Rastrelli, G. et al. (2018) Symptomatic androgen deficiency develops only when both total and free testosterone decline in obese men who may have incident biochemical secondary hypogonadism: prospective results from the EMAS. *Clinical Endocrinology*, 89(4), pp. 459-469.(doi:10.1111/cen.13756)

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Article type : 1 Original Article - UK, Europe

Symptomatic Androgen Deficiency Develops only When Both Total and Free Testosterone Decline in Obese Men Who may have Incident Biochemical Secondary Hypogonadism: Prospective Results from the EMAS

Short title: Hypogonadism: Role of Free Testosterone

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Declaration of interest: GR, RJAHE, TWON, TA, GB, FFC, GF, BK, AG, TSH, JSH, MEJL, NP, MP, LA, JT, DV, MM have nothing to declare. Frederick Wu has acted as a consultant for Bayer-Schering, Eli Lilly and Besins Healthcare and also participated in advisory board meetings and lectured on their behalf. Frederick Wu has received lecture fees from Bayer-Schering and Besins Healthcare. Frederick Wu has received grant support (2010-2014) from Bayer Schering AG and Besins Healthcare. Ilpo Huhtaniemi is a consultant and/or has received grants from Ferring Pharmaceuticals and Novartis.

Funding: The European Male Aging Study is funded by the Commission of the European Communities Fifth Framework Program “Quality of Life and Management of Living Resources” Grant QLK6-CT-2001-00258 and facilitated by the Manchester Biomedical Research Centre and the NIHR Greater Manchester: Clinical Research Network. Additional support was also provided by Arthritis Research UK and the National Institute for Health Research and the Manchester Musculoskeletal Biomedical Research Centre. The Principal Investigator of EMAS is Professor Frederick Wu, MD; Andrology Research Unit, University of Manchester, Manchester, UK. Prof. Dirk Vanderschueren is a senior clinical investigator supported by the Clinical Research Fund of the University Hospitals Leuven, Belgium. Robert J.A.H. Eendebak is supported by a Biotechnology and Biological Sciences Research Council – Doctoral Training Partnership (BBSRC-DTP) PhD-fellowship and is grateful for support received from the Fundatie van de Vrijvrouwe van Renswoude and Scholten-Cordes scholarship foundations.

Author contributions: G.R., R.J.A.H.E. and F.C.W.W. interpreted the data. R.J.A.H.E. analysed the data. G.R. and F.C.W.W. wrote the manuscript. F.C.W.W. developed the concept. G.R. and F.C.W.W. designed the study. F.C.W.W. designed and led the European Male Ageing Study. M.M. assisted with interpretation of the data and preparation of the manuscript. G.F., G.B., F.F.C., J.S.H., M.P., A.G., D.V. collected data. All of the authors reviewed and edited the manuscript.

Summary

Objective: Limited evidence supports the use of free testosterone (FT) for diagnosing hypogonadism when sex hormone binding globulin (SHBG) is altered. Low total testosterone (TT) is commonly encountered in obesity where SHBG is typically decreased. We aimed to assess the contribution of FT in improving the diagnosis of symptomatic secondary hypogonadism (SH), identified initially by low total testosterone (TT), and then further differentiated by normal FT (LNSH) or low FT (LLSH).

Design: Prospective observational study with a median follow-up of 4.3 years.

Patients: 3369 community-dwelling men aged 40-79 years from eight European centres.

Measurements: Subjects were categorised according to baseline and follow-up biochemical status into persistent eugonadal (referent group; n=1880), incident LNSH (eugonadism to LNSH; n=101) and incident LLSH (eugonadism to LLSH; n=38). Predictors and clinical features associated with the transition from eugonadism to LNSH or LLSH were assessed.

Results: The cumulative incidence of LNSH and LLSH over 4.3 years was 4.9% and 1.9% respectively. Baseline obesity predicted both LNSH and LLSH but the former occurred more frequently in younger men. LLSH, but not LNSH, was associated with new/worsened sexual symptoms, including low desire [OR= 2.67 (1.27-5.60)], erectile dysfunction [OR= 4.53 (2.05-10.01)] and infrequent morning erections [OR= 3.40 (1.48-7.84)].

Conclusions: These longitudinal data demonstrate the importance of FT in the diagnosis of hypogonadism in obese men with low TT and SHBG. The concurrent fall in TT and FT identifies the minority (27.3%) of men with hypogonadal symptoms, which were not present in the majority developing low TT with normal FT.

Keywords: Free testosterone, total testosterone, secondary hypogonadism, obesity, sexual symptoms, androgen deficiency, sex hormone binding globulin

Introduction

According to the “free hormone hypothesis”, the biological activity of hormones is assigned to the fraction that is not bound to plasma proteins[1]. This would imply that the total hormone concentration may not always provide an accurate readout of hormone activity, particularly in the presence of altered binding protein concentrations. Although widely accepted in endocrinology, the “free hormone hypothesis” still lacks robust experimental proof. Recently,

support for this hypothesis for sex hormones has been provided using a transgenic mouse model over-expressing the human sex hormone binding globulin (SHBG)[2]. As compared to wild-type controls, SHBG-transgenic animals had similar free T (FT) levels, but significantly higher total testosterone (TT) and luteinising hormone (LH)[2]. Despite the high TT, these animals displayed features consistent with mild androgen-deficiency[2], thus supporting the view that TT does not accurately reflect androgen status in the presence of SHBG abnormalities. Compelling support for the free hormone hypothesis also comes from patients with naturally occurring mutations in the SHBG gene. In a young man who carried a missense mutation in SHBG gene resulting in extremely low/undetectable levels of SHBG[3], TT was very low whereas FT and LH were normal. Despite this, sexual development, secondary sexual characteristics and semen parameters were normal. Conversely, women with normal T and very low SHBG, due to SHBG gene mutations, were reported to have features of hyperandrogenism[4,5].

