Acute Effects of Vitamin D_3 Supplementation on Muscle Strength in Judoka Athletes: A Randomized Placebo-Controlled, Double-Blind Trial

Matthew A. Wyon, PhD,† Roger Wolman, PhD,†‡ Alan M. Nevill, PhD,§ Ross Cloak, MPhil,§ George S. Metsios, PhD,§ Douglas Gould, MSc,§ Andrew Ingham, PhD,¶ and Yiannis Koutedakis, PhD§‖*

Objective: Indoor athletes have been shown to be prone to vitamin D_3 deficiency. The aim of the study was to examine the acute effects of vitamin D supplementation on muscle function using isokinetic dynamometry.

Design: Randomized placebo-controlled, double-blind study.

Setting: Institutional.

Participants: Adult male white national level judoka athletes (n = 22) who were involved in full-time training. Exclusion criteria were vitamin supplementation, overseas travel to sunny climes, and/or an injury incurred during the last 3 months before testing.

Interventions: Subjects were randomly allocated to the treatment (150 000IU vitamin D_3) or placebo and given blinded supplements by an independent researcher. Participants were tested twice, 8 days apart, on a Monday morning before the start of judo training and after 2 days of rest. A 5 to 7 mL of blood sample was collected followed by isokinetic concentric quadriceps and hamstring muscle function assessments on the right leg at 30 and $200^{\circ}\cdot s^{-1}$.

Main Outcome Measures: Repeated-measures analysis of variance was used to analyze isokinetic muscle force and serum 25 $(OH)D_3$. Regression to the mean was used to examine changes in 25 $(OH)D_3$ levels over the study period.

Results: The treatment group demonstrated a significant increase in serum 25(OH)D levels (34%, $P \leq 0.001$) and muscle strength (13%, $P = 0.01$) between days 1 and 8. No significant differences were found for the placebo group for the same period.

Conclusions: A single bolus of 150 000IU vitamin D_3 had a significant positive effect on serum 25(OH)D levels and muscle function in vitamin D insufficient elite indoor athletes.

Clinical Relevance: Serum 25$(OH)D_3$ levels of indoor athletes should be monitored throughout the year and especially during winter months. Beneficial responses, in muscle strength and serum 25 $(OH)D_3$, to 1 dose of vitamin D_3 supplementation can be observed within 1 week of ingestion. Muscle strength is linked to serum 25 (OH)D levels.

Key Words: human, muscle strength, calcium, vitamin D, randomized controlled trial

(*Clin J Sport Med* 2015;0:1–6)

INTRODUCTION

Vitamin D is now increasingly recognized as a complex hormone involved in a wide range of functions from calcium homeostasis[1] to the integrity of the innate immune system.[2] Vitamin D can be obtained through food products, such as fatty fish, vitamin D fortified milk, margarine, and cereals, and the use of multivitamins. However, the main intake is by photoconversion of 7-dehydrocholesterol to previtamin D_3 in the skin when exposed to ultraviolet radiation. It is subsequently hydroxylated to 25$(OH)D_3$ in the liver. Serum concentrations of 25$(OH)D_3$ constitute a storage facility similar to other steroid hormones.[3]

Serum vitamin D insufficiency has been defined when values are between 10 and 30 ng/mL and deficiency for levels below 10 ng/mL[4–6] Holick and Chen[4] highlighted in their review that the increases in worldwide vitamin D (25(OH) D_3) deficiency are due to the use of sunblock and skin coverings to reduce the risk of skin cancer; this is exacerbated for indoor athletes,[5] and dancers[6,7] have been shown to be at risk of deficiency mainly because of decreased sunlight exposure.[8]

Birge and Haddad[9] were the first to confirm the link between muscle function and 25$(OH)D_3$. Improved muscle cell protein synthesis and growth has been demonstrated when 25$(OH)D_3$ binds to the muscle nuclear hormone receptor, vitamin D receptor (VDR).[10] Human muscle biopsy studies have reported atrophic changes of predominantly the type

Submitted for publication July 23, 2014; accepted May 4, 2015.

