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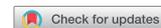
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adam.c.weiss@taylorandfrancis.com](http://www.tandfonline.com/adam.c.weiss@taylorandfrancis.com)

To whom it may concern,

This is to certify that the article “Aging and sex hormones in males” by Maria Chiara Decaroli and Vincenzo Rochira, published in *Virulence* Volume 8 Issue 5 (doi: 10.1080/21505594.2016.1259053), was solicited for a Special Focus on *Geriatric HIV Medicine* by the Guest Editors Drs. Giovanni Guaraldi and Andrea Cossarizza.

Sincerely,  
Adam Weiss, PhD  
Acquisitions Editor  
Virulence

REVIEW



## Aging and sex hormones in males

Maria Chiara Decaroli<sup>a</sup> and Vincenzo Rochira <sup>a,b</sup>

<sup>a</sup>Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy;  
<sup>b</sup>Azienda USL of Modena, Modena, Italy

### ABSTRACT

Several large cohort studies have disclosed the trajectories of sex steroids changes overtime in men and their clinical significance. In men the slow, physiological decline of serum testosterone (T) with advancing age overlaps with the clinical condition of overt, pathological hypogonadism. In addition, the increasing number of comorbidities, together with the high prevalence of chronic diseases, all further contribute to the decrease of serum T concentrations in the aging male. For all these reasons both the diagnosis of late-onset hypogonadism (LOH) in men and the decision about starting or not T replacement treatment remain challenging. At present, the biochemical finding of T deficiency alone is not sufficient for diagnosing hypogonadism in older men. Coupling hypogonadal symptoms with documented low serum T represents the best strategy to refine the diagnosis of hypogonadism in older men and to avoid unnecessary treatments.

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bone mineral density; estradiol; late-onset hypogonadism (LOH); menopause; replacement treatment; sex steroids; testosterone

### Introduction

Aging is associated with changes in the physiological functioning of the endocrine system.<sup>1</sup> Except for the thyrotropin hormone (TSH) that tends to increase with advancing age, the secretion of other pituitary hormones such as growth hormone (GH) and prolactin progressively decreases with aging.<sup>1</sup> The same happens to insulin-like growth factor-1 (IGF-1), which is directly regulated by GH.<sup>1</sup>

The aging of the reproductive function is characterized by the loss of function of gonads that occurs as an on/off phenomenon in females at the time of menopause.<sup>1,2</sup> (Fig. 1). *Vice versa*, in aging males, the reproductive function progressively declines in parallel with the decrease of testicular function year by year.<sup>1</sup> Accordingly, gonadotropins slightly increase in men while definitely soar at the time of menopause in women.<sup>1</sup> In men, however, also a decline of gonadotropic cell function has been documented in a significant percentage of aging men.<sup>3</sup>

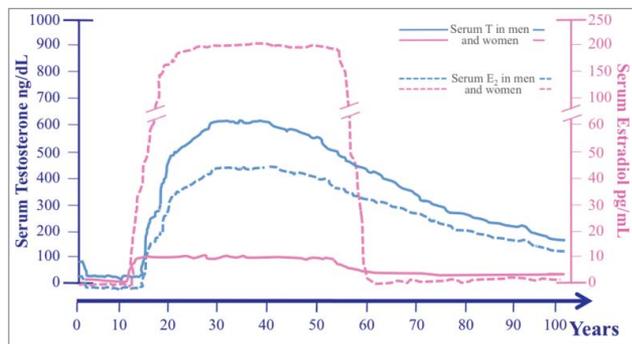
As far as sex hormones are concerned, it should be remarked that their production is different in men and women during adulthood: serum testosterone (T) is 10 folds higher in men than in women, while serum estradiol (E<sub>2</sub>) declines after the fifties in both sexes; thus sex hormones changes during aging differ by gender (Fig. 1).

Gender differences in aging related decline of sex hormones are outlined in Figure 1.

### Physiological decline of serum T with advancing age in men

Serum total T declines with advancing age with a rate that has been estimated to be about 1–2% per year from the third decade onward.<sup>4,5</sup> (Fig. 1). This rate of decline in serum T corresponds approximately to a reduction of 3.2–3.5 ng/dL (0.110–0.121 nmol/L) per year.<sup>4,6</sup> At the age of 75 y a man has already lost about 30% of his circulating quote of T measured at the age of 25 years.<sup>5,7</sup> According to different studies, the prevalence of low total T in men over 60 y is, on average, around 20% to 30% and it constantly increases with aging.<sup>3-5,8-10</sup>

The total amount of circulating serum T corresponds to the total serum T usually assayed into the clinic, which is the sum of both free T and T bound to serum proteins.<sup>11</sup> Serum free T represents the circulating fraction that is completely unbound and promptly available for binding the androgen receptor in target tissues; it is, however, a small part of circulating serum T.<sup>11</sup> A minimal part of circulating T binds albumin and the strength of this binding is weak.<sup>11</sup> For this reason, serum



**Figure 1.** Serum levels of both testosterone (T) (solid lines) and estradiol ( $E_2$ )<sup>\*</sup> (broken lines) across the lifespan in men (blue lines) and women (pink lines). Data used for plotting age-related sex steroids changes in both sexes have been derived from Kaufman & Vermeulen 2005<sup>5</sup>, Zmuda et al. 1997<sup>6</sup> (for men), and from Al-Safi et al. 2000<sup>2</sup> and Veldhuis 2013 (for women). *serum  $E_2$  levels of premenopausal women are represented as the mean of  $E_2$  measured during the different phases of the menstrual cycle*; T: testosterone;  $E_2$ : estradiol.

free T and T bound to albumin—in other words the T not bound to Sex Hormone Binding Globulin (SHBG)—represent the bioavailable T<sup>11</sup>. *Vice versa* T bound to SHBG is not promptly available for binding the androgen receptor due to the high strength of the binding; this represents the amount of circulating T which is not bioavailable.<sup>7,11-14</sup> Since bioavailable serum T is more available to the cells compare with SHBG-bound serum T, information on changes in both serum free and bioavailable T during aging is of relevance.<sup>14</sup> SHBG increases with advancing age of about 2.7% per year,<sup>4,15,16</sup> and accounts for an even larger decline in serum bioavailable T (3 to 4% per year) compare with total T in the elderly man.<sup>5</sup> In addition, several factors (e.g. obesity)<sup>17-20</sup> and/or comorbidities,<sup>21,22</sup> which become common with advancing age, might modify in percentage amount amount of different T fractions.<sup>7</sup>

Several methods are available to measure the different fractions of circulating T. Total serum T is the fraction most commonly measured in clinical practice and is usually assayed with Immunoassays (IAs) in clinical laboratories.<sup>12,23-25</sup> Mass spectrometry (MS), particularly liquid chromatography-tandem mass spectrometry (LC-MS/MS),<sup>26</sup> is considered the gold standard for the measurement of total serum T, but, at present, is mainly used for research purposes.<sup>23,27</sup> In general, results obtained with IAs do not reproduce in a reliable way those obtained with LC-MS/MS<sup>28</sup> leading to the concept that commercially available IAs are widely inaccurate if compared with the gold standard.<sup>24,27-30</sup> Accordingly, LC-MS/MS is able to lessen interferences, has better specificity, and reduce between-method bias.<sup>31</sup> However, different results obtained by using different IAs are unimportant

in the male, eugonadal range, but becomes relevant when measuring T levels falling in the low male range.<sup>23,27</sup> In discordance with other studies,<sup>27</sup> the comparison between serum total T measurements by using IAs or MS, which represents the gold standard for T levels measurement, has shown similar, highly-correlated results in the EMAS cohort.<sup>32</sup> This means that IAs could be considered a good method for the evaluation of total serum T in clinical practice also for distinguishing between eugonadism and hypogonadism in the clinical setting.<sup>32</sup> This issue is crucial for the diagnosis of male hypogonadism and is still controversial (see the paragraph below on the diagnosis of male hypogonadism in aging). In general, for clinical purposes it is important to validate the assay and to perform continuous quality control, independently from the methodology used.<sup>23,29,30,33</sup> Furthermore, in the last few years, LC-MS/MS is becoming more and more available in the clinical setting and this evolving scenario is expected to be implemented, especially in clinical laboratories able to provide high throughput outcomes thanks to the procedural low costs that allows saving money for each assay and writing off expenses due to initial purchase of technological equipments.<sup>23</sup>

The measurement of free serum T might be performed by means of equilibrium dialysis or ultrafiltration methods, which are, however, not manageable in clinical practice or, alternatively, by the use of direct free T assays, which are unreliable and strongly inaccurate.<sup>23,24</sup> The better way to have an esteem of free serum T is to calculate free serum T starting from the value of total serum T and the measurement of both SHBG and albumin.<sup>7,13,23,24</sup> Even though it is well known that calculated free T overestimates the value obtained by equilibrium dialysis,<sup>14</sup> it remains largely employed both for research and clinical purposes.

Techniques for the measurement of bioavailable serum T are based on previous precipitation and separation of SHBG-bound T. For this reason, these methods are not simple and are not routinely employed in clinical laboratories. In addition, it is not clear the advantage of measuring bioavailable T rather than free T<sup>23</sup>. Starting from available data of serum albumin, SHBG, and total T, it is possible to calculate free and bioavailable serum T by using a simple formula and a calculator widely available on the web (<http://www.issam.ch/freetesto.htm>). By taking into account all the issues mentioned before, it becomes clear that the comparison of the results coming from different studies suffers from bias due to methodological differences when measuring serum T and its decline during aging.

