Vitamin D status in hospitalized male patients in Ain Shams University Hospitals and relation to body composition
Menna El Arabya, Heba Y. Kamela, Tomader T. Abdel Rahmana, Wessam S. Sayedb, Ahmed K. Mortagyba

Introduction
Vitamin D deficiency is a pandemic, even though it is still undertreated and underdiagnosed worldwide [1]. Vitamin D deficiency is prevalent irrespective of age, sex, race, and geography. On exposure to UVB rays, vitamin D photosensitizes subcutaneously; sufficient vitamin D levels can be maintained just by exposure to sunlight [2].

One study was carried out on elderly men; serum concentrations of 25-hydroxyvitamin D [25(OH)D] were measured and a deficiency was considered if the level of 25(OH)D less than 20 ng/ml. Deficiency was present in 26% and insufficiency (<30 ng/ml) was present in 72% of the population studied. Deficiency was particularly common among men during the winter and spring seasons (especially in the northern communities) and in the oldest and more obese men. For instance, in White men, in the winter or the spring season, who were more than 80 years old and did not engage in lawn/garden work, had a BMI greater than 25 kg/m², and vitamin D intake below 400 IU/day, the prevalence of vitamin D deficiency was 86% [3].

The most well-known function of 1,25-dihydroxyvitamin D [1,25(OH)2D] is regulation of calcium and phosphorus level, which is involved in bone mineralization and remodeling. The absence of active vitamin D at sufficient levels in blood will lead to decreased calcium...
absorption from the intestine and secondary stimulation of the parathyroid gland and production of parathyroid hormone, which will increase reabsorption of calcium from the tubules of the kidney and resorption of bone to produce enough calcium and this will lead to weak and brittle bones, with increased susceptibility to fracture. Prolonged vitamin D deficiency in adults results in osteomalacia, osteoporosis, muscle weakness, and fracture [4]. Vitamin D deficiency leads to muscle weakness and pain, decreased strength, impaired balance, muscle function [5], increased bone turnover [6], and increased risk of falls and hip fractures [7] in older adults. Elderly individuals have decreased ability to synthesize vitamin D; this may be attributed to the following:

(1) Decreased exposure to sunlight and decreased ability of the skin to synthesize vitamin D, (2) Reduced intake of the inactive forms of D₂ (ergocalciferol) and D₃ (cholecalciferol), (3) Altered ability of absorption, and (4) Decreased hydroxylation by the liver and kidneys [5].

Vitamin D synthesis decreases in the winter months; thus, vitamin D levels in spring may be reduced in western countries [8] among housebound elderly [9] and in inpatients [10]. Vitamin D deficiency in patients with fragility fractures is highly prevalent (varying from 55 to 91.6%) [8].

The vitamin D receptor was found on the surface of skeletal muscle, indicating that it might play a regulatory role in the skeletal muscle [9,10]. Two mechanisms are suggested for how vitamin D affects skeletal muscle. One suggested mechanism is that 1,25(OH)₂D acts on vitamin D receptor (VDRs) within the muscle cells [9,10]. After transportation to the nucleus, this ligand–receptor interaction is altered by various transcription factors and biochemical processes, yielding an active complex [11].

A second explanation is that vitamin D works through its role on calcium, where it increases number and efficacy of binding sites responsible for muscle contraction [10].

Vitamin D supplementation leads to a rapid response, and reversal of pathology cannot be explained by the slow genetic pathway; this is supported by evidence suggesting that vitamin D acts through the vitamin D membrane receptor [12,13]. It binds to a cell membrane receptor with several secondary messengers that are activated from the cell membrane, leading to increased calcium uptake, a response that occurs within minutes through voltage-dependent calcium channels [14,15].

Other study disputes the fact of the presence of the vitamin D receptor [16,17].

Finally, muscle strength is affected by the genotype of the VDR. Several VDR polymorphisms have been determined that will consecutively affect muscle strength according to the type [18].

Prolonged vitamin D deficiency is associated with severe muscle weakness [19,20] and this was found in the elderly [21,22]; deficiency resulted in marked disability [19,21]. This disability and weakness was associated with marked improvement after replacement [19,21,22] and particularly in elderly men [23].

Vitamin D supplementation was associated with improvement in muscle function in those with vitamin D deficiency and because of an increase in the size and amount of type II (fast twitch) muscle fibers [10,24]. It is noteworthy that type II fibers are responsible for power and anaerobic activities [10].

Given this background, the aim of the study was to identify vitamin D status among elderly hospitalized male patients and its relation to parameters of body composition, muscle strength, and performance.

