

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Wu FCW, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. *N Engl J Med* 2010;363:123-35. DOI: 10.1056/NEJMoa0911101.

## Supplementary Appendix- Section 1: Thirty two candidate symptoms suggestive of androgen deficiency elicited by self-completed and interviewer-assisted questionnaires in the European Male Aging Study (EMAS)

Questions	
<i>Sexual symptoms</i>	
EMAS SFQ	How often did you think about sex? How would you rate your level of sexual desire? How satisfied have you been with your overall sexual life? How frequently did you awaken with a full erection?
MMAS	Were you able to get and keep an erection which would be good enough for sexual intercourse?
<i>Physical symptoms:</i>	
SF36:	During a typical day, does your health limit you in these activities? If so, how much: Vigorous activities (such as running, lifting heavy objects, participating in strenuous sports) Moderate activities (such as moving a table, pushing a vacuum cleaner, bowling or playing golf) Lifting or carrying groceries Climbing several stairs Climbing one flight of stairs Bending , kneeling or stooping Walking more than a kilometre Bathing yourself Walking in your own home Getting up from a chair Feeding yourself Cut down on the amount of time spent on work or other activities
<i>Psychological symptom :</i>	
SF36	Have you felt so down in the dumps that nothing could cheer you up? Do you feel downhearted and low? Have you been a very nervous person? Do you feel full of life? Do you have a lot of energy? Do you feel tired? Do you feel worn out?
BDI	How you assess your irritability in the past 2 weeks? Have you lost interest in other people or activities? How you assess your ability to concentrate in the past 2 weeks? Have you experienced any changes in sleeping pattern? Do you have enough energy to do things? Do you feel more tired or fatigued than usual? How you assess your sadness in the past 2 weeks? Do you feel worthless?

EMAS SFQ: EMAS Sexual Function questionnaire, SF36: Short Form 36 health survey questionnaires, BDI: Beck Depression Inventory.

MMAS: Massachusetts Male Aging Study (Derby CA, Araujo AB, Johannes CB, Feldman HA, McKinlay JB. Measurement of erectile dysfunction in population-based studies: The use of a single question self-assessment in the Massachusetts male aging study. Int J Impot 2000;12:197–204.) All BDI items describe a self reported feeling in the past two weeks including the day of the interview.

### ***Symptomatic and asymptomatic response categories:***

The operational definition of symptomatic and asymptomatic response categories (Table 2 in the manuscript) is based on validated criteria (e.g. MMAS erectile dysfunction (1, 2), ESFQ morning erection and sexual thoughts (3), clinical face validity (e.g. morning erection, and sexual thoughts) and construct/criterion validity (e.g. limitation in vigorous activity and fatigue), supported by similarity in prevalence of the symptoms as defined, compared to published studies (external validity)(1-10).

### **References:**

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## Supplementary Appendix- Section 2: Three symptoms related to testosterone: symptom relationships with testosterone and prevalence of symptoms in the training set

### *Sexual symptom - Decreased morning erections*

**Question - How frequently did you awaken with a full erection?**

Response category	Response label	Response prevalence
1	None	444 (28.8)
2	1 last month	170 (11.0)
3	2-3 times month	229 (14.9)
4	1 week	187 (12.1)
5	2-3 times week	325 (21.1)
6	4-6 or more a week	110 (7.1)
7	1+ day	75 (4.9)

POLR<sup>‡</sup>: OR (95% CI)

GOLR<sup>†</sup>: OR (95% CI)

	Total Testosterone (nmol/liter)	Free Testosterone (pmol/liter)
	1.02 (1.005, 1.03)	
7 to 2 vs 1		1.02 (1.01, 1.04)
7 to 3 vs 2 to 1		1.02 (1.004, 1.03)
7 to 4 vs 3 to 1		1.02 (1.01, 1.04)
7 to 5 vs 4 to 1		1.02 (1.01, 1.04)
7 to 6 vs 5 to 1		1.02 (0.99, 1.05)
7 vs 6 to 1		1.01 (0.97, 1.05)

### *Physical symptom – Limitation in vigorous activity*

**Question - Does your health limit you in doing vigorous activities (such as running, lifting heavy objects, participating in strenuous sports)? If so, how much?**

Response category	Response label	Response prevalence
1	Limited	389 (24.7)
2	Limited a Little	684 (43.5)
3	Not limited	501 (31.8)

POLR<sup>‡</sup>: OR (95% CI)

