CLINICAL STUDY

Testosterone but not estradiol level is positively related to muscle strength and physical performance independent of muscle mass: a cross-sectional study in 1489 older men

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Abstract

Objective: To examine the relationship between different measures of testosterone and estradiol (E_2), muscle mass, muscle strength, and physical performance; and to test whether the association of sex hormone level with muscle strength and physical performance was independent of muscle mass.

Design and methods: A cross-sectional survey on 1489 community-dwelling men older than 64 years of age. Serum levels of testosterone and E_2 were measured by mass spectrometry, and sex hormone-binding globulin (SHBG) levels were measured by immunoradioassay. Muscle mass was examined by dual-energy X-ray absorptiometry and physical performance was assessed by hand-grip strength, gait speed, step length and chair-stand test.

Results: Appendicular skeletal mass (ASM) was positively associated with total testosterone (TT; $P < 0.001$), free testosterone (FT; $P < 0.001$), and total E_2 ($P < 0.001$) but not with free E_2 ($P = 0.102$). After adjustment for age, serum SHBG and relative ASM, both TT and FT were significantly associated with grip strength, narrow-walk speed and the composite neuromuscular score. Higher total E_2 , but not free E_2 was associated with lower grip strength ($P < 0.05$) after adjustment for age, FT, SHBG and relative ASM.

Conclusions: Testosterone level was related to both muscle mass, strength and physical performance. Total E_2 level, though related to muscle mass positively, affected muscle strength adversely in older men.

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Introduction

Several studies have examined the inter-relationship between various measures of testosterone level, muscle mass and muscle strength (1–6). In men aged 65 or older, Baumgartner *et al.* (1) reported that free testosterone index, but not total testosterone (TT) level, was modestly associated with muscle mass, whereas Iannuzzi-Sucich *et al.* (2) demonstrated that muscle mass was correlated to bioavailable testosterone (BioT), leg strength and physical performance. However, in another cohort of healthy older men, Van den Beld *et al.* (3) reported that testosterone level, as represented by TT and BioT, was related to muscle strength but not to muscle mass. On the other hand, Roy *et al.* (5) observed that TT and BioT were positively correlated with both the muscle mass and strength, yet after adjustment for muscle mass, they were no longer

independently associated with muscle strength. Szulc *et al.* (4) had compared the muscle mass and physical performance of hypogonadal and eugonadal men and found that hypogonadal men exhibited impaired static and dynamic balance despite relatively preserved muscle mass. Similarly, Orwoll *et al.* (6) have observed that testosterone level was related to physical performance and fall risk but not to muscle mass. Although the effect of testosterone supplementation on muscle mass and strength was less controversial in interventional studies (7–14), whether endogenous testosterone was related to muscle mass alone, muscle strength alone, or both; and which measures of endogenous testosterone best related to muscle mass and strength had been inconsistent.

In addition, the association of muscle mass and strength with endogenous estrogen level in older men has scarcely been examined. It was reported that two

studies observed no correlation between estradiol (E_2) and muscle mass (3, 15), whereas Vandenput *et al.* (16) have recently reported a positive association between lean mass and E_2 in elderly Swedish men.

We have reported that cognitive impairment was adversely associated with muscle strength and physical performance independent of muscle mass, and have postulated that the co-existing sex hormone decline in cognitively impaired older adults (17, 18) might account for this observation (19). In this study, we hypothesized that endogenous sex hormone level, similar to cognitive function, might affect both muscle quantity (lean mass) and quality (muscle strength and physical performance). We therefore, attempted to examine the relationship among different measures of testosterone and E_2 , muscle mass, muscle strength and physical performance; and to test whether the association of sex hormone level with muscle strength and physical performance was independent of muscle mass.

Methods

Study population and measurements

Participants were from the MrOS Hong Kong cohort, which form part of the study of Osteoporotic Fractures in Men (MrOS) (20) together with subjects from the US and Sweden. Details of the Hong Kong cohort have been described elsewhere (21). In brief, 2000 Chinese men aged 65 years and above who were ambulant without assistance from another person were invited to the study center for a comprehensive health check. Stratified sampling was adopted to achieve ~33% of participants in each of the three age groups: 65–69, 70–74, and ≥ 75 years. The method of recruitment consists of notices placed in housing estates and community centers all over Hong Kong.

