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# Is bone tenderness, as measured by manual algometry, associated with vitamin D deficiency?

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Objective: *To explore the relationship between serum 25-hydroxycholecalciferol (25[OH]D3) and pressure-pain thresholds, as measured by algometer, in advance of a main study to determine whether PPT is a potentially cost-effective proxy measure of 25[OH]D3 status in the general population.*
Methods: *The cross-sectional pilot study involved a convenience sample of twenty-two subjects (10 males, 12 females), aged 18 to 67 years. All subjects consented to three trials of pressure-pain threshold readings on both tibiae and the manubrium. Serum 25[OH]D3 levels were determined from blood samples drawn post-algometry.*
Results: *The average pressure pain thresholds were 14.92 (±6.03), 15.07(±6.07), 11.10 (±6.68) for the left and right tibia and sternum, respectively. The stability between the measurements was very high with the interclass correlation coefficient (95% CI) calculated as 0.94 (0.62-1.00), 0.9 (0.81-1.00), 0.96(0.93-1.00). The Pearson correlation coefficients were 0.03 for the left tibia, 0.17 for the right tibia and 0.20 for the sternum,*

Objectif : *Étudier la relation entre le taux sérique de 25-hydroxycholécalciférol (25 [OH] D3) et les seuils de tolérance à la pression, tels que mesurés par un algésimètre, en préparatif d'une étude principale pour déterminer si le STP pourrait être une mesure de remplacement économique de l'état de 25[OH]D3 dans la population générale.*

Méthodologie : *L'étude pilote transversale a porté sur un échantillon pratique de vingt-deux sujets (10 hommes, 12 femmes), âgés de 18 à 67 ans. Tous les sujets ont consenti à trois essais lecture des seuils de tolérance à la pression sur le tibia et le manubrium. Les taux sériques de 25[OH]D3 ont été déterminés à partir d'échantillons de sang prélevés après la mesure par l'algésimètre.*

Résultats : *Les seuils moyens de tolérance à la pression étaient 14,92 (±6,03), 15,07(±6,07), 11,10 (±6,68) pour respectivement les tibias gauche, droit et le sternum. La stabilité entre les mesures était très élevée avec le coefficient de corrélation interclasse (IC à 95 %) calculée comme 0,94 (0,62 à 1,00), 0,9 (0,81 à 1,00), 0,96 (0,93 à 1,00). Les coefficients de corrélation de Pearson ont été de 0,03 pour le tibia gauche, 0,17 pour le tibia droit et 0,20 pour le sternum, montrant une corrélation négligeable pour les tibias gauche et droit,*

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*showing a negligible correlation for the left and right tibia, but a low positive correlation for the sternum.* Conclusion: *We did not find preliminary evidence of a strong or otherwise clinically meaningful correlation between bone tenderness and manual algometry in this pilot study. Only a weak linear relationship between PPT in the sternum and serum 25[OH]D3 concentrations was found. Replication of this study is warranted in larger and more representative study populations of interest. Discussion on a number of feasibility issues is provided to inform those future studies.*

*mais une faible corrélation positive pour le sternum.*

Conclusion : *Nous n'avons pas relevé dans cette étude pilote des preuves préliminaires d'une corrélation forte ou cliniquement significative entre la sensibilité des os et l'algométrie manuelle. Seule une faible relation linéaire entre le SPT dans le sternum et les taux sériques de 25[OH]D3 a été constatée. Une reproduction de cette étude est recommandée dans de plus grandes populations cibles qui seraient plus représentatives. Une discussion portant sur plusieurs questions de faisabilité est offerte pour renseigner ces futures études.*

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KEY WORDS: vitamin D, pain, algometry

