Comment on: "Association of Vitamin D Supplementation in Cardiorespiratory Fitness and Muscle Strength in Adult Twins: A Randomized Controlled Trial"

Nicholas B. Tiller

Institute of Respiratory Medicine and Exercise Physiology, Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance CA, USA.

Correspondence: Nicholas B. Tiller, Ph.D. | 1124 W. Carson Street, CDCRC Building, Torrance, CA 90502 | Email: nicholas.tiller@lundquist.org | Tel: (+1) 310-980-8163 | Orchid: https://orcid.org/0000-0001-8429-658X

Funding Disclosures/conflicts of interest: The author declares no conflicts of interest and no financial disclosures.

To The Editor,

I read with interest the article by Medeiros *et al.* which was published in the journal's January 2022 issue. Therein, the researchers recruited 37 pairs of monozygotic twins and randomized one twin from each pair to an intervention group, which was supplemented with 2,000 IU/day vitamin D (cholecalciferol) for 60 days, or a control group which received no intervention. The pre- to-post-intervention dependent variables were cardiorespiratory fitness (maximal oxygen uptake; $\dot{V}O_{2max}$) and muscle strength (handgrip and scapula dynamometry).

This letter will draw attention to serious irregularities in the quality/integrity of the data and the authors' interpretation and reporting of results; all of which potentially undermine the study's scientific validity. I have focused my critique on the most profound problems while omitting discussion of issues that are relatively trivial by comparison (namely, lack of a placebo arm, lack of participant masking to randomization, unexplained unilateral increases in forearm strength, no correction for familywise error, no reporting of effect size thereby placing undue reliance on *p*-values, and inappropriate and inconsistent reporting of variance).

The authors assessed cardiorespiratory fitness before and after the trial, reporting an increase in absolute $\dot{V}O_{2max}$ from 2.0 to 2.6 L/min ($p < 0.001$) in the vitamin D-supplemented group, with no change in controls. This equates to a ~28% increase in $\dot{V}O_{2max}$ relative to baseline. According to the authors, subjects in both groups "…maintained their usual routine, without dietary, sports, or lifestyle changes". It is assumed, therefore, that the increases in $\dot{V}O_{2max}$ were attributable to vitamin D supplementation: "In the case of the current study, the divergent external influence [in the two groups of twins] will be vitamin D supplementation."

Conspicuously, there was no concomitant change in relative $\dot{V}O_{2max}$ (i.e., values reported relative to body mass; mL/kg/min) in the supplemented group. The authors recognize this disparity and offer the following explanation:

"We observed alterations in oxygen consumption in the group that received supplementation, through improvement in absolute VO_2max; however, when considering body mass, we did not find significant changes in relative VO_2max, suggesting that regardless of genetics, absolute VO_2max can be modified by healthy eating habits and possibly by an increase in serum vitamin D."

Given that relative $\dot{V}O_{2max}$ is mathematically dependent on the absolute values, the only explanation for a disparity between the two is an increase in body mass following supplementation. Remarkably, body

mass was not reported in this study, nor could it be derived from body mass index (BMI) because stature was also not reported. Nevertheless, based on the absolute and relative $\dot{V}O_{2max}$ at baseline (Table 1), we can deduce a mean starting body mass of ~60 kg. An increase in absolute $\dot{V}O_{2max}$ of 0.6 L/min without a change in relative values would require a mean body mass increase of ~18 kg (~40 lbs) during the 60-day supplementation regimen. This is physiologically impossible by means of vitamin D supplementation alone. The authors also assert that "BMI (kg/m^2) of the CG [control group] was 25.2 and of the SG [supplement group] 24.9, with no differences between groups (p = .798) at T_0 and T_{60}". As such, there is a gross and unexplained discordance between BMI, body mass, and $\dot{V}O_{2max}$ in this study that requires urgent clarification.

