PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Vitamin D supplementation in chronic spinal cord injury (VitD-SCI): study protocol for a randomized controlled trial
AUTHORS	Hertig-Godeschalk, Anneke; Brinkhof, Martin; Scheel-Sailer, Anke; Perret, Claudio; Jenny, Andreas; Landmann, Gunter; Wyss, Patrik; Flueck, Joelle

VERSION 1 – REVIEW

REVIEWER	Fushimi, Kazunari Gifu Prefectural General Medical Center, Department of Orthopedic Surgery
REVIEW RETURNED	18-Jul-2021

GENERAL COMMENTS	The authors are investigating the effect of vitamin D supplementation on several parameters in patients with chronic SCI. The study design is good, and concept of the study is interesting. This might be the first study which we have never seen before. There are some concerns in the manuscript. - The authors mentioned in “Abstract” that immune and respiratory systems are affected by a suboptimal vitamin D status. How do you evaluate patient’s immunologic function? Is that included in assessment of outcomes in this study? - In general, severity of paralysis in SCI patients are quite varied. Daily activity and frequency of sun exposure are also different among patients. How the authors standardize those factors? They might be big bias in this study. Please clarify. - Are the authors matching propensity of the patient in each group, including severity of SCI and patients’ character? if so, sample size has to be large enough.

REVIEWER	Garshick, Eric VA Boston Healthcare System, Pulmonary and Critical Care Medicine Section, Medical Service
REVIEW RETURNED	31-Jul-2021

GENERAL COMMENTS	This is a well written protocol that addresses an important question in the SCI population, and it appears the trial is underway. In publication of this protocol, the authors should further address potential weaknesses not mentioned in the protocol. These include.

	1. Lack of any dietary assessment of dietary vitamin D intake. This could be available by a standardized food frequency assessment conducted at baseline, and at least once or twice during follow-up. This would enable the authors to determine if changes in diet impacted on vitamin D levels. 2. It is possible that vitamin D level will vary by AIS score (as a measure of SCI severity). In regards to the secondary outcomes such as ulcers, these would vary significantly. Did the authors consider only including individuals with motor complete SCI with cervical or high thoracic injury, as compared to less severe SCI? 3. In regards to assessing effects on the secondary outcomes, the sample size is small, and this should be acknowledged. 4. Given the potential impact of vitamin D on respiratory illness, it is surprising that a respiratory outcome, such as upper respiratory tract illness symptoms/cold occurrence, will not be tracked. This could be done by standardized questionnaire. This could be added to the calls that are made to to encourage compliance. 5. How completely and frequently will information on medical comorbid conditions, lifestyle habits (smoking, alcohol), and healthcare utilization be assessed? Chronic illness in general has been associated with lower vitamin D levels.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Kazunari Fushimi, Gifu Prefectural General Medical Center Comments to the Author:
The authors are investigating the effect of vitamin D supplementation on several parameters in patients with chronic SCI. The study design is good, and concept of the study is interesting. This might be the first study which we have never seen before.

Dear Dr. Fushimi, thank you very much for this positive feedback. We appreciate your valuable suggestions. Please find our responses below, all changes are highlighted in the manuscript.
There are some concerns in the manuscript.

1.1 The authors mentioned in “Abstract” that immune and respiratory systems are affected by a suboptimal vitamin D status. How do you evaluate patient’s immunologic function? Is that included in assessment of outcomes in this study?

Thank you for this question. Due to financial and feasibility reasons, it is unfortunately not possible to assess biomarkers for immune function or respiratory function during the study. However, we agree with you that this are important aspects to investigate in the future. To acknowledge this, we have added it in the Strengths and Limitations ("For reasons of feasibility, not all supposedly relevant clinical outcomes can be assessed, including respiratory and immune function") and Methods – Outcomes and assessments ("Due to feasibility reasons, no direct measurements of respiratory or immune function will be assessed.") sections. In this study, we do track the incidence of urinary tract infections as well as the incidence of other illness during the intervention phase. All outcomes are summarized in Table 2, we also added another sentence in the manuscript (Methods – Outcomes and Assessments: "Furthermore, the occurrence of any new

illness, injury or disease together with newly taken medication and supplements will be assessed at each visit.").

