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Effect of a Sliding Scale Vitamin D Supplementation Protocol on 25(OH)D Status in Elite Athletes with Spinal Cord Injury

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EFFECT OF A SLIDING SCALE VITAMIN D SUPPLEMENTATION PROTOCOL ON 25(OH)D STATUS IN ELITE ATHLETES WITH SPINAL CORD INJURY

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by

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ABSTRACT

EFFECT OF A SLIDING SCALE VITAMIN D SUPPLEMENTATION PROTOCOL ON 25(OH)D STATUS IN ELITE ATHLETES WITH SPINAL CORD INJURY

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Recent studies suggest that a substantial proportion of elite athletes with SCI (spinal cord injury) have insufficient 25(OH)D status which may be associated with decreased muscle strength. This study: 1) examined the effects of a 16-wk sliding scale Vitamin D supplementation protocol on 25(OH)D concentration and 2) determined whether subsequent 25(OH)D status impacts muscle function/performance in elite athletes with SCI. Thirty-four members of the US Olympic Committee Paralympic program, and the Canadian Wheelchair Sports Association from outdoor and indoor sports participated. Serum 25(OH)D concentrations, lifestyle and dietary factors were assessed during the Winter and Spring. Participants were assigned to a 16-week sliding scale vitamin D3 (cholecalciferol) (KleanAthlete Pittsburg, PA) supplementation protocol based on initial 25(OH)D levels. Participants with deficient 25(OH)D (<50 nmol/L) status received 50,000 IU/wk. for 8 wks., and participants with insufficient status (50-75 nmol/L) received 35,000 IU/week for 4 weeks followed by a maintenance dosage of 15,000 IU/wk. Participants with sufficient status (>75nmol/L) received the maintenance dosage of 15,000 IU/wk. Performance measurements were assessed using a 20 meter wheelchair sprint, and handgrip strength. A paired t-test was used to assess differences in 25(OH)D status and performance before and after supplementation, respectively. 25

(OH)D concentrations increased significantly after supplementation ($p < .001$; 66.3 + 24.3 nmol/L; 111.3 + 30.8 nmol/L; mean + SD) for Winter and Spring, respectively. 26% of athletes had sufficient 25(OH)D concentrations prior to supplementation, and 94% had sufficient concentrations post supplementation. 62% of participants improved handgrip strength post supplementation. However, no change in wheelchair sprint performance time was observed. The 16-week sliding supplementation protocol used in the current study is effective for achieving sufficient vitamin D concentrations during the winter months in elite athletes with SCI.

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CHAPTER I

LITERATURE REVIEW

Background

The National Health and Nutrition Examination Survey (NHANES III) determined the prevalence of vitamin D insufficiency (<75 nmol/L) in over 77% of the population. Factors that influence vitamin D status are sun exposure, diet and lifestyle (Armas, L., & Heaney, R., 2011, Lips, P., van Schoor, N., & de Jongh, R., 2014, Ogan & Pritchett,,2013). Groups whom have an increased risk for compromised vitamin D status include young children, pregnant women, and older people (>65 years old) (Lips et al. 2014; Mithal, A., Wahl, D., Bonjour, J., Burckhardt, P., … & Morales-Torres,J., 2009). Athletes are not immune to insufficient vitamin D status. An estimated 33% to 94% of athletes are vitamin D deficient (Constantini, N., Arieli, R., Chodick, G., Dubnov-Raz, G., (2010); Girgis, C., Clifton-Bligh, R., Turner, N., Lau, S., Gunton, J., 2014; Oleson, C., Patel, P., Wuermser, L., 2012). A substantial portion of elite athletes with a chronic spinal cord injury (SCI) have insufficient/deficient 25(OH)D status that may lead to decreased physical performance (Barbonetti, A., Sperandio, A., Micillo, A., D'Andrea, ... & Francavilla, F., 2016; Flueck, J., Schlaepfer, M., & Perret, C., 2016; Pritchett, K., Pritchett, R., Ogan, D., Bishop, P., ... & LaCroix, M.. 2016).

Physiology and Function

Vitamin D status is obtained from two form of vitamin D: Vitamin D_2 (ergocalciferol) and Vitamin D_3 (cholecalciferol). Vitamin D_2 is the plant life and can be found in many vitamin D fortified foods (cereal, soymilk). Vitamin D_3 is considered the active form found in animals and produced in the skin (Armas & Heaney, 2011).

Vitamin D is the main hormone in calcium homeostasis and mineral bone metabolism via regulation of calcium phosphate (Halfon, Phan & Teta, 2015). A majority of vitamin D is produced through endogenous synthesis when skin is directly exposed to the ultraviolet-B radiation (Lips et al. 2014); however, sunscreen, clothing style, season, geographical location and lifestyle factors intervene to reduce the synthesis of vitamin D at any given time. Vitamin D may also be ingested orally with a diet that includes dairy, fatty fish, egg yolks and fortified cereals (Lips et al. 2014).

Vitamin D receptors (VDR) are located on in virtually every cell type, which controls calcium and phosphate metabolism (Tanner, S., & Harwell, S., 2015). The VDR initiates a positive or negative response once bound with vitamin D to target gene expression, thus affecting the target muscle cell (Feldman, F., Krishnan, A., & Swami, S., 2013; Tanner et al. 2015). In addition to linking vitamin D with muscle weakness, balance, and pain, it is also suggests that fast twitch muscle fiber morphology with an increase in amount and size with increased vitamin D (Campbell, P. & Allain, T., 2006; Cannell, J., Hollis, B., Sorenson, M., Taft, T., & Anderson, J., 2009; Ceglia, L., 2008; Grigis et al. 2014). Further research is needed to conclude vitamin D's effect on muscle fibers.

Vitamin D Status

Vitamin D status is measured in either nanograms (ng/ml) or nanomoles (nmol), (1nmol/L=0.4ng). 25(OH)D serum concentration is the best indicator of Vitamin D status according to the National Institute of Health (NIH) as it has a 15-day half-life in the body. However, it is not, a marker for how much vitamin D is stored in the body. Vitamin D concentrations are constantly changing within the body due to the multiple synthesis methods in the body.