The relative roles of TT and FT in the assessment of androgen deficiency have not been extensively investigated. At present, the diagnosis of androgen deficiency in symptomatic men relies largely on TT[6,7]. The use of FT is tentatively limited to symptomatic men with borderline TT and/or conditions known to affect SHBG[6,7], a recommendation based more on expert opinion than hard data. There is an urgent need for more and better clinical and experimental evidence to assess the role of FT as an independent biomarker of androgen function. Recently a cross-sectional analysis of the European Male Ageing Study (EMAS), reported a higher probability of androgen deficiency symptoms in men with low FT and low TT but not in men with normal FT and low TT[8]. This strongly supports the view that documenting low FT (in addition to low TT) can improve the accuracy of diagnosing symptomatic hypogonadism.

In a prospective analysis of the EMAS[9], we showed that obese eugonadal men are at increased risks for developing biochemical secondary hypogonadism (SH) with a concomitant deterioration in sexual function compatible with symptomatic androgen deficiency. However, SH was defined according to TT and it is unclear whether lower SHBG may have contributed to the observed decline in TT (without a concomitant fall in FT), thereby potentially inflating the incidence/diagnosis of SH in obese men[10].

We hypothesise that the addition of FT to TT in the categorisation of SH can improve the precision in identifying men who develop not only biochemical SH but also accompanying features of androgen deficiency. Hence, the aim of the present study is to compare and contrast the natural history and clinical characteristics of developing SH as defined by low TT with low FT and low TT but normal FT.

Materials and Methods

Participants and study design

The study design and methods of the EMAS have been previously described extensively[11,12]. Briefly, EMAS is a multicentre, prospective, population-based survey on an age-stratified sample of 3369 unselected community-dwelling men aged 40-79 years (mean ± standard deviation 60±11) recruited from population registers in eight European centres: Manchester (United Kingdom), Leuven (Belgium), Malmö (Sweden), Tartu (Estonia), Lodz (Poland), Szeged (Hungary), Florence (Italy) and Santiago de Compostela (Spain). Participants were invited to attend the local research clinic for baseline and follow-up (after a median of 4.3 years; range 3.0-5.7) assessments[11,12]. During this period, 193 men died and 440 were lost to follow-up. At both baseline and follow-up, all participants completed questionnaires dealing with degree of education, smoking habits, alcohol intake and currently treated illnesses[11,12]. Moreover, they completed the EMAS Sexual Function Questionnaire, the Medical Outcomes Study 36-item Short-Form health survey and the Beck Depression

Inventory dealing with sexual, physical and psychological health, respectively. Based on previous data from EMAS[13], three sexual (erectile dysfunction, decrease in sexual thoughts and decreased morning erections), physical (decreased vigorous activity, limited walking and decreased bending) and psychological symptoms (fatigue, loss of energy and sadness) were considered as the most informative for androgen deficiency. Presence/absence of symptoms at baseline or follow-up was defined according to previously identified thresholds[13]. The change in symptoms was evaluated as development/worsening (symptom absent at baseline and present at follow-up or present at baseline but with a lower severity grading than at follow-up)[9,14]. Anthropometric measurements, Reuben's physical performance test, and psychomotor processing speed (digit symbol substitution test) were performed at baseline and follow-up according to standardised methods[11,12]. Ethical approval was obtained according to local regulations in each centre. All men provided a written informed consent.

Hormone measurements

Single fasting morning (before 10:00 AM) venous blood samples were obtained at baseline and follow-up. T was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS), with paired baseline and follow-up samples analysed simultaneously. LH and SHBG were measured by the E170 platform electrochemiluminescence immunoassay (Roche Diagnostics). FT was calculated using the Vermeulen formula[15]. Intra- and interassay coefficients of variation (CVs) were 4.0% and 5.6% for T, 1.7 and 3.2% for SHBG and 1.9 and 3.0% for LH. The lower limit of TT measurement was 0.17 nmol/L (0.05 ng/mL). Insulin was measured by chemiluminescence (CVs 3.9 and 5.0%). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) as calculated by $\frac{insulin\left(\frac{mU}{L}\right)\times glucose\left(\frac{mmol}{L}\right)}{22.5}$. Biochemistry and haematology parameters were assessed by standardised methods in hospital laboratories in each centre.

Gonadal status

In keeping with previous EMAS classifications[9,14,16], eugonadism was defined as TT≥10.5 nmol/L and secondary hypogonadism (SH) as TT<10.5 nmol/L and LH≤9.4 U/L. In the present analysis, SH was further subdivided, according to FT levels, into normal FT (≥170 pmol/L) with low TT (Low TT and Normal FT: LNSH) and low FT (<170 pmol/L) with low TT (Low TT and Low FT: LLSH). Men in the analytical sample were categorised on the basis of TT into two main groups, according to their change in gonadal status: 1) persistent eugonadism (PE; eugonadism at baseline and follow-up; referent group) and 2) incident SH (eugonadism at baseline and SH at follow-up). The second group was further subdivided according to gonadal status at follow-up (incident LNSH or LLSH). The group of men (n = 30 at baseline and n = 2 at follow-up) with normal TT but low FT was not included in the analysis because the research question was aimed at assessing the relevance of introducing FT to the previous definition of SH based on low TT[9].