The magnitude of the reported increase in absolute $\dot{V}O_{2max}$ should also be contextualized against other controlled interventions of similar duration. For instance, untrained subjects undertaking eight weeks of endurance exercise training exhibited increases in $\dot{V}O_{2max}$ in the region of 7 – 18% [1–4], while 11 weeks administration of erythropoietin (EPO) – a potent stimulus for red blood cell production, widely utilized as a doping agent in endurance sport - improved $\dot{V}O_{2max}$ in untrained subjects by 12% [5]. Moreover, the increase in $\dot{V}O_{2max}$ reported by Medeiros *et al.* is ~3-fold greater than would be anticipated given the documented association between serum vitamin D concentrations and cardiorespiratory fitness [6]. Accordingly, while there may be several mechanisms by which vitamin D supplementation may augment cardiorespiratory fitness, the magnitude of the reported change is unprecedented.

Further to these data irregularities, the paper by Medeiros *et al.*, on numerous occasions, cites references that are non-existent or those that do not fully support the assertions. For example, to justify the use of $\dot{V}O_{2max}$ as a main dependent variable, the authors assert that "There is significant evidence that vitamin D is an influential factor for $\dot{V}O_{2max}$ (Bacchetta et al., 2014; Zughaier et al., 2014)." The paper by Bacchetta *et al.*,[7] explored a possible role for vitamin D in iron homeostasis, finding that vitamin D was a regulator of the hepcidin-ferroportin axis in humans as a putative strategy for the management of anemia. The paper by Zughaier *et al.* [8] also explored hepcidin kinetics, showing that high-dose vitamin D influenced systemic hepcidin levels in patients with early-stage chronic kidney disease. Both were clinical studies and neither assessed $\dot{V}O_{2max}$ or any aspect of exercise performance. As such, the stated link between these studies and vitamin D as "an influential factor for $\dot{V}O_{2max}$" is tenuous at best.

To justify their strength assessments, the authors then assert that "Vitamin D supplementation has been gaining prominence as a strategy to control physical fitness, including muscle strength (Mokta et al., 2017; Orces, 2017)". The first paper by Mokta *et al.*,[9] was a clinical case-report with two patients, and Orces (2017) [10] did not assess the effects of vitamin D supplementation on muscle function or fitness. In their discussion, Medeiros *et al.* cite a paper by Gallagher (2014) asserting that "vitamin D can improve aerobic performance through indirect action on $\dot{V}O_{2max}$". However, the referenced article links to a study

from 2004 (not 2014) by the same author which assessed the effect of vitamin D on falls, fractures, and physical performance tests in elderly women [11]. That study found that vitamin D, when taken twice daily for three years, partially mitigated physical decline quantified as “timed rising” and “timed walk over 5 m”. The study makes no mention of aerobic “performance” or $\dot{V}O_{2max}$.

In conclusion, Laplace’s Principle asserts that “The weight of evidence for an extraordinary claim must be proportioned to its strangeness”[12]. This statement embodies the ethos of scientific skepticism and the scientific method. A claim can be deemed “extraordinary” if it falls outside the boundaries of current understanding or scientific consensus, and this is the only appropriate term to describe a 28% increase in $\dot{V}O_{2max}$, and/or an 18 kg (40 lb) increase in body mass, following 60 days of low dose vitamin D supplementation. Yet, the weight of evidence in support of these claims is far from proportional.

Given the above considerations, I invite the authors to address the apparent incongruities in their data and correct the scientific record.

Acknowledgements. NA.

Declarations

Funding. No funding was received in developing this manuscript. Nicholas B. Tiller is funded by a postdoctoral fellowship from the Tobacco-Related Disease Research Program (TRDRP; award no. T31FT1692).

Conflicts of interest. Nicholas B. Tiller declares that he has no conflicts of interest relevant to the content of this letter.

Authors’ contributions. NA.

References

1. Helgerud J, Høydal K, Wang E, Karlsen T, Berg P, Bjerkaas M, et al. Aerobic high-intensity intervals improve VO2max more than moderate training. Med Sci Sports Exerc. 2007;39:665–71.

2. Duffield R, Edge J, Bishop D. Effects of high-intensity interval training on the VO2 response during severe exercise. J Sci Med Sport. 2006;9:249–55.

3. SCRIBBANS TD, VECSEY S, HANKINSON PB, FOSTER WS, GURD BJ. The Effect of Training Intensity on VO2max in Young Healthy Adults: A Meta-Regression and Meta-Analysis. Int J Exerc Sci. 2016;9:230–47.