From the *Research Centre for Sport Exercise and Performance, University of Wolverhampton, Walsall, United Kingdom; †National Institute of Dance Medicine and Science, United Kingdom; ‡Department of Rheumatology and Sport and Exercise Medicine, Royal National Orthopaedic Hospital, Stanmore, United Kingdom; §Research Centre for Sport Exercise and Performance, University of Wolverhampton, Walsall, United Kingdom; ¶School of Pharmacy, School of Life Sciences, Aston University, Birmingham, United Kingdom; and ‖Department of Exercise Sciences, University of Thessaly, Trikala, Greece.

The project was not funded by an outside organisation or charity. SunVit LLP, Essex UK supplied vitamin D_3 tablets for the project.

The authors report no conflicts of interest.

Corresponding Author: Matthew A. Wyon, PhD, Research Centre for Sport Exercise and Performance, University of Wolverhampton, Gorway Rd, Walsall, West Midlands, United Kingdom WS1 3BD (m.wyon@wlv.ac.uk).

II fibers because of vitamin D deficiencies, with increases in fibrosis and glycogen granules.[11] Interventions in older adults, through vitamin D and calcium supplementation, reverse these changes in type II fiber composition.[12,13] The effects of vitamin D supplementation on younger and athletic performance have also been studied but hampered by a lack of muscle biopsy and randomized controlled trials (RCT).[14] Some authors reported improved muscle power[15] and strength[16] in adolescent girls and muscular strength in healthy male adults[17] as a result of vitamin D supplementation; similarly, Wolman et al[6] and Wyon et al[7] indicated improved muscle function in dancers. In contrast, other authors failed to find any effects of vitamin D supplementation on muscular strength[18] and physical performance parameters such as 1 repetition maximum (1-RM) bench and leg press and vertical jump height.[19]

Previous research has focused either on cross-sectional or long-term supplementation intervention designs, and there have been very few RCT studies. Vitamin D_3 supplementation doses have also varied considerably with interventions of 200 to 100 000IU over 3 to 12 month of periods.[14,20] However, the longer the intervention the more likely, it is that other variables could affect the final outcome. Wyon et al[7] noted an improvement in muscle strength and power, and a decrease in injury incidence over a 4-month supplementation intervention period (2000IU/d). The reduction in the observed injury incidence could be linked to the reported improvements in muscle function as reported by Koutedakis et al.[21] Therefore, it could be assumed that if supplementation was beneficial as observed in Wyon et al's study, muscle function adaptations would be observable at the early stages of the intervention. Therefore, the hypothesis for the present study was to examine whether 150 000IU vitamin D supplementation would significantly increase serum 25(OH)D and muscle function, as determined by isokinetic dynamometry.

METHODS

Subjects

Participants were male white national level judoka athletes involved in full-time training (4-6 hours a day), which included competition, mat work, and conditioning (Table). For the period of the study and for 3 weeks previously, all participants engaged in the same training schedule to reduce potential confounding factors. This involved 2-hour strength and conditioning, 1-hour technical training, and 2-hour randori. Exclusion criteria were inability to perform training for more than 48 hours in the previous 3 months, vitamin supplementation, and overseas travel to sunny climes during these last 3 months.

Procedure

Data collection occurred at a single location at latitude of 52°29′N in February 2013. In the previous 3 months, there had been an average of 58.3 hours of sunlight (November, 72.9; December, 58.3 hours; and January, 43.6 hours). An independent researcher, using an online random number generator (www.sealedenvelope.com) allocated all participants to the treatment or placebo group using a 1:1 allocation. This individual also blinded the supplements and dispensed to the participants either active vitamin D_3 (150 000 IU) or placebo. The dispensed material was in tablet form, prepacked in appropriately numbered bottles, and identical in their appearance. Participants, outcome assessors, and data analysts were kept blinded to the allocation, and the independent researcher unblinded the trial postdata analysis. The entire protocol was approved by the University of Wolverhampton's Ethics Committee.