Since a validated reference range for FT is lacking[17], we conducted exploratory analyses on our sample in order to identify an appropriate FT threshold. We modelled logistic regressions using incident low FT as the independent variable (with persistent normal FT as referent group) to predict the development/worsening of androgen deficiency symptoms[9,14]. The definitions of incident low FT were based on FT values only (without reference to TT) and were constructed using various putative thresholds by the stepwise decline of FT (by 10 pmol/L) below 250 pmol/L. FT values of 180 and 170 pmol/L emerged as the best predictors of worsening erectile dysfunction and decreased morning erection (Supplementary Figure 1). This accorded with the lower reference limit of FT of 170 pmol/L suggested by the Endocrine Society guidelines of 2010[18] based on results from several commercial laboratories measuring FT by equilibrium dialysis. In addition, it confirmed our previous cross-sectional analyses[13]. We therefore selected the FT threshold of 170 pmol/L for constructing the operational definition of SH in the present analyses.

Statistical analysis

The differences between the analytical groups were initially assessed by the analysis of variance and chi-squared tests for continuous and categorical variables, respectively. Post hoc analyses were performed using the Tukey–Kramer test and Z-test with correction for multiple pairwise comparisons. Multilevel binary logistic regression models were used to assess the associations between nine putative predictors[9,14] and changes in gonadal status (transition from eugonadism to SH) as outcomes, with PE being the referent category. The relationship between change in gonadal status and change in clinical features of hypogonadism was assessed by binary logistic regression models with nine putative symptoms of hypogonadism as outcomes[9,14]. Results from logistic regressions are presented as odds ratios (ORs) and 95% confidence intervals (CIs). With respect to the *recovery* from LNSH and LLSH to eugonadism, the small sample size allowed only the assessment and comparison of recovery rates. All statistical analyses were conducted using Stata SE 13.1 (StataCorp, College station, TX, USA). A p-value <0.05 was considered as significant.

Results

Gonadal status transition

Figure 1 describes the derivation of the analytical sample from the initial population of 3369 EMAS participants. After excluding those who died (n=193) or were lost to follow-up (n=440), a further 132 and 162 men, with diagnosed pituitary, testicular or adrenal diseases or use of medications known to affect the hypothalamic-pituitary-gonadal axis at baseline and follow-up, were excluded. Gonadal status at baseline or follow-up could not be defined due to missing data in 121 men. A further 53 and 32 men were excluded because of biochemical primary hypogonadism or normal TT but low FT respectively, at baseline or follow-up (Figure 1). This provided 2236 eligible men in the analytical sample, their differences from those who died or were lost to follow-up are shown in Supplementary Table 1. 2019 eugonadal men at

baseline were included in the analytical sample for capturing progression to SH, while 217 men who already had SH at baseline were considered only for describing recovery rates.

Of the 2019 EUG men at baseline, 1880 were classified as PE at follow-up, 139 developed SH, of whom 101 (72.7%) had FT≥170 pmol/L (LNSH) and 38 (27.3%) had FT<170 pmol/L (LLSH) (Figure 1). Incidence for LNSH and LLSH was 4.9% and 1.9% respectively over 4.3 years. Assuming linearity of transition, this translates to an LNSH incidence rate of 114.5 per 10,000 per year, or 1.1% per annum and for an LLSH incidence rate of 43.1 per 10,000 per year, or 0.4% per annum.

Characteristics of the analytical groups

Incident LNSH men were younger than incident LLSH subjects (Table 1). No differences in education level, marital status, smoking habits and alcohol intake at baseline and follow-up were found between the three groups (Table 1).

Hormone levels

At baseline, by definition, all men had TT and FT within the normal range, although their levels were already significantly lower in incident LNSH and LLSH than in PE (Table 1). SHBG at baseline was the lowest in incident LNSH, whereas incident LLSH had intermediate levels and PE men had the highest (Table 1). At follow-up, the differences in SHBG between the three groups followed those found at baseline. SHBG had different trends over time in the three groups, being significantly increased in PE, decreased in incident LNSH and unchanged in incident LLSH.

Anthropometrics and metabolic parameters

At baseline, both incident LNSH and LLSH men had significantly greater BMI, waist circumference (WC) and body weight than PE subjects (Table 1). At follow-up, the differences in BMI and WC between PE and both the incident SH groups were even greater. Incident LNSH was characterised at baseline by higher triglycerides than PE, whereas incident LLSH had lower HDL-cholesterol and higher HOMA-IR than PE, with a trend towards higher fasting glucose (Table 1). At follow-up, the metabolic pattern of incident LNSH men significantly worsened, with higher fasting glucose and HOMA-IR and triglycerides as well as lower HDL-cholesterol compared to PE. In contrast, incident LLSH men maintained their metabolic profile largely unchanged (Table 1). Both the SH groups showed a higher prevalence of cardiovascular diseases (CVDs) than PE, particularly at follow-up (Table 1).