As serum T varies during the day<sup>34</sup> and seasonally,<sup>25,35,36</sup> it should be assayed in the morning and low

values need to be confirmed twice in 2 different serum samples, the second one being obtained at least after 2 to 4 weeks after first T determination.<sup>12,25</sup> Differences in timing serum samples for T determination might also account for discrepant results obtained in different research settings.

In addition, the decline of both total and free serum T shows of course a high degree of interindividual variability<sup>5</sup> accounting for serum T within the normal range in most of the elderly patients (>60 years) and below the normal range in about 20% of them.<sup>37</sup>

Several risk factors have been associated with serum T decline.<sup>38-40</sup> Among them ethnicity, age, anthropometry, lifestyle factors, other concomitant comorbidities, chronic diseases, critical acute illnesses, drugs and/or other treatments should be taken into account.<sup>15,41</sup> The main risk factors or clinical conditions associated with low serum T are listed in [Table 1](#).

### Relationship between T decline and estradiol E<sub>2</sub>

Longitudinal studies have shown that serum E<sub>2</sub> declines in parallel with serum T in men.<sup>3,32,37</sup> It is well known that serum E<sub>2</sub> rather than T is important for bone health.<sup>42</sup> Thus, the decline of serum T in older men becomes particularly harmful for the bone only when also serum E<sub>2</sub> decreases. In particular serum E<sub>2</sub> should decrease below a threshold which is in-between 15 and 25 pg/mL (55.1–91.8 pmol/L).<sup>42,43</sup>

Apart from the role of E<sub>2</sub> on bone, other several physiological functions have been attributed mainly to E<sub>2</sub>. Among them, the inhibitory effect of T on the hypothalamus and the pituitary seems to be due to a greater extent to its conversion into estrogens.<sup>44,45</sup> Furthermore, also sexual function, especially sexual desire, is in part dependent from E<sub>2</sub> too.<sup>45,46</sup> Accordingly, serum E<sub>2</sub> is directly related to sexual desire in men.<sup>47</sup>

**Table 1.** Risk factors involved in serum T decline in aging men.

#### Risk factors and predictors of T deficiency:

**General factors:** Age, ethnicity, poor health status and frailty, progressive decrease of number and function of Leydig cells.

**Metabolic and anthropometric factors:** BMI, weight, waist circumference, visceral adiposity.

**Life-style habits:** excessive alcohol intake, cigarette smoking, sedentary lifestyle.

**Comorbidities:** hypertension, metabolic syndrome.

**Chronic Diseases:** moderate to severe chronic obstructive pulmonary disease, HIV, chronic liver diseases, chronic renal failure (especially end-stage and hemodialysis), type 2 diabetes, hypopituitarism, rheumatoid arthritis, Hodgkin disease.

#### Critical Acute illness

**Pharmacological and other treatments:** opiates, glucocorticoids, inhibitors of T synthesis (e.g., mitotane, ketoconazole), chemotherapy, testicular irradiation, GnRH analogs

Note. T: testosterone; BMI: body mass index; GnRH: gonadotropin-releasing hormone.

At present less is known about the E<sub>2</sub> interindividual differences in serum among men with hypogonadism. However, it is possible to hypothesize that hypogonadal men with a higher aromatase activity might be protected from the decrease of E<sub>2</sub> when their serum T declines.<sup>48</sup> E<sub>2</sub> plays an important role also in the relationship between hypogonadism and obesity since the increased production of estrogens within the fat tissue contributes to inhibit luteinizing hormone (LH) secretion from the pituitary and to reduce T secretion from the testes.<sup>49</sup> This mechanism could be involved not only in older men with hypogonadism, but also in other clinical conditions characterized by fat redistribution (e.g., Human Immunodeficiency Virus [HIV] infection).<sup>50</sup>

The measurement of serum E<sub>2</sub>, however, is not useful in clinical practice since the accuracy of IAs commonly used in the clinical laboratories is poor for the low values typical of the male range.<sup>51</sup>

### Age-related male hypogonadism: Results from large cohorts studies involving middle-aged to older men

One of the first studies investigating serum T in aging men by a longitudinal design was the New Mexico Process Study<sup>52</sup> that followed preliminary data previously obtained by cross-sectional studies.<sup>53-56</sup> Previous data were contradictory and came from observational studies mainly based on inpatients with a poor health status, thus they were not representative on average of an aging population of men.<sup>37,54,57</sup> After the above mentioned study, several other studies on large cohort of aging men were prospectively performed. Of them, the main are: the Baltimore Longitudinal Study of Aging (BLSA), the Massachusetts Male Aging Study (MMAS), the Osteoporotic Fractures in Men Study (MrOS), the European Male Aging Study (EMAS), the Rancho Bernardo Study (RBS), the InCHIANTI Study, the Tromsø Study, the Concord Health and Aging in Men Project (CHAMP), the Health in Men Study (HIMS). The main characteristics of these studies are all summarized in [Table 2](#). All these studies differ each other for several aspects, including the main aim of the study (that not always is specifically addressed to the study of sex hormones decline during aging), the involvement of subjects of both genders, the duration of the study, and the number of participants ([Table 2](#)). As expected, also the methodological approach differ among studies both in terms of methods used for the assessment of circulating sex steroids, the type of sex steroid assayed (estradiol is not available from all the studies), the study design, and the clinical investigations included in the study protocol, the latter varying widely among studies according to their primary and secondary endpoints ([Table 3](#), [Table 4](#)). Even though

**Table 2.** Main characteristics of large cohort studies investigating sex steroids and aging.

Study	Period (yrs)	Study Design	Sex M, F	Subjects (N)*	Age	Span (yrs)	T assay**	Sex Steroids as Main Aim	Primary End Point^^	Country
BLSA	1958-	PLOC-B	M,F	n.a.	>20	58	IAs	no	General overview on aging	Baltimore (USA)
MMAS	1987–2004	PLOC-B	M	1709	40–70	17	IAs	yes	Hormone levels and ED	Boston (USA)
MrOS	2000–2006	MC-PLOC-B	M	~11000	>65	6	LC-MS/MS	no	Fracture risk factors	USA, Sweden, Hong-Kong
EMAS	2003–2010	MC-PLOC-B	M	3369	40–79	7	LC-MS/MS IAs	yes	Anabolic hormones changes and health outcomes	Some EU Countries^
RBS	1972-	PLOC-B	M,F	1094	>30	44	IAs	no	Heart disease risk factors	Southern California (USA)
InCHIANTI	1998-	PLOC-B	M,F	601	>65	18	IAs	no	Physical function in aging	Tuscany (Italy)
Tromsø	1974–2008	PLOC-B	M,F	6595	n.d.	34	IAs	no	CV-related mortality	Tromsø (Norway)
CHAMP	2005-	PLOC-B	M	1705	>70	11	LC-MS/MS	no	Major geriatric syndromes	Sydney (Australia)
HIMS	1996–2004	P-BRT	M	12203	>65	8	IAs	no	Aortic aneurysm in aging	Perth (Australia)

Notes. M: males; F: females; \*Number of male participants at the beginning of the study; \*\*For some studies methods for T assay varied overtime during the long period of study protocol; ^UK, Italy, Belgium, Poland, Sweden, Spain, Hungary, Estonia; EU: European; ^^The main end point to be investigated in relation to aging; PLOC-B: Prospective, Longitudinal, Observational, Community-Based Study; MC-PLOC-B: MultiCenter, Prospective, Longitudinal, Observational, Community-Based Study; P-BRT: Population-Based, Randomized, Trial; n.d.: not detectable at any analysis; CV: cardiovascular; ED: erectile dysfunction.

the comparison among all these studies goes beyond the aim of this review, we will try to report in a critical fashion the aspects that are more relevant for the comprehension of the relationship between sex hormones and aging in males.

### Main characteristics of large cohort studies

The BLSA is a longitudinal study on human aging, which begun in 1958 (<https://www.blsa.nih.gov/about/history/>); it is the oldest study taking into account sex steroids changes during aging in men and is based on enrollment of participants with different ages who are followed afterward all lifelong.<sup>58</sup> This is one of the biggest studies on aging showing T levels decline in men and its relationship with several aspects related to aging<sup>4</sup> (Table 2). Other than BLSA, also the RBS and the Tromsø Study are very old, being started in the 70s (Table 2).<sup>59,60</sup>

Among studies that have specifically investigated the decline of sex steroids, particularly T, in middle-aged to older men, the MMAS is one of the pioneering study, the investigation of sex hormones changes in aging being the main study endpoint (Table 2).<sup>53,61</sup> The MrOS study is a multicenter study enrolling around 6000 men older than 65 y in order to identify risk factors for fractures in older men (Table 2).<sup>62</sup> By enrolling men also from Sweden and Hong Kong, the study has reached a considerable number of participants (Table 2). The main aim of the study, however, was to evaluate the association between sex hormones levels and osteoporosis rather than overtime changes during aging (Table 2).<sup>62</sup>

The EMAS (<http://www.emas.man.ac.uk>) is a multicenter (involving 8 European centers) cohort study that has been pointedly designed in order to evaluate physical, psychological and endocrine changes overtime, with

particular attention to modifications of T secretion occurring with aging.<sup>63,64</sup> (Table 2).