Participants were tested twice 8 days apart on a Monday morning before the start of judo training and after 2 days of rest. Each participant completed informed consent before anthropometric measurements, blood collection, and muscle function tests. The independent technician dispensed the prepacked bottles immediately after the first test session and instructed the participants to consume the contents immediately with water.

Standing height was measured to the nearest 0.5 cm using a Seca stadiometer (Hamburg, Germany), with the participants in bare feet and their heads in Frankfort horizontal plane. Total body mass was measured to the nearest 0.5 kg with a Seca beam balance 710 (Hamburg, Germany), participants wore minimal clothing.

A 5 to 7 mL of blood sample was collected into serum separator EDTA tubes that did not contain anticoagulants between 8:00 and 10:00 AM on each testing day. The blood was left to clot for 30 minutes before being spun in a centrifuge at

TABLE. Pre-and-Post Descriptive Data (Mean ± SD) for Treatment and Placebo Groups

Group	Age, yrs	Height, cm	Mass, kg	Serum 25(OH)D, ng/mL			Isokinetic Speed, °·s^{-1}
				Pre	Post	Δ	
Treatment	29 ± 10.6	175.3 ± 6.5	80.6 ± 19.7	13.16 ± 3.75	16.76 ± 3.21	5.74	30
							200
Placebo	26 ± 7.4	178.3 ± 10.1	76.9 ± 12.1	16.33 ± 2.73	16.33 ± 2.56	0.004	30
							200

Group	Quadriceps, N·m			Hamstrings, N·m		
	Pre	Post	Δ	Pre	Post	Δ
Treatment	232 ± 37.44	265 ± 45.56	33.6	147 ± 27.4	163 ± 3.86	15.6
	163 ± 28.25	183 ± 32.97	20.2	130 ± 25.05	147 ± 28.44	16.4
Placebo	239 ± 65.93	239 ± 63.73	5.5	136 ± 26.35	139 ± 20.71	3.9
	145 ± 29.27	148 ± 28.21	1.9	117 ± 18.85	120 ± 17.07	2.9

1500×*g* for 10 minutes. A 0.5 mL of serum was aliquoted into 2-mL microtubes before being stored at −80°C. The thawed samples were later analyzed for serum 25(OH)D by electrochemiluminscent immunoassays (Tecan Infinite F500, Mannedorf Switzerland) with interassay coefficient of variance of 2.9%.

Concentric quadriceps and hamstring muscle function were measured at 30 and 200°·s^{-1} using an isokinetic dynamometer (Kin-Kom Chattecx Co, Chattanooga, TN). The right leg was tested in a seated position with waist and shoulder straps. Verbal encouragement was given to all participants during the tests. All participants had a familiarization session 3 days before the start of the testing as suggested by Callaghan et al.[22] On testing days, each participant completed a 5-minute 100 Watt cycle ergometer warm-up, before conducting 6 concentric contraction cycles as follows: 3 submaximal and 3 maximal effort contractions. The maximal peak torque was recorded for each muscle group and leg speed. Muscle order and testing speed were randomized for each participant and each testing sessions using a computer-randomization program.

Statistical Analysis

Kolmogorov–Smirnov tests were initially performed to establish distribution of all the variables. Isokinetic muscular function was analyzed with a 4-way analysis of variance (ANOVA) with repeated measures; the between-subject factors were "treatment" (intervention and control), and the within-subject factors were "muscle" (quadriceps and hamstrings), "speed" (30 and 200°·s^{-1}), and "time" (before and after intervention). Serum 25(OH)D_3 levels were analyzed using a 2-way repeated-measures ANOVA with the between-subject factor being treatment and the within-subject factor time. Regression to the mean[23] was used to examine changes in 25(OH)D_3 levels over the study period. Significance was set at $P \leq 0.05$.

