabetic neuropathy, Vitamin B12, Alpha Lipoic Acid, inflammation, hyperglycemia, and diabetes mellitus.
Strengths and limitations of this study.

- This proposed review will comprehensively assess published randomized controlled trials on the primary research question, focusing on glycemic control, inflammation, and lipid profile in diabetic patients on either monotherapy or dual treatment of vitamin B12 and alpha-lipoic acid.

- A comprehensive search for literature will be conducted on the database to source out and retrieve studies relevant to our research question.

- This will be the primary systematic review and meta-analysis conducted to evaluate the single or dual supplementation of these treatments in diabetes mellitus.

- The anticipated limitation includes heterogeneity that may arise due to different dosages, forms of diabetes, dual or monotherapy, and also the duration of intervention.

- However, subgroup and sensitivity analysis will be performed to source the exact cause of heterogeneity.
Introduction.

Diabetic Neuropathy (DN) is a heterogeneous type of nerve damage associated with diabetes mellitus, the condition most often damages nerves in the legs and feet [1]. It presents both clinically and sub-clinically affecting the peripheral nervous system due to an increase in glucose concentration which interferes with nerve signaling [1,2].

Following the discovery of insulin as a treatment for diabetes mellitus (DM), the prevalence of DN has since increased significantly due to DM patients having a longer life expectancy [2]. It has been estimated that at least 50% of DM patients will develop DN in their life, with approximately 20% experiencing neuropathic-related pains [1,2]. Nerves are susceptible to changes in glucose concentrations, and insulin treatment makes it impossible for neurons to continue regulating glucose uptake [3,4].

For many years, vitamin B12 and alpha-lipoic acid (ALA) have been regarded as components that reduce pain and oxidative stress. Vitamin B12 is essential in the metabolism of essential fatty acids involved in the maintenance of nerve myelin and direct scavenging of reactive oxygen species (ROS). However, patients with DN on metformin treatment have been shown to have low vitamin B12; this is because metformin on its own impairs vitamin B12 absorption. When vitamin B12 deficiency is prolonged, it leads to nerve degeneration, causing irreversible nerve damage [5]. Previous researchers have explored the benefits of vitamin B12 and have discovered that it stimulates nerve regeneration by promoting axon growth of neural cells after peripheral nerve damage [6].

On the other hand, the naturally occurring ALA, an organosulfur compound derived from octanoic acid [7], is required to generate energy in the mitochondria by various enzymes. Most importantly, ALA is a potent antioxidant agent that can neutralize ROS and nerve blood flow, resulting in improved distal nerve conductions (1). Additionally, ALA acts as a scavenger for free radicals intra-
and extracellularly to repair oxidative damage [8,9]. Furthermore, ALA has been shown to increase the uptake of glucose to control glucose metabolism [7–9]. Moreover, another study showed an improvement in glucose level and insulin sensitivity following the use of ALA treatment in type 2 diabetes mellitus (T2D) patients [10]. In metabolic syndromes, a previous meta-analysis demonstrated that treatment of ALA improves the marker of inflammation [11]. However, it is still unclear how these compounds impact the markers of inflammation and related glucose parameters in patients with diabetes/diabetic neuropathy. A previous meta-analysis assessing the effect of ALA on lipid profiles revealed a significant decrease in lipid profiles; however, this was conducted in patients with various conditions, including diabetes, schizophrenia, obesity, renal disease, and hyperthyroidism [12]. Therefore, this systematic review and meta-analysis aim to explore the effectiveness of vitamin B12 and ALA as either monotherapy or dual treatment for diabetic mellitus/neuropathy with major emphasis on markers of inflammation, lipid profile, and glucose metabolism.

**METHODOLOGY.**

This protocol for a systematic review and meta-analysis is aimed at evaluating the beneficial impact of vitamin B12 and alpha-lipoic acid in diabetic mellitus with a major focus on markers of inflammation, lipid profile, and glucose metabolism. The review will follow the Preferred Reporting Items for Systematic reviews and Meta-Analysis protocol (PRISMA-P) [13] Supplementary file 1.

**Patient and public involvement.**

No patient involved.
Aim.

The study’s main aim is to explore the effectiveness of vitamin B12 and alpha-lipoic acid as a possible treatment for diabetic mellitus/neuropathy with major emphasis on markers of inflammation, lipid profile, and glucose metabolism.

Questions.