The Rancho Bernardo Study started as a survey of heart disease risk factors in adults who were older than 30 y and lived in the southern California community of Rancho Bernardo, that has progressively accumulated a lot of data (not only on CV system) from this cohort across the last 40 years<sup>59,65</sup> (Table 2).

The InCHIANTI study involves older people living in the Chianti geographic area (Tuscany, Italy) (<http://inchantistudy.net/wp/>). For the majority of men, measurements of total T, E<sub>2</sub> and SHBG serum levels were available, even though the main aim of the study was not strictly related to hormonal changes overtime<sup>66-70</sup> (Table 2).

The Tromsø study ([https://en.uit.no/prosjekter/prosjekt?p\\_document\\_id=80172](https://en.uit.no/prosjekter/prosjekt?p_document_id=80172)) is composed by several surveys performed in sequence and collecting information that have changed overtime across different surveys<sup>60,71,72</sup> The fourth survey was taken between 1994 and 1995; all inhabitants of Tromsø (Northern Norway) older than 24 y were invited and, between the subjects who accepted to take part to the study, all men aged 55–74 y and random samples of the other age groups were involved and hormonal measurements performed<sup>60</sup> (Table 2).

The CHAMP study was originally designed in order to investigate the health status in men older than 70 y living in a defined geographical region near the Concord Hospital in Sydney.<sup>73-75</sup> The study paid particular attention to the effects of age-related sex hormones decline.<sup>73</sup> After a baseline assessment; a subsequent, biennial follow up was then realized.<sup>73</sup> A strength of this project is the use of LC-MS/MS for steroid assays, which represents currently the “gold standard”(Table 2).<sup>74,76,77</sup> The HIMS (<https://www.perkins.org.au/wacha/our-research/mens/>

**Table 3.** Main outcomes from large cohort studies investigating sex steroid changes occurring in aging.

Outcomes	BLSA	MMAS	MrOS	EMAS	RBS	In CHIANTI	Tromsø	CHAMP	HIMS
<b>Total T decline (per year)</b>	Yes (3.2 ng/dl)	Yes (1.6%)	n.a.	Yes	Yes (1.9 pg/ml)	n.a.	Yes	Yes (2% per year)	No
<b>T-free/bioavailable decline (per year)</b>	Yes	Yes (2–3%)	Yes	Yes	Yes (18.5 pg/ml)	n.a.	Yes	Yes	Yes
<b>SHBG increase</b>	Yes	Yes	Yes	Yes	Yes	n.a.	n.a.	Yes	Yes
<b>E2 decline (per year)</b>	n.a.	n.a.	Yes	Yes	n.a.	n.a.	n.a.	No (↑3%)	n.a.
<b>Sexual desire decline</b>	n.a.	Associated to ↓TT and ↓fT	n.a.	n.a.	n.a.	n.a.	n.a.	Associated to ↓TT and ↓fT	n.a.
<b>Erectile function decline</b>	n.a.	Associated to ↓TT and ↓fT*	n.a.	Associated to ↓fT	n.a.	n.a.	n.a.	No association with TT and fT	n.a.
<b>BMD impairment</b>	n.a.	n.a.	Associated to ↓E <sub>2</sub> ; conflicting data about T	Associated to ↓E <sub>2</sub> but not to T	Associated to ↓bioavailable E <sub>2</sub> and T	n.a.	Associated to ↑SHBG, but not with T and E <sub>2</sub>	Associated to ↑SHBG, but not with T and E <sub>2</sub>	n.a.
<b>Increased fracture risk</b>	n.a.	n.a.	Associated to ↓E <sub>2</sub> and ↓T and ↑SHBG	n.a.	n.a.	n.a.	Not associated to TT, fT, E <sub>2</sub>	Not associated to sex steroids	n.a.
<b>Lean mass and muscle strength decline</b>	Associated to ↓fT <sup>†</sup>	n.a.	Associated to ↓fT	Associated to ↓fT	n.a.	Associated to ↓TT	n.a.	Associated to ↓fT and ↓TT	n.a.
<b>Physical performance decline</b>	Associated to ↓fT	Associated (weakly) to ↓TT	n.a.	Associated to ↓fT	n.a.	n.a.	n.a.	n.a.	n.a.
<b>Frailty increase</b>	n.a.	n.a.	Associated to ↓TT	n.a.	n.a.	n.a.	n.a.	Associated to ↓TT, ↓fT, ↓E <sub>2</sub> , not to SHBG	Associated to ↓TT, ↓fT
<b>Overweight and obesity increase</b>	n.a.	Associated to ↓TT and ↓SHBG	n.a.	Associated to ↓TT, ↓fT, and ↓SHBG	n.a.	n.a.	Associated to ↓TT, ↓fT, ↓SHBG, and ↑E <sub>2</sub>	n.a.	Associated to ↓TT (if
<b>Metabolic Syndrome risk</b> (BMI > 25)	Associated to ↓TT and ↓SHBG	Associated to ↓TT and ↓SHBG	n.a.	n.a.	n.a.	Associated to ↓TT and ↓SHBG, and ↑E <sub>2</sub> , not to ↓fT	n.a.	Associated to ↓SHBG, not to ↓TT	Associated to ↓TT, ↓fT, ↓SHBG
<b>Diabetes risk</b>	n.a.	Associated to ↓TT and ↓SHBG	n.a.	n.a.	Associated to ↓TT, ↓fT	n.a.	↑TT and ↑SHBG are protective	n.a.	n.a.
<b>CV risk increase</b>	Arterial stiffness associated to ↓TT	n.a.	Atherosclerosis associated to ↓TT	Hypertension associated to ↓TT	Hypertension associated to ↓TT	Artery disease associated to ↑SHBG, not to T and E <sub>2</sub>	Hypertension and carotid atherosclerosis associated to ↓TT	n.a.	n.a.
<b>Low T association with poor health status and unhealthy habits</b>	Alcohol intake associated to ↓TT	n.a.	n.a.	n.a.	n.a.	n.a.	Smoking associated to ↑TT, ↑fT, ↑SHBG	Self-reported poor status associated to ↓TT, ↓fT,	n.a.
<b>Low T associated to reduced hemoglobin</b>	n.a.	n.a.	n.a.	yes	n.a.	yes	n.a.	n.a.	n.a.

(continued on next page)

Table 3. (Continued)

Outcomes	BLSA	MMAS	MrOS	EMAS	RBS	In CHIANTI	Tromsø	CHAMP	HIMS
Low T associated to dyslipidemia	n.a.	n.a.	n.a.	yes	yes	n.a.	yes	n.a.	n.a.
Increase of all-cause mortality risk with low T	no	Weak association	yes	yes	yes	yes <sup>^^</sup>	yes	yes <sup>^^^</sup>	yes
CV mortality risk increase associated to low T	n.a.	n.a.	n.a.	n.a.	yes	n.a.	No	Associated to ↓E <sub>2</sub>	Associated to ↓fT
Cognitive function decline associated to T	Associated to ↓fT	Not associated to T	Associated to ↑SHBG	n.a.	n.a.	n.a.	n.a.	No associated to E <sub>2</sub> , T, and SHBG levels	Not
Depressive mood increase	Not associated to T	n.a.	n.a.	n.a.	Associated to ↓bioavailable T	n.a.	n.a.	n.a.	Associated to ↓TT

Notes. TT: Total Testosterone; fT: free Testosterone; \*Conflicting data (association with both total and free low T, but only in more recent analyses); ^muscle strength only, not muscle and lean mass; ^^no correlation with low T alone, but with low T together with other hormones deficit; ^^correlation with both total and free low T and with low E<sub>2</sub>.

**Table 4.** Main similarities and differences of large longitudinal, cohort studies.

←-----→			
STRONG DISCREPANCIES	WEAK DISCREPANCIES	WEAK ANALOGIES	STRONG ANALOGIES
<b>Hormonal Trajectories</b>			
-	-	↓ serum total and free E <sub>2</sub> with advancing age	↓ serum total T ↓↓ serum free T ↓↓ bioavailable T ↑ serum SHBG with advancing age
<b>Consequences of Hormonal Changes Related to Male Aging</b>			
<ul style="list-style-type: none"> <li>• Association between low T and low BMD.</li> <li>• Association between low T and depression.</li> <li>• Association between low T and CV-related mortality.</li> <li>• Relationship between T and physical performance.</li> <li>• Relationship between T and prostate cancer.</li> </ul>	<ul style="list-style-type: none"> <li>• Association between T and cognitive functions.</li> <li>• Association between low T and atherosclerosis.</li> <li>• Relationship between hypogonadism and frailty development.</li> <li>• Correlation between serum T measured by LC-MS/MS and by AIs.</li> <li>• Association between low T and impaired pulmonary function.</li> <li>• Association between low T and/or low E<sub>2</sub> and frailty.</li> </ul>	<ul style="list-style-type: none"> <li>• Association between low T and CV risk factors' increase.</li> <li>• Association between low T and increased CV risk.</li> <li>• Association between low T and poor health status.</li> <li>• Association between low T and low hemoglobin.</li> <li>• High T correlates with better lean mass, and muscle strength.</li> <li>• Low T correlates with erectile dysfunction.</li> <li>• Serum E<sub>2</sub> is inversely related to fracture risk.</li> </ul>	<ul style="list-style-type: none"> <li>• Serum T is directly related to sexual function, especially libido.</li> <li>• Association between low T and increased all-causes mortality risk.</li> <li>• Healthy life-style correlates with higher T levels/prevention of T decline.</li> <li>• Association between low T and both overweight and obesity.</li> <li>• Association between low T and both metabolic syndrome and type 2 diabetes.</li> <li>• Serum E<sub>2</sub> correlates with BMD.</li> </ul>

mens-health-1/) was initially realized as a trial of screening for abdominal aortic aneurysms in Perth, Australia, but blood samples were obtained since baseline from all participants, thus it was possible to correlate their gonadal status to different physical, metabolic and psychological aspects.<sup>78</sup> However, the study was not addressed specifically to reach endocrinological endpoints<sup>78</sup> (Table 2).