	Total Testosterone (nmol/liter)	Free Testosterone (pmol/liter)
	1.01 (1.01, 1.02)	1.01 (0.99, 1.02 )

### *Psychological symptom - Fatigue*

**Question - Do you feel more tired or fatigued than usual?**

Response category	Response label	Response prevalence
1	Worse/worst	87 (5.5)
2	Medium	679 (42.9)
3	Ok	817 (51.6)

POLR<sup>‡</sup>: OR (95% CI)

GOLR<sup>†</sup>: OR (95% CI)

	Total Testosterone (nmol/liter)	Free Testosterone (pmol/liter)
	1.01 (0.99, 1.02)	
3 to 2 vs 1		1.05 (1.02, 1.07)
3 vs 2 to 1		1.01 (0.99, 1.03)

POLR: Proportional ordinal logistic regression model GOLR: Generalised ordinal logistic regression model. OR: Odds ratios, CI: Confidence Interval. For total testosterone, OR corresponds to 1 nmol/L change. For free testosterone, OR corresponds to 10 pmol/L change. Models were adjusted for age and center. ‡The proportional odds assumption was verified and the model was found not to violate the proportional odds/parallel-lines assumption. †The proportional odds assumption was not imposed.

*Statistical note:*

Supplementary Appendix- Section 2 shows examples of three symptoms found to be significantly associated with Total Testosterone and/or Free Testosterone. One symptom is presented from each of the three domains (sexual, physical and psychological).

The hierarchy of standardised responses to a question provided ordinal response items. We used ordinal regression models to test the association between response categories and Testosterone adjusted for age and center. The coefficients from ordinal logistic regression models, represented as odds ratios (ORs), can be interpreted in the same way as the ORs from binary logistic regression models.

The most widely used type of ordinal regression model is the proportional odds logistic regression model (POLR). POLR considers the proportional odds (PO) assumption to be valid. This means that the ORs are independent of the cut-off level of the response (i.e. similar ORs are found for each response categories irrespective of cut-off point of the response). This is the case for the symptom “limitation in vigorous activity”. Thus an OR of 1.01 indicates that lower Total Testosterone is associated with an increased likelihood that a subject will be observed in the lower categories (i.e. with more limitation of vigorous activity) as opposed to being in the higher categories, regardless of the level of response.

If PO assumption is not verified, the generalized ordinal logistic regression (GOLR) (McCullagh P, Nelder JA. Generalized Linear Models. Chapman & Hall: London, 1999.) model is more appropriate to use, because it allows for different ORs at different cut-off points of the response. For example, the OR for ‘fatigue’, was significant at 1.05 for each 10 pmol/L decrease in Free Testosterone for the comparison of category 3 to 2 vs 1, but not significant at 1.01 (0.99, 1.03) for the category comparison of 3 vs 2 to 1.