1. Does alpha-lipoic acid increase the uptake of glucose for better glycaemic control?
2. Do vitamin B12 and alpha-lipoic acid improve markers of inflammation?
3. What is the impact of vitamin B12/alpha-lipoic acid on lipid profile amongst diabetes patients?

Participants/Population.

We will consider studies conducted in patients with diabetes/Diabetic Neuropathy on either vitamin B12 or alpha-lipoic acid as supplements.

Intervention.

We will consider studies conducted on patients living with diabetes mellitus/diabetic neuropathy on Vitamin B12 and Alpha lipoic acid supplementations. If vitamin B2 and alpha lipoic acid are used in the same trial, and there is a comparator/control, such studies will be analyzed as dual treatment versus placebo/control. However, if one treatment is used in a trial, it will be subgrouped as monotherapy versus placebo/control.

Design: Randomised controlled trials.

Comparator.

The comparator will be divided into two:

1. Patients with diabetes mellitus/diabetic neuropathy on placebo.
2. Healthy participants not on any treatment (control).
Outcomes.

Improved markers of inflammation such as tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), and C-reactive protein (CRP).

Improved glucose parameters, fasting blood glucose, and insulin.

Improved lipid profile; total cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein.

Eligibility criteria.

Inclusion.

All studies will be reviewed for inclusions, and where there may be uncertainty, the studies in the review will be required to meet the following criteria.

The randomised controlled trials focus on the effects of vitamin B12 and alpha-lipoic acid in diabetic mellitus/ neuropathy.

The studies that use human subjects with diabetes/diabetic neuropathy.

The studies published in English.

Exclusion.

Animal studies and studies that are non-English will be excluded from this review. This is partially because translating studies from other languages end up losing the exact results obtained from such studies. Reviews will not be considered. Cohorts, case-control, case studies, and experimental studies will all be excluded. Those studies that use other drugs will be deemed irrelevant to the current study.

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For peer review only

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If the obtained results are heterogeneous, we will perform a subgroup analysis to find the source of heterogeneity [21] [22], which can arise from study design, race, age, sex, different intervention forms, drug dosage, forms of diabetes, monotherapy or dual therapy and duration of treatment.

Statistical analysis

We will use the Review Manager software Version 5.4 (The Nordic Cochrane Center, The Cochrane Collaboration, 2014, Copenhagen, Denmark) to analyse all data. The mean difference (MD) or standardized MD (SMD) and 95% CI will be estimated for continuous data. If the same scale is used to measure an outcome in different studies, MD will be used. Similarly, we will use SMD if different scales are used to measure the same outcome. If an outcome measure contains less than 2 trials, we will summarize the results descriptively. Meta-analysis will be carried out if at least two or more studies report the same outcome. However, in case of an insufficient number of studies to perform a meta-analysis, a qualitative synthesis will be performed [23]. The statistical level of heterogeneity among included studies will be assessed using the Cochran Q test (x²) and the I² statistical tests. We will classify the heterogeneity using the predefined rules [22]. An I² of 0% to 25% will be classified as low heterogeneity, I² of 25% to 50% represents moderate heterogeneity [24]. An I² of 75% to 100% represents high heterogeneity. We will use the fixed-effects model when the P-value from an x² test is more than 0.10 or I² 50%. A subgroup analysis will be conducted to identify possible sources for
statistical heterogeneity, considering prespecified factors. A descriptive summary of individual studies will be made when a meta-analysis is not possible.

Discussion

Diabetic neuropathy has no existing medical cure, and prevention will be crucial. With tight glucose control, we can see a decrease in the progression of nerve damage. However, this warrants for effective medical intervention. This systematic review and meta-analysis focus on vitamin B12 and ALA as monotherapy or dual treatments in diabetic mellitus/neuropathy. These components have shown properties that decrease oxidative stress and increase nerve myelination. A previous meta-analysis assessing the effect of ALA on lipid profiles revealed a significant decrease in lipid profiles; however, this was conducted in patients with various conditions, including diabetes, schizophrenia, obesity, renal disease, and hyperthyroidism[12]. So, there is a need for evaluation of the effects of both ALA and vitamin B12 on lipid profile, glycemia, and inflammation in diabetes mellitus.

Ethics and dissemination

Ethical clearance is not required. Participants will not be recruited in this study, and data will be obtained from already published studies. The findings will be disseminated through peer-reviewed publications and presented at local and international seminars and conferences. There is a rising rate of cardiovascular disease among patients with diabetes, and the available drugs prove to exacerbate this. This review may highlight the beneficial impact of these treatments in diabetes against secondary complications.