#### Age-related sex steroids changes across cohort studies

Almost all the studies found a decline of total serum T with advancing age (Table 3, Table 4). The BLSA estimated a decline of total serum T around 3.2 ng/dl per year,<sup>4,79</sup> this esteem being around 1.9 pg/ml per year in the RBS,<sup>80</sup> while the first data coming from the MMAS reported a decline of serum total and free T of about 0.4% and 1.2% per year, respectively<sup>53</sup> (Table 3). More recently, the CHAMP study showed a decline of serum total T around 2% per year<sup>81</sup> (Table 3).

The EMAS results have confirmed, in the last years, the data concerning the progressive decline in aging men of serum total T, free T, and E<sub>2</sub><sup>82</sup>, as well as those concerning the rise of SHBG with aging<sup>83</sup> (Table 3).

The CHAMP recently replicated these results except for a surprising increase of E<sub>2</sub><sup>81</sup> (Table 3).

In the HIMS study, serum free T significantly decreased with increasing age, while total serum T levels seemed to remain stable; this is probably due to the

important increase of SHBG in older age, which could justify the persistence of stable total T levels<sup>84</sup> (Table 3).

In line with other studies, also the RBS showed a decline of both total and bioavailable serum T levels with aging<sup>80</sup> (Table 3). In particular, bioavailable serum T levels decreased more (18.5 pg/ml) than serum total T in aging men mainly due to the corresponding increase of SHBG with age,<sup>59,80,85</sup> (Table 3).

Even though the MrOS did not provide data on the trajectories of sex steroids overtime, it indirectly has confirmed that both serum T and E<sub>2</sub> decline with advancing age<sup>86,87</sup> (Table 3). By dividing participants according to quartiles of SHBG and sex steroids, in fact, their age was higher in the higher quartiles of serum SHBG<sup>88</sup> and in the lower quartiles of both serum E<sub>2</sub><sup>86</sup> and free T<sup>87</sup>.

*Vice versa* no data on the decline of sex steroids were available in the InChianti study even though measurements of total T, E<sub>2</sub> and SHBG serum levels were available for the majority of men<sup>67,70,85</sup> in this study hormonal data were mainly used for investigating the association between circulating sex steroids and several conditions that are common in aging<sup>69,68,67,70,66</sup> (Table 3).

#### Male hypogonadism across cohort studies

The prevalence of biochemical hypogonadism seems always to increase with advancing age, being independently from the study considered, at the lowest in men younger than 49 y and progressively increasing through decades, with the highest prevalence being in men in

their 80s.<sup>4,8,82,83,89,90</sup> However, according to a more recent analysis, total serum T levels seem to be quite stable until the age of 70 years, after that there would be a progressive, significant decline, while the bioavailable serum T seems to decline more linearly with aging.<sup>79</sup>

In line with other studies,<sup>3,8,89,90</sup> in the Tromsø study the investigation of hormonal changes with aging resulted in the finding of lower serum T levels and higher gonadotropins levels in older men<sup>60</sup> (Table 3), even though the measurement of gonadotropins in aging men is scarcely helpful in predicting the future development of hypogonadism<sup>91</sup> (Table 3).

Several cutoffs for male hypogonadism have been suggested on the basis of the results of these large cohort studies. The MMAS study has suggested the following age-related cutoffs for serum total T: 251, 216, 196, and 156 ng/dL (8.7, 7.5, 6.8, and 5.4 nmol/L) for men in their 40s, 50s, 60, and 70s, respectively.<sup>61</sup> Many data about aging and gonadal status arose from the EMAS and the identification and definition of the LOH as a clinical condition has been one of the biggest results.<sup>3</sup> LOH has been in fact defined as a condition characterized by 3 sexual symptoms (decreased sexual thoughts, decreased frequency of morning erections and erectile dysfunction) together with total serum T less than 317 ng/dL (11 nml/L) and free T less than 63,4 pg/mL (220 pmol/L).<sup>3</sup> According to the grade of serum T decrease, LOH can be considered as moderate or severe. In particular, total serum T concentrations lower than or equal to 230 ng/dL (8 nmol/L) have been associated with worse sexual functioning and higher rates of erectile dysfunction.<sup>92</sup>

### Main outcomes of cohort studies

The comparison of such kind of large cohort studies clearly remarks the great heterogeneity of outcomes, which differ for each study according to the peculiarity of the study design and the number and type of clinical and laboratory data collected. While data on trajectories of sex steroids are almost available in all studies (see the paragraph above), information about the relationships among circulating sex steroids and/or various clinical aspects and physiological changes related to aging become patchy when moving across the various studies (Table 3).

While changes in circulating total, free, and bioavailable T, E<sub>2</sub>, and SHBG are unidirectional in almost all the studies, several other outcomes were not replicated, thus leading to conflicting results when different studies are compared each other (Table 3, Table 4).

The relationship between the decline of serum T and the worsening of sexual function has been investigated and confirmed by most of these studies (Table 3). The MMAS,<sup>93,94</sup> the CHAMP,<sup>74</sup> and the EMAS.<sup>3,95</sup> provided

a lot of data on the relationship between gonadal status and sexual desire, sexual function, and erectile function in middle-aged to older men (Table 3), suggesting that loss of sexual desire, but not erectile function, is strictly related to low T in aging men<sup>96</sup> and could be predictive for the development of erectile dysfunction (ED)<sup>94</sup> Thus, low serum T (the main component of low desire) is indirectly related to the onset of ED and to impaired male sexual function.<sup>47</sup> In particular, in the EMAS study lower serum free T levels have been more strongly associated with severe sexual dysfunction (such as increased erectile dysfunction and lower masturbation frequency).<sup>83,92</sup> Less data are available on sexual function in the BLSA and MrOS studies<sup>97</sup> (Table 3).

Overweight and obesity are strongly associated to the decline of both total serum T and SHBG in several studies such as the MMAS,<sup>37,98,99</sup> the BLSA,<sup>4</sup> the EMAS,<sup>100</sup> and the Tromsø,<sup>101,102,103</sup> studies (Table 3). Changes in body composition lead to an increased risk of developing both the metabolic syndrome<sup>104</sup> dyslipidemia,<sup>65,105,106</sup> and diabetes<sup>107</sup> (Table 3).

Metabolic syndrome was associated to low serum T and/or low SHBG in the BLSA,<sup>41</sup> HIMS,<sup>108,109</sup> InChianti,<sup>68,67</sup> Tromsø,<sup>110,111,112</sup> and RBS.<sup>65,105,106</sup> studies, but not in the CHAMP study, the latter showing an association between lower SHBG levels, but not T, and the development of metabolic syndrome<sup>76</sup> (Table 3).

The EMAS study showed that weight loss is associated to an increase, while weight gain to a decrease in both total and free serum T, with the same association persisting by considering waist circumference.<sup>82</sup> As previously reported, obesity probably determines an impairment of the hypothalamic-pituitary function, leading to lower LH secretion and finally to hypogonadotropic hypogonadism. This evidence is very important because it suggests that weight management and obesity avoidance could be useful to prevent T decline.<sup>82,100</sup> Furthermore, the relationship between weight and T is probably bidirectional, since hypogonadism promotes fat accumulation and low total and free serum T levels have been associated with an increased risk of developing metabolic syndrome.<sup>18</sup>

Data on the relationship between T and the cardiovascular (CV) system are available from the BLSA,<sup>113</sup> the MrOS,<sup>114</sup> the Tromsø,<sup>110,111,112</sup> and the RBS,<sup>115</sup> showing that hypertension is associated to low serum T (Table 3). As suggested by the Tromsø study, smoking, overweight, and obesity represent possible confounding factors when evaluating the relationship between serum T and CV diseases in cohort studies.<sup>116</sup>

The major role of serum estrogens on bone health has been suggested by almost all the studies,<sup>117</sup> serum E<sub>2</sub> and T being directly correlated with bone mineral density (BMD). This correlation is particularly strong for free

serum  $E_2$ <sup>86,118,119</sup>. Furthermore, the MrOS has shown that low serum T together with high SHBG levels and low serum estradiol levels are associated with increased risk of non-vertebral fracture.<sup>117</sup> On the other hand, data about the effects of serum T on BMD remain conflicting since the association between low serum T and lower BMD has not been demonstrated in all studies, such as the MrOS.<sup>120</sup> In the EMAS study, however, men with overt hypogonadism (total T < 230 ng/dL [8 nml/L]) show worse serum parameters of bone health compared with eugonadal men.<sup>118,121</sup> Probably, T contributes to bone health through its effect on muscle mass (and the consequent mechanical load on bone) and via aromatization to estrogens.<sup>42,48,121</sup> Thus, low circulating levels of T result in parallel in low serum  $E_2$ <sup>42,48</sup>. Differently from the majority of studies, the CHAMP study did not find any association among serum T,  $E_2$ , SHBG, and bone health,<sup>122</sup> on one hand, and, on the other hand, the Tromsø did not find any association between sex steroids and both BMD<sup>71</sup> and fracture risk.<sup>72</sup>

Low total T levels were associated with lower hemoglobin levels in the InChianti study similarly to EMAS,<sup>89</sup> suggesting that the effects of low T on hemoglobin should be carefully evaluated in older men due to the fact that anemia is one of the strongest markers of frailty.<sup>69</sup>

Not all studies investigated physical performance and both the muscular mass and strength in relation to circulating sex steroids.<sup>77,123,124</sup> (Table 3). Physical performance was only weakly associated to serum T in the MMAS<sup>125</sup> In regard to body composition, total serum T is related directly to lean mass, playing a possible protective role in healthy aging, since a better muscular function helps preventing falls, fractures, and frailty to some extent, as suggested by the MrOS study.<sup>126</sup>

Low total and free serum T levels have been also associated with frailty in the HIMS,<sup>127</sup> CHAMP,<sup>128</sup> and the MrOS studies.<sup>129</sup> In addition, men with poor health status, in particular those who had a stroke or a cancer diagnosis, had lower levels of both total and free serum T levels in the Tromsø study,<sup>91</sup> while self reported worse health status was associated to low T in the CHAMP<sup>75</sup> (Table 3). Otherwise, in the MMAS a good health and the absence of chronic illness were both associated with a less important decline of serum T<sup>37</sup> (Table 3).

