Evaluation of sex-specific association of serum testosterone and estradiol levels with frailty in elderly Egyptian men and women

Marwa A.M. Saad[a], Marwa H. Mahmood[b]

Departments of [a]Internal Medicine and, [b]Clinical and Chemical Pathology, Geriatric Unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Correspondence to Marwa A.M. Saad Gouda, MD, Department of Internal Medicine, Geriatric Unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt
Tel: 002/033252668;
e-mail: drmarwasaad74@gmail.com

Received 11 November 2015
Accepted 18 November 2015

Egyptian Journal of Obesity, Diabetes and Endocrinology
2015, 1:121–127

Background
Frailty is an age-associated syndrome characterized by a reduced functional reserve and impaired adaptive capacity. Age-ass ociated decline in sex-hormone levels represent one of the potential mechanisms involved in the development of frailty. We aimed at studying the association of serum testosterone and serum estradiol levels with frailty in elderly Egyptian men and women, and evaluating sex-specific differences in the association between testosterone and estradiol levels with frailty.

Materials and methods
A total of 94 elderly participants (55 men and 39 women), aged 65 years and older, were included in the present study. Participants were divided into three groups according to their frailty status, which was determined according to the Fried criteria. Total testosterone (TT), free testosterone (FT), and total estradiol (E2) were determined.

Results
For men, frailty was significantly correlated with TT and FT but not with E2, whereas, for women, frailty was significantly correlated with FT and E2 but not with TT. In addition, BMI was significantly correlated with frailty for both men and women.

Conclusion
We concluded that lower levels of FT are associated with frailty for both men and women, whereas lower levels of TT are associated with frailty in men but not in women. Estradiol (E2) is correlated with frailty in women but not in men. In light of these findings, men with low levels of testosterone are at an increased risk for physical frailty and could thus benefit from testosterone therapy. In addition, postmenopausal women might also benefit from testosterone administration and estrogen supplementation in the context of a wider hormonal care.

Keywords:
elderly, estradiol, frailty, sex-differences, testosterone

Egyptian Journal of Obesity, Diabetes and Endocrinology 1:121–127

2356-8062

Introduction

Testosterone is a steroid hormone from the androgen group. More than 95% of testosterone is secreted from the Leydig cells of the testis in men under the control of the luteinizing hormone [1], and to a lesser extent in the theca cells of the ovaries in women, placenta during pregnancy, the zona reticularis of the adrenal cortex, and the skin of both sexes [2].The amount of testosterone synthesized is regulated by the hypothalamic–pituitary–testicular axis [3]. Overall, 98% of testosterone in plasma is bound to protein, 65% is bound to sex steroid-binding globulin, and 33% to albumin. A small amount of circulating testosterone is converted to estradiol, but most of the testosterone is converted to 17-ketosteroids, principally androsterone and its isomer etiocholanolone, and excreted through urine [4]. Testosterone effects in humans occur via multiple mechanisms, by activation of the androgen receptor, by conversion to estradiol, and activation of certain estrogen receptors [5]. In men, testosterone is the principle sex-hormone and plays a critical role in the development and maturation of reproductive tissues, and promotion of secondary sexual characteristics [6]. While testosterone production is significantly higher in men than in women, the hormone is important in the health and well-being of both men and women and plays a vital role in preventing osteoporosis [7,8]. On average, in adult men, levels of testosterone are about seven to eight times of that in adult females [9]. As the metabolic consumption of testosterone in men is greater, the daily production is about 20 times greater in men. Women are more sensitive to the hormone; abnormally high levels of testosterone in women have been associated with menstrual irregularities, hirsutism, and polycystic ovary syndrome [10].