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## Introduction

Vitamin D is an essential nutrient that plays an integral role in the maintenance of strong and healthy bones. Recent research also suggests its involvement in immunity, metabolic signaling and in the protection against diabetes, cardiovascular disease, auto-immune disease and cancer.[1,2] The precursors of vitamin D3 are produced in the body by steroidogenesis. The formal name of Vitamin D3 is cholecalciferol, which is derived from the irradiation of 7-dehydrocholesterol in the skin following exposure to UV rays.[2,3] It can also be obtained minimally from fatty fishes and fortified foods in the diet.[4,5] There are many factors that contribute to vitamin D deficiency in a population including season, latitude, age, skin pigmentation, and social/cultural practices.[4,6] These factors limit the availability of vitamin D and predispose at-risk populations to deficient states. Statistics Canada reports that 32% of Canadians (age 6 to 79 years) were vitamin D deficient (according to 1997 IOMS standards).[7] Inadequate concentrations of vitamin D for bone health was found in 10% of the population.[7] Vitamin D deficiency definitions vary, but the symptoms include global bone sensitivity, widespread aches, weakness, and general malaise as well as more focal symptoms that commonly present to health care practices.[5,8,9] Consequently, vitamin D deficiency is often misdiagnosed as arthritis, chronic low back pain, fibromyalgia, or chronic fatigue syndrome due to these diffuse, general symptoms. This presents a concern of particular interest to manual therapists.[6] Plotnikoff and Quigley (2003) demonstrated a link between nonspecific musculoskeletal pain and severe 25-hydroxyvitamin D deficiency (defined as <8.0128 nmol/mL) and have suggested all patients with chronic widespread pain ought to be screened for hypovitaminosis D.[4,10-13] Classification has been proposed offering four categories of Vitamin D deficiency: insufficient (50-100nmol/L), mild (25-50 nmol/L), moderate (12.5-25 nmol/L) and severe (<12.5 nmol/L).[14] Currently, serum analysis of 25-hydroxyvitamin D seems to be the most reliable measure of vitamin D status;[4,15] however, this method comes with disadvantages and mass expense to the public. The government of Ontario discontinued covering Vitamin D analysis in 2010 as costs had increased by 2500% at a cost of $66 million per year.[16] Minimizing the number of lab tests and doctors visits required would reduce some of the economic burden created by chronic pain problems thus, a simpler and less expensive means of assessing vitamin D status should be explored.

Manual algometry is a safe and inexpensive method of clinically assessing tissue pain that can be easily utilized in most health care settings. Algometers provide a reliable and credible method for measuring pressure-pain thresholds (PPT) in patients without pain[17] and in patients with bone sensitivity[18,19]. This global bone discomfort can be elicited with gentle pressure on superficial bones such as the sternum, anterior tibia, radius and ulna.[4,11] Intraclass correlation (ICC) coefficients for PPT within days

are reportedly 0.93 to 0.96 and 0.88 to 0.90 between days.[17] Jones et al, found PPT to be highly consistent and repeatable over four days of testing at eight locations in young healthy women.[20] Errors associated with algometry originate from variation in the application of pressure by practitioners, subjective bias in the understanding of pressure versus pain by patients, application location, as well as angulation of the algometer.[17] Should a relationship exist between PPT and vitamin D status, the algometer could serve as a reliable proxy measure and be a more accessible and less expensive means of assessing vitamin D status in patients.

A proposed mechanism for the bone pain associated with vitamin D deficiency is poor bone quality from insufficient calcium phosphate available to mineralize the expanding collagen matrix of bone. The rubbery matrix found in people deficient of cholecalciferol does not provide sufficient trabecular and collagenous support but instead hydrates and expands the bone, causing an outward pressure on the internal Haversian canals and external periosteal covering, richly innervated with sensory pain fibres.[11] Long bones have hypertrophic chondrocytes that increases the stress and deformation tolerance within the bone[21] to help support and absorb shock from ambulation and weight bearing. Flat bones, on the other hand, have less trabeculation and collagen content and as such are more sensitive to deformation and pain, as they are structured to contain red bone marrow.[22] With these features taken into consideration, it is reasonable to assume that the sternum will be more sensitive to changes in circulating 25-hydroxycholecalciferol and in pain sensation.[23,24]

The purpose of this pilot study is to assess the association between algometer readings taken from the sternum or tibia and 25-hydroxycholecalciferol D status. If good correlation can be found, algometry could potentially be used as a safer, simpler means of investigating vitamin D status as a possible cause of chronic widespread muscle and bone pain in patients presenting to health care practices. It is not meant to provide conclusive evidence, but to determine feasibility and direction of future research.

## Methods

### *Participant Recruitment.*

Adults aged 18 years or older without any major health concerns were considered eligible for this study. Subjects were recruited from a private, fee-for-service chronic pain clinic. Patients entering the clinic were recruited through posters in the office advertising free vitamin D testing in return for participation in a research study. Participants were then sampled based on convenience. On the day of testing, the first twenty-two patients scheduled for appointments at the clinic were asked to participate in the study, all of whom agreed to enroll in our study. Exclusion criteria included a history of uncontrolled rheumatic condition, uncontrolled diabetes, active cancer, skin lesions near regions of testing, and individuals under 18 years. This information was obtained through a questionnaire provided as part of patient intake. The questionnaire asked questions pertaining to health status, diet, and medication/supplementation.