4. Mahdiabadi J. The effect of 8 weeks moderate-intensity continuous training on central hemodynamics and VO2max in non-athlete male. Phys Educ Stud. 2021;25:172–7.

5. Thomsen JJ, Rentsch RL, Robach P, Calbet J a. L, Boushel R, Rasmussen P, et al. Prolonged administration of recombinant human erythropoietin increases submaximal performance more than maximal aerobic capacity. Eur J Appl Physiol. 2007;101:481–6.

6. Marawan A, Kurbanova N, Qayyum R. Association between serum vitamin D levels and cardiorespiratory fitness in the adult population of the USA. Eur J Prev Cardiol. 2019;26:750–5.

7. Bacchetta J, Zaritsky JJ, Sea JL, Chun RF, Lisse TS, Zavala K, et al. Suppression of iron-regulatory hepcidin by vitamin D. J Am Soc Nephrol JASN. 2014;25:564–72.

8. Zughaier SM, Alvarez JA, Sloan JH, Konrad RJ, Tangpricha V. The role of vitamin D in regulating the iron-hepcidin-ferroportin axis in monocytes. J Clin Transl Endocrinol. 2014;1:19–25.

9. Journal of the Association of Physicians of India - JAPI [Internet]. [cited 2022 May 14]. Available from: https://www.japi.org/t28484/vitamin-d-and-muscle-weakness

10. Orces CH. Prevalence of clinically relevant muscle weakness and its association with vitamin D status among older adults in Ecuador. Aging Clin Exp Res. 2017;29:943–9.

11. Gallagher JC. The effects of calcitriol on falls and fractures and physical performance tests. J Steroid Biochem Mol Biol. 2004;89–90:497–501.

12. Gillispie CC, Grattan-Guinness I. Pierre-Simon Laplace, 1749-1827: A Life in Exact Science. Princeton University Press; 2000.

13. Medeiros JFP, Borges MV de O, Soares AA, de Sousa EC, da Costa JRR, Silva WAC, et al. Association of Vitamin D Supplementation in Cardiorespiratory Fitness and Muscle Strength in Adult Twins: A Randomized Controlled Trial. Int J Sport Nutr Exerc Metab. 2022;32:2–7.

TABLES

Table 1. All dependent variables reported in the study by Medeiros *et al.* Data replicated verbatim from 'Table 2' in the original manuscript [13]. Note the large (~28%) increase in absolute $\dot{V}O_{2max}$ (L/min) in the supplemented group, without any change in relative values (mL/kg/min).

	Controls (n=37)			**Controls (n=37)**		
	T_0	T_{60}	*p*-value	T_0	T_{60}	*p*-value
$\dot{V}O_{2max}$ (L/min)	2.2 (1.8 - 2.6)	2.1 (1.2 – 2.4)	0.359	2.0 (1.6 – 2.6)	2.6 (2.1 – 3.2)	<0.001
$\dot{V}O_{2max}$ (mL/kg/min)	34.5 (31.0 – 40.0)	34.5 (31.0 – 40.8)	0.414	33.5 (29.0 – 38.8)	33.5 (29.3 – 47.0)	0.118
RERmax	1.1 (1.0 – 1.2)	1.1 (1.1 – 1.2)	0.098	1.1 (1.0 – 1.2)	1.1 (1.0 – 1.1)	0.071
Right hand grip (kgf)	32.5 (29.5 – 38.0)	36.0 (31.0 – 37.0)	0.631	32.0 (28.5 – 39.0)	34.0 (29.0 – 40.0)	0.163
Left hand grip (kgf)	29.0 (25.3 – 38.0)	31.0 (27.0 – 37.3)	0.606	28.0 (24.0 – 32.5)	33.0 (29.0 – 40.5)	0.007
Scapular force (kgf)	23.0 (19.5 – 28.0)	23.0 (18.5 – 28.3)	0.952	24.0 (17.5 – 30.0)	23.0 (18.8 – 28.3)	0.441

Note. Variables are shown as median (percentile 25 – percentile 75) and compared by Wilcoxen test, $\dot{V}O_{2max}$ = maximum oxygen consumption; RER = respiratory exchange ratio; T_0 = first analysis; T_{60} = analysis 60 days after the first; p-values < 0.05 were considered significant.