Vitamin D, Mitochondria, and Muscle

Roger Bouillon and Annemieke Verstuyf

Clinical and Experimental Endocrinology, Katholieke Universiteit Leuven, 3000 Leuven, Belgium

Vitamin D was initially identified as the cure for rickets. Its metabolism and its action on bone and growth plate are now well recognized. 1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D$) probably has, however, a much wider spectrum of activities because the vitamin D receptor (VDR) is expressed in virtually all nucleated cells, the activating CYP27B1 or 1α-hydroxylase is expressed in at least 10 different tissues outside the kidney, and a very large number of genes (maybe even 3% of the whole mouse or human genome) are directly or indirectly regulated by this hormone (1–3). This should not be a surprise because most ligands for nuclear receptors, such as estrogens or androgens, glucocorticoids, or peroxisome proliferator-activated receptor ligands, also regulate a large number of genes and modify cell fate in many tissues. The muscle is a special potential target for the vitamin D endocrine system. Severe myopathy is well recognized as a major consequence of severe rickets, as already described in the 17th century, and is still a potential problem in patients with severe vitamin D deficiency due to chronic renal failure or in patients with a genetic deficiency of CYP27B1 (pseudovitamin D deficiency). This myopathy can be rapidly and impressively corrected by the appropriate vitamin D therapy (2).

Sinha et al (4) report in this issue of the *JCEM* a beneficial effect of vitamin D supplementation of severely deficient but otherwise healthy adults on muscle weakness, and they explain this by improved mitochondrial function as measured in vivo using 31-phosphate nuclear magnetic resonance (NMR) spectroscopy . Phospho-creatinine (P-creatinine) is an important storage mechanism of energy in muscle to satisfy rapid and extensive energy needs during exercise. Three parameters, muscle content of inorganic phosphate, P-creatinine, and the ratio of P-creatinine over inorganic phosphate, were measured in vivo with state-of-the-art technology. All parameters did not differ between vitamin D-deficient subjects and controls and did not change after vitamin D supplementation. The time needed to recover P-creatinine stores after exercise (and the disappearance rate of the substrate ADP), a parameter of efficacy of ATP production and thus of oxidative function of mitochondria, however, was markedly better/shorter after vitamin D repletion and correlated with serum 25-hydroxyvitamin D (25OHD) concentrations. These data indicate that the energy production during the recovery phase of modest exercise by muscle mitochondria is impaired in subjects with severe vitamin D deficiency. This situation is likely to be persistent throughout rest and exercise, but this would be hard to measure in vivo because of technical reasons. Such slower energy generation in skeletal muscle mitochondria could likely contribute to decreased muscle strength and a rapid feeling of fatigue during moderate exercise. There are, however, several limitations in this nonrandomized study because the number of subjects is small, only a single dose (20 000 IU of vitamin D_3 every other day for 12 wk) was used, and the control subjects were not fully vitamin D replete (mean serum 25OHD, 18 ng/ml) and were not studied again after similar vitamin D supplementation. Moreover, the half-life of recovery of P-creatinine after vitamin D supplementation was greater in the supplemented group than in the baseline control group, but this may in fact be due to their nonoptimal vitamin D status. Finally, a more direct comparison between changes in energy recovery and muscle function or fatigue sensation is needed. The mitochondrial dysfunction could be due to a wide variety of reasons such as decreased vascular or oxygen supply (unlikely), decreased mitochondrial number (not observed in vitamin D-deficient animal muscle), or deficient enzyme function of the oxidative pathway (eg, by direct effect of the vitamin

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2013-1352 Received February 8, 2013. Accepted February 12, 2013.

For article see page E509

Abbreviations: NMR, nuclear magnetic resonance; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D_3; 25OHD, 25-hydroxyvitamin D; P-creatinine, phospho-creatinine; ROS, reactive oxygen species; VDR, vitamin D receptor.

D hormone on enzyme gene or protein expression). Intramitochondrial calcium deficiency is, however, a more likely culprit. Indeed, cardiac muscle mitochondria obtained from vitamin D-deficient chicks consume much less oxygen (−30 to 40% depending on the choice of substrate) and thus produce less energy when compared to control chicks (5). This default was ascribed to lower calcium content of cardiac muscle and cardiac muscle mitochondria (−48%).

The Sinha et al manuscript (4) generates two major additional questions: what is the role of vitamin D for: 1) mitochondria in general; and 2) muscle?