Ethical approval was provided by the College REB (No. 1103A01)

### *Compensation.*

Participants were given an honorarium of $20 in the form of a Shoppers Drug Mart® gift card for completing the study. If a participant chose to withdraw from the study after having completed one, but not both, components of the study (pressure-pain readings and blood test) the subject was compensated with $10 for their willingness to cooperate.

### *Protection of Patient Anonymity.*

Prior to the day of data collection, all forms were coded with a letter of the alphabet. Each form was placed into an envelope with the corresponding letter. The envelope contained a general intake form, an informed consent form, and a letter-labeled test tube. The researchers had prepared a script to explain the research procedure to prevent coercion. Subjects filled out all forms with the assistance of a researcher who reviewed the informed consent form with subjects and answered all questions. After filling out all forms, subjects were escorted to an examination room where two researchers performed algometry testing. Results were recorded on a form labeled with the letter corresponding to the letter on the patient's envelope. After testing, the participant was notified they would receive their blood analysis results in a letter mailed to them when results became available. Researchers placed the result form in the envelope such that the patient's identity remained anonymous. The intake researcher did

Table 1.
*Demographic Characteristics.*

| | | **Participants (n =21)** |
|---|---|---|
| Age (yr) | | 47.6 ± 13.7 |
| Height (cm) | | 167.2 ± 9.6 |
| Weight (kg) | | 68.9 ± 13.8 |
| Female | | 57.2% |
| Serum 25-(OH) D (nmol/L) | | 80.5 ± 38.9 |
| Ethnicity | Caucasian | 61.9% (13) |
| | Asian | 28.5% (6) |
| | Other | 0.09% (2) |

Table 2.
*Serum Vitamin D levels mean pressure-pain thresholds (PPT) and standard deviations, interclass coefficient and Pearson correlation coefficients (n=19).*

| | Left Tibia PPT | Right Tibia PPT | Sternum PPT | Serum Vitamin D level |
|---|---|---|---|---|
| Average | 14.92 | 15.07 | 11.10 | 80 |
| Standard Deviation | 6.03 | 6.07 | 6.68 | 37.89 |
| ICCs for multiple readings within subjects (95% CI) | 0.94 (0.62-1.00) | 0.90 (0.81-1.00) | 0.96 (0.93-1.00) | |
| Pearson Correlation Coefficients (PPT versus vitamin D level) | 0.03 | 0.17 | 0.20 | |

not view subject algometry results until data analysis was completed. Test tubes were sent to the laboratory with no identifying information besides their letter code.

### *Algometry.*

Pressure-pain thresholds were measured by a Wagner Instrument Pain Test manual algometer at three standardized landmarks: 5cm distal to the medial joint line of the knee bilaterally and 5cm distal to the sternal notch. Three readings were taken at each landmark in a consecutive manner, as research shows that the highest inter-rater ICC coefficients improve to the highest levels with three trials (ICC=0.74 to 0.89).[17,20] The algometer was calibrated the day before data collection. One researcher was responsible for briefing the participants as they began the study procedure. She also measured and marked the landmarks used on each patient prior to testing. The second researcher administered the algometer, while remaining blinded to the results. This was decided due to enhanced reliability when measurements are taken by one examiner.[17] The other researcher then read and recorded the results. During the pressure-pain threshold testing, subjects communicated to the practitioner the moment they felt the slightest sensation of pain.

After the algometer component, each participant underwent venipuncture by a registered nurse at the centre. In order to measure 25-hydroxycholecalciferol, one serum-separator tube (SST) was used to contain the sample of approximately 7 mL of blood. Participants were discharged at this point following a short debrief by the first researcher who was also responsible for the remuneration. The samples were stored at room temperature for ten days and then refrigerated for two weeks at an off-site lab for radioimmunoassay analysis of 25-hydroxycholecalciferol serum concentration.

## Follow Up

After the blood results were analyzed, letters to the participants were mailed to them regarding their vitamin D status. The letters indicated their status and provided nutrition education and feedback appropriate to their vitamin D status. There were three standard letters created, addressing either low, normal or high vitamin D levels.

## Statistical Methods

Stata Software 10.0 ™ was used to perform all statistical evaluations. Mean, standard deviation, confidence intervals, interclass correlation coefficients and Pearsons correlation coefficients were assessed.