Not all the studies investigated neuropsychological correlates of age-related T decline. The BLSA studied the possible correlation between androgens and cognitive function leading to a possible role of T in preventing cognitive dysfunction and depressive mood.<sup>130-132</sup> These data on mood<sup>105</sup> and cognition were also replicated by the RBS study,<sup>133</sup> and the HIMS<sup>134</sup>; in particular the latter showed an increased risk of developing depressive

symptoms in association to low serum total T<sup>135</sup> (Table 3). Conversely, no relationship between sex steroids and cognition was found in other studies, such as the MMAS,<sup>136</sup> the CHAMP,<sup>137</sup> and the MrOS.<sup>97</sup>

In these large cohort studies, low serum free T was almost constantly associated with an increased all-causes mortality risk, this association was found by the HIMS,<sup>138</sup> CHAMP,<sup>81</sup> MMAS,<sup>139</sup> EMAS,<sup>95</sup> RBS,<sup>119</sup> Tromsø,<sup>140</sup> and MrOS<sup>141</sup> studies. In the MrOS the mortality risk appeared to be higher when also low serum  $E_2$  was present.<sup>141</sup> *Vice versa*, the InChianti study did not find any association between low baseline free serum T levels and 6-years mortality risk.<sup>66</sup> The association between low serum T and mortality due to CV events remains less known since this association was proven only by the HIMS study.<sup>138</sup>

### Lesson learnt from large cohort studies

The integrated analysis of data provided by these large longitudinal studies allows highlighting several issues that are of concern for research advancement, methodological outcome and finally for clinical practice.

At the beginning most of these longitudinal studies provided first analyses based on cross-sectional data.<sup>53,142</sup> The longitudinal design of the studies came up progressively as the studies aged together with their participants. First cross-sectional data did not find changes in circulating sex steroids between older and younger participants.<sup>142</sup> Subsequently, the decline of sex steroids was unequivocally proved when the data from longitudinal analyses became available. Accordingly, the most important result reached by all the studies is to demonstrate the serum T decline with advancing age. Particularly, both free and total serum T levels tend to decrease; the latter decreases lesser than the former as a consequence of the progressive increase of SHBG with aging (Table 4). Also the modification of SHBG has been confirmed by all the studies (Table 4).

In all studies that have investigated male sexuality, serum T resulted inversely related to sexual desire and to some extent to sexual activity and erectile function too (Table 4). Another concordant result is the association between low serum T and obesity, metabolic comorbidities such as the metabolic syndrome, and type 2 diabetes (Table 4).

Among other clinical aspects that are associated to serum T lowering with advancing age, all-cause mortality, body composition changes, especially overweight and obesity as well as metabolic syndrome and diabetes, all have been over and over again associated to T decline in aging (Table 4).

The methodological approach used for T measurement has changed overtime according to advancements

in this field in all the studies (Table 4). At present, the gold standard LC-MS/MS is being used in all studies, while at the beginning of the studies sex steroids were measured by IAs. This implies that the comparison of the results coming from different studies as well as the results of the same study obtained in different periods by using different assay methods remain challenging. There is not consensus about the correlation between total serum T measured by MS and IAs among studies since some studies find a good correlation while others find discordant measurements (Table 4). This issue is important when data coming from research studies like these are transposed and applied to daily clinical practice since IAs are commonly employed, at present, in clinical laboratories.

A lesser degree of certainty has been reached regarding the association between T lowering with advancing age and CV diseases, increase in CV risk, ED, and impaired bone health (Table 4).

The most controversial issues concern the association of T decline to impaired physical performance, neuropsychological issues, CV-related mortality, and cancer risk (Table 4).

Table 4 summarizes in detail the main analogies and discrepancies regarding the issues that have been mostly investigated by large cohort studies till now (Table 4).

### Significance of age-related serum T changes in aging men

The decline of serum T with advancing age has been traditionally interpreted as the male counterpart of menopause. In other words, the decrease of T was considered as a condition due to the aging of gonadal function. In this old view, the term andropause was considered as a physiological event like menopause in females<sup>143-145</sup> with the unique difference that the ovaries stop to function suddenly while the testes lose their function gradually (Fig. 1).

At present, it has not been defined whether the decline of serum T in the elderly represents a physiological process of aging or whether this event should be considered as pathological. In 2004 Snyder stated that '*an essential but still unanswered question is whether this decrease in the testosterone concentration is physiologic, perhaps conveying a benefit, or pathologic, causing harm*'.<sup>146</sup> Today, this point remains still crucial in order to establish if a strategy that is effective in counteracting serum T decline should be set up or if treating serum T decline maybe potentially harmful. With this in view, the identification of patients with low serum T that might benefit of replacement therapy on one hand and the recognition of those for which therapy is not indicated is challenging.

All these issues usually converge on the practical point concerning how to diagnose LOH.<sup>147</sup>

### The clinical diagnosis of LOH: lights and shadows

Recently, there is consensus among endocrinologists, andrologists, and urologists about the fact that the finding of biochemical T deficiency does not necessarily correspond to LOH in middle-aged-to-older men.<sup>12,25,38-40,49,147,148</sup>

Accordingly, lower serum T in older men should not be considered "*per se*" significant, but it acquires a clinical value in men with concomitant signs and symptoms of T deficiency, a condition known as hypogonadism.<sup>3,12,25,38,40,49,148</sup>

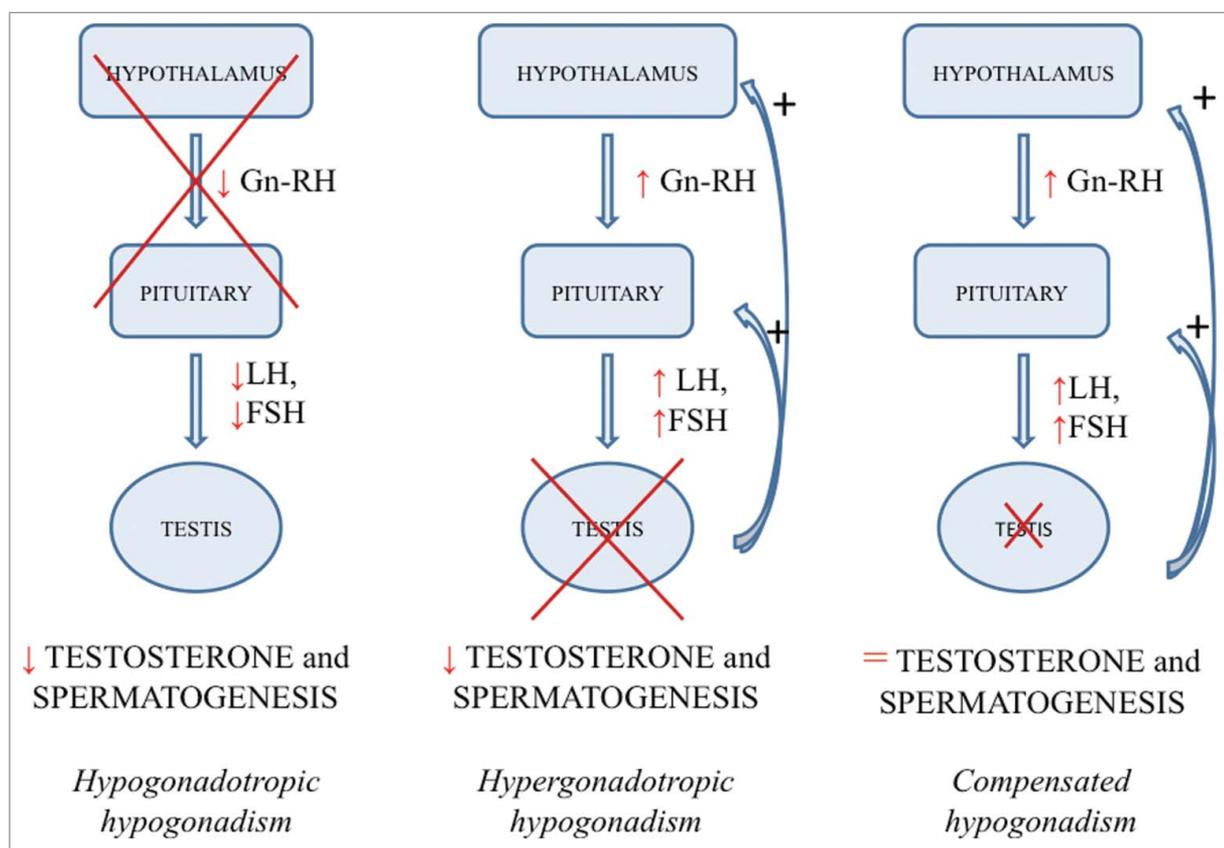
Some aspects related to the diagnosis of hypogonadism do not differ according to the patients' age (e.g., categories of hypogonadism), while for other aspects (e.g., causes of hypogonadism, signs and symptoms, and serum T thresholds) the patients' age does matter.<sup>40,49,149,150</sup>