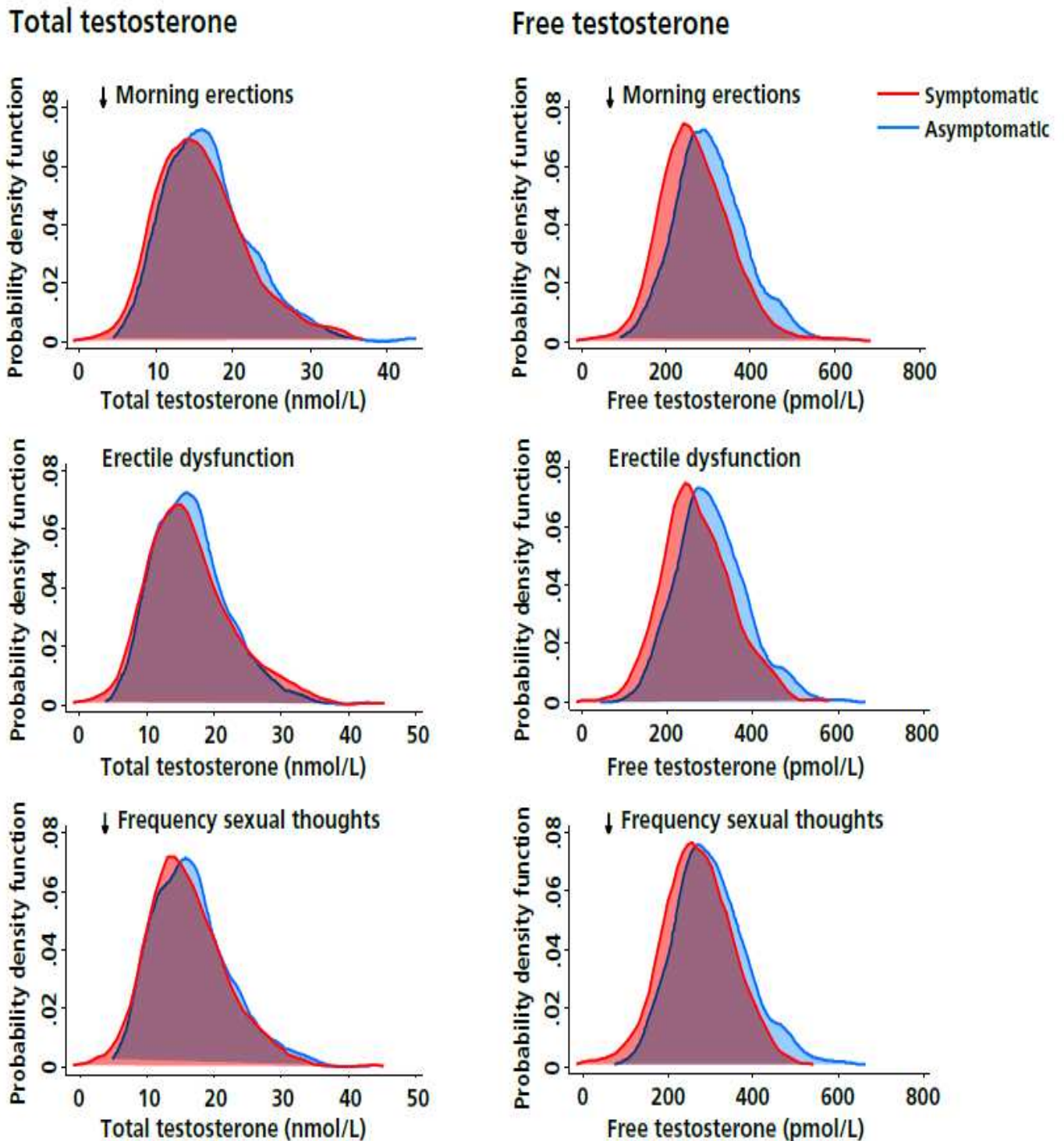
### Supplementary Appendix- Section 3: Identification of testosterone thresholds using linear spline logistic regression models from the training dataset

Symptoms	Total Testosterone (nmol/liter)		Free Testosterone (pmol/liter)	
	Linear splines	OR (95% CI)	Linear splines	OR (95% CI)
<i>Sexual</i>				
↓ Morning erections	< <b>11</b>	<b>1.10 (1.02, 1.19)</b>	< <b>280</b>	<b>1.05 (1.02, 1.07)</b>
	≥11	1.01 (0.99, 1.04)	≥280	1.00 (0.98, 1.02)
Erectile dysfunction	< <b>8.5</b>	<b>1.23 (1.06, 1.42)</b>	< <b>280</b>	<b>1.03 (1.01, 1.05)</b>
	≥8.5	0.99 (0.97, 1.01)	≥280	0.99 (0.97, 1.01)
↓ Frequency sexual thoughts	< <b>8</b>	<b>1.48 (1.20, 1.83)</b>	< <b>160</b>	<b>1.16 (1.01, 1.34)</b>
	≥8	1.01 (0.99, 1.03)	≥160	1.01 (0.98, 1.03)
<i>Physical</i>				
↓ Vigorous activity	< <b>13</b>	<b>1.11 (1.03, 1.19)</b>	<280	1.03 (0.99, 1.07)
	≥13	0.99 (0.97, 1.00)	≥280	1.00 (0.98, 1.02)
Limited in walking >1 kilometer	<13	1.03 (0.92, 1.15)	<280	1.02 (0.96, 1.08)
	≥13	1.03 (0.99, 1.07)	≥280	1.02 (0.95, 1.09)
Unable to bend	<13	0.99 (0.87, 1.13)	<280	1.02 (0.98, 1.07)
	≥13	1.01 (0.98, 1.04)	≥280	0.99 (0.95, 1.03)
<i>Psychological</i>				
Downheartedness	<10	1.16 (0.96, 1.40)	< <b>160</b>	<b>1.13 (1.08, 1.20)</b>
	≥10	0.98 (0.96, 1.00)	≥160	1.03 (0.99, 1.07)
Loss of energy	<10	0.95 (0.72, 1.26)	<160	0.99 (0.84, 1.18)
	≥10	1.00 (0.97, 1.03)	≥160	1.03 (0.99, 1.06)
Fatigue	<11	1.14 (0.84, 1.53)	<160	1.00 (0.86, 1.16)
	≥11	1.01 (0.96, 1.06)	≥ <b>160<sup>T</sup></b>	<b>1.06 (1.04, 1.08)</b>