Estradiol – a steroid – is the primary female sex hormone. Estradiol is essential for the development and maintenance of female reproductive tissues, development of secondary sexual characteristics, and for the regulation of female menstrual cycles [11]. Fat structure and skin composition are modified by estradiol. Bone structure is affected by estrogen deficiency, resulting in early osteopenia and osteoporosis [12]. In addition, estrogen is considered to play a significant role in women's mental health, mood, and well-being. Sudden drops or fluctuations in or long periods of sustained low levels of estrogen may be correlated with significant mood-lowering [13]. The role of estrogens in men physiology has become more evident, as a consequence of the discovery of human models of estrogen deficiency, such as estrogen resistance or aromatase deficiency [14]. In men, testosterone is the major source of plasma estradiol, only 20% of which is secreted by the testis. Because ~80% of the circulating estradiol in men derives from androgens [15], serum levels of estradiol and testosterone are significantly associated [16]. The plasma concentration of estradiol in men is significantly higher than in postmenopausal women [14]. Studies investigating aromatase or estrogen receptor deficiency in men have demonstrated that estradiol has important physiological effects on bone maturation and peak bone mass in younger men [17]. Free and bioavailable estradiol levels do decrease modestly with age, as does the ratio of free testosterone (FT) to free estradiol [14], the latter testifying to the age-associated increased aromatization of testosterone. Estrogen in men play an important role in the regulation of the gonadotropin feedback, several brain functions, bone maturation, regulation of bone resorption, and in lipid metabolism.

Testosterone gradually declines as men age [15]. Decreased testosterone levels may contribute to the symptoms and signs of aging, such as decreased muscle mass and strength, impaired physical performance, cognitive function, and lack of energy [15]. Men with low serum testosterone are at an increased risk for falls, low bone mineral density, and fractures [16,18]. Moreover, low serum testosterone associates with increased fat mass, an adverse metabolic risk profile, and atherosclerosis [15,19]. The role of estradiol in elderly men remains more unclear, and few studies have explored the relationship between estradiol levels in elderly men and health-related outcomes [16].

In the last few decades, the concept of frailty has emerged as one of the main conditions preceding the development of disability. Frailty is an age-associated syndrome characterized by a reduced functional reserve and impaired adaptive capacity [20]. In their study, Fried *et al.* [20] considered someone frail if he or she met three or more of the following criteria: weight loss, exhaustion, weak grip strength, slow walking speed, and low physical activity. Frailty has shown a strong association with increased risks of disability and other adverse outcomes such as institutionalization, hospitalization, falls, and mortality [21]. Many factors have been implicated to the development of frailty, such as hormones, inflammation, oxidative stress, and mithochondrial DNA [22]. Among hormones, low levels of endogenous testosterone, which are linked to the muscle mass and strength, is one of the potential mechanisms involved in the development of frailty [23]. Although the age-associated decline in testosterone occurs both in men and women, this decline does not arise to the same extent in both sexes, suggesting a possible differential impact on frailty according to sex [24]. Many studies have reported an association between low testosterone levels and frailty in men [25,26]. However, although women represent around two-third of individuals with frailty, little is known about the impact of low testosterone on frailty or its components in women. Only two recent cross-sectional studies have examined this issue in postmenopausal women [27].

The decline in estrogen associated with menopause has long been suspected to be the cause of multiple aspects of health deterioration in women, including loss of muscle mass and strength, which represent the core of the frailty syndrome. Although a good body of evidence suggests a beneficial role of estrogen on muscle mass, biological hypotheses regarding the link between estrogen and muscle strength are still unclear [28]. Few studies have examined the association of estradiol with components of the frailty syndrome [29,30].

In the present study, we aimed at investigating the association of serum testosterone and serum estradiol levels with frailty in elderly Egyptian men and women, and evaluating sex-specific association between testosterone and estradiol levels with frailty.

Materials and methods

The present study included 94 elderly participants of both sexes (55 men and 39 women), aged 65 years and older, who attended the geriatric outpatient clinic at the main Alexandria University Hospital. The purpose and benefits of the study were explained to all participants and an informed written consent was obtained. The proposal was accepted by the ethical committee of Faculty of Medicine, Alexandria University.

All participants were subjected to a thorough history-taking, and complete physical examination. After

overnight fasting, blood samples were collected from all participants and sent for basic laboratory investigations. Blood samples for total testosterone (TT), FT, and total estradiol (E2) were sent to the laboratory within 2 h of blood sampling. Hormone levels were determined using Quantitative ELISA kits (Biosense Laboratories AS Thormøhlensgt. 55 Bergen, N-5008, Norway) [31].