The skin can synthesize vitamin D through ultraviolet B (UVB) radiation, but variables like duration of exposure, season and distance from the equator affect the rate of absorption. Seasonal variations inhibit vitamin D synthesis for individuals located in latitudinal areas greater than 35-37 degrees during winter months, as UVB radiation is unavailable (Cannell et al. 2009, Holick, M., 2007). Vitamin D deficiency also is a risk for individuals located in regions with excessive pollution and or cloud cover.

Sunscreen usage and clothing choice also are factors in which the sun is unable to reach skin contact to be metabolized. There is a 99% decrease with the use of sun protection factor (SPF) 15 of vitamin D absorption (Holick et al. 2007). Protective clothing and increased indoor activates decrease the availability of skin exposure to the sun as well. SCI athletes are at a greater risk for vitamin D deficiency due to increased sensitivity below the lesion level and use of protective skin covering, bedrest and immobility (Flueck et al. 2016).

Additionally, individuals with dark pigmented skin (e.g., Hispanic, non-Hispanic black individuals) are also at risk individuals for developing vitamin D deficiency, as it requires extended sun exposure time to absorb as much UVB radiation as a lighter skin individual (Grober, U., Reichrath, J., & Holick, M., 2015, Holick et al. 2007). According to Mithal et al. (2009), vitamin D insufficiency and deficiency is widespread across the world and is “re-emerging as a major health problem”.

Vitamin D is a fat-soluble vitamin and has been linked to having an inverse relationship with body adiposity, putting obese individuals at risk for low vitamin D status (Larson-Meyer, E. & Willis, K., 2010; Ogan et al. 2013). In addition to the metabolism of vitamin D via sun exposure, oral intake of vitamin D rich foods is a contributing factor of vitamin D status. Vitamin D rich foods include dairy products, fortified breakfast cereals, fatty fish, orange juice, egg yolks and cod liver oil (Halliday, T., Perterson, N., Thomas, J., Kleppinger, K., ... & Larson-Meyer, D., 2011). However, SCI athletes tend to digest less dairy products due to gastrointestinal discomfort, and intestinal malabsorption due to anticonvulsant medications (Flueck et al. 2016; Krempien & Barr 2011; Nemunaitis, G., Mejia, M., Nagy, J., Johnson, T., ... & Roach, M., 2010; Pritchett et al. 2016,).

Vitamin D Recommendations

According to the World Health Organization and Endocrine Society (WHO/ES) serum 25(OH)D standards, vitamin D deficiency is defined as <50 nmol/L (<20ng/ml), insufficiency as 50-75 nmol/L (20-30ng/ml), and >75nmol/L (>30ng/ml) would be considered sufficient (Holick, M., Binkley, N., Bischoff-Ferrari, H., Gordon, C.,... & Weaver, C., 2011; Dubnov-Raz, G., Livne, N., Raz, R., Rogel, D., ... & Constantini, N., 2014). The Institute of Medicine’s (IOM) recommended dietary allowance (RDA) for individuals 1-70 years old is 600 International Units (IU/day) of vitamin D per day (Ross, A., Manson, J., Abrams, S., Aloia, J., ... & Kovacs, C., 2011). The IOM places Upper

Limits (UL) of vitamin D intake at 4,000IU for individuals 9 years or older while the ES recommends an UL of 10,000IU for individuals 19 years of age from the 4,000IU for 9 to 18 years old (Hollick et al. 2011). Recommendations also take into consideration pregnancy and lactation with NIH recommending 600IU for pregnancy/lactation and the ED specifying that for pregnant/lactating individuals aged 14-18 years to consume 600-1000IU/day and 1500-2000IU/day for 19-50 year olds.

Populations for which there are no current vitamin D daily intake recommendations are athletes. More specifically SCI athletes, may need a greater optimal serum 25(OH)D level in comparison to able-bodied athletes and individuals to increase calcium absorption and suppress parathyroid hormone (PTH) (Hummel et al. 2012).

Athletes and Vitamin D Status

Recent research has suggested that adequate vitamin D status may increase performance in a number of ways. Notably, by maintaining an adequate vitamin D status, athletes may reduce the risk for stress fractures, impaired muscle function, inflammation and illness (Hildebrand, R., Miller, B., Warren, A., Hildebrand, D., & Smith, B., 2016; Larson-Meyer, E. & Willis, K., 2010; Maroon, J., Mathyssek, C., Bost, J., Amos, A., … & Norwig, J., 2015).

Current research is examining performance measures and the effect of vitamin D status may have for athletes. In a recent study, Koundourakis, N., Androulakis, N., Malliaraki, N., & Margioris, A., 2014 found that all performance measures (squat jump, VO_2max, sprint and countermovement jumps) were significantly correlated ($p<0.001$)

with vitamin D concentrations. Koundourakis et al., (2014) used male professional soccer players, where as Hildebrand et al. (2016) used collegiate athletes of both genders yet found comparable effects in performance measures (vertical jump, shuttle run, triple hop for distance and 1 rep max squat) and vitamin D concentrations.

However, other studies have not found similar results. Dobnov-Raz et al. (2014) did not find a significant correlation between vitamin D concentration and grip strength, balance and swimming performance in a group of competitive adolescent swimmers of both genders. Likewise Ceglia, L., Chiu, G. R., Harris, S. S., & Araujo, A. B., 2011 examined a large cohort of non-athletes (*n*=1219) to find no correlation in handgrip strength or physical function score. A lack of consistency in studies when comparing likewise performance measures within a wide variety of athletic skill.