Predictors of incident LNSH

Multiple logistic regression analysis showed that overweight and obesity at baseline were both significant risk factors for transition from eugonadism to LNSH (OR=2.12[1.06-4.22], p=0.033 and 4.18[2.00-8.71], p<0.001 respectively) (Table 2). Younger age was a further significant predisposing factor (OR=3.24[1.40-7.47], p=0.006) for LNSH. Substituting waist circumference (WC) for BMI confirmed the association with LNSH (OR=2.66[1.36-5.22], p=0.004 and 4.48[2.35-8.55], p<0.001 for WC 94-102 and >102 cm, respectively).

Predictors of incident LLSH

Obesity (Table 2) and WC≥102 cm significantly predicted the development of LLSH (OR=5.85 [1.86-18.37], p=0.003 and OR=3.36[1.26-9.01], p=0.016 respectively).

Symptoms associated with incident LNSH

There was no difference in sexual, physical or psychological symptoms between incident LNSH and PE men (Table 1) at baseline or follow-up. This was confirmed after adjusting for confounders (age, centre, BMI, comorbidity and smoking) (Figure 2). Accordingly, there was also no difference in the change (development/worsening) of symptoms during follow-up.

Symptoms associated with incident LLSH

At baseline, prevalence of symptoms was not different between incident LLSH and PE men before (Table 1) and after adjustment for confounders (Figure 2). However, at follow-up, incident LLSH men reported all three sexual symptoms (erectile dysfunction, decrease in sexual thoughts and decreased morning erections) more frequently. Accordingly, development/worsening of sexual symptoms also occurred more frequently in incident LLSH men. Lack of vigour was found also to be increased over time in incident LLSH but this attenuated after adjustment for comorbidity and smoking (Supplementary Table 2). No other associations between LLSH and physical or psychological symptoms were found (Figure 2).

Recovery from LNSH or LLSH

Of the 224 men with prevalent SH at baseline, 173 (77.2%) were LNSH and 51 (22.8%) LLSH (Table 3). Recovery from LNSH to eugonadism during 4.3 years occurred in 46.2% men (80/173), which gives a recovery rate of 10.8% per annum, assuming linearity of transition. Recovery from LLSH to eugonadism was significantly less frequent (27.5%, 14/51; p=0.017 vs. recovery from LNSH), with a recovery rate of 6.4% per annum.

Analyses on possible predictors or clinical correlates of recovery from LNSH or LLSH did not yield meaningful information due to the small sample size.

Discussion

In a previous longitudinal analysis of the EMAS population, we found increased sexual symptoms in men who developed SH as defined by the decline in TT[9]. We have also shown previously, in a cross-sectional analysis of the entire EMAS cohort, that adding FT to TT yielded stronger associations with clinical feature of androgen deficiency than TT alone, which can be influenced by variations in SHBG[8]. Using subnormal TT alone may therefore over-diagnose SH in obese men with low SHBG, when FT can remain normal. The present evaluation shows that most men (72.7%) developing apparent biochemical SH, as defined by low TT, in fact had normal FT levels (LNSH), whereas low FT (LLSH) was found only in a minority of these men (27.3%). Men with LNSH and LLSH have clear differences in clinical characteristics. The most important is that sexual symptoms are associated only with the development of LLSH, but not with LNSH. Despite different clinical pictures, both LLSH and LNSH are predicted by obesity. This confirms our previous results showing adiposity as the most important predisposing factor for development of SH[9]. In the present analysis, we were able to show additionally that LNSH typically develops in younger men, whereas age is not a significant predictor for the development of LLSH. Furthermore, a different natural history was revealed, with a significantly higher proportion of men recovering to eugonadism in LNSH than LLSH (46.2% vs. 27.5%, p=0.017) over 4.3 years.

Since sexual symptoms are considered the cardinal features of adult hypogonadism[13,19], our findings indicate that low FT, in combination with low TT, provides the best discriminatory metric for detecting androgen deficiency. Furthermore, the association with sexual symptoms suggests that LLSH only should be regarded as a genuine form of hypogonadism. In contrast, the clinical features associated with the development of LNSH are indistinguishable from that of PE, suggesting that it is a specious biochemical state (reflecting low SHBG) rather than true hypogonadism. The present prospective data confirm and extend our previous cross-sectional results[8] showing that androgen deficiency features are most commonly encountered when both TT and FT are low, highlighting the potential

pitfall of mis-diagnosing SH in obese men which can be avoided by the addition of FT. Given that our previous cross-sectional results provided the only available evidence showing the importance of FT in the diagnosis of hypogonadism to date[17], the new longitudinal data presented here furnish important additional evidence to support the use of FT when evaluating the gonadal status in conditions of altered SHBG, such as in obesity.

The present findings have wider clinical relevance in the context of the huge increase in the number of testosterone prescriptions worldwide (particularly in North America) over the last two decades[20]. Our data suggest that genuine symptomatic hypogonadism associated with obesity has a relatively low prevalence and incidence in the general population in Europe, and may well also be the case in North America, thus suggesting that only a small proportion of obese men presenting with low TT would merit T therapy. Instead, it can be inferred that weight-reducing lifestyle measures may represent the most appropriate management to promote recovery to eugonadism in obese patients with borderline-low TT levels. Previous longitudinal data from the EMAS[21] have shown that 10-15% weight loss over 4.3 years was associated with an increase in both TT and FT, together with LH, thus suggesting recovery of hypothalamus-pituitary-testis (HPT) axis function. Similar results were observed in the meta-analysis of clinical trials, which evaluated hormone changes upon managed intentional weight loss[22], where both diet and bariatric surgery were able to reverse obesity-related SH with an improvement directly proportional to the extent of weight loss. It should be also noted that, in the present study, a high recovery rate (46.2%) to eugonadism was observed among younger obese men with LNSH. This suggests that symptomatic young obese men with low TT levels should be cautiously evaluated with repeated hormone testing (preferably after losing weight) before a final diagnosis of hypogonadism is established.