The average grip strength was measured using both hands (Jamar Hand dynamometer 5030 J1, Sammons Preston, Inc., Bolingbrook, IL, USA). We performed four other neuromuscular tests. Subjects were asked to stand up with folded arms from a chair five times and the time required was recorded. The time to walk 6 m at normal pace, with and without a 20 cm wide boundary, was measured. The number of steps to complete the 6-m walk without the 20 cm boundary was counted to derive the step-length. Subjects who failed to perform or complete a particular neuromuscular test had to be excluded from the analysis of that individual test. To include these subjects who might represent the worst neuromuscular function group (being unable to complete a test), a performance-based physical function scoring method was adopted from Wang *et al.* (22). Each neuromuscular test result was assigned a score of 0 if the subject was not able to complete the test, and 1–4 according to the quartiles of the neuromuscular performance. The scores of the five tests were summated

into one neuromuscular composite score which ranged from 0 to 20, with 20 representing the best performance.

Stature and body weight were measured to calculate body mass index (BMI; body weight in kilograms divided by the square of stature in meters). The subjects were asked to stand upright without shoes and look straight ahead with standing heights measured by the Holtain Harpenden Stadiometer (Holtain Ltd, Crosswell, UK). Body weight was measured, with the subjects wearing a light gown, by the Physician Balance Beam Scale (Healthometer, Alsip, IL, USA).

We measured muscle mass using dual-energy X-ray absorptiometry (DXA) using a Hologic QDR 2000 densitometer (Hologic, Waltham, WA, USA). Appendicular skeletal mass (ASM) was calculated by the summation of muscle mass measured in the four limbs, with the operator adjusting the cut lines of the limbs according to specific anatomical landmarks as described by Heymsfield *et al.* (23). Calibration with a Hologic body composition step phantom was performed at least three times per week. The method has been described in detail in other reports of the same cohort of older adults (19, 24). Muscle mass was corrected for body size by dividing the ASM with the square of stature (1, 2, 25) and was termed relative ASM.

A random subpopulation consisting of 1489 men had fasting venous sampling from 0800 to 0900 h for assay of sex steroid hormones. Serum was stored at -80°C and then transported by courier service to Quebec, Canada, for assay in dry ice (-78.5°C).

Laboratory methods

A validated gas chromatography mass spectroscopy system (26, 27) was used for analysis of sex steroids at the CHUL Medical Research Center, Quebec, Canada: testosterone (limit of detection, 0.05 ng/ml; intra-assay coefficient of variation (CV), 2.9%; inter-assay CV, 3.4%) and E_2 (limit of detection, 2.00 pg/ml; intra-assay CV, 1.5%; inter-assay CV, 2.7%). A 50% phenyl-methyl polysiloxane (DB-17HT) capillary column (30 m $\times$ 0.25 mm internal diameter; 0.15–1.0 μm film thickness) with helium as carrier gas was used. The analytes and the internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source. Serum sex hormone-binding globulin (SHBG) was measured using IRMA (Orion Diagnostics, Espoo, Finland; limit of detection 1.3 nmol/l, intra-assay CV, 3%; inter-assay CV, 7%). Free fractions of testosterone were calculated as described by Van den Beld *et al.* (3), taking the concentration of TT, total E_2 and SHBG into account, and assuming a fixed albumin concentration of 43 g/l.

Statistical analysis

The relationship between muscle mass (ASM/height²) and testosterone level (FT and TT) was analyzed by

Table 1 Sex hormone level, muscle mass and physical performance of the participants.

	Mean	S.D.
Age (years)	72.52	4.99
Height (cm)	163.01	5.71
Weight (kg)	62.13	9.41
BMI	23.35	3.15
ASM (kg)	19.10	2.62
Relative ASM	7.17	0.82
Total testosterone (ng/ml)	5.47	2.05
Free testosterone (ng/dl)	10.60	3.05
Total E ₂ (pg/ml)	24.63	14.37
Free E ₂ (pg/ml)	0.58	0.40
SHBG (nmol/l)	51.17	20.36
Grip strength (kg)	30.98	6.57
Timed 5 chair-stand (s)	12.69	3.93
Step-length (m)	0.57	0.08
Walk speed (m/s)	1.02	0.22
Narrow-walk speed (m/s)	0.91	0.25
Neuromuscular composite score	12.12	4.09

multiple linear regression with adjustment for age, SHBG and BMI, since BMI is a major determinant of muscle mass (19, 24). In analyzing E₂ (total and free E₂), additional adjustments were undertaken for FT since E₂ was transformed from testosterone aromatization. In analyzing the association between grip strength and physical performance tests with testosterone and E₂ level, multiple linear regressions were employed with the test performance as the dependent variable and different measures of sex hormone as the independent predictor. The regression was then repeated with adjustment for age alone and then for age, SHBG and muscle mass (ASM/height²) for testosterone; and additional adjustment for FT for total and free E₂. All tests were two-sided and a *P* value <0.05 was taken as statistically significant.