**Participants and methods**

**Study design**

This was a cross-sectional study.

**Participants**

Data collected from a previous cross-sectional study, where systematic random sampling technique where every third patient (skip interval) and patient who measured vitamin D, were selected for inclusion in the study on the basis of the exclusion criteria, which might have affected the parameters measured.

**Exclusion criteria**

(1) Patients on dialysis, patients with a history of liver cell failure, and severe malnutrition.
(2) Pace maker, diuretics use, and dehydration.
(3) Manifested degenerative joint disease, lumbar spinal canal stenosis, neurogenic, or vascular claudication pain.
(4) Cerebrovascular disease.
(5) Any amputations.
(6) Hand osteoarthritis.
(7) Patients taking the following drugs were excluded: antacids, calcium channel blockers, cholestyramine, phenobarbital, phenytoin, anticonvulsant, glucocorticoids.
(8) Kidney diseases (renal insufficiency, elevated creatinine, reduced glomerular filtration rate (GFR)).

Methods
All participants were subjected to a complete assessment of medical history including history of physical activity [25,26], assessment of BMI [27], and assessment of body composition by bioelectrical impedance analysis [28] and measurement of vitamin D levels.

The survey was conducted between June 2012 and June 2013. This study was approved by the Ain Shams Medical Ethics Committee.

Assessment of muscle mass
Assessment of body composition was performed using the bioelectrical impedance analysis [28] (Geratherm body analyzer scale, Germany), where the patient stood on the scale after entering details of age, sex, height, and physical activity. Feet had to be dry and each foot had to touch the two electrodes for at least 5 s until the end of the analysis. Results are obtained as weight, BMI, fat mass, fat-free mass, bone mass, and body water.

Assessment of muscle strength
Assessment of hand grip strength was performed using a Jamar handheld dynamometer (Jamar hydraulic hand dynamometer, JI, USA), where three trials were performed by both hands with a 1 min interval between each trial, and the average of the dominant hand was calculated [29].

Assessment of physical performance
This was done using the timed get up and go test, where the patient first received an explanation for the test and then he/she sat on a stool about 45 cm from the ground and was asked to stand without support. He/she could use an assistive device and was asked to walk at his/her own average speed for 3 m and to turn back and sit down again on the chair; the time is calculated from the moment the physician ask him to start till he/she sits down [30].

Physical activity
Physical activity refers to any bodily movement produced by skeletal muscles that increases energy expenditure above a basal level. It includes exercise or nonexercise physical activity. Exercise physical activity involves structured and repetitive bodily movements such as walking. Nonexercise physical activity involves standing or gardening. Thirty minutes or more of moderate physical activity 5 days a week is required to consider a participant physically active [26].

Laboratory measurement
Five milliliters of venous blood was collected under completely aseptic conditions from each participant. After clotting, samples were centrifuged at 1000g for 15 min, and sera were separated and stored at −20°C for assay of serum 25(OH)D. Hemolyzed samples were discarded and only one freeze and one thaw cycle was performed. Measurement of total 25(OH)D (vitamin D₃ and vitamin D₃) in serum was carried out by an enzyme immunoassay using reagents provided by DRG 25(OH)D (total) enzyme-linked immunosorbent assay (ELISA, EIA-5396; DRG International Inc., Springfield Township, New Jersey, USA). The DRG 25(OH)D total ELISA kit is a solid-phase ELISA based on the principle of competitive binding. In the first step, samples have to be pretreated in separate vials, with denaturation buffer to extract the analyte, as most circulating 25(OH)D is bound to vitamin D-binding protein in vivo. After neutralization, biotinylated 25(OH)D (enzyme conjugate) and peroxidase-labeled streptavidin–enzyme complex are added. After careful mixing, the solution is transferred to the wells of a microtiter plate. Endogenous 25(OH)D of a sample competes with a 25(OH)D₃–biotin conjugate for binding to the (vitamin D-binding globulin) vitamin D binding globulin (VDBG) that is immobilized on the plate. Binding of 25(OH)D–biotin is detected by peroxidase-labeled streptavidin. Incubation is followed by a washing step to remove unbound components. The color reaction is started by the addition of an enzyme substrate and stopped after a defined time (15 min). The color intensity is inversely proportional to the concentration of 25(OH)D in the sample [31]. To deduce the concentration of 25(OH)D in serum samples and control material, a standard curve was constructed by plotting the absorbance obtained from each standard against its concentration with the absorbance value on the vertical (y) axis and concentration on the horizontal (x) axis using a linear graph paper. 25(OH)D status less than 10 ng/ml indicates deficiency and 10–29 ng/ml in case of insufficiency, whereas 30–100 ng/ml is considered sufficient [32].