Funding statement

There are no funders to report for this submission.
Competing of interests

No, there are no competing interests for any author

Data Availability Statement

Raw data involving literature search, data extraction, and statistical analysis will be made available from the corresponding author on request after the article has been published.

Contributorship

KM and PKL conceptualised, designed, and initiated the protocol. PLK and KM drafted the original manuscript. KM revised and edited the final version of the protocol. All authors approved the final version of the manuscript submitted for publication. Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Abbreviations

DN: Diabetic neuropathy
DM: Diabetes mellitus
ROS: Reactive oxygen species
ALA: Alpha lipoic acid
TC: Total cholesterol
TG: Triglyceride
LDL: Low-density lipoprotein
HDL: High-density lipoprotein
IL-6: Interleukin-6
TNF-α: Tumour necrosis factor-alpha
References

1 Mayo Clinic. Mayoclinic.org. 2022. Symptoms and causes - Mayo Clinic. [online] Available at: <https://mayoclinic.org/diseases-conditions/diabetic-neuropathy/symptoms-causes/syc-20371580?p=1> [Accessed 13 April 2022]. 2022.

2 Nascimento OJM do, Pupe CCB, Cavalcanti EBU. Diabetic neuropathy. Revista Dor 2016;17. doi:10.5935/1806-0013.20160047

3 Didangelos T, Karlafti E, Kotzakioulafi E, et al. Efficacy and Safety of the Combination of Superoxide Dismutase, Alpha Lipoic Acid, Vitamin B12, and Carnitine for 12 Months in Patients with Diabetic Neuropathy. Nutrients 2020;12:3254. doi:10.3390/nu12113254

4 Okdahl T, Brock C. Molecular Aspects in the Potential of Vitamins and Supplements for Treating Diabetic Neuropathy. Current Diabetes Reports 2021;21:31. doi:10.1007/s11892-021-01397-1

5 Melese H, Alamer A, Temesgen MH, et al. Effectiveness of exercise therapy on gait function in diabetic peripheral neuropathy patients: A systematic review of randomized controlled trials. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2020;13:2753–64. doi:10.2147/DMSO.S261175

6 Julian T, Syeed R, Glaucow N, et al. B12 as a treatment for peripheral neuropathic pain: A systematic review. Nutrients. 2020;12:1–16. doi:10.3390/nu12082221

7 Elbadawy AM, Abd Elmoniem RO, Elsayed AM. Alpha lipoic acid and diabetes mellitus: potential effects on peripheral neuropathy and different metabolic parameters. Alexandria Journal of Medicine 2021;57:113–20. doi:10.1080/20905068.2021.1907961

8 Okdahl T, Brock C. Molecular Aspects in the Potential of Vitamins and Supplements for Treating Diabetic Neuropathy. doi:10.1007/s11892-021-01397-1/Published

9 Didangelos T, Karlafti E, Kotzakioulafi E, et al. Vitamin B12 supplementation in diabetic neuropathy: A 1-year, randomized, double-blind, placebo-controlled trial. Nutrients 2021;13:1–14. doi:10.3390/nu13020395

10 Kamenova P. α-lipoic acid increases insulin sensitivity Improvement of insulin sensitivity in patients with type 2 diabetes mellitus after oral administration of alpha-lipoic acid.

11 Akbari M, Ostadmohammadi V, Tabrizi R, et al. The effects of alpha-lipoic acid supplementation on inflammatory markers among patients with metabolic syndrome and related disorders: A systematic review and meta-analysis of randomized controlled trials. Nutrition and Metabolism. 2018;15. doi:10.1186/s12986-018-0274-y
Mousavi SM, Shab-Bidar S, Kord-Varkaneh H, et al. Effect of alpha-lipoic acid supplementation on lipid profile: A systematic review and meta-analysis of controlled clinical trials. Nutrition. 2019;59:121–30. doi:10.1016/j.nut.2018.08.004

Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Revista Espanola de Nutricion Humana y Dietetica 2016;20:148–60. doi:10.1186/2046-4053-4-1

by Julian Higgins EP, Savović J, Page MJ, et al. Revised Cochrane risk of bias tool for randomized trials (RoB 2.0). 2016.

Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration’s tool for assessing risk of bias in randomised trials. BMJ (Online) 2011;343. doi:10.1136/bmj.d5928

Mathur MB, VanderWeele TJ. Sensitivity analysis for publication bias in meta-analyses. Journal of the Royal Statistical Society Series C: Applied Statistics 2020;69:1091–119. doi:10.1111/rssc.12440

Moreno SG, Sutton AJ, Ades A, et al. Assessment of regression-based methods to adjust for publication bias through a comprehensive simulation study. BMC Medical Research Methodology 2009;9. doi:10.1186/1471-2288-9-2

Atkins D, Eccles M, Flottorp S, et al. Systems for grading the quality of evidence and the strength of recommendations I: Critical appraisal of existing approaches. BMC Health Services Research 2004;4. doi:10.1186/1472-6963-4-38

Granholm A, Alhazzani W, Møller MH. Use of the GRADE approach in systematic reviews and guidelines. British Journal of Anaesthesia. 2019;123:554–9. doi:10.1016/j.bja.2019.08.015

Balshem H, Helfand M, Schünemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. Journal of Clinical Epidemiology 2011;64:401–6. doi:10.1016/j.jclinepi.2010.07.015

Sedgwick P. Meta-analyses: Heterogeneity and subgroup analysis. BMJ (Online). 2013;346. doi:10.1136/bmj.f4040

Schroll JB, Moustgaard R, Gøtzsche PC. Dealing with substantial heterogeneity in Cochrane reviews. Cross-sectional study. BMC Medical Research Methodology 2011;11. doi:10.1186/1471-2288-11-22

Popay J, Roberts H, Sowden A, et al. Guidance on the Conduct of Narrative Synthesis in Systematic Reviews A Product from the ESRC Methods Programme Peninsula Medical School, Universities of Exeter and Plymouth. 2006.

Imrey PB. Limitations of Meta-analyses of Studies with High Heterogeneity. JAMA Network Open. 2020;3. doi:10.1001/jamanetworkopen.2019.19325
# Reporting checklist for protocol of a systematic review and meta analysis.

Based on the PRISMA-P guidelines.

**Instructions to authors**

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the PRISMA-Preporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

| Reporting Item | Page Number |
|----------------|-------------|
| **Title**      |             |
| Identification | #1a         | Identify the report as a protocol of a systematic review | 1 |
| Update         | #1b         | If the protocol is for an update of a previous systematic review, identify as such | 1 |
| **Registration** |               | If registered, provide the name of the registry (such as PROSPERO) and registration number | 2,4 |
| **Authors**    |             |
| Contact        | #3a         | Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author | 1 |
| Contribution   | #3b         | Describe contributions of protocol authors and identify the | 10, 11 |

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Amendments

#4 If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments

N/A

Support

Sources #5a Indicate sources of financial or other support for the review 9

Sponsor #5b Provide name for the review funder and / or sponsor 9

Role of sponsor or funder #5c Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol 9

Introduction

Rationale #6 Describe the rationale for the review in the context of what is already known 3-4

Objectives #7 Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO) 4-5

Methods

Eligibility criteria #8 Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review 6

Information sources #9 Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage 6-7

Search strategy #10 Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated 7

Study records - data management #11a Describe the mechanism(s) that will be used to manage records and data throughout the review 6-7

Study records - #11b State the process that will be used for selecting studies (such
selection process as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)

Study records - data collection process #11c Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators

Data items #12 List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications

Outcomes and prioritization #13 List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale

Risk of bias in individual studies #14 Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis

Data synthesis #15a Describe criteria under which study data will be quantitatively synthesised

Data synthesis #15b If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall’s τ)

Data synthesis #15c Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)

Data synthesis #15d If quantitative synthesis is not appropriate, describe the type of summary planned

Meta-bias(es) #16 Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)

Confidence in cumulative evidence #17 Describe how the strength of the body of evidence will be assessed (such as GRADE)
The PRISMA-P elaboration and explanation paper is distributed under the terms of the Creative Commons Attribution License CC-BY. This checklist was completed on 10. June 2022 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai
### PubMed on the 30 June 2022

| Item | Mesh terms                                           | Hits   |
|------|-----------------------------------------------------|--------|
| 1    | Alpha-lipoic acid                                   | 4,509  |
| 2    | Type 2 diabetes                                     | 159398 |
| 3    | Alpha-lipoic acid AND type 2 diabetes mellitus      | 36     |

| Item | Mesh terms                                           | Hits   |
|------|-----------------------------------------------------|--------|
| 1    | Vitamin B12                                         | 23248  |
| 2    | Cobalamin                                           | 23248  |
| 3    | cyanocobalamin                                      | 23248  |
| 4    | Type 2 diabetes mellitus                            | 159398 |
| 5    | 1,2,3, and 4                                        | 30     |