Results

Table 1 shows the sex hormone levels, relative ASM, and physical performance of the participants. Relative ASM was positively related to TT, FT, and total E₂ but not to free E₂ level with adjustment for age, BMI and SHBG (Table 2). Higher FT level was consistently associated with stronger grip strength, better performance in all physical tests, and higher composite neuromuscular score before adjustment (Table 3). After adjustment, the associations remained significant between higher FT and grip strength, narrow-walk speed, and the composite neuromuscular score (Table 3). Higher TT level was associated with shorter step-length and faster narrow-walk speed before adjustment (Table 3). After adjustment, TT was associated with stronger grip strength, faster narrow walk speed and higher neuromuscular score (Table 3). Despite a positive

association between total E₂ and relative ASM (Table 2), total E₂ was negatively associated with grip strength after adjustment for age, SHBG, FT, and relative ASM (Table 4).

Discussion

Compatible with the results of other reports (1–6), we observed a positive correlation between endogenous testosterone level and stronger muscle strength and better physical performance. The effect size was modest (Table 3), and it is therefore not certain whether they may be translated into clinical benefit, such as reduction in fall risk, improved quality of life or better survival. However this favourable effect of testosterone on muscle strength and physical performance was independent of muscle mass, implying that testosterone affected not only the quantity but also the quality of muscles.

We have demonstrated that cognitive impairment could adversely affect muscle strength and physical function independent of muscle mass (19). As at least two of the physical performance tests, grip strength (28) and gait speed (29) have been observed to be closely related to cognitive function in past studies, and that sex hormone decline and cognitive function may be closely related (17, 18), it is therefore plausible that testosterone decline may be the common factor that links cognitive impairment with muscle loss and weaker strength in older men. However, the relative importance of sex hormone and cognition in determining muscle mass and muscle performance remained elusive. In addition, the effect of androgen on motor neuron has been demonstrated in some animal studies, in which motor neuron size was reduced in castrated mice. It is therefore plausible that the efficiency or quality of muscle contractility can be adversely affected by the co-existing motor neuron atrophy related to sex-hormone decline (30, 31).

Age-adjusted TT was negatively associated with weaker grip strength. This paradoxical relationship reverted after adjustment for SHBG, which had a significant negative effect on muscle strength (β -coefficient: -0.044 , $P < 0.001$; data not shown).

Table 2 Relationship between sex hormone level and relative ASM.

	Unit (S.D.)	Difference in relative ASM (kg/m ²) per unit (S.D.) change (95% CI) ^a	P value
Total testosterone ^a	2.05 ng/ml	0.1 (0.06, 0.13)	<0.0001
Free testosterone ^a	3.05 ng/dl	0.08 (0.05, 0.1)	<0.0001
Total E ₂ ^b	14.37 pg/ml	0.04 (0.02, 0.07)	0.0009
Free E ₂ ^b	0.40 pg/ml	0.02 (−0.01, 0.05)	0.1028

^aAdjusted for age, BMI, and SHBG.

^bAdjusted for age, BMI, FT, and SHBG.

Table 3 Relationship between testosterone level, grip strength and physical performance.