Obesity, especially when severe, can be associated with SH, characterised by low/normal LH and low FT despite the low SHBG[10]. The mechanisms involved in gonadotrophin suppression are complex and poorly understood. These may include central leptin- and

insulin-resistance as well as the inhibitory effect of pro-inflammatory cytokines on KNDY/GnRH neurons, which can impair directly or indirectly the HPT axis[23–25]. The hypothesised role of oestradiol excess in enhancing the negative feedback on HPT axis is controversial[16,26]. Indeed, the putative increase of aromatase activity due to the expanded adipose tissue is not supported by data from our[16] and other studies[27], which show that oestradiol, concurrently with T decline, is lower rather than higher, in obese men. There is also some evidence from in vitro studies that testicular steroidogenesis can be directly affected by adipocytokines, including leptin, TNF-α and decreased adiponectin[28,29]. The mechanisms leading to the reduction in SHBG observed in obese men are also debated. Hyperinsulinemia and hyperglycaemia have been shown to inhibit SHBG synthesis by hepatocytes[30,31]. Recent studies indicated that SHBG can be also down regulated by TNFα and IL1β and up regulated by adiponectin[32–34]; these mechanisms, arising from low grade chronic inflammation, may contribute to the lower serum SHBG associated with obesity

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Strength and limitations

The main strengths of this study are the large sample of men representative of the European general population and the longitudinal design, which is less affected by confounders than cross-sectional studies. The relatively small size of some of the analytical groups reflects the low incidence of hypogonadism in the general population, which may have been inflated by less rigorous studies. Another strength is the use of standardised instruments throughout the EMAS centres and at both study phases. Testosterone was measured by LC-MS/MS, with baseline and follow-up samples analysed simultaneously representing a further strength. However, some limitations should be acknowledged. As in previous EMAS published reports, FT was calculated, rather than directly measured in the present study. Although equilibrium dialysis is considered the most reliable and accurate determination for FT, it is impractical for an epidemiological study of this scale. Using the Vermeulen formula[15], which has been well-validated against directly measured FT[35], our results, based on internal comparisons of the same subjects over time, should not be affected by any potential

differences between alternative methods for calculating FT. By using the Vermeulen formula, the most commonly used algorithm in hospital clinical biochemistry laboratories and in clinical practice, to derive calculated FT in this study, our results are potentially more generalizable to real life clinical practice. The study interval of a median of 4.3 years may be relatively short for observing spontaneous changes in hormones and unfolding of clinical characteristics over time. A single measurement at each time point was available for TT and LH. Although important for individual patient diagnosis in clinical practice, multiple blood sample collection is impractical in large population-based epidemiological surveys[36,37]. In this regard, T has been confirmed as a stable analyte without significant fluctuation within the same subject when measured serially over many months[38–41]. Furthermore, the consistency of TT and LH levels between baseline and follow-up (respectively r=0.764 and 0.794, both p<0.001) and the agreement between LH and FSH (r=0.695 and 0.766, both p<0.01 respectively at baseline and follow-up) adds credence to our results, making it unlikely that a single hormone determination would have introduced substantial misclassification of gonadal status.

Conclusion

These prospective data show that a subgroup of obese men characterised by low TT and low FT are prone to develop multiple sexual symptoms suggestive of androgen deficiency, in contrast to the majority of obese men with low TT (and low SHBG) but normal FT, who do not develop symptoms of hypogonadism. The latter group, which frequently remits spontaneously to eugonadism, is often incorrectly diagnosed to have (and treated for) hypogonadism. The use of FT in obesity can contribute to clinical practice by preventing the over-diagnosis of hypogonadism and minimising inappropriate testosterone treatment.

Acknowledgements: The authors wish to thank the men who participated in the eight countries, the research/nursing staff in the eight centres: C. Pott (Manchester), E. Wouters (Leuven), M. Nilsson (Malmö), M. del Mar Fernandez (Santiago de Compostela), M. Jedrzejowska (Lodz), H-M. Tabo (Tartu), A. Heredi (Szeged) for their data collection, and C. Moseley (Manchester) for data entry and project co-ordination. The authors wish to particularly thank Mr. Joseph Finn for his invaluable contribution to EMAS.

The European Male Ageing Study (EMAS) Group includes the following: Florence (Gianni Forti, Luisa Petrone, and Giovanni Corona), Leuven (Dirk Vanderschueren, Steven Boonen, and Herman Borghs), Łódź (Krzysztof Kula, Jolanta Slowikowska-Hilczer, and Renata Walczak-Jedrzejowska), London (Ilpo Huhtaniemi), Malmö (Aleksander Giwercman), Manchester (Frederick Wu, Alan Silman, Terence O'Neill, Joseph Finn, Philip Steer, Abdelouahid Tajar, David Lee, and Stephen Pye), Santiago (Felipe F. Casanueva and Mary Lage), Szeged (György Bartfai, Imre Földesi, and Imre Fejes), Tartu (Margus Punab and Paul Korrovitz), and Turku (Min Jiang).

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Figure legends:

Figure 1. Flowchart showing derivation during follow-up and distribution of study sample by gonadal status and exclusions.

