Received Date : 03-Dec-2015
Revised Date : 19-Feb-2016
Accepted Date : 14-Mar-2016
Article type : Original Articles

Handling Associate Editor: Stanislas Pol

High circulating estrone and low testosterone correlate with adverse clinical outcomes in men with advanced liver disease

Marie Sinclair[1, 2], Paul J Gow[1, 2], Peter W Angus[1, 2], Rudolf Hoermann[2], David J Handelsman[3], Gary Wittert[4], Sean Martin[4], Mathis Grossmann[2, 5]

1. Austin Health, Gastroenterology and Hepatology
2. The University of Melbourne
3. ANZAC Research Institute, University of Sydney
4. The University of Adelaide
5. Austin Health, Endocrinology

Corresponding author: Dr Marie Sinclair. marie.sinclair@austin.org.au
Ph: (+613)94965353. Fax: (+613)94963487
Austin Hospital, Melbourne, Victoria, Australia, 3084

Word count: 4988. Tables: 4. Figures: 1

Abbreviations: MELD: model for end-stage liver disease; TUG: timed up-and-go; SF36: short-form health survey; SHBG: sex hormone binding globulin; DHT: dihydrotestosterone; DHEA: dehydroepiandrostenedione; BMI: body mass index; E1: estrone, E2: estradiol; T: testosterone

Financial support included an Australian Postgraduate Award. Bayer pharmaceuticals provided funding for investigations. There are no other conflicts of interest to report.

Australian New Zealand Clinical Trials Registry trial number ACTRN 12614000526673. https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=366086&isReview=true

Abstract

Background & Aims

Circulating testosterone is usually reduced in men with cirrhosis but there has not been a comprehensive analysis of androgen status or circulating estrogens. Little is known about associations between circulating sex steroids with aspects of health in this population.

Methods

We report data from men with cirrhosis and low serum testosterone (<12nmol/L or calculated free testosterone <230pmol/L). Comprehensive circulating sex steroid profiles were measured by liquid chromatography-mass spectrometry and compared with age-matched controls. Relationships between sex hormone levels, severity of liver disease, biochemistry, and clinical outcomes were assessed.

Results

Serum estrone and estradiol were significantly elevated in men with cirrhosis compared to controls (median 869.1pmol/L vs 133.8pmol/L and 166.7pmol/L vs 84.6pmol/L respectively). Serum estrone correlated with MELD score (correl +0.306, p<0.001), and inversely correlated with serum sodium (correl -0.208, p=0.004) and haemoglobin (correl -0.177, p=0.012). No such correlations were observed for estradiol. Serum testosterone levels inversely correlated with MELD score (correl -0.294, p<0.001), and positively with handgrip strength (correl +0.242, p<0.001), physical activity (correl +0.276, p=0.012), haemoglobin (correl +0.282, p<0.001), and serum sodium (+0.344, p<0.001)). Dihydrotestosterone inversely correlated with MELD score (correl -0.225, p=0.002) and shared similar significant relationships to testosterone.

Conclusion

Low serum androgens and elevated serum estrone (but not estradiol) are associated with higher MELD and individual adverse health outcomes in cirrhotic cohort of men selected for low testosterone. Serum estrone may be a novel marker of ill health in this population. Whether low androgens are markers or mediators of ill health requires further investigation.

Word count: 250. Keywords: cirrhosis, estrone, estradiol, testosterone

KEY POINTS

- Serum estrone and estradiol are elevated in men with cirrhosis despite the reduction in serum testosterone levels
- Circulating serum estrone but not estradiol correlates with individual adverse health outcomes
- Low testosterone, DHT and DHEA each correlate with individual adverse health outcomes
- Abnormalities in sex hormone profile worsen with increasing severity of liver disease

Introduction

Both androgens and estrogens have important but distinct functions in men. Estrogenic activity is mostly mediated by estradiol, whereas circulating estrone is considered mainly a pro-estrogen with low bioactivity. Estradiol in men is predominantly from aromatisation of testosterone in adipose tissue, muscle and brain, and it is metabolised by the liver. In men both androgens and estradiol are necessary to maintain bone mineral density with estradiol having a dominant effect (1). Androgens are important anabolic hormones produced predominantly in the testes, with effects on muscle, haematopoiesis, metabolism and sexual function. Low circulating testosterone levels in men are associated with anaemia, osteoporosis, sarcopenia, insulin resistance, and increased all-cause mortality (2-4) however, whether low circulating testosterone is a cause or a biomarker in these relationships remains controversial. Little is known about the impact of circulating androgens and estrogens on health and quality of life in men with cirrhosis.

Serum testosterone is low in up to 90% of cirrhotic men in the pre-liver transplant setting (5) and inversely correlates with severity of liver disease as measured by MELD score (6). The literature regarding circulating estradiol levels in cirrhosis however is conflicting, with reports of both normal and elevated estradiol (6, 7). Despite the lack of strong supportive evidence, clinical features such as gynecomastia and altered body hair distribution have been conventionally attributed to elevated estradiol levels. Given that testosterone is frequently reduced, however, a raised estradiol-to-testosterone ratio may be responsible.

