A 15-week vitamin D supplementation and indoor cycling intervention reduces exercising heart rate, with no effect on glycaemic control in healthy adults: A pilot investigation

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ABSTRACT

Significant positive correlations are often observed between vitamin D concentrations and physical activity levels. Whilst this may be due to individuals who are physically active spending time outdoors (i.e. increased opportunity for vitamin D synthesis), there is growing evidence to suggest a more complex relationship between vitamin D status, physical fitness and health outcomes. To explore this further thirty-nine healthy adults were randomly allocated to 15 weeks of exercise training (Ex), no training (NoEx), 2000 IU/day vitamin D (VitD) and/or placebo (Pla) supplementation (giving four possible allocations: NoEx+VitD; NoEx+Pla; Ex+VitD; Ex+Pla). Vitamin D status, glycaemic control and exercise responses were measured pre- and post-intervention. A series of 2 x 2 ANOVAs failed to find any effect of supplementation or exercise on any of the measures except heart rate during low intensity exercise, and vitamin D status. Heart rate was significantly reduced (6%, p < 0.05) in the Ex+VitD group. Vitamin D status was significantly raised (28%, p < 0.05) in the supplementation groups (NoEx+VitD and Ex+VitD) at a time of year (August-November) when a seasonal decline was observed in the placebo groups (33%, p < 0.05). These findings indicate that vitamin D supplementation (2000 IU/day) may have an enhancing role alongside exercise in inducing cardiorespiratory adaptations to exercise training. Further investigations are required to confirm these findings and identify the mechanisms involved.

KEY WORDS: Physical activity, cholecalciferol, heart rate, glycaemic control

INTRODUCTION

Once only recognised for its role in bone and mineral homeostasis, there is emerging evidence that vitamin D exerts a range of effects on many aspects of both human health (19) and physical
performance (9). Positive correlations have frequently been observed between vitamin D status, physical activity and physical fitness (7). Whilst there is still much work to be done in this area it is becoming apparent that the higher levels vitamin D observed in those who are more physically active may not simply be due to increased sun exposure alone.

Whether orally ingested or obtained via sunlight, vitamin D is biologically inert and requires two consecutive hydroxylations to form its biologically active form; 1,25 dihydroxyvitamin D3. The first part of this process occurs in the liver which produces 25 hydroxyvitamin D (25(OH)D), a pre-hormone which is used worldwide as a measure of vitamin D status (19).

Exercise training results in increased muscle mass and induces increases in mitochondrial mass, volume, and mitochondrial enzymes (2). The mitochondria are essential sites for the biosynthesis of steroid hormones with the mitochondrial P450 enzymes being crucial for the activation and degradation of vitamin D (12), consequently any alterations in mitochondrial volume and enzymatic actions could affect vitamin D metabolism and status. Exercise training also results in a reduction in fat mass (6), and inverse associations have been observed between adiposity and 25(OH)D concentrations (5). When vitamin D is sequestered in the adipose tissue it is unavailable for biological processes within the body (18, 4), therefore any reductions in adipose tissue could result in an increase in circulating 25(OH)D.

Several plausible mechanisms have been suggested (12, 19) by which increasing physical activity and increasing 25(OH)D status could have additive effects working together to increase cardiorespiratory fitness. A reduction in both resting and exercising HRs are frequently observed when individuals engage in a regular physical activity program of an adequate volume (1). There is some evidence that increasing vitamin D status may also result in a reduction in heart rate (13), potentially mediated via a vascular smooth muscle mechanism as this is a target organ for vitamin D action (13).

It is well recognised that physical activity can reduce insulin resistance in skeletal muscle (8). It has also been suggested that via its effects on muscle metabolism vitamin D has an effect on insulin sensitivity (10). Vitamin D receptors and the enzymes required for its actions have been identified in numerous tissues including skeletal muscle where approximately 85% of insulin and calcium mediated glucose uptake takes place. Vitamin D regulates calcium homeostasis, and calcium plays a vital role in exercise induced glucose uptake (10). Therefore, it is plausible that vitamin D may affect glucose uptake in skeletal muscle (15), independently and/or in combination with exercise.

It is hypothesised that vitamin D supplementation and physical activity will exert both an independent and interactive effect on body composition, glycaemic control, and exercise response in healthy adults.
METHODS

Participants
The experimental protocol was approved by Aberystwyth University Ethics Committee and was performed during 2012 (August – November) in accordance with the Declaration of Helsinki (2008).