Frailty status was determined according to the Fried criteria. Participants were classified as frail if they had three or more of the following parameters; prefrail, if they had one or two of the following parameters; and nonfrail, if they had none of the following parameters [20,32]: unintentional weight loss of greater than or equal to 4.5 kg in the previous year; weakness (i.e. low handgrip strength), evaluated using a Jamar hand-held dynamometer (the cutoff points adjusted for BMI were: ≤32 kg for BMI>28; ≤30 kg for BMI 24.1–28; and ≤29 kg for BMI ≤24); poor endurance (i.e. self-reported exhaustion), evaluated by using two statements from the center for epidemiological studies depression scale [(a) 'I felt that everything I did was an effort' and (b) 'I could not get going')]; slowness, evaluated using the timed get up and go test, which requires the participant to stand up from a chair, walk a distance of 6 m, turn around, and return and sit down again, thus serving as an assessment of dynamic balance (balance function was observed and scored (normal value 17 s); and low physical activity level.

Levels of sex steroid hormones were recorded, compared in both sexes, and correlated with the frailty score for both men and women.

Statistical methods

Data were collected and fed into a personal computer. Statistical analysis was carried out using statistical package for social sciences (version 20) software. For comparison between two studied groups, arithmetic mean and standered deviation (SD) were used. To find the correlation between the two variables, Pearson's correlation coefficient was used. The level of significance was set at 0.05.

Results

The present study included 94 participants: 55 men (58.51%) and 39 women (41.48%). They were divided into three groups according to their frailty status. Among men, 39 (70.9%) were frail, 13 (23.6%) were prefrail, and three (5.5%) were nonfrail. The mean age of the three groups is shown in Table 1, with no statistical significant difference between the studied groups (P = 0.103). The BMI was significantly lower in the frail and prefrail groups compared with the nonfrail group (P = 0.013). Levels of TT, FT, and E2 were significantly lower in frail participants compared with prefrail and nonfrail participants (P = 0.001, 0.013, and 0.002, respectively) Table 1.

Among women, 30 (76.9%) were frail, six (15.4%) were prefrail, and three (7.7%) were nonfrail. Frail participants were significantly older than the prefrail and nonfrail participants (P = 0.013). Prefrail participants had slightly higher BMI, although there was no statistical difference between the three groups (P = 0.98). No statistical difference was detected between the three groups regarding TT level (P = 0.124). FT and E2 levels significantly decreased in frail participants compared with prefrail and nonfrail participants (P = 0.025, and 0.001, respectively) Table 2.

For men, a high statistical significant negative correlation was detected between TT and FT and frailty score (P =

Table 1 Comparison between the frailty groups regarding different studied parameters in men

Male	Frail	Prefrail	Nonfrail	*P*
n (%)	39 (70.9)	13 (23.6)	3 (5.5)	
Age (years)				
Range	67.00–79.00	65.00–75.00	71.00–75.00	0.103
Mean ± SD	72.49 ± 3.46	70.23 ± 3.24	73.33 ± 2.08	
BMI (kg/m^2)				
Range	24.30–28.30	25.80–29.20	28.90–30.00	0.013*
Mean ± SD	26.30 ± 1.29	27.25 ± 1.02	29.53 ± 0.57	
TT (ng/ml)				
Range	2.60–6.40	4.70–10.00	6.90–7.80	0.001*
Mean ± SD	4.34 ± 1.06	7.18 ± 1.78	7.33 ± 0.45	
FT (pg/ml)				
Range	55.00–179.00	82.00–218.00	200.00–240.00	0.013*
Mean ± SD	112.10 ± 34.65	148.00 ± 48.14	216.67 ± 20.82	
E2 (pg/ml)				
Range	10.00–27.00	10.00–27.00	21.00–27.00	0.002*
Mean ± SD	17.79 ± 4.92	18.15 ± 5.47	23.00 ± 3.46	

E2, total estradiol; FT, free testosterone; TT, total testosterone; *Statistical significance $P \leq 0.05$.

0.000, and 0.001, respectively), whereas the correlation with E2 levels did not reach a statistically significant level (P = 0.437). In addition, a high statistically significant negative correlation was detected between BMI and frailty score (P = 0.000). BMI was positively correlated with FT (P = 0.026) but not correlated with TT or E2 (P = 0.099, and 0.324, respectively) Table 3.