RESULTS

Twenty-two volunteers met the current inclusion criteria and were randomly assigned to either the treatment or placebo group (Table 1). Although the participants were randomly assigned to the treatment or placebo group by an independent researcher, the treatment group had a significantly lower pretreatment serum 25(OH)D levels than the placebo group ($P < 0.05$); there were no significant difference between the groups for the other variables. The treatment group demonstrated a 34% increase in serum 25(OH)D levels between days 1 and 8, whereas the placebo group remained constant; ANOVA of the blood data reported a main effect of time ($P \leq 0.001$) and more importantly "treatment by time" interaction ($P \leq 0.001$) (Figure 1). Regression of the mean analysis of the whole cohort reported a nonsignificant trend (r = −0.337, $P = 0.125$) with evidence of posttest shrinkage in the SD compared with the pretest. This was achieved by participants with the lowest preintervention serum 25(OH)D levels saw the greatest increase over the 1-week monitoring period (Figure 2).

Muscle strength data identified an overall 13% mean increase in muscle strength between days 1 and 8 for the treatment group (Figure 3), the placebo group's strength increased by 3% over the same period. Statistical analysis

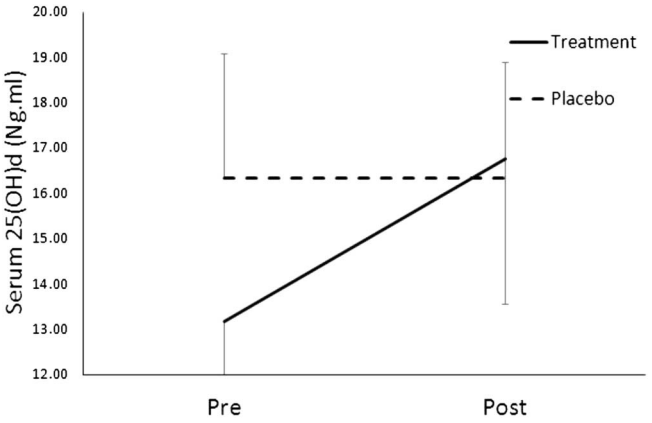

FIGURE 1. Mean ± SD serum 25(OH)D_3 levels for the treatment and placebo groups pre-and-post intervention.

reported a main effects for muscle ($P \leq 0.001$), speed ($P \leq 0.001$), and time ($P = 0.001$); most importantly time by treatment ($P = 0.01$) interaction, see (Figure 3), and "muscle-by-time by treatment" ($P = 0.027$) interaction.

DISCUSSION

The previously reported prevalence of serum 25(OH)D insufficiency and deficiency in indoor athletes[5,6,8,10] have also been confirmed by the present cohort of judoka athletes who were found to have insufficient levels at baseline. The aim of this study was to examine the acute effects of vitamin D supplementation on selected muscle function parameters. The intervention group significantly increased serum 25 (OH)D levels, 1 week after ingestion, but still remained clinically insufficient (10-30 ng/mL). A review on high-dose vitamin A supplementation on deficient patients[24] suggested