1.2 In general, severity of paralysis in SCI patients are quite varied. Daily activity and frequency of sun exposure are also different among patients. How the authors standardize those factors? They might be big bias in this study. Please clarify.

Thank you for this comment. In light of the randomization we respectfully disagree that the factors daily activity and frequency of sun exposure represent a "big" risk of "bias" in the present study, which investigates the effect of vitamin D supplementation (experimental exposure) on vitamin D status (primary outcome). Presuming that the reviewer hints at remaining confounding and accepting that these factors may affect vitamin D status, there is no reason to assume that daily activities or sun exposure affect vitamin D supplementation. Thus confounding bias is not an issue:

vitD supplementation ◄—╫— contextual factor (daily activity, sun exposure) —►vitD status
Although we do not consider physical activity and sun exposure as major source of bias, these parameters are systematically evaluated by questionnaire at each study visit (see Table 2). We will assess the duration and type of physical activity. Sun exposure will be assessed as a function of time spent in the sun and relative surface of skin exposed. Data on skin phototype and BMI (as an equivalent for obesity/subcutaneous fat) will also be collected. Repeated measurements of these contextual factors may be used to verify effectiveness of randomization. Furthermore, these factors provide time-updated information to statistically control for within- and between-person variation in investigating the efficacy of vitamin D supplementation on vitamin D dynamics, as part of a sensitivity analysis.

We have added a sentence about this in the Strengths and Limitations ("The recurrent assessment of contextual parameters, including sun exposure and secondary health conditions, for use as time-updated covariates in the data analysis, will further support unbiased inference of the efficacy of vitamin D supplementation.") as well as the Methods – Outcomes and Assessments section ("The recurrent assessment of contributory parameters, including sun exposure and secondary health conditions, provide time-updated information to statistically control for withinand between-person variation in investigating the efficacy of vitamin D supplementation on vitamin D dynamics.").

1.3 Are the authors matching propensity of the patient in each group, including severity of SCI and patients' character? if so, sample size has to be large enough.

Thank you for these important questions. Patients' characteristics will not be matched for each group. Since the study has a within-participant repeated-measures design, changes in the primary outcome will also be assessed within an individual. Therefore, the heterogeneity between participants will not influence the effect of vitamin D supplementation as strongly, which is also justified by our sample size calculation (Supplement 2). We have added sentences about this in the Strengths and Limitations ("The longitudinal study design facilitates control for within- and between-person variation in investigating the temporal efficacy of vitamin D supplementation on vitamin D dynamics.") as well as the Methods – Sample size ("Sample size calculation did not take secondary outcome parameters into account. Since long-term studies among the chronic SCI population are rare, this study may inform the minimal sample size needed for future studies targeting the dynamics of secondary outcome parameters in response to vitamin D supplementation.") sections.

Reviewer: 2

Dr. Eric Garshick, VA Boston Healthcare System, Brigham and Women's Hospital and Harvard Medical School, Boston Comments to the Author:
This is a well written protocol that addresses an important question in the SCI population, and it appears the trial is underway.
Dear Dr Garshick, thank you very much for your positive and constructive feedback which helped us to improve our manuscript.

In publication of this protocol, the authors should further address potential weaknesses not mentioned in the protocol. These include…..
We appreciate your recommendations and included those in the limitations section. Please find our responses below, all changes are highlighted in the manuscript.
2.1 Lack of any dietary assessment of dietary vitamin D intake. This could be available by a standardized food frequency assessment conducted at baseline, and at least once or twice during follow-up. This would enable the authors to determine if changes in diet impacted on vitamin D levels.