Participants were recruited via posters displayed around the local area and study information was included in the weekly University email. Eligible participants were aged ≥ 18 years and did not meet any of the exclusion criteria: (1) diagnosis of diabetes, (2) current use of vitamin D supplements and (3) any contraindication for moderate intensity exercise - as assessed by physical activity readiness questionnaire. Written informed consent was obtained from thirty-nine individuals who were not taking part in any form of structured exercise training.

Protocol
Thirty-nine participants were randomised (computer-generated random allocation sequence) to one of four groups: (1) vitamin D supplementation (NoEx+VitD, males = 2; females = 8), (2) placebo supplementation (NoEx+Pla, males = 2; females = 9), (3) exercise plus vitamin D supplementation (Ex+VitD, males = 2; females = 8) and (4) exercise plus placebo (Ex+Pla males = 2; females = 6).

All participants were instructed to take one capsule (size 00; 100% HIDE gelatine derived from the bovine skin, Blackburn Distributions LTD, Lancashire, UK) twice a day. Vitamin D capsules contained 25 µg of vitamin D (1000 IU) (Healthspan, Gurnsey, UK) and microcrystalline cellulose powder, 99.12% purity (Blackburn Distributions LTD, Lancashire, UK); the placebo contained only the microcrystalline cellulose. Participants were invited back to the laboratory every 35 days to return used capsule pots and collect a new supply, enabling adherence to the supplementation protocol to be monitored.

Prior to commencing the exercise training program, the exercise groups participants completed a sub-maximal exercise test (see “measures” below) used to establish heart rate (HR) response and determine program workloads. Participants were required to attend a minimum of two exercise sessions a week (on different days). All sessions consisted of a 10-minute warm up and a 5-minute cool down which were performed at 40-50% of the HR reserve. Exercise sessions were alternated between interval and continuous exercise. During week one the continuous sessions consisted of 10 minutes’ steady state cycling at 60-75% HR reserve, which increased by ten minutes in week two, and a further ten minutes in week three. Participants continued to cycle for 30 minutes at 60-75% HR reserve in these sessions for the remainder of intervention period. The interval phase started at 10 minutes in week one and increased by five minutes each week until 30 minutes was reached. The interval sessions began with a 2-minute low intensity phase at 40-50% HR reserve alternated with two minutes of high intensity periods at 80-90% HR reserve. Once built up, each exercise session lasted approximately 45 minutes.
Participants in the exercise groups completed an additional sub-maximal exercise test mid-way through the exercise program for recalculation of exercise workloads.

All measures were completed at baseline and at 15 weeks (2-3 days post intervention). Participants attended the laboratory at each time point, one visit included venous blood sampling and an oral glucose tolerance test, and the second visit, completion of the International Physical Activity Questionnaire (www.ipaq.ki.se.) and a submaximal exercise test.

Following an overnight fast, participants attended the laboratory where they remained seated for 10 minutes prior to blood being drawn from an antecubital vein (BD Vacutainer Precision Glide Multi Sample Needle, Plymouth, UK) and collected in to one 6 ml BD 367873 plastic serum tube (red top) and one 6 ml K2EDTA plasma tube (lavender top).

The 6 ml BD serum tube was kept at room temperature for 1.5 hours prior to being centrifuged at 4°C for 10 minutes at 1300 g to separate serum which was stored at -80°C. Samples were transported to Merthyr Tydfil biochemistry laboratories (United Kingdom) for analyses of 25(OH)D (Agilent 6410 Series Triple Quadrupole LC-MS/MS). 0.5 ml of whole blood was removed from the 6 ml K2EDTA plasma tube and refrigerated in an Eppendorf tube prior to analyses for glycated haemoglobin concentrations (D-10 HbA1c analyser, Bio-Rad Laboratories Ltd). Remaining blood was centrifuged at 4°C for 10 minutes at 1500 g to separate plasma, 0.5 ml aliquots were stored at -80°C. Following thawing at room temperature the frozen 0.5 ml plasma samples were analysed for insulin concentrations using an Invitron Insulin Assay (IV2-001; Invitron Ltd, Monmouth, UK).