Footnote: *29 men missing from the original PE group (reference #9) due to normal TT and low FT at baseline or at follow-up, **1 men missing from the original incident SH group (reference #9) due to normal TT and low FT at baseline, † 2 missing men from the original recovery from SH group (reference #9) due to normal TT and low FT at follow-up. ‡ 32 men with normal TT and low FT at baseline or at follow-up accounts for the men missing from the previously studied analytical group (reference #9), as reported above.

Abbreviations: LH, luteinising hormone; TT, total testosterone; FT, free testosterone; PE, persistent eugonadism; SH, secondary hypogonadism; LNSH, low total testosterone/normal free testosterone secondary hypogonadism; LLSH, low total testosterone/low free testosterone secondary hypogonadism.

Figure 2: Figure 2. Relationship between change in gonadal status and clinical symptoms.

Footnote: Logistic regression analysis of clinical symptoms (outcome variables) in relation to incident SH groups using persistent eugonadism as referent. Adjustments were made for BMI, age, comorbidity burden (≥1 comorbidities), centre and smoking. Data are expressed as odds ratio ±95% confidence interval. The black, white and grey symbols denote the relation between incident SH and respectively the symptoms at baseline, at follow-up and their change (worsening/development) over time during follow-up. Level of statistical significance is denoted by: * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

Abbreviations: SH, secondary hypogonadism; LNSH, low total testosterone/normal free testosterone secondary hypogonadism; LLSH, low total testosterone/low free testosterone secondary hypogonadism, BMI, Body Mass Index.

Table 1. Baseline and Follow-Up Characteristics of Men with Persistent Eugonadism (PE), those with Incident Secondary Hypogonadism with low total and normal free testosterone (LNSH) and those with Incident Secondary Hypogonadism with both low total and low free testosterone (LLSH)

	Baseline				**Follow-Up**			
Parameter	PE	Incident LNSH	Incident LLSH	P[1]	PE	Incident LNSH	Incident LLSH	P[2]
Sample size (N)	1,880	101	38		1,880	101	38	
Age, years	58.1 ±10.4	55.7 ±10.3	60.6 ±9.4[b]	**0.025**	---	---	---	---
Pre-degree Education[3], n (%)	1291 (70.2)	74 (77.1)	28 (73.7)	0.323	---	---	---	---
Living with partner, n(%)	1610 (87.5)	83 (86.5)	33 (86.8)	0.950	1491 (85.0)	80 (86.0)	30 (79.0)	0.564
Smoking, n (%)	383 (20.6)	18 (17.8)	6 (16.2)	0.658	331 (18.2)***	8 (8.6)	6 (16.7)	0.060
Frequent Alcohol, n (%)	470 (25.1)	22 (21.8)	7 (18.4)	0.493	579 (35.5)***	29 (36.3)**	12 (38.7)*	0.924
Poor health, n (%)	375 (20.1)	22 (22.0)	10 (27.0)	0.537	424 (23.4)***	23 (24.5)	15 (39.5)	0.070
≥1 illness, n (%)	705 (37.5)	46 (45.5)	18 (47.4)	0.132	984 (52.3)***	68 (67.3)***[a]	30 (79.0)**[a]	**<.001**
≥2 illnesses, n (%)	275 (19.0)	11 (16.7)	8 (28.6)	0.387	532 (37.3)***	30 (47.6)**	16 (66.7)	**0.004**
Diabetes, n (%)	95 (5.1)	5 (5.0)	2 (5.4)	0.947	116 (6.4)***	8 (8.5)	6 (15.8)	0.052
CVD, n (%)	553 (29.8)	35 (35.0)	16 (42.1)	0.155	710 (40.1)***	51 (54.8)***[a]	23 (63.9)[a]	**<.001**
Cancer, n (%)	81 (4.3)	6 (5.9)	6 (15.8)[a]	**0.003**	149 (8.4)***	9 (9.7)	11 (29.0)[ab]	**<.001**
Prostate disease, n (%)	164 (8.9)	4 (4.0)	2 (5.4)	0.203	181 (10.0)*	3 (3.2)	5 (13.5)	**0.039**
Overall Sexual Function	22.2 ±6.5	23.1 ±6.4	19.9 ±6.9	0.073	21.4 ±6.8***	21.3 ±6.8**	16.2 ±7.9**[ab]	**<.001**
SF-36 physical	51.2 ±7.5	51.8 ±7.6	51.9 ±8.0	0.609	50.6 ±8.1**	49.8 ±8.9**	48.0 ±10.1*	0.128
Beck's Depression Inventory	6.3 ±5.9	6.1 ±5.5	7.3 ±6.4	0.550	6.2 ±6.3	5.5 ±5.8	8.2 ±7.7	0.090
SF-36 mental	52.1 ±8.6	53.2 ±7.4	52.5 ±9.4	0.438	52.0 ±9.1	52.9 ±7.8	50.2 ±10.2	0.328
DSST	29.0 ±8.3	29.4 ±8.8	28.5 ±8.3	0.827	28.1 ±8.9***	28.2 ±9.8**	25.1 ±9.9**	0.113