Cause of admission
Patients were admitted because of various conditions such as uncontrolled diabetes, hypertension, heart failure, chronic obstructive lung disease, decompensated liver disease, and transient ischemic attack, but they were included after their condition was treated.

Comorbidities
Comorbidities included diabetes, hypertension, ischemic heart disease, compensated heart failure,
chronic obstructive pulmonary disease, chronic kidney disease, and liver disease.

Statistical analysis
Data were collected and analytical statistics were obtained using the 15th version of the statistical package for the social sciences (SPSS; SPSS Inc., Chicago, Illinois, USA).

Descriptive statistics
Numerical data were described as mean, SD, median, and minimum and maximum values (range). Non-numerical data were described as frequency and percentage.

Correlation analysis (using Pearson’s method)
To assess the strength of association between two quantitative variables, correlation analysis was carried out. The correlation coefficient, denoted symbolically as ‘r’, defines the strength and direction of the linear relationship between two variables. The P-value was always set at 0.05.

Results
Our study included 88 elderly men aged 60 years and older. The mean age of the participants studied was 64.74 ± 4.6 years. In total, 86.3% were married, 22.7% were physically active, and 15.9% were nonsmokers. The mean BMI was 26.4 ± 7.2 kg/m². The mean vitamin D level was 12.1 ± 5.5 ng/ml (Table 1).

The prevalence of vitamin D deficiency was 19.3%, whereas insufficiency was 79.5% (Table 2). Vitamin D was not significantly correlated with age, smoking, physical activity, BMI, fat-free mass, fat mass, hand grip, and timed get up and go test (Table 3).

Discussion
The current study was carried out to assess the prevalence of vitamin D deficiency and insufficiency status in hospitalized patients and its relation to parameters of body composition, muscle strength, and performance.

In the current study the prevalence of vitamin D deficiency was 19.3% and that of vitamin D insufficiency was 79.5%; deficiency was considered when vitamin D was less than 10 ng/dl and insufficiency was defined as levels between 11 and 30 ng/dl [32]; this is comparable with a study carried out in southern Australia, where the prevalence of vitamin D deficiency was found in 22.7% of the population, with deficiency diagnosed at a serum 25(OH)D level below 50 nmol/l (<20 ng/ml) [3]. However, in Shanghai, China, in a study on 5470 Chinese patients with various diseases, who were hospitalized between May 2012 and August 2013 (deficiency was defined as serum concentrations <15 ng/ml), only 53.31% would have been nondeficient because the 25(OH)D concentrations of most patients were between 10 and 15 ng/ml [33]. In a study in San Paolo, Brazil, it was found that the prevalence of vitamin D insufficiency was 85.7% in a group with a mean age of 71 years [34].

It has been estimated that one billion individuals worldwide have vitamin D deficiency or

| Table 1 Participants’ characteristics |
|--------------------------------------|
| Characteristics                      | N (%)                      |
| Age                                  | 64.74 ± 4.6                |
| Marital status                        |
| Married                               | 76 (86.3)                  |
| Single                                | 8 (13.7)                   |
| Occupation                            |
| Manual                                | 29 (33)                    |
| Nonmanual                             | 59 (67)                    |
| Physical activity                     |
| Sedentary                             | 68 (77.2)                  |
| Active                                | 20 (22.7)                  |
| Smoking                               |
| Nonsmoker                             | 14 (15.9)                  |
| Current smoker                        | 36 (40.9)                  |
| Ex-smoker                             | 38 (43.2)                  |
| Number of comorbidities               |
| No                                    | 11 (12.5)                  |
| 1–2                                   | 47 (53.4)                  |
| ≥3                                    | 30 (34)                    |
| Height (cm)                           | 167.8 ± 7.5                |
| Weight (kg)                           | 74.4 ± 21.3                |
| BMI                                   | 26.4 ± 7.2                 |
| Body fat                              | 20.46 ± 10.7               |
| Body water (%)                        | 58.58 ± 7.5                |
| FFM (kg)                              | 50.5 ± 9.8                 |
| Bone mass                             | 2.6 ± 3.2                  |
| FFMI                                  | 26.3 ± 9.5                 |
| Hand grip                             | 26.31 ± 9.5                |
| TGUGT (s)                             | 16.1 ± 6.6                 |
| Vitamin D (ng/ml)                     | 12.15 ± 5.5                |

FFM, fat-free mass; FFMI, fat-free mass index; TGUGT, timed get up and go test.

| Table 2 Prevalence of vitamin D deficiency and insufficiency in the studied participants |
|-----------------------------------------------|
| Descriptive data and parameter of body composition | Frequency (%) |
| Vitamin D deficiency                          | 17 (19.3)    |
| Vitamin D insufficiency                        | 70 (79.5)    |
| Vitamin D sufficiency                          | 1 (1.2)      |
| Total                                         | 88 (100)     |
insufficiency [35–38]. According to several studies, 40–100% of the US and European elderly men and women still living in the community (not in nursing homes) have vitamin D deficiency [35–38].

