**The impact of vitamin D supplementation on resting metabolic rate, body composition, and strength in physically active adults**

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1. **Abstract**

Vitamin D (VitD) inadequacy induces muscle fibre atrophy, slow twitch peak, long periods of muscle relaxation, increased risk of chronic musculoskeletal pain, may decrease resting metabolic rate, and increases the risk of developing sarcopenia. Although some evidence suggests that VitD supplementation may affect energy metabolism, and improve muscle strength, resting metabolic rate (RMR) and body composition in VitD deficient individuals, these outcomes in VitD sufficient individuals are still conflicting. The aim of this study is to determine the effects of VitD supplementation on RMR, body composition, strength and oxidative stress in physically active adults. Participants will complete pre-supplementation testing to assess RMR, body composition, whole body strength, diet, exercise training load and sunlight recall before being matched for sunlight exposure and then randomly allocated in a counterbalanced manner to the VitD or placebo group. Blood samples will also be collected to identify markers connected with VitD status and muscle metabolism. Following 12 weeks of supplementation with VitD, participants will repeat the pre-supplementation testing. We hypothesise that higher levels of VitD may increase RMR, and strength, and enhance body composition when compared to a placebo.

**Keywords:** Calcitriol,energy metabolism**,** muscle strength, body fat

1. **Literature Review**

*Vitamin D Overview*

Vitamin D (VitD), is a pro-steroid hormone that is described as potentially the first hormone to exist on earth (1). In the last 20 years, the number of scientific studies reporting the importance of VitD production from exposure to ultraviolet (UV) radiation, dietary intake and supplementation for skeletal muscle metabolism and function has increased dramatically (2-5). Deficiency of this vitamin has emerged as a worldwide public health problem and is associated with the development of many diseases, such as osteoporosis, cancer, infertility, type two diabetes mellitus, coronary artery disease and also impacts significantly on the immune system (6-9). It is estimated that more than one billion individuals are VitD insufficient or deficient (5). In Australia one in four adults have a suboptimal VitD status (serum 25-hydroxyvitamin D [25(OH)D] levels < 50 nmol∙L-1)(5).Vitamin D exists in two forms: VitD2 and VitD3. Sources of dietary VitD2 include fatty fish, eggs, and dairy products; however, the consumption and intestinal absorption of VitD is a minor part of satisfying total VitD requirements (32). Exposure of the skin to UV radiation permits the conversion of 7-dehydrocholesterol to cholecalciferol (pre-vitamin D3) and represents 80-90% of total VitD production(10-12)..The active form of VitD, 1α,25(OH)2D3, also known as calcitriol, is a steroid hormone that regulates the expression of more than 1,000 genes in humans through vitamin D receptors (VDR).

It is described in the literature as a potential skeletal muscle modulator, via different pathways not well established in the literature (13), such as improvements in skeletal muscle function, energy production and inflammation (14). Typically, the effects of 1α,25D are intermediated by its interaction with a nuclear VDR, which is part of the nuclear receptor superfamily of ligand-activated transcription factors. Vitamin D receptors can also be translocated into the mitochondria of certain cell types, including the skeletal muscle cells, and potentially act directly on cellular bioenergetics (15). It has been proposed that VitD biological activity involves unbound or free fractions of the vitamin (16). The ‘free-hormone hypothesis’ is considered a different pathway for cellular uptake of steroid hormones, as these molecules are highly lipophilic and therefore have the potential to quickly and passively diffuse across cell membranes (16). It seems to be important to differentiate total VitD (measured as 25(OH) D) and bioavailable VitD as the latest evidence suggests that VitD binding protein (DBP) inhibits certain actions of VitD, since the bound fraction is unavailable to act on target cells (17, 18).

*Vitamin D and Resting Metabolic Rate (RMR)*

The maintenance of body-mass depends on the balance between energy intake and energy utilisation (i.e., resting metabolic rate + diet-induced thermogenesis and physical activities). Resting metabolic rate represents 60–75% of total energy expenditure and can be defined as the energy production of the respiring tissue mass at rest. Fat-free mass (FFM) and exercise are the major modifiable predictors of RMR (19). As VitD also has non-calcaemic activities (e.g., inhibition of adipocyte differentiation that impacts energy metabolism)(20), it has been suggested that VitD may impact RMR.