All regression models adjusted for age and centre. For Total Testosterone, odds ratio (OR) corresponds to 1 nmol/L decrease in each Total Testosterone spline (bin). For Free Testosterone, OR corresponds to 10 pmol/liter decrease in each Free Testosterone spline (bin), CI: Confidence Interval. Testosterone threshold highlighted in bold. <sup>T</sup>10 pmol/L decrease above 160 pmol/L is associated with an increased OR of 1.06.

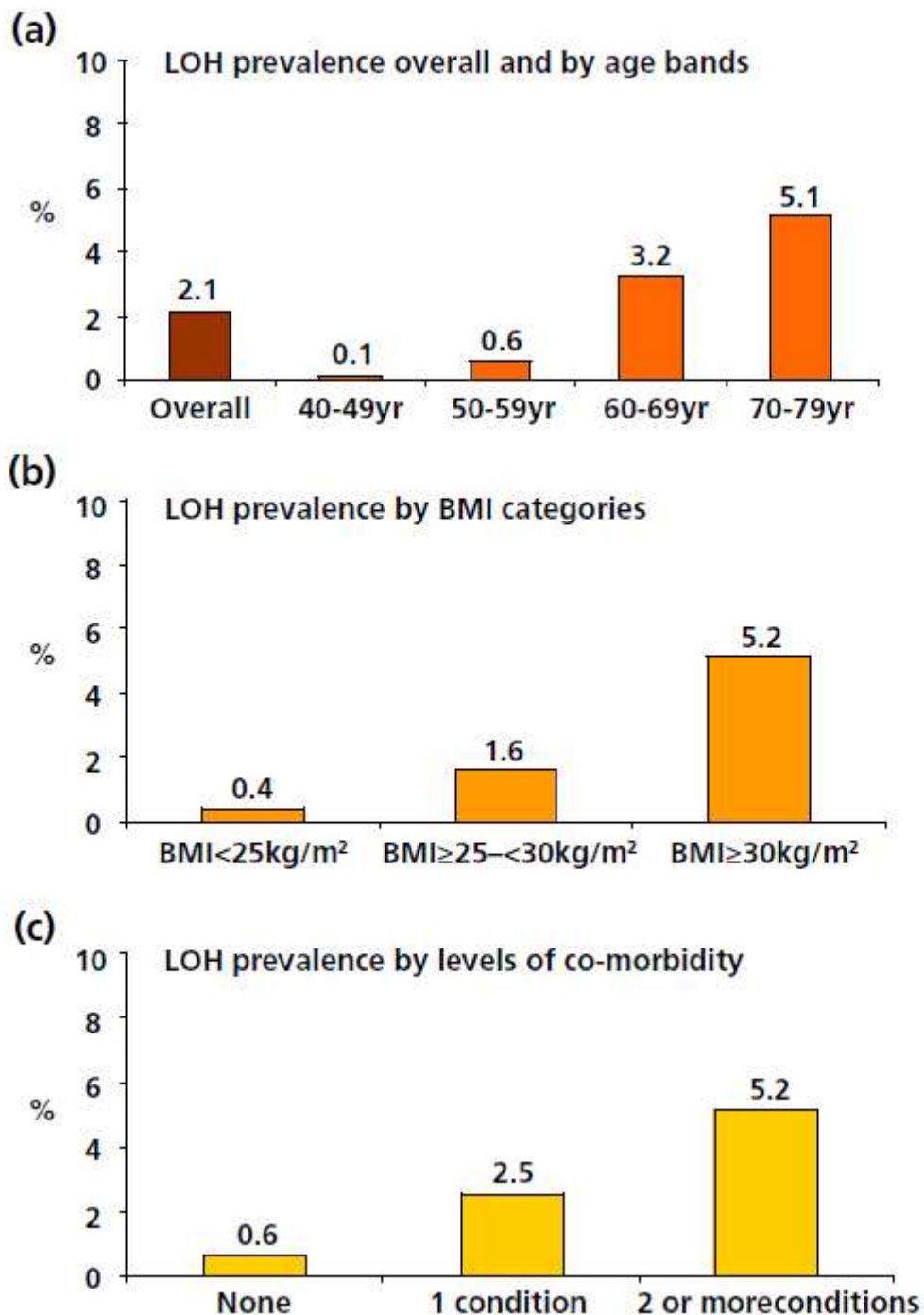
## Supplementary Fig. 1: Testosterone distributions by symptom categories