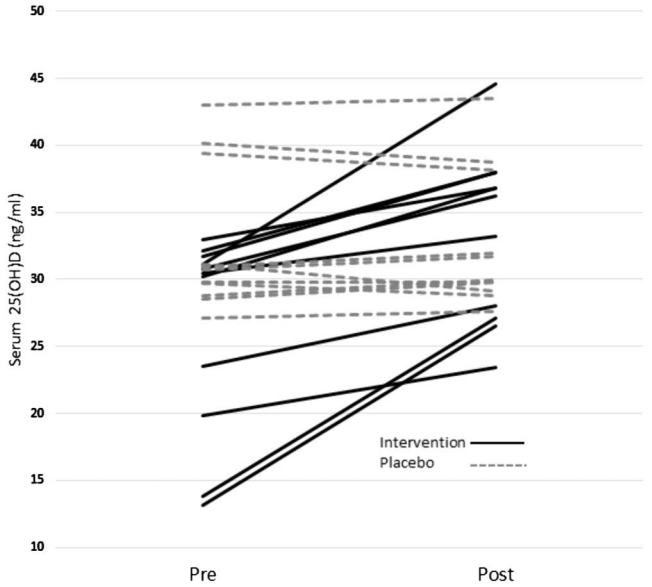

FIGURE 2. Individual changes in serum 25(OH)D_3 levels for the treatment and placebo groups.