### Classification of LOH

In general, 2 main forms of hypogonadism can be distinguished: (a) primary hypogonadism (or hypergonadotropic hypogonadism), where the site of origin is the testis and which is characterized by low T levels with high gonadotropin levels, and (b) secondary hypogonadism (or hypogonadotropic hypogonadism), in which there is hypothalamic and/or pituitary failure causing low gonadotropin production and consequent low T levels<sup>12,25,49,148</sup> (Fig. 2). Furthermore, among aging men is quite common a condition of compensated hypogonadism, represented by normal T levels with high gonadotropin levels, whose prevalence increases with aging and which seems to be a subclinical condition that could develop in overt, primary hypogonadism overtime<sup>90</sup> (Fig. 2). Recently, data coming from the EMAS study confirm that age is correlated with the development of primary hypogonadism, but not with secondary hypogonadism, the latter being strictly associated to comorbidities rather than age.<sup>3,151,152</sup>

### Causes of LOH

In younger men hypogonadism (both hypergonadotropic and hypogonadotropic) is usually due to well-known causes, which can be congenital or acquired, and the site of origin is easily recognizable.<sup>40,49,149,150</sup> Conversely, many different factors, some of which are still not well defined, contribute to the decrease of T production in aging men (Table 1). For example, aging "*per se*" is known to induce a progressive dysfunction of Leydig cells, but age-advanced typical chronic comorbidities (e.g., cancer, chronic obstructive pulmonary disease, chronic renal failure and chronic liver failure) are also associated with low serum T (Table 1).<sup>6,153</sup> Accordingly, age and chronic diseases resulted associated to primary



**Figure 2.** Classification of hypogonadism according to its pathogenesis.

hypogonadism in the EMAS cohort.<sup>151</sup> Thus, low serum T of the elderly man is often due to both primary and secondary failure of T secretion. Other factors involved in serum T declining include bad lifestyle habits, such as smoking and excessive alcohol intake, and chronic therapies with opiates and glucocorticoids, increasingly used to treat elderly population (Table 1).<sup>6</sup> Several clinical conditions (e.g., chronic or acute diseases) are associated to hypogonadism in aging men, some of them cause secondary hypogonadism, which is not related to age and might be reversible.<sup>15,152</sup> In particular, diabetes mellitus, obesity and metabolic syndrome seem to play a major role especially in decreasing gonadotropin levels, leading to secondary hypogonadism (Table 1).<sup>90,147</sup> Other factors involved in serum T declining include bad lifestyle habits, such as smoking and excessive alcohol intake, and chronic therapies with opiates and glucocorticoids, increasingly used to treat elderly population (Table 1).<sup>6</sup> Here we summarize the main clinical conditions and diseases linked to hypogonadism.

### **Hypogonadism and non-infectious comorbidities**

**Chronic non-infectious diseases.** Hypogonadism might occur in several chronic conditions at any age, but it is self-

evident that this association increases with advancing age as a consequence of higher probability to develop and/or accumulate new morbidities during aging.<sup>15,91,154</sup>

Overweight, obesity, and metabolic disorders (metabolic syndrome, diabetes, dyslipidemia) are more frequent in older men and are all associated to low serum T in men, as already discussed in this article.<sup>20,22,40,41,102,103,105,107,152</sup>

Chronic liver diseases, especially cirrhosis and fatty liver disease,<sup>155</sup> are associated to hypogonadism as a consequence of increased liver production of SHBG and of estrogen excess and its inhibitory effect on gonadotropins.<sup>22</sup>

The prevalence of hypogonadism seems to be high in men with chronic obstructive pulmonary disease (COPD), especially in middle-aged and elderly men,<sup>22,153</sup> ranging from 22 to 69%.<sup>156,157</sup> In particular, secondary hypogonadism is associated to COPD and the severity of COPD seems to be directly correlated with the degree of hypogonadism.<sup>157</sup> An interesting, not widely investigated association has been found between low total and free serum T levels and impaired pulmonary function evaluated by spirometry in the Tromsø study.<sup>116</sup> Considering that oxygen therapy is known to increase T levels in men with respiratory failure, it seems more likely that poor, pulmonary function is responsible of lower T levels and not vice-versa, may be by altering the hypothalamic-

pituitary function.<sup>158</sup> The studies on COPD, however, are scanty and have been performed on small samples of patients, thus more data from more studies are still needed to better characterize hypogonadism in older men with COPD.<sup>22</sup>

Male hypogonadism is highly prevalent also in patients with chronic kidney disease as a consequence of testicular damage (primary hypogonadism).<sup>21,22,159</sup> In patients who undergo hemodialysis for the first time hypogonadism do not normalize and tends to worsen, only renal transplantation being able to restore normal serum T.<sup>21,22,159,160</sup>

Several congenital systemic diseases might induce hypogonadism in the young, adult man and hypogonadism is intended to worsen with advancing age as in the case of  $\beta$ -thalassemia<sup>161</sup> and hemochromatosis.<sup>162</sup>

Finally, treatments used for systemic chronic diseases might induce hypogonadism as in the case of rheumatoid arthritis.<sup>22,153</sup> or treatment for cancer.<sup>22</sup>

The issue concerning the relationship existing between morbidities and LOH is relevant since might to be bidirectional.<sup>40</sup> On one hand, LOH sustains or exacerbates morbidities, while, on the other hand, LOH might be caused by comorbidities and might be reversible if comorbidities are treated.<sup>40</sup> The same hormonal change occurs in patients with other acute illnesses different from infectious diseases.<sup>163-165</sup> These changes seem to be part of a mechanism, which is adaptive to poor health status aiming to counteract the high catabolic state due to the acute, severe clinical condition. By decreasing the activity of the pituitary-gonadal axis it is possible to reduce some body functions (e.g., physical and sexual activity), thus allowing sparing energy and avoiding reproduction during a unhealthy condition.<sup>165</sup> Since chronic diseases are associated to hypogonadism in the male,<sup>153,163</sup> it has been postulated that in a part of men developing T deficiency with advancing age LOH maybe the consequence of multimorbidity that accumulates in the aging patient.<sup>40</sup> With this in view, a considerable quote of LOH are not consistent with a clinical condition of hypogonadism, but represents an epiphenomenon of a poor health status, which is a common condition in the elderly.<sup>38,40</sup> With this in view, hypogonadism could be considered as a functional, adaptive mechanism enrolled in presence of a condition of poor health status,<sup>40,50,165,166</sup> as it happens in case of acute, severe illness.

**Acute non-infectious diseases.** Serum T might fall down in patients with acute, critical illnesses and normal serum T levels are restored by the resolution of the acute clinical condition.<sup>164,167,168</sup> This condition occurs regardless the nature of the acute illness,<sup>163,164,169,170</sup> it is associated to

the severity of patients's clinical condition,<sup>163,168</sup> and might be transitory and reversible.<sup>167,171</sup> This mechanism based on a strict relationship between patient's poor health status and low serum T operates especially in aging men and share a mechanism involving the hypothalamus and the pituitary resulting in secondary hypogonadism.<sup>3,9,100,152</sup>

**Aging, hypogonadism, and frailty.** As mentioned above, patient's poor health status in terms of deteriorated clinical conditions might influence T secretion. In aging men, however, the more complicated, multidimensional concept named frailty should be also considered.<sup>172</sup> Frailty depends not only by the clinical condition of the patients, but encompasses also all the physiological and pathological changes accumulating during aging that impair patients' physical performance (e.g., sarcopenia, osteoporosis, walking disabilities, impairment of visual acuity) as well as his pshychological status (loss of memory, depressed mood) and well-being.<sup>172-174</sup> All these factors contribute to frailty and expose the aging man to experiment harmful events (e.g., falls, progressive reduction of physical activities).<sup>172-174</sup>

Frailty is inversely related to serum T in older men, as substantiated by several studies.<sup>15,17,154,175-177</sup> Furthermore, sarcopenia is directly related to low serum T<sup>178</sup> and takes part to the physiological process of aging itself.<sup>179-181</sup> Even chronic illnesses are able to induce sarcopenia.<sup>180,182</sup> Thus it is evident that sarcopenia should be considered as a central player in the relationships among LOH, frailty, and comorbidities.<sup>181</sup>