Distributions of total and free testosterone for symptomatic and asymptomatic groups for the three sexual symptoms (decreased frequency of morning erections, erectile dysfunction and decreased frequency of sexual thoughts) using kernel probability density function in the training dataset. This shows only small differences in mean testosterone levels between symptomatic and asymptomatic men.



## Supplementary Fig. 2: The prevalence of the syndrome in EMAS, overall and stratified by age, BMI and co-morbidity

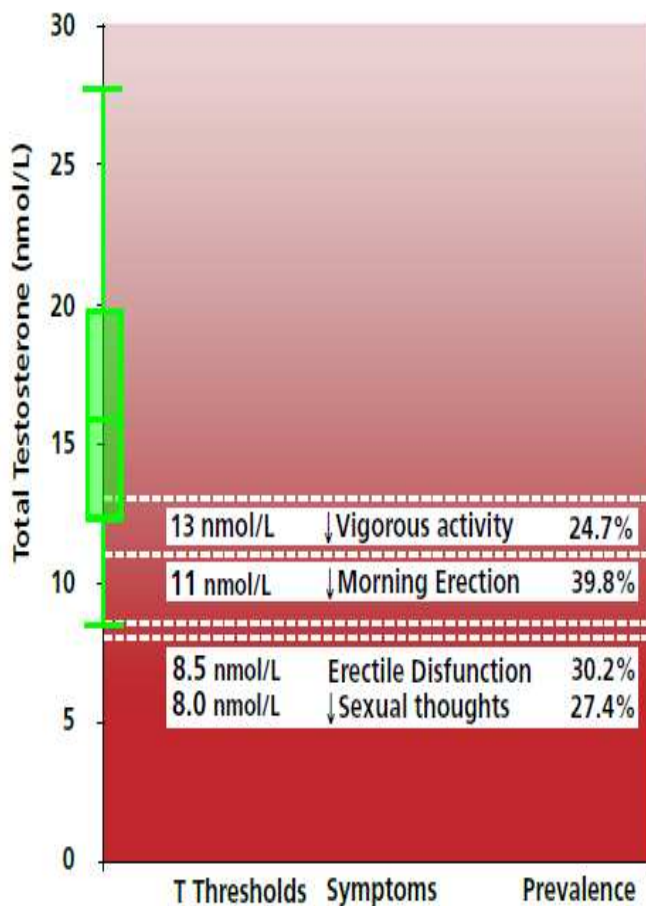
The syndrome of late-onset hypogonadism as defined by at least three sexual symptoms associated with total testosterone levels of  $<11$  nmol/L and free testosterone of  $<220$  pmol/L. The overall prevalence of late-onset hypogonadism is 2.1% (a), the prevalence of late-onset hypogonadism increased with age from 0.1%, 0.6%, 3.2% to 5.1% at 40–49, 50–59, 60–69 and  $>70$  yr respectively (a). The prevalence of late-onset hypogonadism also increased with BMI (b) and co-morbidity (number of coexisting illnesses) (c).



### Supplementary Fig. 3: Testosterone thresholds and symptom prevalence

Testosterone thresholds below which the prevalence of the respective symptoms increased significantly. This shows different testosterone thresholds for various symptoms and summarises data from Figure 1 (of the main manuscript) and Supplementary Appendix Section 3. The distribution of total testosterone (a) and free testosterone (b) in the EMAS cohort (training dataset) is represented by the green box plot in the Y axis, where the median total testosterone is 15.9, inter-quartile range, 5th and 95th percentiles are 19.8 - 12.3, 8.5 and 27.8 nmol/L respectively, and for free testosterone the median, 236.1, inter-quartile range, 5th and 95th percentiles are 343.2 - 236.1, 164.4 and 450.7 pmol/L, respectively.

(a) Total testosterone



(b) Free testosterone