## Results

The results are shown in Tables 1 and 2. Twenty-one patients enrolled in this study, consisting of 9 males and 12 females (57.2% female). The average age of the partici-

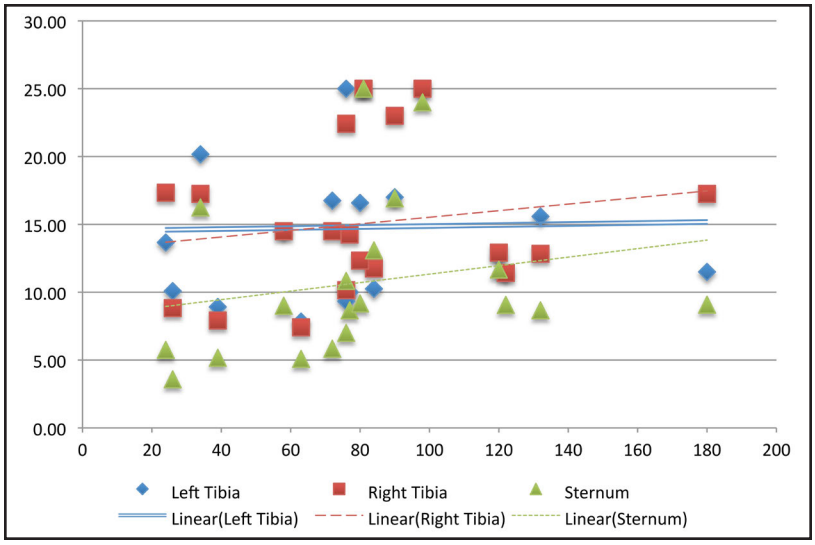


Figure 1.
*Comparison of Serum Vitamin D levels and pressure pain thresholds of right tibia, left tibia and sternum.*

pants was 47.6 years (±13.7y). The average height was 167.2 cm (±9.6cm) and weight was 68.9kg (±13.8kg). The average PPTs were 14.92 (±6.03), 15.07 (±6.07), 11.10 (±6.68) for the left and right tibia and sternum, respectively. During the blood analysis, three samples were unable to be analyzed. As such, results of nineteen subjects are reported. The relationship between serum levels and bone tenderness is plotted in Figure 1. The stability between the measurements was very high with the ICC (95% CI) calculated as 0.94 (0.62-1.26), 0.9 (0.81-1.00), 0.96(0.93-1.00). The Pearson correlation coefficients calculated were 0.03, 0.17, 0.20 indicating negligible correlation for both left and right tibia, but a weak association between PPTs in the sternum and serum vitamin D levels. There may be benefit in exploring this relation further. These findings are intended to be used descriptively to identify possible direction of future research.

## Discussion.

This is the first study, to our knowledge, to explore the relationship between pressure-pain thresholds, as measured by algometry, and vitamin D status. The results of this study did not reveal a clear correlation between PPT and 25-hydrocholecalciferol levels at the locations tested however as this was a pilot study our results are not definitive. Our main purpose in the current study was to collect preliminary data to inform future hypothesis testing studies.

There are challenges associated with determining accurate values of vitamin D status in individuals, as there is no reported 'gold standard' that adequately and consistently is able to determine a picture of the bioavailable supply in the body. This is due to a number of different assay techniques that have different standards of normal. Further, it is currently not possible to delineate between a true vitamin D deficiency and a deficiency due to comorbidities, such as hyperparathyroidism or bone-related cancers[25,26], as these conditions can create artificially normal or elevated vitamin D levels in testing. This study attempts to determine the feasibility of using alternative, non-invasive testing as a measure of vitamin D status.

Perhaps the most important role of cholecalciferol is in aiding the intestinal absorption of calcium. A proposed mechanism for the bone pain associated with vitamin D deficiency is that there is insufficient calcium phosphate to mineralize the expanding collagen matrix of bone. Furthermore, 1,25-dihydroxycholecalciferol has been found to increase type I collagen production in a dose dependent manner.[27,28] This is important, as the quality of bone is interrelated with collagen and mineral concentrations. The rubbery matrix found in people deficient of cholecalciferol does not provide sufficient trabecular and collagenous support but instead hydrates and expands the bone, causing an outward pressure under the periosteal covering, richly innervated with sensory pain fibres.[11]

There has been research to also indicate the nutrient rich Haversian canals within bone can act as pain-sensitive structures[29], allowing for deep-seated bone pain in daily activities.