**Aging, hypogonadism, and infectious diseases.** Data available on the relationship among aging, hypogonadism, and infectious diseases are scanty. It is well known that sepsis induces a fall of serum T in men together with a decrease of serum LH, consisting with a condition of hypogonadotropic hypogonadism.<sup>169,170</sup> (Fig. 2). The same hormonal change occurs in patients with other acute illnesses different from infectious diseases.<sup>163-165</sup> These changes seem to be part of a mechanism, which is adaptive to poor health status aiming to counteract the high catabolic state due to the acute, severe clinical condition. By decreasing the activity of the pituitary-gonadal axis it is possible to reduce some body functions (e.g., physical and sexual activity), thus allowing sparing energy and avoiding reproduction during a unhealthy condition.<sup>165</sup> Since chronic diseases are associated to hypogonadism in the male,<sup>153,163</sup> it has been postulated that in a part of men developing T deficiency with advancing age LOH maybe the consequence of multimorbidity that accumulates in the aging patient.<sup>40</sup> With this in view, a considerable quote of LOH are

not consistent with a clinical condition of hypogonadism, but represents an epiphenomenon of a poor health status, which is a common condition in the elderly.<sup>38,40</sup>

Among infectious diseases that may interfere with circulating T, rare testicular infections causing orchitis usually induce a temporary or permanent primary hypogonadism (hypergonadotropic hypogonadism).<sup>183</sup> Conversely, chronic viral hepatitis are often related to an increase of serum total T rather to T deficiency; this condition is due to the rise of SHBG and does not modify free serum T.<sup>184</sup>

**Hypogonadism and HIV infection.** Among HIV infected-men a process of accelerated aging seems to take place, deriving from a multifactorial etiology (immunosenescence, inflammation, multimorbidity); therefore, HIV infection can be considered a model of premature aging.<sup>185</sup> Accordingly, a premature decline of serum T has been described in this population.<sup>186</sup> The prevalence of biochemical hypogonadism in HIV-infected men is around 25% in young-to-middle-aged men<sup>50</sup> and serum T decline in HIV-infected men aged 45–60 y seems to parallel what happens in older men after 60.<sup>50,186</sup> In HIV-infected patients secondary hypogonadism is more common and is associated to fat redistribution and visceral adiposity.<sup>50,186</sup> Furthermore, low levels of serum T are associated with to higher number of comorbidities and are inversely related to frailty.<sup>166</sup> The latter finding does not help in establishing if these patients are truly hypogonadic or if their low T is related only to their poor health status.

The diagnosis of hypogonadism in these patients shares all the problems physicians have with older men and it is even more difficult due to the fact that symptoms and signs are much more less specific than in the general population. Sign and symptoms, especially those related to sexuality and body composition changes, overlap, in fact, with those of HIV infection.<sup>50</sup>

Finally, SHBG measurements is mandatory in men with HIV since SHBG alterations are very common in these patients and might lead to underestimation of hypogonadism.<sup>50</sup>

**Serum T thresholds for LOH diagnosis.** The cut-off of serum total T below which the diagnosis of overt biochemical hypogonadism could be formulated remains to be determined.<sup>3,187,188</sup> Different studies,<sup>12,25,33,148,154</sup> clinical guidelines (<https://uroweb.org/guideline/male-hypogonadism/>), expert panels,<sup>49</sup> and extensive literature revision.<sup>38-40,147</sup> provided different cutoffs. However, all the cutoffs fall within the interval of serum total T comprised between 180 ng/dL and 300 ng/dL (Table 5).

Since T secretion follows a circadian rhythm, with peak values in the morning, T measurement should be performed in the morning, preferably in 2 different occasions<sup>12,25,33,38,49,148,154</sup> (<https://uroweb.org/guideline/male-hypogonadism/>). When serum total T levels are borderline evaluation of free T can be useful, especially among older men who physiologically tend to have increased levels of SHBG.<sup>33,154</sup> In particular, SHBG measurement is indicated in obese patients and patients with chronic diseases (e.g., HIV-infection, viral hepatitis).<sup>25</sup>

**Table 5.** Serum testosterone (T) cutoffs suggested for the diagnosis of LOH.

Cutoff Provider	Biochemical Hypogonadism	Gray Area (Mild Biochemical Hypogonadism)	Normal T
Serum total T ng/dL (nmol/L)			
Endocrine Society Guidelines	<300 (<10.4)	–	>300 (>10.4)
AACE Guidelines	202(<7)	–	>202 (>7.0)
ISA, ISSAM, EAU, EAA, & ASA Guidelines	<231(<8)	231 < T < 346 (8 < T < 12.0)	>346 (>12.0)
EMAS Study	<317(<11)	–	>317(>11.0)
HIMS Study (<5 <sup>th</sup> percentile)	<185(<6.4)	–	>185(>6.4)
MMAS Study			
40–49 yrs	<251 (<8.7)	–	>251 (>8.7)
50–59 yrs	<216 (<7.5)	–	>216 (>7.5)
60–69 yrs	<196 (<6.8)	–	>196 (>6.8)
>70 yrs	<156 (<5.4)	–	>156 (>5.4)
Australian Clinical Pathology Laboratories	<185 (<6.4)	–	>185 (>6.4)
Serum free T pg/mL (pmol/L)			
ISA, ISSAM, EAU, EAA, & ASA Guidelines	<64.8 (<225)	–	>64.8 (>225)
EMAS Study	<63,4 (<220)	–	>63.4(>220)

<https://uroweb.org/guideline/male-hypogonadism/>

Notes. T: testosterone; AACE: American Association of Clinical Endocrinologists; ISA: International Society of Andrology; ISSAM: International Society for the Study of the Aging Male; EAU: European Association of Urology, EAA: European Academy of Andrology; ASA: American Society of Andrology; EMAS: The European Male Aging Study; MMAS: The Massachusetts Male Aging Study.

It is important to remark, however, that common laboratory assays used for free T measurement are not reliable and calculation of free T starting from total T, albumin and SHBG with equations that have been validated should be preferred.<sup>3,13,14,25,148</sup> Some guidelines<sup>12</sup> or large longitudinal studies<sup>3</sup> have provided thresholds also for calculated serum free T (<http://www.issam.ch/freetesto.htm>) (Table 5).

**Signs and symptoms of hypogonadism.** The diagnosis of hypogonadism requires both biochemical measurements and clinical features<sup>12,25,33,38,49,148,154</sup> (<https://uroweb.org/guideline/male-hypogonadism/>). Since very often the symptoms suggestive of low T are attributed to age among older men, recognizing hypogonadism is not easy.<sup>15,148</sup>

The interview is essential in order to disclose symptoms of hypogonadism, which belong mainly to sexual and physical functions.<sup>12,25,33,38,49,148,154</sup> (Fig. 3). The most specific symptoms related to the decrease of serum T are: reduced sexual desire (libido) and activity, decreased spontaneous erections in the morning, erectile dysfunction, reduced shaving frequency, decreased energy and vitality, and occasionally hot flushes<sup>3,12,25,38-40,49,147,148</sup> (Fig. 3). Other

symptoms are less specific (e.g., depressed mood and reduced muscle strength)<sup>12,25,33,38,49,148,154</sup> (Fig. 3).

At physical examination, signs suggestive of hypogonadism should be investigated, such as reduced pubic and axillary hair, reduced testicular size and volume of ejaculation<sup>12,25,33,38,49,148,154</sup> Figure 3 summarizes sign of hypogonadism according with to their specificity.<sup>3,12,25,38-40,49,147,148</sup>

In conclusion, the best way for the diagnosis is to combine serum T (total or free) with the symptoms,<sup>3,38</sup> regarding at LH as an adjunctive information useful to obtain a comprehensive picture of the pituitary gonadal axis and to find patients at higher risk to develop hypogonadism in the next future (compensated hypogonadism)<sup>90</sup> (Figure 2).

**Other examinations.** If low serum total T is confirmed, measurement of serum LH and follicle-stimulating hormone (FSH) is recommended in order to identify primary or secondary hypogonadism (Figure 2).<sup>12,25,33,38,49,148,154</sup> Both the conditions can be found in older men without specific endocrinological causes, nonetheless when LH and FSH levels are very low and suggest a central origin of hypogonadism, a global evaluation of pituitary function should be taken to exclude hypopituitarism.<sup>25,148</sup>