Spinal Cord Injured Athletes and Vitamin D Status

The prevalence of vitamin D deficiency with in the SCI community has been documented and although some research shows similar percentages to overall population, the limited research is still inconclusive and further research is warranted. Barbonetti et al. (2016) found 78% of SCI individuals in a rehabilitation setting had vitamin D deficiency with increased weakness and "lower functional independence in activities of daily living". Pritchett et al. (2016) found 56.4% of their 39 elite SCI athletes were insufficient/ deficient following the winter months. Swiss elite wheelchair athletes showed comparable deficiencies with 73.2% insufficiency/deficiency (Flueck, J., Hartmann, K., Strupler, M., & Perret, C., 2016).

Few studies have looked at performance measurements in SCI athletes but rather individuals with SCI in general. Performance measurements include ADL, and leisure time physical activity (LTPA) in SCI individuals (Barbonetti et al. 2016). Barbonetti et al. (2016) concluded that insufficient vitamin D levels "represents an independent predictor of poor physical function". As for SCI athletes, performance measurements are similar to those used for their able-bodied counterparts. Handgrip strength, 20M-sprint test, Wingate test, and isokinetic dynamometer have been used to assess performance in SCI athletes (Flueck et al. 2016; Pritchett et al. 2016). As measuring performance in SCI athletes is not well documented, further research is needed to determine the most effective measurements of strength and speed for this specialized population.

Pritchett et al. (2016) used handgrip strength and 20M sprints to measure performance while observing vitamin D status from Autumn to Winter in elite SCI athletes. Vitamin D status did not see a seasonal decline nor was there a correlation to performance measures during change in season. It is possible to speculate that if there was a significant seasonal decline in vitamin D status within these subjects that there would have been a significant change in performance as well. Further research is needed to conclude the relative effect on vitamin D status and performance measures. Few studies have controlled for vitamin D status through supplementation.

One of the first studies to take SCI athletes into consideration was in conjunction with elite able-bodied boxers (Magee, P., Pourshahidi, L., Wallace, J., Cleary, J., ... &

Madigan, S., 2013). Vitamin D supplementation protocol was based on their abilities and was only provided to those who were insufficient or deficient during the winter season. Boxers received a one or two time vitamin D supplement of 50,000IU and Paralympic SCI athletes were provided 5,000IU of vitamin D daily. Magee et al. (2013) concluded that their vitamin D supplementation protocol during the winter months significantly (p = .001) increased 25(OH)D levels in insufficient/deficient vitamin D status athletes.

Flueck et al. (2016) followed the their previous study which examined elite Swiss wheelchair athletes and vitamin D deficiency with a 12 week vitamin D supplementation to observe any changes in performance within the same population. Supplementation was based off the initial vitamin D status with insufficient and deficient receiving 6,000IU of vitamin D per day and those with sufficient status receiving a placebo. Their performance measures included a Wingate test and elbow isokinetic dynamometer test. However, all of the 20 athletes were not sufficient. With the 12-week supplementation, Flueck concluded that all athletes significantly increased their vitamin D status. The only significant change in performance measures was an increase in elbow flexion within the non-dominant hand.

Conclusion and Study Objective

The purpose of this study is to examine the effects of a 16-week, vitamin D supplementation protocol based on initial values of 25(OH)D concentration on 25(OH)D concentration and determine whether subsequent 25(OH)D status impacts muscle function/performance in elite athletes with SCI. Further research is needed is a number of areas surrounding SCI athletes. Most importantly, recommendations surrounding optimal vitamin D status for SCI individuals needs to be addressed, as they are more susceptible to vitamin D insufficiency. It is still unclear the relationship in which vitamin D has on muscle development and performance. Continued research on SCI athletes and optimal measures of performance are needed as well. By replicating the performance measures of Pritchett et al. (2016), the hope of producing reliable performance testing for SCI athletes as well. As the study of athletic performance and vitamin D continues to grow, it is valuable to understand how specific athletic populations like SCI athletes can apply current findings.

CHAPTER II

JOURNAL ARTICLE

EFFECT OF A SLIDING SCALE VITAMIN D SUPPLEMENTATION PROTOCOL ON 25(OH)D STATUS IN ELITE ATHLETES WITH SPINAL CORD INJURY

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b. Average handgrip strength change from pre to post 25(OH)D supplementation by subject and supplementation protocol

ABSTRACT

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Recent studies suggest that a substantial proportion of elite athletes with SCI (spinal cord injury) have insufficient 25(OH)D status which may be associated with decreased muscle strength. This study: 1) examined the effects of a 16-week sliding scale Vitamin D supplementation protocol on 25(OH)D concentration and 2) determined whether subsequent 25(OH)D status impacts muscle performance in elite athletes with SCI. Thirty-four members of the US Olympic Committee Paralympic program, and the Canadian Wheelchair Sports Association from outdoor sports participated. Serum 25(OH)D concentrations, lifestyle and dietary factors were assessed during the Winter and Spring. Participants were assigned a 16-week sliding scale vitamin D3 (cholecalciferol) (KleanAthlete Brand) supplementation protocol based on initial 25(OH)D levels. Participants with deficient 25(OH)D (<50 nmol/L) status received 50,000 IU/wk. for 8 wks., and participants with insufficient status (50-75 nmol/L) received 35,000 IU/week for 4 weeks followed by a maintenance dosage of 15,000 IU/wk. Participants with sufficient status (>75nmol/L) received the maintenance dosage of 15,000 IU/wk. 25 (OH)D concentrations increased significantly after supplementation

(p <.001; 66.3 + 24.3 nmol/L; 111.3 + 30.8 nmol/L) for Winter and Spring, respectively. 26% of athletes had sufficient 25(OH)D concentrations prior to supplementation, and 91% had sufficient concentrations post supplementation. 62% of participants improved handgrip strength post supplementation. No change in 20-meter wheelchair sprint performance time was observed. The 16-week sliding scale supplementation protocol used in the current study is effective for achieving sufficient vitamin D concentrations during the winter months in elite athletes with SCI.