PASE score	208.1 ±87.5	200.0 ±82.9	200.3 ±103.1	0.611	183.8 ±93.8***	162.4 ±93.7**	182.3 ±103.3	0.101
50-ft walk, sec	13.0 ±2.7	12.8 ±2.2	13.2 ±2.2	0.707	13.7 ±2.8***	13.8 ±2.6***	14.1 ±2.9*	0.719
PPT rating	24.3 ±2.4	24.7 ±2.4	24.2 ±2.4	0.339	23.8 ±2.5***	23.4 ±2.5***	23.2 ±3.1*	0.149
Weight, kg	82.5 ±13.1	87.6 ±12.9[a]	90.5 ±15.4[a]	**<.001**	82.4 ±13.4	90.2 ±16.0**[a]	92.5 ±18.4[a]	**<.001**
BMI, kg/m^2	27.1 ±3.8	29.0 ±3.7[a]	29.7 ±4.3[a]	**<.001**	27.2 ±3.9***	30.1 ±4.5***[a]	30.7 ±5.3*[a]	**<.001**
Waist circumference, cm	96.7 ±10.4	101.6 ±8.1[a]	103.3 ±12.5[a]	**<.001**	98.0 ±10.8***	105.0 ±11.9***[a]	106.1 ±14.9*[a]	**<.001**
FPG	5.5 ±1.0	5.5 ±0.8	5.9 ±0.9	0.055	5.5 ±1.3	5.8 ±1.5*[a]	5.7 ±1.1	**0.032**
HOMA-IR	2.8 ±3.6	3.3 ±3.0	4.3 ±5.7[a]	**0.012**	2.8 ±3.0	4.1 ±4.8*[a]	4.4 ±3.4[a]	**<.001**
Total cholesterol, mmol/L	5.6 ±1.0	5.8 ±1.1	5.6 ±1.1	0.158	5.3 ±1.0***	5.2 ±1.1***	4.8 ±1.2***[a]	**0.029**
HDL-cholesterol, mmol/L	1.4 ±0.3	1.4 ±0.5	1.3 ±0.3[a]	**0.010**	1.4 ±0.4***	1.2 ±0.4*[a]	1.3 ±0.4	**<.001**
LDL-cholesterol, mmol/L	3.5 ±0.9	3.6 ±0.9	3.7 ±1.0	0.265	3.3 ±1.0***	3.1 ±1.1**	3.0 ±1.1**	0.067
Triglycerides, mmol/L	1.5 ±1.1	1.9 ±1.2[a]	1.5 ±0.9	**0.001**	1.5 ±1.9	2.0 ±1.6[a]	1.5 ±1.0	**0.024**
PSA, ng/ml	1.6 ±2.3	1.4 ±1.7	3.4 ±7.7[ab]	**<.001**	2.2 ±7.0***	1.8 ±2.4**	1.3 ±1.1	0.649
PSA >10 ng/mL, n(%)	20 (1.1)	1 (1.0)	2 (5.3)a	0.128	35 (1.9)	3 (3.0)	0 (0.0)	0.706
PSA after exclusion of values >10 ng/ml	1.4±1.4	1.3±1.2	1.4±1.3	0.736	1.6±1.6***	1.5±1.2***	1.3±1.1	0.264
Hb, g/L	150.4 ±10.4	150.4 ±10.0	147.1 ±9.0	0.157	150.2 ±11.4	148.0 ±10.8	143.3 ±15.5[a]	**0.001**
Total T, nmol/L	18.4 ±5.5	12.6 ±1.9[a]	13.2 ±2.3[a]	**<.001**	18.1 ±5.4**	9.3 ±0.9***[a]	7.5 ±2.5***[a]	**<.001**
Free T, pmol/L	323.7 ±79.3	279.5 ±45.6[a]	255.8 ±47.6[a]	**<.001**	309.4 ±76.9***	211.4 ±28.9***[a]	138.5 ±44.9***[ab]	**<.001**
SHBG, nmol/L	44.0 ±17.9	27.3 ±9.0[a]	35.9 ±11.2[ab]	**<.001**	46.5 ±19.0***	25.2 ±7.6**[a]	38.2 ±14.5[ab]	**<.001**
LH, U/L	5.7 ±3.0	4.7 ±2.0[a]	4.7 ±2.0	**<.001**	6.0 ±3.6***	4.5 ±1.8[a]	4.0 ±1.8[a]	**<.001**
FSH, U/L	7.3	6.0	6.4	**0.044**	7.7 ±6.8***	5.8 ±3.6[a]	5.9 ±3.5	**0.008**

	±5.9	±3.7	±4.3					
Decreased morning erections, n(%)	596 (32.5)	30 (31.6)	14 (36.8)	0.834	586 (33.8)*	35 (38.0)	22 (59.5)[a]	**0.004**
Decreased sexual thoughts, n(%)	409 (22.3)	21 (21.9)	11 (29.0)	0.616	452 (25.8)**	27 (29.4)	17 (44.7)	**0.026**
Erectile dysfunction, n(%)	444 (24.3)	19 (20.0)	14 (36.8)	0.123	509 (30.2)***	25 (27.5)	21 (56.8)[ab]	**0.002**
Decreased vigorous activity, n(%)	352 (18.9)	15 (15.0)	6 (15.8)	0.561	419 (23.2)***	23 (24.5)*	12 (32.4)	0.406
Limited walking, n(%)	74 (4.0)	5 (5.0)	2 (5.4)	0.613	132 (7.3)***	7 (7.5)	7 (18.9)[a]	**0.030**
Inability to bend, n(%)	78 (4.2)	4 (4.0)	3 (7.9)	0.416	85 (4.7)	5 (5.3)	3 (8.1)	0.480
Fatigue, n(%)	67 (3.6)	4 (4.0)	2 (5.3)	0.634	78 (4.3)	8 (8.3)	3 (7.9)	0.080
Loss of energy, n(%)	68 (3.6)	3 (3.0)	1 (2.6)	1.000	97 (5.3)**	5 (5.2)	5 (13.2)	0.110
Sadness, n(%)	65 (3.5)	2 (2.0)	0 (0)	0.657	69 (3.9)	3 (3.2)	1 (2.8)	1.000

Data are expressed as mean±standard deviation for continuous variables or as number (percentage) for binary categorical variables.