	Grip strength (kg) (95% CI)	Timed 5 chair-stand (s) (95% CI)	Step-length (m) (95% CI)	Walk speed (m/s) (95% CI)	Narrow-walk speed (m/s) (95% CI)	Neuromuscular composite score (95% CI)
Total testosterone						
Unadjusted	-0.19 (-0.52, 0.14)	-0.08 (-0.28, 0.12)	0.01 (0.003, 0.01)*	0.01 (-0.01, 0.02)	0.02 (0.01, 0.04)*	0.19 (-0.02, 0.4)
Adjusted for age	-0.35 (-0.66, -0.04) [‡]	-0.03 (-0.23, 0.16)	0.01 (0.002, 0.01) [†]	0.002 (-0.01, 0.01)	0.02 (0.01, 0.03)*	0.08 (-0.11, 0.27)
Adjusted for age and SHBG	0.66 (0.23, 1.1) [†]	-0.03 (-0.31, 0.25)	0.01 (-0.001, 0.01)	0.002 (-0.01, 0.02)	0.03 (0.02, 0.05)*	0.27 (0.01, 0.54) [‡]
Adjusted for age, SHBG and relative ASM	0.69 (0.27, 1.11) [†]	-0.03 (-0.31, 0.25)	0.01 (-0.001, 0.01)	0.002 (-0.01, 0.02)	0.03 (0.02, 0.05)*	0.28 (0.01, 0.54) [‡]
Adjusted for age, SHBG, height and ASM	0.67 (0.28, 1.07)*	-0.03 (-0.31, 0.25)	0.01 (-0.001, 0.01)	0.002 (-0.01, 0.02)	0.03 (0.02, 0.05)*	0.27 (0.01, 0.54) [‡]
Free testosterone						
Unadjusted	0.68 (0.35, 1.01)*	-0.2 (-0.4, -0.001) [‡]	0.01 (0.01, 0.01)*	0.02 (0.01, 0.03) [†]	0.04 (0.03, 0.05)*	0.53 (0.33, 0.74)*
Adjusted for age	0.17 (-0.14, 0.49)	-0.04 (-0.24, 0.16)	0.005 (0.001, 0.01) [‡]	0.002 (-0.01, 0.01)	0.03 (0.01, 0.04)*	0.18 (-0.01, 0.37)
Adjusted for age and SHBG	0.49 (0.16, 0.81) [†]	-0.04 (-0.25, 0.17)	0.004 (-0.001, 0.01)	0.002 (-0.01, 0.01)	0.03 (0.01, 0.04)*	0.22 (0.02, 0.42) [‡]
Adjusted for age, SHBG and relative ASM	0.51 (0.2, 0.83) [†]	-0.04 (-0.25, 0.17)	0.004 (-0.001, 0.01)	0.002 (-0.01, 0.01)	0.03 (0.01, 0.04)*	0.23 (0.03, 0.42) [‡]
Adjusted for age, SHBG, height and ASM	0.51 (0.21, 0.8)*	-0.04 (-0.25, 0.17)	0.004 (0, 0.01)	0.002 (-0.01, 0.01)	0.03 (0.01, 0.04)*	0.22 (0.03, 0.42) [‡]

* $P < 0.001$, [†] $P < 0.01$, and [‡] $P < 0.05$. Values are difference in grip strength or physical performance per one s.d. change in testosterone level.

In contrast to two previous reports that failed to demonstrate any association between endogenous estrogen and muscle mass (3, 15), we found a positive correlation between total E₂ but not free E₂ and relative ASM. This agreed partially with the recent work done by Vandenput *et al.* (16), in which muscle mass was observed to be associated positively with both total and free E₂. In addition, this positive relationship was unrelated to the testosterone level as the association persisted after its adjustment (Table 2). The role of estrogen receptor activation in the modulation of muscle mass remains unclear although some animal studies have revealed that estrogen receptor activation was involved in this process (32–34). Our results also revealed that total E₂ level, though positively related to muscle mass, was negatively related to muscle strength (Tables 2 and 4), in contrary to testosterone that was associated favorably with both muscle quantity and quality. Total E₂ had no effect on grip strength before adjustment but had a negative effect after adjustment for age, SHBG, FT, and relative ASM. The negative effect of total E₂ on grip strength might have been confounded by its positive association with muscle mass, which could only be revealed after adjustment for ASM. The mechanistic explanation of this negative effect of E₂ on muscle quality remains elusive.

The testosterone level in our subjects was higher than the Swedish MrOS cohort (16). This observation is consistent with the racial and geographical variations in sex steroid levels reported among Asians from Japan (35). It is likely that Chinese in Hong Kong, also had higher testosterone level than the Caucasian and Black populations due to the same reason. In addition, our sample was recruited by voluntary participation, in contrary to the Swedish MrOS subject, which was recruited by population-based random sampling. Our subjects might therefore be healthier and more active. Contrary to the Swedish MrOS cohort (16), but in consistent with other studies (1–6), we have demonstrated a positive relationship between testosterone and muscle mass. We have used appendicular lean mass corrected for body size instead of the un-corrected total body lean mass and leg lean mass in the Swedish MrOS. The inclusion of trunk lean mass, which may be less testosterone dependent, and the use of lean mass un-corrected for body size might have diluted the testosterone effect in the Swedish study.

Our study had several limitations. The participants were all independent community-living older men. Our results, therefore, cannot be extrapolated to the frail or institutionalized older adults, in whom sex hormone decline may be disease associated rather than age-associated, and in whom the relationship of sex hormone with muscle mass, muscle strength and physical performance may be different. The presence of intramuscular adipose tissue renders DXA measurement of muscle mass less accurate when compared with the magnetic resonance imaging method (36).