The influence on energy metabolism by VitD involves the regulation of uncoupling proteins (UCPs) and enzymes involved in oxidation and lipolysis (21). Uncoupling proteins are associated with a wide range of physiological processes, such as adaptive thermogenesis (22), and the regulation of fatty acid oxidation (23) and body-mass (24). Clinically, higher dietary VitD intake and serum 25(OH)D levels are associated with a reduction in omental adipocyte size and lower visceral adiposity in women (25). Cross-sectional studies report a negative relationship between overweight and/or obesity and serum levels of 25(OH)D (26), and prospective studies have reported that low 25(OH)D plasma levels may contribute to the development of obesity (27, 28). A recent study investigated the association between 25(OH)D and body composition in elderly participants and found that individuals in the lowest serum 25(OH)D quartile (4.7 – 17.5 ng∙mL-1) had a higher fat mass (9.3 kg∙m2 ) compared with participants in the third (8.40 kg∙m2; Q3 = 26.1–34.8 ng∙mL-1; p = 0.049) and highest (8.37 kg∙m2; Q4 = 34.9–62.5 ng∙mL-1; p = 0.04) quartile (29). In-vitro experiments have suggested that VitD induced increases in intracellular calcium concentrations within adipocytes, resulting in decreased lipogenesis and increased lipolysis (30). However, this area has not been well explored, with only one study finding an association between VitD and RMR in obese adults (11) (observational study). The model produced by Calton et al. predicts that for every 10 nmol∙L-1 increase in serum 25OHD, RMR would increase by 56.5 kJ∙day-1 (11). However, randomised clinical trials examining the influence of VitD on energy expenditure are rare. To the best of our knowledge, only one study with a very short supplementation protocol (1 week) reported no influence of VitD on energy or substrate utilisation and this topic has not been further explored in a physically active population (31). Slight changes in energy expenditure over time can have a large impact on fat and muscle mass (32). Furthermore, identifying variables that directly impact RMR is essential to calculate total energy expenditure, which may have a direct impact on practice for dietitians and other health scientists.

*Vitamin D and Strength*

Importantly, if VitD potentially impacts pathways related to muscle function, it could potentially impact muscle structure, function and strength. The effects of VitD supplementation on exercise and muscle health have recently been reported in soccer and rugby players, elite ballet dancers, and active adult males that were a mixed sample of VitD deficient or insufficient at baseline (33-40). Results are equivocal, with four studies (33, 34, 36, 40) reporting that supplementation with VitD significantly increased strength (increase in isometric force peak, 1-RM bench press, 1-RM back squat and weighted reverse-grip chin up), and three studies reporting no significant effect of VitD on any physical parameter (35, 37, 38). However, variability in participant populations, VitD status, and the type, duration and type of VitD used for supplementation makes contrasts difficult. Another limitation is that only one study has included males and females to allow for the analysis of any gender effects, and they showed that VitD increased isometric strength in male and female and it was positively associated with gender (p < 0.001) and “time” – pre vs. pos supplementation period (p = 0.01) (36). Although males had higher isometric strength and vertical jump heights, and a significant main effect for time suggested that VitD supplementation lead to improvements in these variables, there was no difference in the change between genders. (36).

Vitamin D supplementation in healthy subjects with low serum levels of 25(OH)D activates the VDR in skeletal muscle, which may stimulate protein synthesis, improve muscle strength and prevent stress fractures in healthy young adults (14, 41, 42)[53, 54]. Several authors have suggested that supplementation with VitD also results in an increase in size and number of type II muscle fibres in VitD deficient individuals (13, 43, 44), however, few studies have tested these associations in humans directly by muscle biopsy. A recent systematic review summarised the effects of supplementation with VitD on muscle strength in insufficient VitD athletes and identified that four of six studies found that VitD supplementation increased muscle strength by 1.4 to 18.8% (45), nevertheless, more studies are needed to support this outcome.