The International Physical Activity Questionnaire (IPAQ) short version captures both volume and number of physical activity sessions/days. Participants may then be categorised to one of three physical activity levels: (1) inactive (does not meet criteria for categories 2 or 3), (2) sufficiently active (3 or more days of vigorous activity of at least 20 minutes per day OR 5 or more days of moderate-intensity activity or walking of at least 30 minutes per day OR 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week) or (3) active (vigorous-intensity activity on at least 3 days achieving a minimum of at least 1500 MET-minutes/week OR 7 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week).

Following the venous blood sample participants ingested 75 grams of glucose in 300 ml of water. Finger prick capillary blood samples were taken pre-ingestion and 20, 40, 60, 80, 100, and 120 minutes post ingestion and analysed for glucose (2300 STAT PLUS, YSI, Yellow Springs, USA). Participants remained seated during this process. From the blood glucose values at each time point total area under the curve (AUC) was calculated.

Participants were seated in the laboratory for 15 minutes to obtain resting HR. To establish each individual’s HR - work rate relationship participants performed a sub-maximal exercise test on
a cycle ergometer (Monark, 874E, Varberg, Sweden). The protocol consisted of four, four-minute work stages at 60, 90, 120 and 150 W. At the end of each stage HR was recorded and a linear regression equation was established between workload and HR. Individualised exercise workloads were then determined from the specific percentages of HR reserve (3).

**Statistical Analysis**

Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Data were checked for normal distribution using the Shapiro-Wilk test prior to statistical analysis. Statistical comparisons were made using a 2-way mixed ANOVA. The four groups were the between subjects factor, and the time points the within subjects factor. Where appropriate, t-tests with Bonferroni corrections were conducted.

**RESULTS**

Two participants from the NoEx+VitD group and one from the NoEx+Pla group withdrew from the study; their data were removed from all analyses. All of the participants in the exercise groups completed the 15-week intervention program. Adherence to the two cycling sessions per week over 15 weeks was 92%.

There were no significant differences in baseline characteristics between the four groups (Table 1). When recruited all participants verbally reported that they were inactive; however, when they completed the IPAQ the results identified 19% of participants as being inactive, 53% were sufficiently active and 28% were categorised as being active.

<table>
<thead>
<tr>
<th>Table 1. participant characteristics (mean±SD).</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Ex+VitD</td>
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<tr>
<td>NoEx+VitD</td>
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<td>Ex+Pla</td>
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<td>NoEx+Pla</td>
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A two-way mixed ANOVA revealed a significant main effect of vitamin D supplementation on 25(OH)D concentrations in the vitamin D supplementation groups ($F(1,30) = 36.276, MS_e = 617.520, p < 0.001$), however there was no main effect of exercise ($F(1,30) = 0.228, p = 0.64$) and no interaction of vitamin D and exercise ($F(1,30) = 0.58, p = 0.81$). Dependant t-tests identified significant differences (increase) in 25(OH)D concentration from pre to post intervention in vitamin D supplementation groups (figure 1).

A series of 2 x 2 ANOVAs failed to find an effect of vitamin D or exercise alone, or in combination on either measures of glycaemia (HbA1c and insulin) ($p > 0.05$). Neither was there a main effect of, or interaction between, vitamin D supplementation and exercise on body mass ($p > 0.05$). Pre and post values are reported in Tables 2, 3, 4 and 5.
There was a significant group x time interaction (F(3,31) = 4.855, p = 0.01, partial $n_2 = 0.320$) for exercising HR at 60 W (figure 2). A significant reduction in exercising HR post- compared to pre-intervention was identified in the Ex+VitD group (F(1,9) = 6.533, p = 0.03, partial $n_2 = 0.421$), but not between pre and post HR at 60 W in NoEx+VitD group (F(1,6) = 4.531, p = 0.07, partial $n_2 = 0.430$), Ex+Pla group (F(1,7) = 0.307, p = 0.59, partial $n_2 = 0.042$) or NoEx+Pla group (F(1,9) = 1.420, p = 0.22, partial $n_2 = 0.127$) (figure 2).
Table 4. Non-significant pre and post measures for Ex+Pla group (mean ± SD).

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<tr>
<td>HbA1c (mmol/mol)</td>
<td>33.1±10.7</td>
<td>34.2±11.3</td>
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<tr>
<td>Insulin</td>
<td>35.1±18.7</td>
<td>29.7±12.5</td>
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<tr>
<td>Body mass (kg)</td>
<td>71.1±9.6</td>
<td>71.4±9.6</td>
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<tr>
<td>HR (bpm) 90 W</td>
<td>133±26</td>
<td>125±17</td>
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Table 5. Non-significant pre and post measures for NoEx+Pla group (mean ± SD).