The density and arrangement of collagen fibres differ between the sternum and the tibia, with the tibia having more hypertrophic chondrocytes and a greater trabeculation density. Stromal cells are connective cells of any organs. In bones, the stroma consists of bone marrow, immune cells, inflammatory cells and fibroblasts.[21] The composition of stroma in different bones varies. In adult long bones, such as the humerus or tibia, the majority of the medulla is filled with yellow bone marrow composed primarily of adipose tissue. This marrow fills the centre of long bones to protect and lighten the bone. Long bones also have hypertrophic chondrocytes that increases the stress and deformation tolerance within the bone.[21] This is necessary as the tibia absorbs shock from ambulation, weight bearing and offers support to the entire body. On the other hand, flat bones, such as the sternum and the ilium, are filled with red bone marrow that functions to produce hematopoietic cells (such as erythrocytes, leukocytes, platelets). Flat bones have less trabeculation and collagen content and as such are more sensitive to deformation and pain.[22] With these histological, physiological and mechanical features taken into consideration, it is reasonable to assume that the sternum will be more sensitive to changes in circulating 25-hydroxycholecalciferol and in pain sensation.[23,24]

## Limitations

While this study provides some insight to future research possibilities, by definition a pilot study does not allow for definitive conclusions to be drawn from the results. While we had 100% compliance, there were a number of issues. Feasibility issues that were raised in the current study will inform future definitive studies on the relationship between PPT and vitamin D levels. One significant shortcoming that we encountered was a miscue between researchers and the blood lab, resulting in unrefrigerated storage of the blood samples for ten days. The blood was not analyzed for another ten days thereafter. While there is research that indicates this does not compromise the quantity of stable 25-hydroxycholecalciferol concentrations in the blood or the quality of the sample[30,31], it cannot be guaranteed that the results provided are an accurate representation of the circulating serum concentration of the participants. Another limitation of the study was the order in which the study design was carried out. There are inherent limitations to convenience sampling. Drawing from a non-randomized, non-diversified population prevents representative conclusions and the ability to extrapolate from those results. Further, reproducing the research in such a limited sample will be difficult to recreate the reported findings. There was no standardization or randomization of data collection such that the intended order (algometer readings followed by venipuncture), at times was not always feasible. The sample population was taken from a specific chronic pain clinic and had no inclusion or exclusion criteria. We consider this a limitation because the results could be confounded by previous pain disorders or medications/supplements and therefore cannot be assumed to represent the general public. There was technical error in the lab with the analysis of three blood samples. Lastly, the inducement of a reimbursement was a limitation in the study as there was an incentive for the participants to participate in the entire study process.

## Considerations for Future Research

Proper care should be taken when handling blood samples. A detailed and specific standardization of data collection will minimize variance within the data set. By randomizing data collection, the feasibility concern and contamination bias can be reduced. Inclusion and exclusion criteria may be considered in order to identify factors which may affect the correlation such as medications (i.e. analgesics), sun exposure (i.e. use of sunblock or travel), conditions which alter pain sensitivity (i.e. fibromyalgia, hypothyroidism, diabetes), dietary considerations (i.e. artificial sweeteners, supplementation), physical activity level, previous trauma, and psychosocial disorders (i.e. conversion disorders, somatization disorders). Meticulous ascertainment and measurement of these variables would permit some control over these potential confounders through the use of multivariate statistical methods.

Recruiting participants from a primary care medical facility, as opposed to using a pain clinic, may minimize selection bias. This would capture a more representative sample of the general population.

The highest correlation coefficient that was found in this pilot study was 0.20; this means that the linear relationship between PPT and serum vitamin D level ac-

counted for only 4% (i.e., r-squared = 0.20 squared) of the total variance in the data. Based on this weak level of correlation, PPT cannot be considered a useful proxy measure for serum vitamin D level, at least in the current study population. Multiple replications of this study are needed to determine whether PPT is better (i.e., more strongly) correlated with serum vitamin D levels in other, larger, and more representative, study populations of interest.

## Conclusions:

This pilot study found no correlation between serum 25-hydroxyvitamin D and mean pressure-pain threshold as measured by manual algometry in a limited sample. Future studies should include larger samples of patients from primary medical centers, as opposed to specialty pain clinics, in order to target more representative study populations of interest. Unless evidence of stronger correlations are revealed in future studies, pressure-pain threshold determinations cannot be regarded as a useful proxy measure for serum 25-hydroxyvitamin D levels.

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