While plasma values of 25(OH)D > 50 nmol∙L-1 have been demonstrated to improve bone health and immune system function and assist with disease prevention, it is believed that different tissues have distinct responses to different concentrations of VitD. Therefore, the optimal 25(OH)D concentration for muscle metabolism, protein synthesis and function may not be the same considered sufficient for other organ systems (46-53). It has been proposed by Heaney and Holick (54) serum total 25(OH)D concentrations of ~ 120 - 225 nmol∙L-1 may be required for optimal skeletal muscle function in adults. The majority of studies into the effects of VitD on muscle strength have examined VitD deficient athletes and not those with adequate or supraphysiological concentrations of VitD. Further studies are necessary to elucidate if supraphysiological dosages of VitD have an ergogenic effect in VitD replete athletes in different sports disciplines. In summary, VitD has the potential to regulate energy metabolism and lean and fat mass, which in combination can impact body composition and may consequently influence muscle strength. Importantly, there is a lack of literature on the impact of VitD supplementation on RMR, body composition and strength in physically active adults that are sufficient in VitD.

**3.1 Main Aim: To investigate the association between serum levels of VitD and resting metabolic rate, body composition and strength in physically active adults**

Research questions:

1) Are VitD serum levels associated with RMR, body composition and strength in physically active adults?

2) Do optimal levels of serum VitD (≥ 100 nmol∙L-1) increase RMR, and improve body composition and strength in physically active adults?

We hypothesise that higher levels of VitD may increase RMR, enhance body composition and strength when compared to placebo.

**3.2 Specific Aims:**

1. To assess the effect of VitD levels on RMR pre- and post-VitD supplementation.
2. To assess the effect of VitD levels on lean and fat mass pre- and post-VitD supplementation.
3. To identify if VitD levels are correlated positively with functional strength.
4. To correlate levels of free 25(OH)D (bioavailable vitamin) post-supplementation with lean and fat mass, RMR and functional strength.

**3.2 Significance**

Vitamin D is recognised as an important factor in optimising muscle function, which may consequently optimise RMR and strength. Many studies have assessed the impact of VitD on muscle strength and performance, showing positive effects when athletes or physically active adults (55, 56) are deficient at the baseline. On the other hand, it is not clear what the effects of VitD supplementation may be when physically active adults are not deficient in VitD regarding RMR, body composition and strength. Recent studies in this area have many limitations, for example it is not known if females will respond in the same way as males to VitD supplementation, the method of assessing VitD was not the gold standard technique (i.e., liquid chromatography–tandem mass spectrometry or automated immunoassays validated in the literature) (57), the dose of VitD was not calculated relative to body-mass and participants were not monitored for toxicity symptoms. These limitations will be addressed in our proposed study. To our knowledge this will be the first study to utilise a VitD dosage that is corrected for body-mass and the first study that will assess resting metabolic rate.

1. **Materials and Procedures**

4.1 Participants

Thirty physically active males and females aged 18 - 35 years, who exercise at least 3 times per week with at least 2 of those sessions involving resistance training, with no history of VitD supplementation in the last month, no current injuries that would prevent them from completing strength testing, and with no current use of multivitamins, medication or other supplements that are related with VitD metabolism and body composition (including calcium, thyroxine, whey protein, creatine and thermogenic supplements) will be recruited to take part in this study. Based on previous research achieving an effect size of *d* = 0.60 (34, 58), a sample size of 30 provides a power of 0.99 at an α value of 0.05.