For women, a high statistically negative correlation was detected between E2 level and frailty score (P = 0.000). A statistically significant negative correlation was detected between FT and frailty score (P = 0.043), whereas no correlation could be detected between TT and frailty score (P = 0.595). BMI was significant negatively correlated with frailty score (P = 0.047). BMI did not show a statistically significant correlation with TT, FT, or E2 (P = 0.078, 0.700, and 0.069, respectively). FT was positively correlated with the levels of E2 (P = 0.012). E2 levels significantly decreased with advancing age (P = 0.006) Table 4.

Discussion

Frailty is an age-associated syndrome characterized by a reduced functional reserve and impaired adaptive capacity [20]. Different hormones were implicated in the pathogenesis of frailty; among these, serum testosterone was extensively examined regarding its correlation with frailty. In the present study, we aimed at investigating the association between serum testosterone and serum estradiol levels with frailty in elderly Egyptian men and women, and evaluating sex-differences in the association between testosterone and estradiol levels with frailty.

Table 2 Comparison between the frailty groups regarding different studied parameters in women

Female	Frail	Prefrail	Nonfrail	*P*
n (%)	30 (76.9)	6 (15.4)	3 (7.7)	
Age				
Range	70.00–82.00	67.00–74.00	65.00–68.00	0.013*
Mean ± SD	75.93–3.89	70.00–2.68	66.33–1.53	
BMI				
Range	25.10–29.20	27.90–30.80	25.70–29.40	0.098
Mean ± SD	27.10–1.31	28.88–1.09	27.07–2.03	
TT				
Range	0.22–0.60	0.40–0.55	0.28–0.47	0.214
Mean ± SD	0.40–0.12	0.49–0.06	0.36–0.10	
FT				
Range	0.43–1.84	0.54–2.78	0.86–3.13	0.025*
Mean ± SD	1.07–0.42	1.73–0.80	1.79–1.19	
E2				
Range	2.00–22.00	14.00–28.00	21.00–29.00	0.001*
Mean ± SD	11.50–6.04	21.50–5.68	23.67–4.62	

E2, total estradiol; FT, free testosterone; TT, total testosterone; *Statistical significance at $P \leq 0.05$

Table 3 The correlation between different studied parameters in men

Correlation parameters	Age	BMI	Frailty score	TT	FT
BMI					
r	−0.035				
P	0.799				
Frailty score					
r	0.140	−0.541**			
P	0.308	0.000			
TT					
r	−0.141	0.225	−0.671**		
P	0.303	0.099	0.000		
FT					
r	−0.083	0.299*	−0.451**	0.510**	
P	0.549	0.026	0.001	0.000	
E2					
r	−0.117	−0.136	−0.107	0.129	−0.052
P	0.397	0.324	0.437	0.350	0.705

E2, total estradiol; FT, free testosterone; TT, total testosterone; *Correlation is significant at the 0.05 level [two tailed]; **Correlation is significant at the 0.01 level [two tailed].

Table 4 The correlation between different studied parameters in women

Correlation parameters	Age	BMI	Frailty score	TT	FT
BMI					
r	−0.383*				
P	0.016				
Frailty score					
r	0.740**	−0.320*			
P	0.000	0.047			
TT					
r	0.082	0.286	−0.088		
P	0.618	0.078	0.595		
FT					
r	−0.168	0.064	−0.326*	−0.034	
P	0.306	0.700	0.043	0.838	
E2					
r	−0.433**	0.295	−0.622**	0.116	0.399*
P	0.006	0.069	0.000	0.483	0.012

E2, total estradiol; FT, free testosterone; TT, total testosterone; *Correlation is significant at the 0.05 level [two tailed]; **Correlation is significant at the 0.01 level [two tailed].