The two most important enzymes responsible for activation or inactivation of 25OHD, CYP27B1 (1α-hydroxylase) and CYP24A1 (24-hydroxylase), are located in the mitochondria, but there are no data that isolated damage to mitochondria apart from global cell damage or genetic abnormalities are the cause of problems of vitamin D metabolism. An intriguing observation is the constitutive presence of abundant VDR and its heterodimer partner retinoid X receptor in mitochondria of platelets and their precursor megakaryocytes as well as in the proliferating human keratinocytes cell line HaCaT (6). Two different anti-VDR antibodies were used for VDR protein identification, and VDR gene silencing eliminated the presence of mitochondrial VDR. Moreover, a specific mitochondrial import mechanism known to be involved in cholesterol import or cytochrome C export is needed for VDR localization in mitochondria. The functional consequences of mitochondrial VDR are not yet clearly identified but could be related to mitochondrial gene expression, cation regulation, oxidative function, or for tissue-specific functions in platelet or muscle. The same, however, is also true for the potential action of many other nuclear receptors (estrogen receptor α, glucocorticoid receptor, and thyroid hormone receptor) located in mitochondria (7). A gene microarray analysis of MC3T3 cells after exposure to $1,25(OH)_2D$ revealed 3 up-regulated and 2 down-regulated genes coding for proteins known to be located or functional in mitochondria (our unpublished data). Mitochondria are well known for their contribution to the generation of reactive nitrogen species and reactive oxygen species (ROS) and for the balance between the oxidant and antioxidant system. In children with rickets, an increased pretreatment oxidant and decreased antioxidant defense mechanisms have been observed (8). Inducible nitric oxide synthase production is inhibited by $1,25(OH)_2D$ in mouse monocytes but enhanced in bovine monocytes, whereas in human monocytes, $1,25(OH)_2D$ only up-regulates inducible nitric oxide synthase in combination with phorbol 12-myristate 13-acetate (9). Oxidative stress is associated with all major diseases of aging, including the metabolic syndrome or osteoporosis (10). $1,25(OH)_2D$ is a well-known stimulator of the expression FoxO3a and of sestrins (SESN1), which together are potent first-line defense mechanisms against oxidative stress by inducing ROS detoxifying proteins (11). A widely used vitamin D analog, paricalcitol, has cytoprotective effects during obstructive or other types of nephropathy, and this effect is mediated by antagonizing the angiotensin-mediated mitochondrial oxidative stress (12, 13). In other circumstances, such as in the breast cancer cell line MCF7, however, $1,25(OH)_2D$ seems to increase ROS levels, and this contributes to the proapoptotic action of $1,25(OH)_2D$ (14). Therefore, depending on tissues and other stimuli, $1,25(OH)_2D$ can stimulate or suppress ROS production while at the same time also enhancing defense mechanisms against ROS.

There is an extensive literature on an association between poor vitamin D status and muscle function or strength and the risks of falls. Proof of causality and identification of a clear mechanism, however, are less convincing. A Cochrane meta-analysis (15) and another systematic review (16) indicated that muscle strength in general is not significantly improved, except for improvement of proximal muscle strength in a subgroup of subjects with very low vitamin D status at baseline. Therefore, it would be highly relevant to perform in vivo 31-P NMR spectroscopy studies in more proximal muscles by using adequate NMR instruments with bigger coil diameter. There is already more evidence that the risk of falls in elderly subjects and especially in nursing home residents can be reduced by about 20% when given a vitamin D supplement of about 800 IU/d. The interpretation of these randomized controlled trials is a matter of dispute because the inclusion of more trials in the analysis of the Institute of Medicine (17) generates a conclusion that differs from a meta-analysis of "better" randomized controlled trials (18). The (potential) molecular or cellular mechanisms for these extraskeletal actions of vitamin D are even more disputed because there is conflicting evidence about the shear presence of VDR protein in mature skeletal muscle. Indeed, the VDR protein could not be detected when highly specific rather than less specific antibodies were used (19). VDR null mice, however, develop a clear muscle phenotype with smaller muscle fibers and prolonged expression of immature muscle genes. Mice with cardiomyocyte-specific deletion of VDR also develop a clear clinical phenotype of cardiac hypertrophy (20).

Moreover, a large number of studies show clear genomic and rapid signaling changes in response to $1,25(OH)_2D$ in in vitro myoblast or myocyte cultures. The negative regulation of the major inhibitory myokine, myostatin, is a further indication of the positive effects of vi-

tamin D on muscle (21). Because muscle tissue has a very high turnover (1% per day) and probably needs even greater repair mechanisms than bone microdamage repair, it may well be that the expression of VDR in muscle satellite cells and myoblasts is more important than its presence in mature muscle cells to explain its potential function. So, a phenotype can be observed in the absence of VDR in skeletal or cardiac muscle, but smooth muscle cells, the third type of muscle cells, are also well-known targets of vitamin D action.

The manuscript of Sinha et al (4) is thus an interesting human in vivo study that opens up more questions than answers. If mitochondria would indeed be a direct target of the vitamin D endocrine system, then it would help to explain the large number of associations between poor vitamin D status and age-related diseases because mitochondrial dysfunction may be an additional common pathway. Moreover, in patients requiring intensive care, vitamin D deficiency, clear mitochondrial dysfunction, and rapid muscle loss are all present (22). Secondly, the Sinha et al (4) manuscript provides ideas for studying the in vivo consequences of vitamin D status on muscle function in patients with a variety of situations with abnormal vitamin D supply, metabolism, or action.