4.2 Study Overview

Participants will complete 4 testing sessions (2 pre-supplementation and 2 post-supplementation) over approximately 14 weeks. First, participants will complete some initial assessments including an assessment of RMR, a few haematological markers including 25(OH)VitD, calcium and parathyroid hormone, and body composition, followed 36 – 72 h later by a functional strength assessment. At the initial session they will be familiarised with a daily training diary, which will be used to assess training load using the sessional rating of perceived exertion method (59). This diary also contains a three-day food diary, calcium quiz and sunlight recall which will be completed at the start, middle, and end of the supplementation period. In the second session individuals will complete a battery of strength tests that will be completed during a single session. The study will start after the summer season (March/April 2019) to increase the chance of individuals being VitD sufficient at the baseline and optimise the chance of participants reaching higher serum levels (≥ 100 nmol∙L-1) at the end of the study. Following pre-testing participants will be matched for sunlight exposure and randomly allocated in a double-blind and counterbalanced manner to the VitD (n = 15; 50 IU∙kg-1BM∙day-1 VitD3) or placebo (n = 15; dextrose) supplement group for 12 weeks. All doses will be concealed in opaque gelatin capsules to ensure that participants remain blinded to their dose.

This dosing strategy was selected because it is generally applied to studies performed in physically active adults (2500 - 4000 IU) (60-62) and it has been associated with a positive effect on training capacity and muscle recovery (36, 63). It is predicted that after summer participants will have sufficient baseline levels of VitD and for each week of supplementation VitD levels will increase ~ 7 nmol∙week-1. Following 12 weeks of supplementation with VitD, participants will repeat the pre-supplementation testing (Figure 1). Low, adequate and optimal VitD status will be defined as 25(OH)D < 50 nmol∙L-1, ≥ 50nmol∙L-1, and > 100 nmol∙L-1 (estimated as the best health skeletal muscle outcomes), respectively (54, 64).

4.3 Procedures

*4.3.1 Resting Metabolic Rate*

Subjects will be requested to abstain from any strenuous exercise for 24 h prior to the measurement. Subjects will arrive at the laboratory after waking up from a 12 h overnight fast, and will empty their bladder before being weighed. They will rest in a supine position for 30 min, in a quiet room without noise and strong lights; before having their oxygen consumption assessed in the final 5 min of the period by metabolic cart (Parvo medics TrueOne 2400, Parvo medics, USA), using a mouthpiece and mixing chamber. These measurements will be used to determine RMR.

*4.3.2 Haematological markers*

Participants will attend an external pathology laboratory (PathWest Laboratories, Perth, Western Australia) pre- and post-supplementation, where a venous blood sample (~10 ml) will be drawn to assess VitD status, calcium and parathyroid hormone. Vitamin D status [25(OH)D] will be determined using the Immunoassay method (Abbott Architect i2000sr analyser, USA). Calcium, parathyroid hormone, and [25(OH)D] will be analysed using the Arsenazo III method and chemiluminescent microparticle immunoassay methodology respectively (Abbott, Barcelona-Spain). Additionally, three small serum aliquots (3 ml) separated in the PathWest laboratory will be stored at –80°C in the freezer room at building 305 for as long as experiments are being conducted and saved for at least two years after the completion of this study.

*4.3.3 Body composition assessment*

Lean and fat mass will be assessed using dual energy X-ray absorptiometry (DXA; GE Lunar Prodigy, General Electric, USA) pre- and post-supplementation.

*4.3.4 Strength and power assessment*

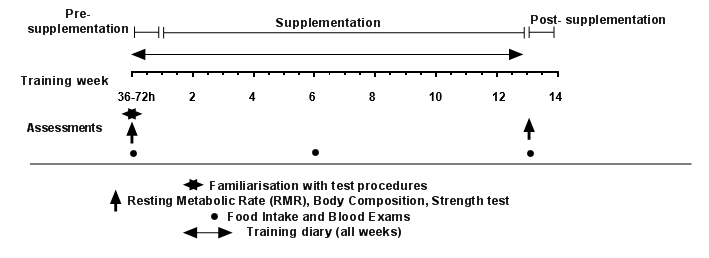
A battery of strength tests will be completed during a single session. Participants will complete a self-selected warm-up, which will be recorded and replicated for the post-test session. First, they will complete the test of 1-repetition maximum (1RM) for the bench press and back squat exercises following the procedures set out by Baechle and Earle (65). Briefly, participants will lift progressively greater weights until a mass is identified that can only be lifted for one repetition for that exercise. Second, participants will complete a counter-movement vertical jump test, (Vertec Yardstick Jumping Device, Swift Performance, Australia) to assess leg power.