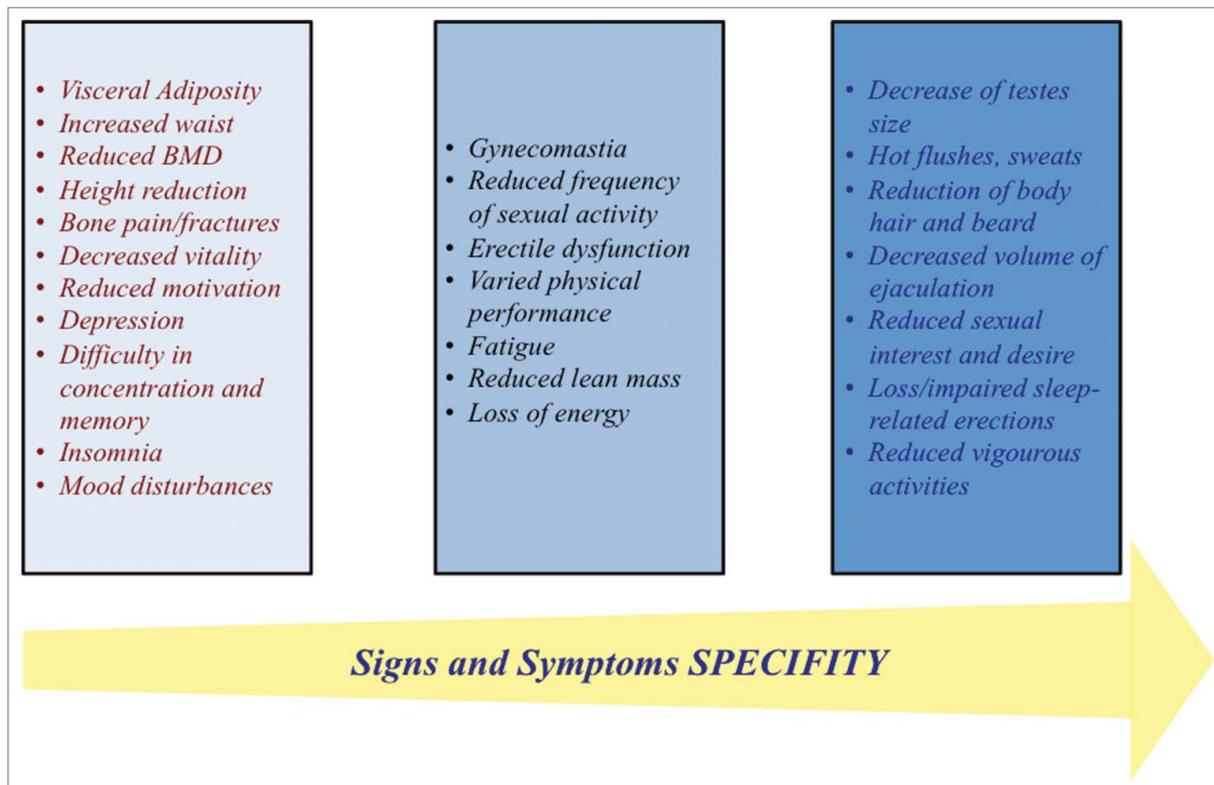


Figure 3. Signs and symptoms of late-onset hypogonadism according to their specificity.

Finally, bone densitometry should be performed in case of low T, because hypogonadism often is responsible for osteopenia, osteoporosis, and increased fracture risk in older men.<sup>48</sup>

### **Testosterone replacement treatment: Certain knowledge and controversies**

Since many factors involved in low T onset are reversible, life-style modifications, such as smoke abolition and moderate alcohol consumption, physical activity and weight loss should be suggested not only to avoid hypogonadism, but also for general well-being in older age.<sup>147</sup> Similarly, the resolution of acute illnesses,<sup>40,164,165</sup> or the improvement of chronic diseases that might cause hypogonadism<sup>153</sup> generally improve serum T as well as clinical condition in older men<sup>171</sup>

Patients who are candidate to receive T replacement are those with documented LOH, concomitant symptoms and/or signs of low T (especially sexual symptoms) and no contraindication to T treatment.<sup>12,25,33,38,49,148,154</sup> Treatment should be tailored according with to patient's health status and expectations after having considered the potential risk and after having informed the patients about possible risk and benefits.<sup>12,25,33,38,49,148,154</sup> Evidence coming from randomized trials in men older than 60 y is, in fact, still lacking and future large, prospective studies are required and worthwhile.<sup>189</sup>

At present, there is consensus against T treatment in men with a serum total T above 346 ng/dL (12 nmol/L).<sup>12,38,49,154</sup> or values of serum T very close to 346 ng/dL (<https://uroweb.org/guideline/male-hypogonadism/>).

T treatment is effective in improving sexual function, but this effect is not evident in men with normal serum T<sup>190</sup>. Other beneficial effects are related to the improvement of body composition and metabolic outcome<sup>40</sup>

It has been supposed that T treatment might increase CV risk (<http://www.fda.gov/Drugs/DrugSafety/ucm436259.htm>) on the basis of CV events reported in some of the T trials.<sup>189,191-194</sup> However, at present 4 different meta-analyses have not found this association to be consistent,<sup>194-197</sup> while only one confirmed an increased CV risk due to T treatment.<sup>198</sup>

Contraindications are documented prostate and breast cancer, and strong caution should be used when hematocrit is increased (>50%).<sup>12,25,33,38,49,148,154</sup> Other aspects that should be considered are congestive heart failure, sleep apnea, benign prostatic hyperplasia and a prostate specific antigen (PSA) >4 ng/mL.<sup>12,25,33,38,49,148,154</sup> Furthermore, patients with a recent (within 6 months) history of CV events should not be considered as candidate to receive T replacement treatment.<sup>38,40,49,189</sup>

The patient should be informed about potential benefits that involve mainly sexual desire and function, vigor, body composition changes and prevention of bone loss. At the same time, patient should be aware that T treatment in older men might be harmful and that evidence in favor of its safety is not of good quality.

### **Unresolved issues**

Sexual symptoms are the most useful to prompt the diagnosis of LOH, but they are also difficult to unmask in the consistent percentage of patients (especially older men) who are reluctant to talk about them with physicians.<sup>199</sup> In addition, even physicians are less prone to interview aging men about their sexuality.<sup>200</sup>

We know that among men with a diagnosis of LOH there are false positive and that this diagnosis is probably overlooked in other men (false negative). However, it is very difficult to have precise data concerning the accuracy of LOH diagnosis due to the lack of certain diagnostic criteria.

Concerning the diagnosis of LOH, there is still a gap of knowledge about the significance of great serum T decrease from the baseline overtime (e.g., 50%) leading to a serum value still in the range of eugonadism, but much lower than in the past. It is possible that patients with this kind of serum T decline trajectory become truly hypogonadal and already display sign and symptoms of hypogonadism.<sup>38</sup> Furthermore, data on the natural history of LOH are scanty.<sup>171</sup>

From literature we know that LOH might be reversible in about half of the patients,<sup>171</sup> but at present we are not able to predict, among patients with LOH, who will return to normal androgenization, except for those patients who are obese or overweight and loose weight.<sup>147</sup>

In the field of geriatrics is getting growing importance the concept of frailty, which is defined as a condition of vulnerability to stressing events. The condition of frailty derives from accumulation of deficits in multiple physiological systems and can be quantified by a frailty index. At present, the impact of T decline on frailty remains to be determined. Accordingly, it is not clear if T treatment is able to improve or worsen frailty in older men. Similarly, the potential for T treatment to be harmful in sick older men or in men with HIV infection and a poor health status remains an unsolved issue.<sup>189</sup>

At present, no association between serum T and life expectancy was found by comparing long-lived with short-lived subjects.<sup>201</sup> However, differences in lifespan between men and women suggest a putative role of sex hormones on longevity.<sup>202</sup> What we know is that lifespan was not different between singers castrated before puberty and non-castrated singers, suggesting that T does not influence longevity.<sup>203</sup> T,

probably might protect centrally from the effects of stress by mitigating the central cascade of events induced by the exposure to stress, while women are more exposed the negative effect of stress.<sup>204</sup> How much gender differences in the exposure to sex steroids at any age are involved in determining longevity and the response to stress remains to be established in detail.<sup>202,204</sup>

## Conclusions

The decline of sex steroids with advancing age depends on gender and interindividual differences. In men physiological changes should be differentiated from pathological low serum T and the coupling low serum T with hypogonadism-related signs and symptoms is a good strategy to diagnose LOH and to identify patients who might benefit from T replacement. However, the specificity of symptoms of hypogonadism is low and measurements of serum T insidious, thus all these factors increase the risk of misdiagnosis. The presence/absence of concomitant acute or chronic illnesses could lead the physician avoiding misdiagnosis and overtreatment. Patients' health status, in fact, should be taken into account prior to start T treatment to prevent adverse events that are more common in men with poor clinical conditions. Replacement treatment might be effective in improving sexual function, body composition, metabolic outcome, and vitality. In older men T treatment is not based on good evidence and the patient should be informed about the lack of proven beneficial effects as well as on the possible side effects and adverse events.

## Abbreviations

AACE	American Association of Clinical Endocrinologists
ASA	American Society of Andrology
BLSA	The Baltimore Longitudinal Study of Aging
BMD	bone mineral density
BMI	body mass index
CHAMP	The Concorde Health and Aging in Men Project
COPD	chronic obstructive pulmonary disease
CV	cardiovascular
E <sub>2</sub>	estradiol
EAA	European Academy of Andrology
EAU	European Association of Urology
ED	erectile dysfunction
EMAS	European Male Aging Study
F	females
FSH	follicle-stimulating hormone

fT	free testosterone
GH	growth hormone
GnRH	gonadotropin-releasing hormone
HIMS	The Health in Men Study
HIV	Human Immunodeficiency Virus
IAs	Immunoassays
IGF-1	insulin-like growth factor-1
ISA	International Society of Andrology
ISSAM	International Society for the Study of the Aging Male
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LH	luteinizing hormone
LOH	late-onset hypogonadism
M	males
MC-PLOC-B	MultiCenter, Prospective, Longitudinal, Observational, Community-Based Study
MMAS	The Massachusetts Male Aging Study
MrOS	The Osteoporotic Fractures in men Study
MS	mass spectrometry
n.a.	not available
P-BRT	Population-Based, Randomized, Trial
PLOC-B	Prospective, Longitudinal, Observational, Community-Based Study
PSA	prostate specific antigen
RBS	The Rancho Bernardo Study
SHBG	sex-hormone binding globuline
T	testosterone
TSH	thyrotropin hormone
TT	total testosterone
US	ultrasonography.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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## ORCID

Vincenzo Rochira  <http://orcid.org/0000-0001-8169-0696>

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