INTRODUCTION

The National Health and Nutrition Examination Survey (NHANES III) determined the prevalence of vitamin D insufficiency (<75 nmol/L) in over 77% of the population. According to the World Health Organization and Endocrine Society (WHO/ES) serum 25(OH)D standards, vitamin D deficiency is defined as <50 nmol/L, insufficiency as 50-75 nmol/L, and >75nmol/L would be considered sufficient (Holick et al. 2011; Dubnov-Raz et al. 2014). The Institute of Medicine's (IOM) recommended dietary allowance for individuals 1-70 years old is 600 International Units (IU/day) of vitamin D per day (Ross et al., 2011). The IOM places Upper Limits (UL) of vitamin D intake at 4,000IU for individuals 9 years or older while the ES recommends an increase to 10,000IU for individuals 19 years of age from the 4,000IU for 9 to 18 years old (Holick et al. 2011).

Research has correlated low vitamin D status with increased incidence of depression, hypertension, cardiovascular disease, multiple sclerosis, and decreased bone density (Holick, M.,2004, 2007, Zitterman, A., 2003. As recent studies have suggested, an estimated 33% to 94% of athletes are vitamin D deficient (Constantini et al. 2010; Girgis et al. 2014, Oleson et al. 2012). Sport season and geographical location may limit exposure to specific UVB wavelengths needed for adequate absorption of vitamin D (Cipriani, C., Pepe, J., Pieminte, S., Colangelo, L., ... & Minisola, S., 2014). A higher risk of vitamin D deficiency is also linked to indoor athletes or those participating in sports that require uniforms or equipment that minimize skin exposure (Constantini et al. 2010). Additionally, recent studies suggest that a substantial portion of elite athletes with a chronic spinal cord injury (SCI) have insufficient/deficient 25(OH)D status that may lead to decreased physical performance (Barbonetti et al. 2016; Flueck et al. 2016; Pritchett et al. 2016). Individuals with a SCI have a high risk of vitamin D deficiency due to medications that induce vitamin D metabolism, a reduction of sunlight exposure, and inadequate vitamin D dense diet (Flueck et al. 2016; Pritchett et al. 2016). The muscle atrophy seen in individuals with SCI may have an impact on vitamin D receptors located in skeletal muscle, which in turn may impact physical performance (Barbonetti et al. 2016).

Due to the recent discovery of vitamin D receptors in the muscle, vitamin D status and its affect on athletic performance is of growing interest (Campbell et al. 2006, Cannell et al. 2009, Ceglia et al. 2008). Thus, recent research has focused its attention on the effects of vitamin D supplementation regimen for maintaining adequate vitamin D status, or preventing vitamin D insufficiencies and deficiencies during the winter months (Ogan et al. 2013; Larson-Meyer E & Willis KS 2010). Mixed results have been reported regarding the relationship between vitamin D status and performance measures (Todd et al. 2015). Ward et al. (2009) found a positive relationship between vitamin D status and skeletal muscle function, while others have found no relationship (Barker et al. 2013; Marantes et al 2011; Pritchett et al. 2016). Therefore, excess vitamin D supplementation may not always result in increased performance (Close et al. 2013).

A lack of consensus remains regarding the optimal supplementation protocol for athletes (Close et al. 2013; Wyon et al. 2014). Recent randomized supplemental trials reported increased performance measures (isometric strength, vertical jump, and 10 meter sprint time) when supplemented with daily dosages of vitamin D between 2,000-5000 IU over the period of 8 weeks to 4 months during the winter season (Close et al. 2013; Wyon et al. 2014). Although, Knutsen et al. (2014) concluded that daily vitamin D supplementation (both 400IU and 1000IU of vitamin D) was enough to increase blood serum levels to an optimal range, there was no improvement in handgrip test, jump test, or chair-raising test. Therefore, one may hypothesize that an increased supplement dosage for a shorter regimen may not only improve serum levels but increase performance measures as well. However, baseline vitamin D status differed in each study.

Few studies have addressed the effect of vitamin D supplementation in SCI athletes. Magee et al. (2013) examined a small subgroup of Irish paralympians (n = 27), 12 of which were wheelchair bound. Of the 12 subjects, 50% were found to be insufficient/deficient at baseline and given the optional supplement of 5,000IU of vitamin D per day for 10-12 weeks during the wintertime. Magee et al. (2013) concluded that the vitamin D supplementation protocol was effective because it significantly increased vitamin D status and corrected any insufficiencies/deficiencies within the subgroups. However, there were no performance measures taken or compliance reported. Flueck et al. (2016) examined the effectiveness of 6,000IU of vitamin D daily during a 12-week period on vitamin D status in a group of 20 Swiss indoor wheelchair athletes with SCI, all of which had insufficient or deficient vitamin D status at baseline. A moderate correlation ($r_s = 0.564$) was observed in elbow flexion strength in the non-dominant arm and the increase in vitamin D status. Each subject reached and maintained sufficient vitamin D status for up to 12 weeks post supplementation (Flueck et al. 2016).

Recent studies suggest that a substantial proportion of elite athletes with SCI have insufficient 25(OH)D status that may be associated with decreased muscle strength

(Oleson et al. 2012, Hildebrand et al. 2016). Therefore the purpose of this study is to: 1) examine the effects of a 16 week, vitamin D sliding- scale supplementation protocol based on initial values of 25(OH)D concentration on 25(OH)D concentration and 2) determine whether subsequent 25(OH)D status impacts muscle function/performance in elite athletes with SCI.

METHODOLOGY

Subjects

Elite male and female athletes were recruited from the US Olympic Committee Paralympic program and Canadian Wheelchair Sports Association. Athletes were required to have an impairment of their spinal cord (e.g. spina bifida, spinal cord injury) in order to participate. Diagnosis of kidney, thyroid, bone or malabsorption disease excluded athletes. All eligible athletes were of at least 18 years of age. Represented sports included rugby, tennis, basketball, and track and field. The Central Washington University Human Subjects Review Committee approved methods and protocol. All subjects provided informed consent prior to participation.

Study Design

Participants provided a blood sample for 25(OH)D analysis and completed a Lifestyle and Diet Questionnaire that addressed UVB exposure, dietary intake, current supplement usage, age, gender, ethnicity, history and level of injury during the Spring data collection session (Pritchett et al. 2016). Current height and weight were recorded as well.