*4.3.5 Training diary and questionnaires*

A daily training diary will be completed during the 12 weeks of supplementation so that training load can be calculated using the sessional rating of perceived exertion (sRPE) method proposed by Foster (59). Briefly, the participant will describe the type of exercise, duration of session and will choose the rating of perceived exertion for the session (sRPE; CR1 – 10 scale)(66). This value will be multiplied by the duration of the session and it will provide us with a training load value in arbitrary units (AU).

As part of the training diary, participants will also complete a 3 day food record, calcium quiz and sunlight exposure questionnaire on three occasions (baseline, 6 and 12 weeks). First, participants will record the amount of food, fluid and supplements consumed during 3 consecutive days (2 weekdays and 1 day of the weekend), after having received detailed instructions about how to complete their dietary intake. This diet monitoring period is considered adequate for the estimation of habitual energy and macronutrient consumption (67). Each food record will be briefly reviewed by a nutritionist together with each participant to ensure that sufficient detail is captured. Food records will be analysed using Foodworks® (V9, Xyris Australia). Each individual will be encouraged to follow similar eating patterns throughout the study to minimise deviations in macronutrients, and vitamin and mineral intake. Second, they will complete a five minute questionnaire (Dairy Council of California (68); baseline, 6 and 12 weeks) to assess calcium intake. The quiz contains 34 calcium-containing foods and fluids along with a serving size, and participants select how many servings of each listed food were consumed the previous day. Additionally, participants will complete a sunlight exposure diary on three occasions (baseline, 6 and 12 weeks), which will ask them about the amount of time spent outdoors each day on average in the previous week (0 = ≤ 5min, 1 = 5 – 30 min, and 2 = ≥ 30 min) and what areas of their body got exposed while outdoors (1 = face and hands only; 2 = face, hands and arms; 3 = face, hands and legs; and 4 = bathing suit). The score to calculate a mean weekly sun exposure will be: the product of the amount of time spent outdoors and the amount of skin exposed for each day to create a daily Sun Exposure Score (SES; min = 0, max = 8). All seven days’ SES will be summed to equal the weekly SES (min = 0, max = 56) (69).

**Figure 1. Study Design**



**5. Statistical Analysis**

We will conduct a split-plot ANOVA (SPANOVA) to assess differences within and between groups. Statistical significance will be as accepted at p < 0.05.These analyses will be conducted using IBM SPSS Statistics for Windows (Version 23.0, IBM Corporation, New York, U.S.A.).Additionally, Cohen’s *d* effect sizes will be calculated and classified as small < 0.49, moderate 0.50 – 0.79, or strong ≥ 0.80. Only moderate and strong effect sizes will be reported.

**6. Ethical Issues and Data Storage**

All data will be kept confidential and will be organised and filed according to the date, sample, and number in the backed-up research drive (R drive). Extracted data will be organised and sorted using Microsoft Excel and SPSS Statistics Package. This project will be submitted to the Curtin University Human Research Ethics Office for approval. All participants will be requested to sign a consent form. All data will be kept confidential and data will be de-identified.

Body composition assessment using DXA involves a small dose of ionising radiation (~ 0.74 μSv) that is equivalent to about one thousandth of the background radiation dose that a person would receive whilst living in Perth, Western Australia for one year. All participants will complete a DXA pre-scan questionnaire to assess their suitability to complete the scan. Any female that is pregnant or may be pregnant will be excluded from participating. Occupational radiation exposure to the operators of this equipment is sufficiently low that screening is not required. Nonetheless, all operators will be required to wear a radiation monitoring badge while using the DXA.

The risks of taking blood include the chance of infection or a bruise at the point where the blood is taken, redness and a rare risk of fainting. Participants will have blood sampled by Pathwest phlebotomists that will be using gloves and will follow hygienic procedures to protect for any contamination. Participants will be reminded to keep the wound clean and dry after blood sampling to minimise the risk of infection at the sample site. To minimise the risk of injury from fainting, individuals will be seated in a chair during blood sampling. As we will be working with human blood, there is a small risk of infection by pathogens that might be present in the blood. The researcher will be trained at the safe handling of biohazardous items.