Acknowledgments

Address all correspondence and requests for reprints to: Roger Bouillon, Clinical and Experimental Endocrinology, Katholieke Universiteit Leuven, Gasthuisberg Campus, Herestraat 49, O&N1, Box 902, 3000 Leuven, Belgium. E-mail: roger.bouillon@med.kuleuven.be.

Funding was provided by a grant from The Science Research Foundation Flanders (FWO-V) G.0587.09 and G.0859.11.

Disclosure Summary: The authors have nothing to declare.

References

1. **Bouillon R, Carmeliet G, Verlinden L, et al.** Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev*. 2008;29:726–776.
2. **Bouillon R.** Vitamin D: from photosynthesis, metabolism and action to clinical applications. In: Jameson JL, De Groot LJ, eds. *Endocrinology*. 6th ed. Philadelphia: Saunders Elsevier; 2010:1089–1110.
3. **Holick MF.** Vitamin D deficiency. *N Engl J Med*. 2007;357:266–281.
4. **Sinha A, Hollingsworth KG, Ball S, Cheetham T.** Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle. *J Clin Endocrinol Metab*. 2013;98:E509–E513
5. **Mukherjee A, Zerwekh JE, Nicar MJ, McCoy K, Buja LM.** Effect of chronic vitamin D deficiency on chick heart mitochondrial oxidative phosphorylation. *J Mol Cell Cardiol*. 1981;13:171–183.
6. **Silvagno F, Consiglio M, Foglizzo V, Destefanis M, Pescarmona G.** Mitochondrial translocation of vitamin D receptor is mediated by the permeability transition pore in human keratinocyte cell line. *PLoS One*. 2013;8:e54716.
7. **Psarra AM, Solakidi S, Sekeris CE.** The mitochondrion as a primary site of action of steroid and thyroid hormones: presence and action of steroid and thyroid hormone receptors in mitochondria of animal cells. *Mol Cell Endocrinol*. 2006;246:21–33.
8. **Dogan M, Cesur Y, Zehra Dogan S, Kaba S, Bulan K, Cemek M.** Oxidant/antioxidant system markers and trace element levels in children with nutritional rickets. *J Pediatr Endocrinol Metab*. 2012;25:1129–1139.
9. **Liu PT.** The role of vitamin D in innate immunity: antimicrobial activity, oxidative stress and barrier function. In: Feldman D, Pike JW, Adams J, eds. *Vitamin D*. 3rd ed. Amsterdam, The Netherlands: Elsevier Academic Press; 2011:1811–1823.
10. **Manolagas SC.** From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. *Endocr Rev*. 2010;31:266–300.
11. **Eelen G, Verlinden L, Meyer MB, et al.** 1,25-Dihydroxyvitamin D(3) and the aging-related Forkhead Box O and Sestrin proteins in osteoblasts [published online ahead of print September 16, 2012]. *J Steroid Biochem Mol Biol*. doi:10.1016/j.jsbmb.2012.09.011.
12. **Garcia IM, Altamirano L, Mazzei L, et al.** Role of mitochondria in paricalcitol-mediated cytoprotection during obstructive nephropathy. *Am J Physiol Renal Physiol*. 2012;302:F1595–F1605.
13. **Dong J, Wong SL, Lau CW, et al.** Calcitriol protects renovascular function in hypertension by down-regulating angiotensin II type 1 receptors and reducing oxidative stress. *Eur Heart J*. 2012;33:2980–2990.
14. **Byrne BM, Welsh J.** Altered thioredoxin subcellular localization and redox status in MCF-7 cells following 1,25-dihydroxyvitamin D3 treatment. *J Steroid Biochem Mol Biol*. 2005;97:57–64.
15. **Gillespie LD, Robertson MC, Gillespie WJ, et al.** Interventions for preventing falls in older people living in the community. *Cochrane Database Syst Rev*. 2009;2:CD007146.
16. **Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL.** Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int*. 2011;22:859–871.
17. **Institute of Medicine.** *Dietary reference intake for calcium and vitamin D*. Washington, DC: National Academies Press; 2011
18. **Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, et al.** Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. *BMJ*. 2009;339:b3692.
19. **Wang Y, DeLuca HF.** Is the vitamin D receptor found in muscle? *Endocrinology*. 2011;152:354–363.
20. **Chen S, Law CS, Grigsby CL, et al.** Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy. *Circulation*. 2011;124:1838–1847.
21. **Szulc P, Schoppet M, Goettsch C, et al.** Endocrine and clinical correlates of myostatin serum concentration in men—the STRAMBO study. *J Clin Endocrinol Metab*. 2012;97:3700–3708.
22. **Vanhorebeek I, Gunst J, Derde S, et al.** Mitochondrial fusion, fission, and biogenesis in prolonged critically ill patients. *J Clin Endocrinol Metab*. 2012;97:E59–E64.