Based on participant's initial 25(OH)D levels, subjects were assigned to a 16-week sliding scale of vitamin D3 (cholecalciferol) (Klean Athlete, Pittsburg, PA) supplementation based on the United States Olympic Committee (USOC) protocol. Deficient status (<50 nmol/L) participants received 50,000 IU/week for eight weeks followed by eight weeks of maintenance dosage of 15,000 IU/week. Insufficient status (50-75 nmol/L) participants received 35,000 IU/week for four weeks followed by the maintenance dosage for the remainder 12 weeks. Sufficient status (>75nmol/L) participants received the maintenance dosage for the entire 16-week duration. Participant performance was assessed by handgrip strength and 20-meter wheelchair sprints.

25(OH)D Assay

Blood spot assay has been suggested to provide valid and reliable data in correlation (R = .97) with liquid chromatography/tandem mass spectrometry assay (Newman et al. 2009). While using sterile procedures, after the use of a lancet, 5-10 drops of blood were sampled from fingertips to assess 25(OH) D (vitamin D) during data collection sessions. Blood was pipetted onto blood spot cards, air dried for at least 30 minutes and sent to a certified laboratory (ZRT Laboratory, Beaverton, OR) in batches.

Diet and Lifestyle Questionnaire

Following supplementation, a lifestyle questionnaire was completed by participants, which addressed factors that may influence their current vitamin D status. Factors including current supplementation (vitamin D, calcium, multivitamin), dietary intake of vitamin D rich foods, injury/illness, UVB exposure and sunscreen use. The questionnaire was replicated with permission (Halliday et al. 2011). Participants were asked to rate the amount of dietary intake of vitamin D rich foods and supplements (never or <1 per month, 1-3 per month, 1 per week, 2-4 per week, 5-6 per week, 1 per day, 2-3 per day, 4-5 per day or 6 or more per day). UVB exposure was also inquired upon with frequency of sunscreen usage and SPF type. Frequency of time spent outdoors (none or <1 hour per week, 1-3 hour per month, 1 hour per week, 2-4 hour per week, 5-6 hour per week, 30 minutes-1 hour per day, >2 hours per day) and at which hours of the day, type of clothing worn while outdoors, tanning bed usage (never or <10 minutes per week, 10-20 minutes per week, 20-30 minutes per week, 30-40 minutes per week, 40-50 minutes per week, 50-60 minutes per week, >60 minutes per week) and average frequency of illness per year was self-reported (Pritchett et al. 2016). A 24-hour diet recall was provided during both sessions as well.

Supplementation

The supplementation protocol was derived from the USOC standards for vitamin D supplementation with permission. Subjects were divided into three categories depending on their initial vitamin D status: sufficient (>75 nmol/L), insufficient (50-75 nmol/L) and deficient (<50 nmol/L). Participants with a sufficient 25(OH)D status received the maintenance dosage (15,000 IU per week of vitamin D) for 12- 16 weeks. Participants with insufficient status received 35,000 IU per week of vitamin D for the first four weeks and the maintenance dose (15,000 IU per week of vitamin D) for the remainder of the study. Participants with deficient status received 50,000 IU per week of vitamin D for the first eight weeks and the maintenance dosage (15,000 IU per week of vitamin D) for the remaining eight weeks (Larson-Meyer, E. & Willis, K., 2010; Patton

C., Powell A., & Patel A., 2012). Supplement adherence was self-reported with subjects by providing documentation of each day supplements were taken on a blank calendar.

Performance Tests

Participants completed three 20-meter wheelchair sprints and their average sprint times were calculated for analysis. Warm up trials were matched to previous self-regulated regimens. 20m-sprint tests were completed with a handheld stopwatch; otherwise 4 sets of timing lights to measure 5m, 10m, and 20m splits were used. Typically, athletes with SCI use a 20m-sprint test to assess anaerobic performance. 20m sprints were completed during both data collection sessions (Pritchett et al. 2016).

A handgrip dynamometer (model 68812 Country Technology INC, Gays Mills, WI) was used to assess handgrip strength. The dominant hand was measured first with the grip width adjusted to where the second joint of the participant's forefinger was bent at 90 degrees during the grasp and the indicator was set to zero before each trial. In a seated position and relaxed, participants gripped the dynamometer with their elbow at a 90 degree angle. The participants gripped and exerted as much force as possible while keeping from bracing their elbow against their body or resting on any part of the wheelchair. The procedure was repeated with both hands for a total of two trials per hand. The highest score recorded for each hand was combined to produce the total sum of the two hands strength in kilograms (Pritchett et al. 2016).

Data Analysis

All statistical analysis was conducted using IBM *SPSS* for Windows version 23.0 software (SPSS Inc., Chicago, IL) with $p < 0.05$ considered statistically significant. A paired t-test was used to assess differences in 25(OH)D status and performance measures before and after supplementation, respectively. One-way analysis of variance (ANOVA) with a Bonferroni post hoc was used to assess differences between baseline and post supplementation vitamin D status, and performance measures between groups (deficient, insufficient, and sufficient). An Analysis of Covariance (ANCOVA) was applied in the case that vitamin D status was correlated with a particular lifestyle variable to determine whether supplementation was a covariate.

RESULTS

Subjects (n = 35) with spinal cord injury (age: 33 ± 15 years, weight: 69.6 ± 28.2kg, height: 170.2 ± 25.4cm) participating in track and field (n = 10) and rugby (n = 25) volunteered for the study. Of the thirty-five subjects, one did not complete the final 25(OH)D analysis. Initial 25(OH)D concentrations suggested n = 9 (26%) had sufficient 25(OH)D status, and n = 26 (74%) with insufficient/deficient vitamin D status. Of those with less than optimal vitamin D status, n = 17 (50%) were insufficient and n = 8 (24%) were deficient. Vitamin D status was measured on week 16 of the study on average.