Vitamin D supplementation is considered safe at doses < 10,000 IU∙day-1 and toxicity has not been reported until doses exceed 10,000 IU∙day-1, which is much higher than the dose that will be prescribed in this study (e.g., for a person with a body mass = 70 kg, the dose will be 50 IU x 70 kg = 3500 IU∙day-1). The main pathology associated with hypervitaminosis D is hypercalcaemia and hypercalciuria, as VitD regulates the metabolism of calcium. Hypercalcaemia does not occur unless serum 25(OH)D levels reach 220 nmol∙L-1 and is typically not observed until plasma levels reach 500 nmol∙L-1 (70). Calcium and parathyroid hormone will be assessed to monitor participants for toxicity. It is not expected that participants will be deficient in VitD at the baseline assessment (as the tests will be performed after the summer season), however, if participants are found to be deficient pre- or post-supplementation, they will be recommended to consult their physician.

Strength testing does involve a small risk of musculoskeletal injury. The testing will be conducted by a trained researcher who will ensure that the participants are warmed up and ready to participate in the tests and will monitor the activity for safety at all times. Also, these tests may cause delayed onset muscle soreness. As participants must be currently completing at least two days per week of strength training they will be experienced in the sort of discomfort that this type of exercise can cause. Prior soreness is also protective of future post-exercise soreness.

**7.** **Facilities and Resources**

Molecularcellular researchequipment will be provided by the School of Pharmacy and Biomedical Sciences and CHIRI at Curtin University. The body composition assessments will be conducted in the Inter-Professional Health and Wellness Centre at Curtin University, specifically in the DXA room (404.329). All RMR and strength testing will take place in the School of Physiotherapy and Exercise Science’s Strength and Conditioning Laboratory (400.366) and Exercise Physiology Research Laboratory (400.116). Blood sampling will be conducted at a Pathwest collection centre that is located in a convenient location for the participant, and will be analysed at the Pathwest pathology laboratory at Fiona Stanley Hospital.

This project will be supported as follows: $2000 p.a. provided by GRS and $3000 p.a. provided by the School of Biomedical Sciences. Reagents and items of equipment will be purchased if necessary (Table 1) and any additional funds will be provided from my supervisor’s (Prof Newsholme) cost centre.

**Table 1. Study estimated budget.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Materials, tests and products** | **Unit Cost ($)** | **Units** | **Total Cost ($)** |
| **DXA** | **$ 10.00** | **60** | **$ 600.00** |
| **Radiation safety course** | **$ 200.00** | **1** | **$ 200.00** |
| **VitD supplementation** | **$ 27.00** | **15** | **$ 405.00** |
| **Placebo supplementation** | **$ 15.00** | **15** | **$ 225.00** |
| **Calcium** | **$ 0.10** | **90** | **$ 9.00** |
| **25(OH)D serum test Immunoassay** | **$ 6.00** | **90** | **$ 540.00** |
| **Parathyroid hormone** | **$ 5.10** | **90** | **$ 459.00** |
| **\* Free 25(OH)D – immunoassay (ELISA)** | **$ 975.00** | **2** | **$ 1,950.00** |
| **Phlebotomy course** | **$ -** | **0** | **$ -** |
| **Sample storage** | **$ 10.00** | **90** | **$ 900.00** |
| **Sample management/collection** | **$ 10.00** | **90** | **$ 900.00** |

**Table 2. Timeline of the project**

|  |  |  |
| --- | --- | --- |
| **Proposed time schedule** | **Year 1 (2019)**  **S1 S2** | **Year 2 (2020)**  **S1 S2** |
| Analyse literature and develop literature review | **✓** | **✓** |
| Ethics submission and approval | **✓** | **✓** |
| Recruitment of physically active people | **✓** |  |
| Familiarisation with test procedures | **✓** |  |
| Assessments | **✓ ✓** |  |
| Analyse data | **✓ ✓** | **✓** |
| Preparation of papers (review/data) | **✓** | **✓ ✓** |
| Thesis writing & submission | **✓ ✓** | **✓ ✓** |

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