The abundant sunlight in our country make us believe that vitamin D deficiency due to sufficient sunlight is restricted to countries of higher altitude. However, Lips et al. [39] and Van der Wielen et al. [40] reported a higher prevalence of hypovitaminosis D in southern Europe than in northern Europe.

It was found that the prevalence of vitamin D deficiency is high in Egypt even though it is considered a sunny country; this may be attributed to the life style of the population and the amount of exposure to sun light and we studied elderly individuals, who have decreased ability to synthesize vitamin D and decreased ability of absorption.

We believe that the cut-off value used by the US Endocrine Society guideline for White patients may not be clinically appropriate for other ethnic groups.

In the present study, it was found that age was not associated with vitamin D levels; this is not in agreement with a study carried out in Brazil, where the percentage of deficiency was 40.3% between the ages of 17 and 35 years, whereas between the ages of 91 and 100 years, it was 100% [34].

It is known that vitamin D synthesis decreases with age because of a decrease in subcutaneous fat and decreased gastrointestinal absorption, but this can be attributed to the fact that most of the studies compared adulthood with elderly not between different decades of the elderly population.

Table 3 Correlation between vitamin D and descriptive data and parameters of body composition

| Vitamin D               | R     | T     |
|-------------------------|-------|-------|
| Age                     | 0.123 (NS) | -0.164 |
| Smoking duration        | 0.905 (NS) | -0.014 |
| Duration of stoppage    | 0.893 (NS) | 0.067  |
| Quantity                | 0.119 (NS) | -0.187 |
| Physical activity       | 0.795 (NS) | -0.028 |
| Height                  | 0.974 (NS) | -0.004 |
| Weight                  | 0.168 (NS) | -0.147 |
| BMI                     | 0.167 (NS) | 0.147  |
| Fat percentage          | 0.663 (NS) | 0.047  |
| Water                   | 0.359 (NS) | -0.098 |
| FFM                     | 0.160 (NS) | 0.149  |
| FFMI                    | 0.127 (NS) | 0.162  |
| Bone mass               | 0.984 (NS) | 0.002  |
| Hand grip               | 0.586 (NS) | -0.058 |
| TGUGT                   | 0.136 (NS) | -0.158 |

FFM, fat-free mass; FFMI, fat free mass index; TGUGT, timed get up and go test.

Smoking exerts a significant effect on calcium and vitamin D metabolism, which is not likely to be explained by other confounding lifestyle factors [41,42]. However, in the current study, smoking was not associated with vitamin D levels, which could be explained by the high prevalence rate of deficiency and insufficiency, which might have led to masking of this relation.

Vitamin D supplementation increased muscle strength without the need for regular physical activity [39]. Others found that calcium and vitamin D decreased the risk of falls documented by quadriceps, body sway and time needed to perform timed up and go test (TUG) test [43]. Also, hand grip strength was better with higher levels of vitamin D assessed by a handheld dynamometer [44,45] and also with supplementation [23,46], which was not clear in our study because of the high percentage of deficiency and insufficiency among the study participants.

Conclusion
Vitamin D deficiency and insufficiency is highly prevalent among elderly Egyptian men and it not related to parameters of body composition, muscle function, or performance.

Recommendation
Larger studies are needed to identify vitamin D status in elderly Egyptian men, especially among community-dwelling elderly; also, larger studies are needed to assess its relation to muscle function and strength. To assess changes that would occur in muscle mass, strength and performance after supplementation of vitamin D.

Acknowledgements
Conflicts of interest
None declared.