We acknowledge, that the assessment of the food frequency at baseline as well as during the study would give further insight into nutritional intake with potential influence on vitamin D status. Nevertheless, this would have only a marginal effect compared to other factors such as mobility, sun exposure or seasonality. Nutritional intake of vitamin D accounts for around 10 to 15% of the daily needs of vitamin D. In Switzerland, we do not have vitamin D fortified products to account for. Energy intake among individuals with SCI is even lower as compared to the general population, which further reduces the influence of nutritional vitamin D intake on vitamin D status (see Farkas GJ, Pitot MA, Berg AS, Gater DR. Nutritional status in chronic spinal cord injury: a systematic review and meta-analysis. Spinal Cord. 2019 Jan;57(1):3-17.). For example, Perret and StoffelKurt (2011) found a low intake of vitamin in individuals with acute and chronic spinal cord injury (Perret C, Stoffel-Kurt N. Comparison of nutritional intake between individuals with acute and chronic spinal cord injury. J Spinal Cord Med. 2011 Nov;34(6):569-75.).

We included the lack of any dietary assessment of vitamin D intake in the Strengths and Limitations ("Dietary vitamin D will not be assessed over the study period, which precludes scaling of vitamin D efficacy to between- and within-person variation in nutrition intake."), as well as in the Methods – Outcomes and Assessments ("Since nutritional intake of vitamin D only accounts for a limited intake and vitamin D fortification of nutrition in Switzerland is rare,34 no dietary assessments are planned, which precludes scaling of vitamin D efficacy to between- and withinperson variation in nutrition intake.") section.

2.2 It is possible that vitamin D level will vary by AIS score (as a measure of SCI severity). In regards to the secondary outcomes such as ulcers, these would vary significantly. Did the authors consider only including individuals with motor complete SCI with cervical or high thoracic injury, as compared to less severe SCI?

Thank you for this important question and topic. The review article by Flueck and Perret (2016) discusses this topic in detail. To date, it seems inconclusive whether the lesion or the AIS score are linked to the vitamin D status. Beside the level of completeness other factors such as mobility and functional independence as well as physical activity might have an impact on vitamin D status. Therefore, we did not narrow the inclusion criteria further down but will collect data on lesion level, AIS score, SCIM, physical activity, mobility and sun exposure to be able to conduct a co-variate analysis.

We have added sentences in the Methods – Participants section ("No further eligibility criteria,

such as the completeness or the lesion level, were set, to allow a generalization of the study results as well as to increase the feasibility. The recurrent assessment of contextual parameters for use as time-updated covariates in the data analysis will further support unbiased inference of the temporal efficacy of vitamin D supplementation.").

2.3 In regards to assessing effects on the secondary outcomes, the sample size is small, and this should be acknowledged.

Thank you for this suggestion. We acknowledged this in the manuscript under the Methods – Sample Size section ("Sample size calculation did not take secondary outcome parameters into account. Since long-term studies among the chronic SCI population are rare, this study may inform the minimal sample size needed for future studies targeting the dynamics of secondary outcome parameters in response to vitamin D supplementation."). As mentioned in the manuscript, our sample size estimation is based on our primary outcome parameter which is the vitamin D status.

Given the potential impact of vitamin D on respiratory illness, it is surprising that a respiratory outcome, such as upper respiratory tract illness symptoms/cold occurrence, will not be tracked. This could be done by standardized questionnaire. This could be added to the calls that are made to to encourage compliance.

Thank you for this comment. We agree with you that the occurrence of respiratory illness might be related to vitamin D status. We track all the illnesses, which occur between the different study visits. Furthermore, we do also assess the occurrence of urinary tract infections as well as intake of any new medication or supplements (Table 2). We added a sentence in the Methods – Outcomes and Assessments section ("Furthermore, the occurrence of any new illness, injury or disease together with newly taken medication and supplements will be assessed at each visit."). As mentioned in the comments above, the sample size estimation was not calculated for the primary outcome, vitamin D status.

2.4 How completely and frequently will information on medical comorbid conditions, lifestyle habits (smoking, alcohol), and healthcare utilization be assessed? Chronic illness in general has been associated with lower vitamin D levels.

For each visit (every 3 months), the occurrence of any illness, disease or injury, new medication, physical activity, pain, fatigue, sun exposure and SCIM will be assessed using questionnaires (Table 2). The collection of these parameters will give further insight into lifestyle habits during the last 3 months as well as the occurrence of any medical comorbidities.