1. *P*-values were calculated using *baseline* parameters and either the analysis of variance (ANOVA) for continuous variables or chi-squared test for categorical variables.

2. *P*-values were calculated using *follow-up* parameters and either the analysis of variance (ANOVA) for continuous variables or chi-squared test for categorical variables.

3. Educated to less than a university degree level

a = data differ significantly ($p<0.05$) from the PE group using the Tukey–Kramer test for continuous variables or the z-test for categorical variables with correction for multiple pairwise comparisons.

b = data differ significantly ($p<0.05$) from the incident LNSH group using the Tukey–Kramer test for continuous variables or the z-test for categorical variables with correction for multiple pairwise comparisons

*, **, *** Data differ significantly ($p<0.05$, $p<0.01$ and $p<0.001$ respectively) from baseline values within the same group when analysed using paired t-tests (for continuous variables) or the McNemar test (for categorical variables).

Abbreviations: PE, Persistent Eugonadism; SH, Secondary Hypogonadism; CVD, CardioVascular Disease; SF-36, Short-Form 36 Questionnaire; DSST, Digital Symbol Substitution Test; PASE, Physical Activity Scale for the Elderly; PPT, Physical Performance Test; BMI, Body Mass Index; FPG, Fasting Plasma Glucose concentration; HOMA-IR, HOmeostatic Model of Insulin Resistance; PSA, Prostate Specific Antigen concentration; Hb, Haemoglobin; T, serum Testosterone concentration; SHBG, Sex Hormone Binding Globulin concentration; LH, Luteinising Hormone concentration; FSH, Follicular Stimulating Hormone Concentration

Table 2. Predictors of change in gonadal status from eugonadal to incident secondary hypogonadism

Predictor		Odds ratio [95% confidence interval]	
		Incident LNSH	**Incident LLSH**
Age (years)	60-69	1.58 [0.69-3.60]	0.59 [0.17-2.00]
	50-59	1.62 [0.70-3.76]	0.93 [0.33-2.61]
	40-49	*3.24 [1.40-7.47]* **	0.59[0.17-2.00]
≥1 comorbidities		1.55 [0.93-2.60]	1.21 [0.55-2.63]
BMI (kg/m^2)	25-30	*2.12 [1.06-4.22]* *	1.86 [0.60-5.81]
	≥30	*4.18 [2.00-8.71]* ***	*5.85 [1.86-18.37]* **
Chronic pain		0.66 [0.26-1.70]	0.28 [0.04-2.12]
Current smoking		0.78 [0.41-1.45]	1.23 [0.48-3.12]
Frequent alcohol intake		1.21 [0.70-2.09]	0.61 [0.23-1.62]
Pre-degree education		0.85 [0.64-1.14]	1.05 [0.68-1.62]
Living with partner		1.35 [0.91-1.98]	1.28 [0.73-2.26]
PASE≤78		1.18 [0.48-2.87]	1.57 [0.52-4.73]

Multiple logistic regression analysis of incident secondary hypogonadal groups (using persistent eugonadism as referent) in relation to risk factors. Data are expressed as odds ratio ±95% confidence interval. BMI categories considered were, BMI: 25–30 (overweight) and BMI≥30 kg/m^2 (obese).

Referent categories for predictors included in the analysis: Age >70 years; No comorbidities; BMI <25 kg/m^2; No chronic pain; No current smoking; Infrequent alcohol intake: alcohol intake for less than 5 days per week; Educated to a university degree level; No partner or not living together with a partner; PASE >78.

Abbreviations: LNSH, low total testosterone/normal free testosterone secondary hypogonadism; LLSH, low total testosterone/low free testosterone secondary hypogonadism; BMI, Body Mass Index; PASE, Physical Activity Scale for the Elderly

Level of statistical significance is denoted by: * = p <0.05, ** = p <0.01 and *** = p <0.001

Table 3. Gonadal status transition in men with secondary hypogonadism (SH) at baseline

		Baseline		Total
		LNSH	LLSH	
Follow-up	**Eugonadism**	80	14	217‡
	LNSH	70	10	
	LLSH	21	22	
	Primary hypogonadism	2**	3**	7
	Normal TT and Low FT	-	2*‡	
	Total	173	51	224

* These men were part of the original recovery from SH group (reference #9) and now excluded due to normal TT and low FT at follow-up.

** These men are part of those excluded from the analytical group either in the present or in the previously published (reference #9) analytical groups due to primary hypogonadism at follow-up.

‡ The sum of 217 men (with persistent SH or recovery from SH to eugonadism) and 2 men (with SH at baseline and normal TT and low FT at follow-up) makes altogether the 219 men in the original recovery from SH group (reference #9)

Abbreviations: TT, total testosterone; FT, free testosterone; SH, secondary hypogonadism; LNSH, secondary hypogonadism with low total and normal free testosterone; LLSH, secondary hypogonadism with low total and low free testosterone.