References
1 Van Schoor NM, Lips P. Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab 2011; 25:671–680.
2 Ritu G, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients 2014; 6:729–775.
3 Orwoll E, Nielsen CM, Marshall LM, et al. Vitamin D deficiency in older men. J Clin Endocrinol Metab 2009; 94:1214–1222.
4 Hazell TJ, DeGuire JR, Weiler HA. Vitamin D: an overview of its role in skeletal muscle physiology in children and adolescents. Nutr Rev 2012; 70:520–533.
5 Horlick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266–281.
6 Mezquita-Rayta P, Muñoz-Torres M, Luna JD, Luna V, Lopez-Rodriguez F, Torres-Vela E, Escobar-Jiménez F. Relation between vitamin D insufficiency, bone density, and bone metabolism in healthy postmenopausal women. J Bone Miner Res 2001; 16:1408–1415.
7 Cauley JA, Lacroix AZ, Wu L, Horwitz M, Danielson ME, Bauer DC, et al. Serum 25-hydroxyvitamin D concentrations and risk for hip fractures. Ann Intern Med 2008; 149:242–250.
8 Nummi I, Kaukonen JP, Löhöte P, Naboulsi H, Tanninen S, Kataja M, et al. Half of the patients with an acute hip fracture suffer from hypovitaminosis D: a prospective study in southeastern Finland. Osteoporos Int 2005; 16:2018–2024.
9 Campbell PMF, Allain TJ. Muscle strength and vitamin D in older people. Gerontology 2006; 52:335–338.
10 Ceglia L, Harris SS. Vitamin D and its role in skeletal muscle. Calcif Tissue Int 2013; 92:151–162.
11 Dusso AS, Brown AJ. Mechanism of vitamin D action and its regulation. Am J Kidney Dis 1998; 32:S13–S24.
12 Nemere I, Dormanen MC, Hammond MW, Okamura WH, Norman AW. Identification of a specific binding protein for 1 alpha,25-dihydroxyvitamin D3 in basilar-lateral membranes of chick intestinal epithelium and relationship to transcellanchia. J Biol Chem 1994; 269:23750–23756.
13 Nemere I, Schwartz Z, Pedrozo H, Sylvia VL, Dean DD, Boyan BD. Identification of a membrane receptor for 1,25-dihydroxyvitamin D3 which mediates rapid activation of protein kinase C. J Bone Miner Res 1998; 13:1353–1359.
14 Massheimer V, Fernandez LM, Boland R, de Boland AR. Regulation of Ca2+ uptake in skeletal muscle by 1,25-dihydroxyvitamin D3: role of phosphorylation and calcumin. Mol Cell Endocrinol 1992; 84:15–22.
15 De Boland AR, Boland RL. Non-genomic signal transduction pathway of vitamin D in muscle. Cell Signal 1994; 6:717–724.
16 Wacker M, Holick MF, Wacker M, Holick MF, Wacker M, Holick MF, Sunlight and Vitamin D: a global perspective for health. Dermatoendocrinol 2013; 5:1–108.
17 Wang Y, De Luca HF. Is the vitamin D receptor found in muscle? Endocrinology 2011; 152:354–363.
18 Geusens P, Vandevyver C, Vanhoof J, Cassiman JJ, Boonen S, Raus J. Quadriceps and grip strength are related to vitamin D receptor genotype in elderly nonobese women. J Bone Miner Res 1997; 12:2082–2088.
19 Mingrone G, Greco AV, Castagneto M, Gasbarrini G. A woman who left her wheelchair. Lancet 1999; 353:806.
20 Ziambaras K, Dagogo-Jack S. Reversible muscle weakness in patients with vitamin D deficiency. West J Med 1997; 167:435–439.
21 Prabhala A, Garg R, Dandona P. Severe myopathy associated with vitamin D deficiency in western New York. Arch Intern Med 2000; 160:1199–1203.
22 Rimaniol JM, Authier FJ, Chariot P. Muscle weakness in intensive care patients: initial manifestation of vitamin D deficiency. Intensive Care Med 1994; 20:591–592.
23 Kenny AM, Biskup B, Robbins B, Marcella G, Burleson JA. Effects of vitamin D deficiency and calcium metabolism. Eur J Clin Nutr 1999; 53:520–526.
24 Yoshimura N, Muraki S, Oka H, Morita M, Yamada H, Tanaka S, et al. Profiles of vitamin D insufficiency and deficiency in Japanese men and women: association with biological, environmental, and nutritional factors and coexisting disorders: the ROAD study. Osteoporos Int 2013; 24:287–297.
25 World Health Organization. Regional office for the western pacific region pacific physical activity guidelines for adults: framework for accelerating the communication of physical activity guidelines. Manila: World Health Organization Regional Office for the Western Pacific Region; 2009.
26 American College of Cardiology/ American Heart Association. Methodology manual for ACC/AHA guideline writing committees. American College of Cardiology Foundation and the American Heart Association Inc.; 2006.
27 World Health Organization. World Health Organization physical status: the use and interpretation of anthropometry: report of a WHO Expert Committee Technical Report Series (1995) 854. Geneva: WHO; 1995.