25(OH)D Status

25(OH)D concentrations significantly increased after supplementation (p <.001; 66.3 ± 24.3 nmol/L; 111.3 ± 30.8 nmol/L; mean ± SD) for pre-supplementation and post-supplementation, respectively. Following the supplementation protocol, 91% of subjects achieved sufficient vitamin D concentrations with an average of 45 ± 12.3nmol/L increase in 25(OH)D. Pre-supplementation, 25(OH)D serum concentrations were significantly different (p = 0.000) between supplementation groups (sufficient, insufficient, and deficient), and post-supplementation resulted in no significant difference (p = 0.562) between groups (Table 1).

Table 1. 25(OH)D Concentrations by Supplementation Group

25(OH)D (nmol/L)	**Sufficient (*n* = 9)**	**Insufficient (*n* = 17)**	**Deficient (*n* = 8)**	***p*= Value**
Pre-Supplementation	98.0 ± 19.3	62.8 ± 8.3	38.8 ± 6.0	0.000*
Post- Supplementation	118.5 ± 25.5	104.0 ± 36.5	103.0 ± 41.5	0.562

Note: Mean ± SD, all such values; *p for difference between groups (p< .05)

Sufficient subjects increased their vitamin D status by 21% (p = 0.036) from pre to post-supplementation 98 ± 19.3nmol; 118.6 ± 25.4nmol, respectively (Table 2).

Table 2. Percent Change in 25(OH)D Within Supplement Groups

25(OH)D Status	**Initial**	**Final**	**% Increase**
Sufficient	26% (*n* = 9)	91%* (*n* = 31)	21%
Insufficient	50% (*n* = 17)	9%*(*n* = 3)	66%
Deficient	24% (*n* = 8)	0%*	167%

Note: *p for significant difference from initial to final vitamin D status (p< 0.001)

Insufficient subjects increased their vitamin D status by 66% ($p = 0.000$) from, from 62.8 ± 8.3nmol, to 103.9 ± 36.4nmol. Deficient subjects increased their vitamin D status by 167% ($p = 0.003$) from pre to post supplementation, 38.8 ± 6; 103.5 ± 41.5nmol respectively (Figure 1).

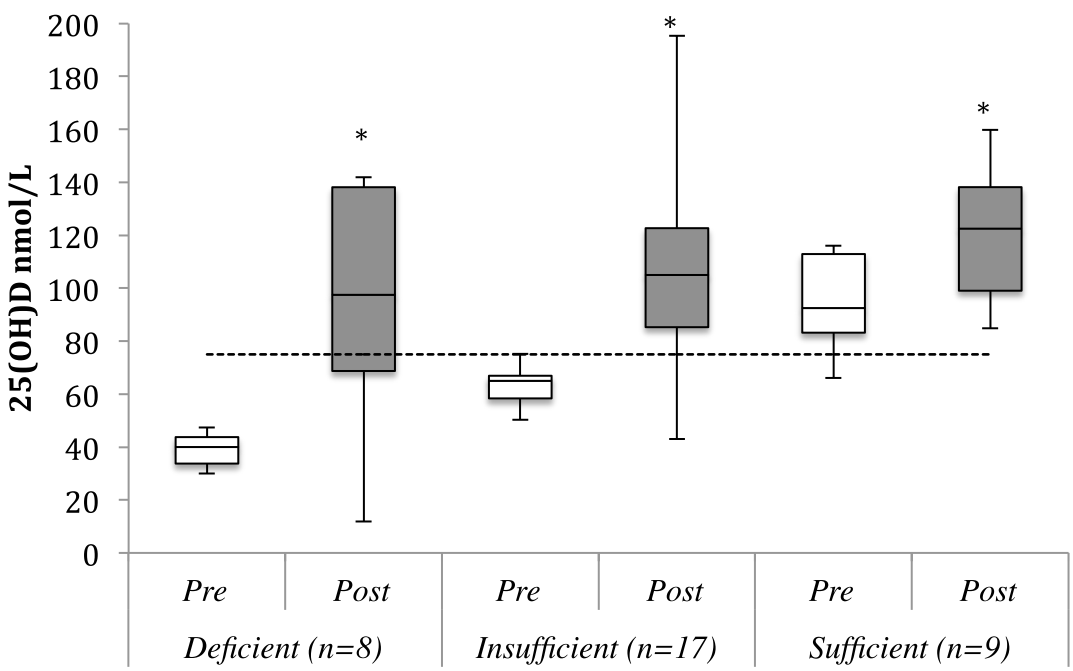

Figure 1: Effect of 25(OH)D sliding scale- supplementation in SCI athletes. Boxplots show change in median (25th, 75th percentile) vitamin D status over time. Dashed line denotes cut off for vitamin D insufficiency and deficiency (<75nmol/L). *p = <0.001 significant difference between pre and post within each supplementation group.

Performance Measures

Subjects ($n = 31$) completed both the initial and final 20M-sprint test. No significant difference ($p = 0.443$) in sprint times was observed post supplementation (Table 3).

Table 3. 20M Sprint Times by Supplementation Protocol

Sprint Times (seconds)	**Sufficient** (***n* = 8**)	**Insufficient** (***n* = 16**)	**Deficient** (***n* = 7**)	***p* = Value**
Pre-Supplementation	7.2 ± 1.0	7.4 ± 1.0	6.6 ± 0.8	0.201
Post- Supplementation	7.4 ± 1.0	7.6 ± 1.1	6.4 ± 1.0	0.065

Note: Mean ± SD, all such values; *p* for difference between groups (ANOVA, $p < .05$)

However, it is important to note that the subjects that were deficient pre supplementation ($n = 7$) improved their sprint times on average from 6.6 ± 0.8s to 6.4 ± 1.0s, while the sufficient ($n = 8$) and insufficient ($n = 16$) subjects' times did not change.