In our study, TT and FT showed high statistically negative correlation with frailty in men. Recent studies have found a cross-sectional association between low levels of FT and frailty or the severity of its components [25,26,32], whereas results of the association of TT with frailty were less consistent across studies, some of them suggesting an association [26,32] and others not [25,33]. We also detected an increase in the number of frailty elements as the levels of TT and FT became lower. In the Third National Health and Nutrition Examination Survey [34], FT of less than 243 pmol/l was associated with prevalent frailty, measured using the Fried scale. Similar results were reported in the Massachusetts Male Aging Study [35]. The Longitudinal Aging Study Amsterdam [36] reported that FT was associated with grip strength and a short physical performance battery (SPPB), whereas estradiol was not associated with any of these physical measures. Several other studies confirmed the association of TT and FT with muscle strength [25,32,33], walking speed [25,33], and weight loss [32]. In our study, E2 did not show statistically significant correlation with frailty.

In our study, low levels of FT but not TT were significantly associated with frailty in women. A study by Cappola *et al.* [27] suggested a possible (but not significant) linear association between decreasing testosterone and frailty. A cross-sectional study from Taiwan [26] showed that the lowest testosterone tertile was defined as less than 0.3 nmol/l in women, as opposed to less than 15.7 nmol/l in men. For men and women, being in the lowest tertile was associated with three to six times greater adjusted odds of frailty as measured using the Fried scale. Other studies have produced negative or peculiar results [37,38].

In a cross-sectional analysis [33], every 1-SD decrease in FT was associated with a 0.13-U lower SPPB score and 0.02 m/s slower gait speed at baseline. In a longitudinal analysis, every 1-SD decrease in FT was associated with a 22% increase in mobility limitation after 6.6 years of follow-up but not with change in SPPB or gait speed. Whereas a combined analysis of the Longitudinal Ageing Study Amsterdam and Health, Aging and Body Composition cohorts [39] analogously reported that baseline FT was not associated with change in SPPB, gait speed, grip strength, or leg extensor strength after 3 years of follow-up.

We found some differences in the relationship between testosterone and frailty according to sex. First, we showed that the probability of frailty linearly increased with TT and FT declined in men, whereas this relationship was detected with FT only in women. Second, both TT and FT were correlated with frailty in men, while FT but no TT was correlated with frailty in women. Finally, BMI correlated with FT in men but not in women.

In our study, lower levels of estradiol (E2) were significantly associated with components of frailty in women but not in men. In contrast, a study by Carcaillon *et al.* [40] found that higher levels of estradiol were associated with frailty in postmenopausal women not undergoing hormonal replacement therapy. In their study, Van Geel *et al.* [29] did not find any association between estradiol, muscle mass, or muscle strength in 329 women. However, these women were younger (as regards their mean age) than women in our study, and they had a higher mean level of estradiol. Together with the fact that muscle strength and muscle mass were evaluated as single measurements in contrast to the composite frailty measure used in our study, these differences may explain the discrepancy with our findings.

The implication of testosterone in body-mass regulation, muscle function and growth, and regulation of bone mineral density [41,42] gives biological support to the relation between testosterone decline and frailty. In addition, testosterone may be linked to weight loss through its effect on appetite [43].

The disparity between sexes could be the implication of different biological mechanisms in the relation between sex hormones and frailty. While testosterone plays a prominent role in frailty in old men, its role in women, although present, seems less relevant. Other hormonal axes and mediators may be of major importance in the relation between testosterone and frailty in women. This is supported by data from the Women's Health and Aging Study where a multiple hormonal burden was found to be more strongly associated with frailty than with the type of hormonal deficiency [37]. Moreover, if testosterone plays a crucial role in men's health, estrogen is the most important sex hormone in women. Considering that the main source of estradiol in postmenopausal women comes from the conversion of testosterone by aromatase in the adipose tissue, and that estrogen therapy and endogenous estrogen have been suggested to respectively have a positive effect on muscle strength [44], it can be hypothesized that estradiol may play a substantial role in the relation between FT and frailty in women.

Conclusion

Lower levels of FT are associated with frailty in both men and women, whereas lower levels of TT are associated with frailty in men but not in women. Estradiol (E2) is correlated with frailty in women but not in men. In light of these findings, men with low levels of testosterone are at an increased risk for physical frailty and could thus benefit from testosterone therapy. In addition, postmenopausal women might also benefit from testosterone administration, and also estrogen supplementation in the context of a wider hormonal care.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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