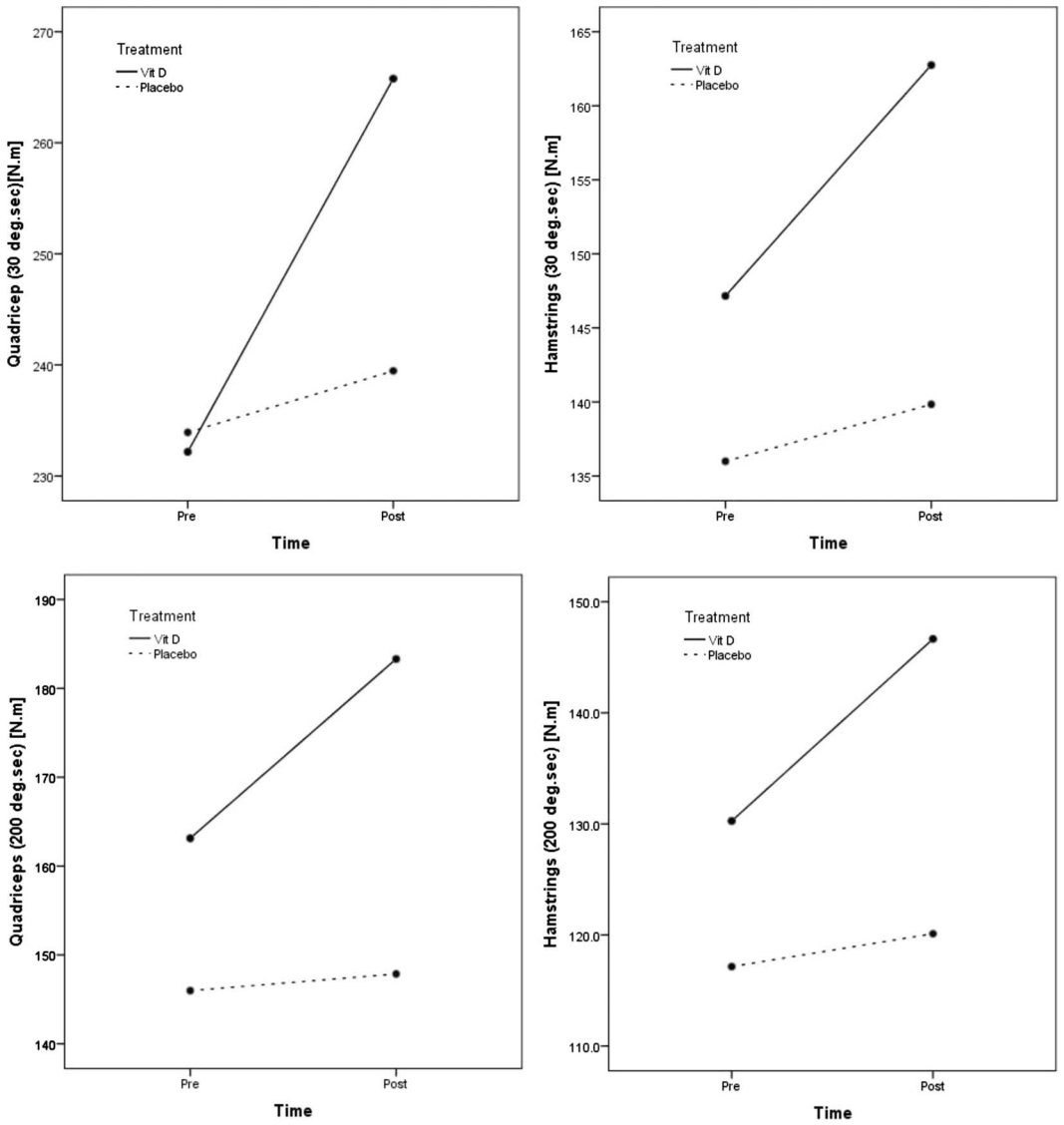

FIGURE 3. Mean ± SD muscle strength changes for the treatment and placebo groups' pre-and-post intervention.

that short-term serum levels are only increased moderately as replenishing cellular storages take precedence. A similar phenomenon could have occurred in this study with serum levels continuing to rise in the subsequent weeks. Regression of the mean analysis also indicated that participants with the lowest pretreatment serum 25(OH)D revealed most of the benefits to the supplementation; similar effects have been previously noticed with different physiological parameters.[19] However, direct comparisons with published data are not possible as this is the first known study to examine the acute effects of vitamin D_3 supplementation.

Early work by Birge and Haddad[9] emphasized the importance of serum 25(OH)D_3 on muscle intracellular phosphate levels. Further studies highlighted the direct effect serum 25(OH)D_3 had on metabolic muscle activity[25] including increased cell protein synthesis and growth through combining with the VDR to promote gene transcription.[26] Animal studies have shown that vitamin D supplementation increased protein synthesis and muscle mass through the activation of the mitogen-activated protein kinase signaling pathway.[27] These long-term adaptations are unlikely to cause the observed acute muscle strength improvements in this study. The 13% increase in strength for the treatment group over a 1-week period is a significant enhancement for elite athletes and highlights the importance of monitoring serum 25(OH)D_3 levels before competition. These increases in strength are possibly due to vitamin D interacting with a specific cell surface receptor,[28] which activates messenger pathways that are believed to directly influence muscle calcium transport and regulation in muscle cells. The authors know of no theoretical basis for the observed greater strength improvements in quadriceps compared to hamstrings for the treatment group. The recent study by Close et al[19] on the effect of different supplementation doses failed to note improved muscle performance in club-level athletes over a 12-week period, despite increases in serum 25(OH)D_3. These authors

suggested that this could be due to skeletal muscle needing a higher serum level than other tissues to illicit a beneficial response. Heaney and Holick[29] suggested that serum 25(OH) D_3 levels of 120 to 225 nmol/L may be required to activate this response.

Previous studies have shown a negative correlation between muscle strength and injury incidence.[21,30] The benefits of prolonged, 3 to 12 months, vitamin D_3 supplementation on improved muscle function[7,31] and decrease in injuries or falls[14] in a variety of populations have already been reported in the literature. This study has demonstrated for the first time that muscle strength significantly improved even within 1 week of vitamin D_3 ingestion. These data highlight the short-term benefits of supplementation on muscle strength of deficient elite sportsmen who not only could have a possible direct effect on their sport performance but also an indirect effect on injury occurrence. Unlike previous studies, that have mainly used isometric strength[18] or dynamometry (handgrip or pinch),[31] we used isokinetic dynamometry at 30 and $200° \cdot s^{-1}$ in an attempt to determine whether vitamin D_3 adaptation is fiber specific; Thorstensson et al[32] reported a correlation between % fast twitch fibers and peak torque at angular velocities above $180° \cdot s^{-1}$. The percentage improvements in peak torque at the 2 angular velocities used in this study were of similar magnitude. Therefore, the present data could not bring greater clarity as to whether muscle function improvements, due to vitamin D_3 supplementation, are muscle fiber specific. Nor we are able to compare the present findings with existing data on elderly, which suggested a type II fiber focused response to vitamin D_3 supplementation.[1,11] Based on the fact that muscle strength indices were assessed by isometric testing, and that within elderly populations' type II fiber size degenerates at a faster rate than type I fibers, these conclusions need to be treated with caution.