Based on the degree of disability, not all subjects were able to complete the handgrip dynamometer test. *Thirteen* subjects were able to complete initial and final handgrip tests using both hands. *Three* additional subjects were able to complete both rounds of testing with a single hand. The group was comprised of *seven* sufficient, *six* insufficient and *three* deficient subjects. No significant difference ($p = 0.562$) was observed in average handgrip strength between pre to post supplementation. There was an improvement in handgrip strength between groups, however, not statistically significant ($p = 0.086$) (Figure 2).

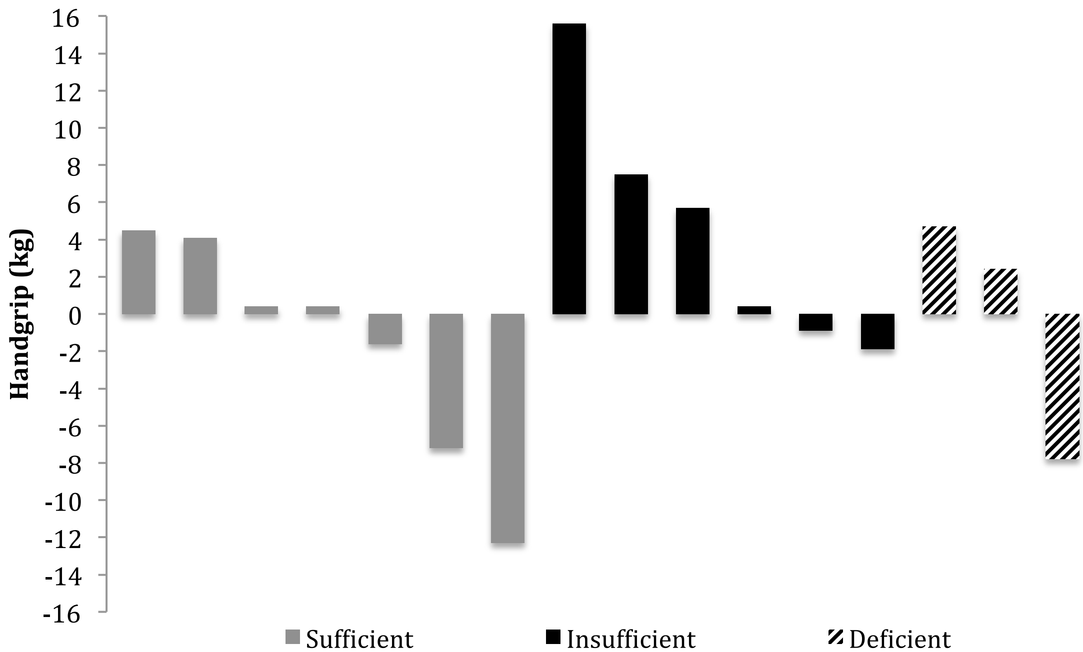

Figure 2 – Average handgrip strength change from pre to post 25(OH)D supplementation by subject and supplementation protocol.

Participants increase their strength by 62% an average of 4.6kg post supplementation (sufficient: 66.4 ± 25.5kg, insufficient: 34.1 ± 33.5kg, deficient: 77.8 ± 31.0kg).

Lifestyle Questionnaire

The lifestyle questionnaire was completed by n = 33 subjects during the final data collection. Estimated vitamin D intake from food sources during supplementation, averaged 212 $\pm$ 103 IU/d^{-1}. Median milk consumption was 5 to 6 servings per week (r = 0.029, p = 0.877), which was not significantly correlated with vitamin D. Median orange juice consumption was 1 to 3 times per month (Table 4), which was significantly correlated with vitamin D status (r = -0.411, $p = 0.019$) post supplementation.

Table 4. Reported Frequency of Consumption of Dietary Vitamin D Sources during Supplementation

Food*	Vitamin D (IU)	Number of Respondents	
Milk (8 oz.), *n* = 32	100	Never, <1/Month =	4
		1-3/Month =	4
		1/Month =	1
		2-4/Week =	6
		5-6/Week =	4
		1/Day =	8
		2-3/Day =	4
		4-5/Day =	1
Soymilk/Rice milk (8 oz.), *n* = 31	102	Never, <1/Month =	25
		1-3/Month =	3
		1/Month =	1
		2-4/Week =	1
		5-6/Week =	1
Cereal (6-8 oz.), *n* = 33	40	Never, <1/Month =	1
		1-3/Month =	13
		1/Month =	4
		2-4/Week =	4
		5-6/Week =	3
		1/Day =	8
Orange Juice (8 oz.), *n* = 32	100	Never, <1/Month =	9
		1-3/Month =	11
		1/Month =	4
		2-4/Week =	6
		1/Day =	1
Egg (1 Whole), n=33	18	1-3/Month =	1

There was a significant difference in vitamin D status [F(2,28) = 6.439, $p = 0.02$] between the supplementation groups, while adjusting for orange juice consumption during the trial. Therefore, the change in 25(OH)D status can be attributed to the supplementation rather than orange juice consumption. No other correlations were found in vitamin D rich foods addressed in the lifestyle questionnaire.

Thirty of the subjects reported multivitamin usage other than the prescribed vitamin D protocol (r = -0.205, $p = 0.277$). In addition, time spent outside was not correlated with vitamin D status (r = -0.082, $p = 0.654$).

DISCUSSION

This study investigated the effectiveness of a 12-16 week sliding scale vitamin D supplementation protocol on 25(OH)D status and performance measures in elite athletes with SCI. Our findings suggest that the sliding scale vitamin D supplementation protocol used in the current study was effective for both maintaining sufficient vitamin D status as well as eliminating vitamin D deficiencies and insufficiencies during in the winter months. A 167% increase in 25(OH)D levels in deficient subjects, 66% increase and 21% increase in 25(OH)D levels for insufficient and sufficient subjects, respectfully was observed utilizing this protocol. To our knowledge, this is the first study to examine the efficacy of a sliding scale vitamin D supplementation protocol in not only SCI athletes but athletes in general.