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<tr>
<td>HbA1c (mmol/mol)</td>
<td>24.8±14.8</td>
<td>35.0±5.0</td>
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<tr>
<td>Insulin</td>
<td>43.4±27.6</td>
<td>56.0±49.0</td>
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<tr>
<td>Body mass (kg)</td>
<td>78.9±25.9</td>
<td>78.6±25.4</td>
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<tr>
<td>HR (bpm) 90 W</td>
<td>142±15</td>
<td>146±18</td>
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Figure 2: Pre and post HR at 60 watts (*significantly different from pre intervention (p < 0.05).

DISCUSSION

There were no significant effects of 15 weeks of vitamin D supplementation (2000 IU/day) or a 15-week exercise training (cycling) intervention on measures of glycaemic control or body mass in healthy participants. There was a significant reduction in exercising HR (60 watts) in the exercise and vitamin D supplementation group only. Vitamin D concentrations in the supplementation group were raised to what many experts in the field would consider to be necessary (98.1±19.0 nmol/l) for benefits to human health (17) at a time of year (mid-August to mid-November) when a seasonal decline would be expected. The placebo groups demonstrated
a significant seasonal decrease of 33%, whilst a significant increase in 25(OH)D of 28% was observed in the vitamin D supplementation groups. The vitamin D dose in the current study was 2000 IU/day, supplementing with a lower dose in line with current recommendations (600 IU/day: 14) may not have achieved this.

The absence of any independent effects of the exercise training program on any of the measures was unexpected and possibly attributable to the characteristics of the participants and/or the volume of exercise provided. Despite verbally self-reporting being “inactive” during recruitment many of the participants were classified “sufficiently active” or “active” (IPAQ) during the first lab visit leaving less scope for adaptation. The lowered HR in the exercise and vitamin D group suggests an enhancing role of vitamin D alongside the exercise program in reducing exercising HR. It is not possible to speculate whether a higher dose of vitamin D would have achieved this independently as the evidence in this area implies a synergistic effect of vitamin D in combination with exercise in the current study or with calcium in earlier work (13).

It was suggested that the mechanism by which vitamin D exerted its effect on HR when co supplemented with calcium over 8 weeks was mediated via a vascular smooth muscle mechanism as this is a target organ for vitamin D (13). An improvement in endothelial function has been observed in participants with type 2 diabetes following vitamin D supplementation (Sugden et al., 2008), which may subsequently reduce peripheral resistance (19) and potentially HR for a given work rate as observed in the current study. These findings not only have relevance to cardiovascular adaptations in relation to exercise performance, but may well be of clinical significance as micro-vascular events are the cause of many of the complications of diabetes (16).

The absence of a vitamin D supplementation effect on glucose control was not totally unexpected as many of the participants (n = 28) in the current study had baseline 25(OH)D concentrations greater than 50 nmol/l. It has been suggested that for vitamin D supplementation to exert any effect on glucose metabolism, 25(OH)D initial concentrations would need to below 50 nmol/l (11). Only eight of the participants in the current study had 25(OH)D concentrations below this level.

The non-significant effects of exercise on measurements of glycaemia and body mass were unexpected as regular exercise training results in a decrease of adipose tissue storage. In participants that are already “sufficiently active” or “active” it is likely that a higher volume of exercise training would be required to induce any kind of significant training adaptations. The degree of insulin resistance at baseline would affect the responsiveness to exercise training (8) and the study participants showed no signs of insulin resistance at baseline. This would result in limited scope for improvement in glucose in response to any intervention. Duration of supplementation may also be a contributing factor as (11) reported the effect of time in relation to vitamin D and glucose control. Despite a significant increase in vitamin D status at three months an effect on glucose control was only observed at six months (11).
While the dose of vitamin D in the current study significantly raised 25(OH)D concentrations, no independent or interactive effects were observed when supplementation was combined with exercise on any of the measures of glycaemia or body mass. The identification that the exercise program induced a significant reduction in exercising HR when combined with vitamin D is of relevance to both an athletic and clinical population. Further investigations are required to confirm these findings, identify optimum vitamin D concentrations in relation to outcomes beyond that of bone health, and explore the mechanisms involved.

ACKNOWLEDGEMENTS

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