It is reasonable to assume that although we have reported novel data, the present results may have been influenced by methodological limitations such the significantly lower serum 25(OH)D_3 levels shown by the intervention participants at baseline and the seasonality of serum 25 (OH)D_3, since the data were collected at a specific month of the year. Another limitation might be associated with the single 150 000 IU dose of vitamin D_3 and whether this dose was actually appropriate. Future studies should look at serial measurements of serum 25(OH)D_3 and muscle strength on a weekly basis and compare the differences between a single bolus dose of vitamin D_3 with weekly or daily supplementations.

CONCLUSIONS

In conclusion, and within the study's limitations, we demonstrated the beneficial acute effects of a single bolus of 150 000IU vitamin D_3 on serum 25(OH)D levels and isokinetic muscle strength in vitamin D deficient elite indoor athletes during the winter months. However, despite significant improvements, serum 25(OH)D deficiency still remains an issue for these athletes, probably because of a reduce exposure to sunlight. Further research is required into the optimal supplementation methods and serum 25(OH)D in elite athletes.

Clinical Implications

- Serum 25(OH)D levels of indoor athletes should be monitored throughout the year and especially during winter months.
- Muscle strength is linked to serum 25(OH)D levels.
- Beneficial responses to 1 dose of vitamin D_3 supplementation can be observed within 1 week of ingestion.
- Athletes might require higher serum 25(OH)D levels (120-225 nmol/L) than the general population.

REFERENCES

1. Janssen H, Samson M, Verhaar H. Vitamin D deficiency, muscle function, and falls in the elderly. *Am J Clin Nutr.* 2002;75:611–615.
2. Laaks I, Ruohola J, Mattila V, et al. Vitamin D supplementation for the prevention of acute respiratory tract infection: a randomized, double-blinded trial among young Finnish men. *J Infect Dis.* 2010;202:809–814.
3. Norman A. Receptors for 1α,25(OH)2D3: past, present and future. *J Bone Miner Res.* 1998;13:1360–1369.
4. Holick M, Chen T. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr.* 2008;87(suppl l):1080S–1086S.
5. Lovell G. Vitamin D status of females in an elite gymnastics program. *Clin J Sport Med.* 2008;18:159–161.
6. Wolman R, Wyon M, Allen N, et al. The vitamin D status of professional dancers in the winter and in the summer. *J Sci Med Sport.* 2013;16: 388–391.
7. Wyon M, Koutedakis Y, Wolman R, et al. The influence of winter Vitamin D supplementation on muscle function and injury occurrence in elite ballet dancers: a controlled study. *J Sci Med Sport.* 2014;17:8–12.
8. Angeline M, Gee A, Shindle M, et al. The effects of vitamin D deficiency in athletes. *Am J Sports Med.* 2013;41:461–464.
9. Birge S, Haddad J. 25-Hydroxycholecalciferol stimulation of muscle metabolism. *J Clin Invest.* 1975;65:1100–1107.
10. Constantini N, Arieli R, Chodick G, et al. High prevalence of vitamin D insufficiency in athletes and dancers. *Clin J Sport Med.* 2010;20: 368–371.
11. Bartoszewska M, Kamboj M, Patel D. Vitamin D, muscle function, and exercise performance. *Pediatr Clin N Am.* 2010;57:849–861.
12. Moreira-Pfrimer L, Pedrosa M, Teixeira L, et al. Treatment of vitamin D deficiency on increases lower limb muscle strength in institutionalized older people independently of regular physical activity: a randomized double-blind controlled trial. *Ann Nutr Metab.* 2009;54:291–300.
13. Ward K, Das G, Roberts S, et al. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. *J Clin Endocrinol Metab.* 2010;95:4643–4651.
14. Redzic M, Lewis R, Thomas D. Relationship between 25-hydoxyvitamin D, muscle strength, and incidence of injury in healthy adults: a systematic review. *Nutr Res.* 2013;33:251–258.
15. Ward K, Das G, Berry J, et al. Vitamin D status and muscle function in post-menarchal adolescent girls. *J Clin Endocrinol Metab.* 2009;94: 559–563.
16. Foo L, Zhang Q, Kun Zhu K, et al. Low vitamin D status has an adverse influence on bone mass, bone turnover, and muscle strength in Chinese adolescent girls. *J Nutr.* 2009;139:1002–1007.
17. Gupta R, Sharma U, Gupta N, et al. Effect of cholecalciferol and calcium supplementation on muscle strength and energy metabolism in vitamin D-deficient Asian Indians: a randomized, controlled trial. *Clin Endocrinol (Oxf).* 2010;73:445–451.
18. Barker T, Martins T, Hill H, et al. Different doses of supplemental vitamin D maintain interleukin-5 without altering skeletal muscle strength: a randomised, double-blind, placebo-controlled study in vitamin deficient adults. *Nutr Metab.* 2012;9:1–12.
19. Close G, Leckey J, Patterson M, et al. The effets of vitamin D3 supplementation on serum total 25[OH]D concentration and physical performance: a randomised dose-response study. *Br J Sports Med.* 2013;47: 692–696.
20. Stockton K, Mengersen K, Paratz J, et al. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int.* 2011;22:859–871.