Similar studies have been conducted using a standard vitamin D supplementation dosage for insufficient and sufficient subjects to determine the effectiveness of the supplement regimen for SCI athletes (Magee et al. 2013, Flueck et al. 2016), and able bodied athletes (Close, G. L., Leckey, J., Patterson, M., Bradley, W., ... & Morton, J. P., 2013, Wyon, M., Koutedakis, Y., Wolman, R., Vevill, A., Allen, N., 2014). However, in the current study the intervention was prescribed according to the initial vitamin D status of the subject. At the beginning of the study, 26% of the subjects had sufficient vitamin D status in comparison to Pritchett et al. (2016) that had 33% of the subjects at sufficient levels during autumn. Another comparable study conducted with SCI athletes reported $n = 0$ subjects with sufficient vitamin D status prior to supplementation (Flueck et al. 2016). Flueck et al. (2016) provided n = 20 subjects with 6000IU of vitamin D for 12 weeks and found that all subjects significantly ($p<0.05$) increased their 25(OH)D status but did not report what time of year their data was collected (2016). Similar to the current study, Magee et al. (2013) reported sufficient initial vitamin D status in 71% of boxers ($n = 17$) and 73% of paralympians ($n = 33$) participating in the study. Following supplementation (Fall to Winter) in the insufficient/deficient subjects, Magee et al. (2013) reported a significant ($p<0.001$) increase in 25(OH)D status in the paralympians ($n = 12$) who received 5,000 IU/day for 10-12 weeks, and in boxers who received one vitamin D dosage of 50,000 IU ($n = 5$) or a two time dosage of 50,000 IU($n = 10$).

It has been well documented that insufficient vitamin D status may increase the risk of injury or illness and therefore, negatively impacting performance (Halliday et al. 2011, Hildebrand et al. 2016, Maroon et al. 2015, Ogan et al. 2013). Maroon et al. (2015) concluded that prevalence of bone fracture is inversely related to deficient vitamin D status in NFL football players greatly affecting performance by restricting participation. However, Dubnov-Raz et al. (2014) cited no significant difference in performance measures (including handgrip strength, $p = .37$) of competitive adolescent swimmers (n

= 80) between deficient, insufficient and sufficient vitamin D status athletes. The effect of vitamin D status on performance is inconclusive and further research is warranted to determine the optimal supplementation protocol in both able-bodied and SCI athletes.

Furthermore, it has been suggested that having a sufficient vs. insufficient/deficient status may benefit performance by reducing stress fracture risk, chronic fatigue and illness (Oleson et al. 2010). It has been theorized that if an athlete were to consume an increased amount of vitamin D in addition to having a sufficient vitamin D status, their performance would increase (Holick et al. 2011; Willis, K.S., Peterson, N.J., & Larson-Meyer, D.E., 2008). Vitamin D status was not associated with sprint time or handgrip strength after supplementation in the current study. Improvements in performance measures may have been hindered due to the nature of highly trained, elite athletes used as subjects (Pritchett et al. 2016). There was a large variety of strength in the small sample size of subjects able to complete handgrip strength testing, which may have influenced the significance. This may be attributed to the variation in level of lesion among subjects. However, 62% of subjects improved their handgrip strength from pre to post-supplementation. Similar studies have also reported no relationship between vitamin D status and performance measures (Flueck et al. 2016, Pritchett et al. 2016). Contrary to our findings, Close et al. (2013) noted a significant (p = 0.008) increase in 10-m sprint times and vertical height jump (p = 0.008) in (n = 61) professional able-bodied, insufficient/deficient, athletes who supplemented with 5,000IU daily of vitamin D_3 for 8 weeks. Further research is needed to address the amount of vitamin D supplementation needed to produce an ergogenic benefit on exercise performance measures.

Interestingly, only a single athlete (<1%) in this study met the current IOM recommended dietary allowance (RDA) of 600IU of vitamin D from food alone (Ross et al., 2010). Similarly, Halliday et al. (2011) reported only 5% able-bodied athletes meeting the RDA (600IU) of vitamin D through food alone, with the average vitamin D intake of 242 ± 161IU/day in Fall, 282 ± 206IU/day in Winter and 204 ± 171 IU/day. A previous study conducted in our lab with the same population of athletes suggested an average vitamin D intake among SCI athletes to be 121 ± 9.8 IU/day in autumn and 115 ± 12.25IU/day in winter (Pritchett et al. 2016). In comparison, the average dietary intake of vitamin D from food alone during the supplementation period for this study was 263 ± 167IU/day. With few foods available for consumption that contain a large amount of vitamin D (fatty fish, milk, orange juice, eggs, fortified cereals etc.), it is no surprise that the RDA is not consumed on a daily basis. It was intriguing to discover a correlation between orange juice consumption and vitamin D status as the athletes consumed a majority of their daily vitamin D intake from milk and salmon on average. However, reported dietary intake of vitamin D was inadequate in the current study participants and would warrant supplementation regardless to reach and maintain sufficient vitamin D status in the absence of sun exposure. Due to the lack of dietary vitamin D intake and sun exposure during the winter months, vitamin D supplementation appears to be necessary for obtaining a sufficient vitamin D status in SCI athletes.

Limitations

Originally, 35 participants began the study, but one was unable to provide a second 25(OH)D measurement. The aim of this study was to examine the effectiveness of a 12 to16- week supplementation protocol. Physical limitations due to the variation in level of lesion is also a factor, with 15 of the 34 subjects able to complete the handgrip test performance measures during pre and post supplementation trials.

CONCLUSIONS

In conclusion, these findings suggest that the 12 to 16-week sliding supplementation protocol is an effective and practical protocol for elite athletes with SCI to reach or maintain sufficient vitamin D concentrations during the winter months due to insufficient dietary vitamin D intake and reduced exposure to sunlight. Based on the improvement in handgrip strength in the majority of the subject group (62%), reaching an optimal vitamin D status may provide performance benefits. Further research is needed to identify specifically how 25(OH)D serum levels impact performance and level is optimal for providing a desired performance outcome in both able-bodied and SCI athletes.

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