Table 4 Relationship between estradiol (E₂) level, grip strength and physical performance.

	Grip strength (kg) (95% CI)	Timed 5 chair-stand (s) (95% CI)	Step-length (m) (95% CI)	Walk speed (m/s) (95% CI)	Narrow-walk speed (m/s) (95% CI)	Neuromuscular composite score (95% CI)
Total E ₂						
Unadjusted	-0.12 (-0.46, 0.21)	0.12 (-0.08, 0.32)	0.002 (-0.002, 0.01)	0.003 (-0.01, 0.01)	0.01 (-0.01, 0.02)	-0.01 (-0.22, 0.2)
Adjusted for age	-0.10 (-0.41, 0.21)	0.11 (-0.09, 0.31)	0.002 (-0.002, 0.01)	0.004 (-0.01, 0.01)	0.01 (-0.004, 0.02)	0.01 (-0.18, 0.19)
Adjusted for age, FT, SHBG	-0.14 (-0.46, 0.19)	0.14 (-0.07, 0.34)	0.001 (-0.003, 0.01)	0.004 (-0.01, 0.02)	0.00 (-0.01, 0.01)	-0.05 (-0.25, 0.15)
Adjusted for age, FT, SHBG and relative ASM	-0.4 (-0.71, -0.08) [‡]	0.15 (-0.06, 0.36)	0.00 (-0.004, 0.01)	0.002 (-0.01, 0.01)	0.00 (-0.01, 0.01)	-0.11 (-0.31, 0.09)
Adjusted for age, FT, SHBG, height and ASM	-0.41 (-0.71, -0.11) [‡]	0.15 (-0.06, 0.36)	0.00 (-0.004, 0.004)	0.002 (-0.01, 0.01)	-0.001 (-0.01, 0.01)	-0.11 (-0.31, 0.08)
Free E ₂						
Unadjusted	-0.18 (-0.54, 0.18)	-0.04 (-0.26, 0.18)	0.00 (-0.01, 0.01)	-0.001 (-0.01, 0.01)	0.003 (-0.01, 0.02)	-0.01 (-0.24, 0.22)
Adjusted for age	-0.17 (-0.51, 0.16)	-0.04 (-0.25, 0.17)	0.00 (-0.004, 0.005)	-0.001 (-0.01, 0.01)	0.003 (-0.01, 0.02)	-0.002 (-0.21, 0.21)
Adjusted for age, FT and SHBG	-0.13 (-0.47, 0.21)	-0.05 (-0.28, 0.17)	0.00 (-0.004, 0.01)	0.00 (-0.01, 0.01)	0.004 (-0.01, 0.02)	0.02 (-0.19, 0.24)
Adjusted for age, FT, SHBG and relative ASM	-0.12 (-0.45, 0.21)	-0.05 (-0.28, 0.17)	0.00 (-0.004, 0.01)	0.00 (-0.01, 0.01)	0.01 (-0.01, 0.02)	0.03 (-0.19, 0.24)
Adjusted for age, FT, SHBG, height and ASM	-0.08 (-0.39, 0.23)	-0.05 (-0.28, 0.17)	0.001 (-0.004, 0.005)	0.00 (-0.01, 0.01)	0.01 (-0.01, 0.02)	0.04 (-0.17, 0.25)

†P<0.01, and ‡P<0.05. Values are difference in grip strength or physical performance per one s.d. change in E₂ level.

The physical performance tests that we employed may have a ceiling effect and cannot differentiate subtle functional impairment, therefore a modest relationship between sex-steroids and physical performance could have been missed. The use of calculated free sex-steroids instead of directly measured ones may lack accuracy, particularly in large population studies (37). However, the Vermeulen method that we employed has been demonstrated to have generally small deviations from the multi-ligand model method proposed by Mazer *et al.* (38). The validity of E₂ as a biological variable has never been tested in men, though calculated free E₂ (39, 40) and FT (40) were closely related to directly measured sex-steroids and were valid predictors of cancer risk in women. Being cross-sectional, the association observed does not imply any causal relationship between testosterone decline and poor muscle performance.

Conclusion

Testosterone was related to both muscle quantity and quality. It was positively correlated with muscle mass and was associated with muscle strength and physical performance independent of muscle mass. Total E₂ level, though related to muscle mass positively, was associated with weaker muscle strength in older men.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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