21. Koutedakis Y, Khalouha M, Pacy P, et al. Thigh peak torques and lower-body injuries in dancers. *J Dance Med Sci.* 1997;1:12–15.
22. Callaghan M, McCarthy C, Al-Omar A, et al. The reproducibility of multi-joint isokinetic and isometric assessments in a healthy and patient population. *Clin Biomech.* 2000;15:678–683.
23. Nevill AM, Holder R, Atkinson G, et al. The dangers of reporting spurious regression to the mean. *J Sports Sci.* 2004;22:800–802.
24. Penniston K, Tanumihardjo S. The acute and chronic toxic effects of vitamin A1,2,3,4. *Am J Clin Nutr.* 2006;83:191–201.
25. Simpson R, Thomas G, Arnold A. Identification of 1,25-dihydroxyvitamin D3 receptors and activities in muscle. *J Biol Chem.* 1985;260: 8882–8891.
26. Cannell J, Hollis B, Sorenson M, et al. Athletic performance and vitamin D. *Med Sci Sports Exerc.* 2009;41:1102–1110.
27. Buitrago C, Vazquez G, De Boland A, et al. The vitamin D receptor mediates rapid changes in muscle protein tyrosine phosphorylation induced by 1,25(OH)(2)D(3). *Biochem Biophys Res Commun.* 2001; 289:1150–1156.
28. Ceglia L. Vitamin D and its role in skeletal muscles. *Curr Opin Clin Nutr Metab Care.* 2009;12:628–633.
29. Heaney R, Holick M. Why the IOM recommndations for vitamin D are deficient. *J Bone Miner Res.* 2011;26:455–457.
30. Raschner C, Platzer H, Patterson C, et al. The relationship between ACL injuries and physical fitness in young competitive ski racers: a 10-year longitudinal study. *Br J Sports Med.* 2012;46:1065–1071.
31. Goswami R, Vatsa M, Sreenivas V, et al. Skeletal muscle strength in young Asian Indian females after vitamin D and calcium supplementation: a double-blind randomised controlled clinical trial. *J Clin Endocrinol Metab.* 2012;97.
32. Thorstensson A, Grimby G, Karlsson J. Force-velocity relations and fiber composition in human knee extensor muscles. *J Appl Physiol.* 1976;40: 12–16.