A major limitation of prior studies examining sex hormones in cirrhosis is the use of insensitive and/or inaccurate automated direct (non-extraction) steroid immunoassays which usually lack specificity against structurally similar steroids and their precursors or metabolites. These assays have low accuracy and poorly quantify the low circulating estradiol levels observed in men (8). The gold standard for steroid measurement is now mass spectrometry (9) but it has not previously been performed cirrhosis.

Men with cirrhosis commonly have features similar to hypogonadism, including anaemia, osteoporosis, sarcopenia and insulin resistance. We therefore hypothesized that the circulating sex hormone profile in men would correlate with sequelae of cirrhosis. We quantified extensive circulating sex hormone profiles via mass spectrometry in a selected group of cirrhotic men with low testosterone levels and investigated their relationships with commonly observed features of cirrhosis. Patients and Methods

We report baseline data from a randomized clinical trial of testosterone therapy in men with cirrhosis (ACTRN12614000526673, Australian New Zealand Clinical Trials Registry). Of the 102 patients enrolled in this trial, 95 had hormone analysis via mass spectrometry. Men were recruited from outpatient hepatology clinics at the Austin Hospital, Melbourne, Australia. The study was approved the Austin Hospital Human Research Ethics Committee and patients provided written informed consent.

Subjects were men with cirrhosis of any aetiology and baseline total testosterone <12nmol/L by hospital immunoassay or calculated free testosterone <230pmol/L using Vermeulen's formula (10). Exclusion criteria included age over 70 years, active cancer (including hepatoma), prostate disease, severe renal failure (estimate glomerular filtration rate <30ml/min), heart failure (New York Heart Association classification III or IV), obstructive sleep apnoea requiring continuous

positive airway pressure, or haematocrit >55%. Given administration of study drug was intramuscular, patients with platelets <30 x 10^6 were excluded.

Clinical data collection included non-quantitative information on gynecomastia (via patient examination to identify palpable breast tissue) and altered body hair distribution (patient self-report of loss of male pattern body hair). The presence and severity of encephalopathy and ascites were assessed from clinical examination and hospital medical records and included episodes in the 3 months preceding assessment. Due to its known effects on sex hormone action, the administration of spironolactone was recorded (11).

Hand-grip strength was assessed by Jamar hand dynamometer and the timed-up-and-go (TUG) was performed to assess mobility. Quality of life questionnaires included the Short Form Health survey (SF-36), the Androgen Deficiency of the Ageing Male (ADAM) and the short International Physical Activity Questionnaire (IPAQ) (12-14). Bone mineral density was assessed at the hip and spine using a single Dual-Energy X-ray Absorptiometry machine. Single slice CT scans were performed at the L4 level to calculate muscle area using Tomovision® software as previously described (15).

In-house laboratory assays were used to measure routine haematology and biochemistry parameters. SHBG levels were determined with the Immulite 2000 analyser (Diagnostics Products Corporation, Los Angeles, CA, USA) with reference range 13-71 nmol/L. Sex steroid profile measured in extracts of serum by liquid chromatography-tandem mass spectrometry (LCMS/MS) included serum dihydrotestosterone (DHT), dehydroepiandrostenedione (DHEA), testosterone, estradiol and estrone as described (16). Lower limits of quantification were 0.035 nmol/L for testosterone (8.0% CV), 0.170 nmol/L (19% CV) for DHT, 5.5 pmol/L (8% CV) for E1, 2.5 pmol/L (10% CV) for E2, and 0.344 nmol/L (11% CV) for DHEA. Accuracy was consistent and reproducible over three spiking levels tested, ranging between 93-118% for each steroid, with all CVs <14%.

A cohort of 256 age and body mass index (BMI) matched men were sourced from the Florey Adelaide Male Ageing Study (17) who were free from significant comorbidities with normal liver function tests, and sex steroid profile measured using the same LCMS/MS assay. This cohort included men irrespective of testosterone levels, to describe a reference range for serum estrogen levels.

Statistics

The median and inter-quartile ranges (IQR) (25th and 75th percentiles) are reported for all data due to non-normal distribution of parameters. Between-group differences were tested for significance by means of Wilcoxon rank sum test for comparison of two groups, Kruskal-Wallis rank sum test for more than two groups and chi-squared test with Yates' continuity correction in case of frequency distributions. Tables were considered explanatory and not corrected for multiple testing. Correlation of sex hormones with other variables was assessed using Kendall's tau rank correlation coefficient because it involved non-normal bivariate distributions and scores. p value <0.05 conferred significance throughout. The odds ratio for binary outcomes was determined by logistic regression (generalized linear model with a binomial link function). The statistical software packages R 3.2.2 for Mac with Deducer 0.7-7 were used for the data analyses (18, 19).

Role of the Funding Source

Bayer Pharmaceuticals AG (Berlin, Germany) provided financial support to conduct investigations, but had no role in trial design, data analysis or interpretation, manuscript preparation or the decision to submit the manuscript for publication.

Results

Demographics

Of 268 cirrhotic men screened, 152 (57%) fell below the testosterone threshold. Low testosterone patients were of similar age to normal testosterone patients (55.5 years [50; 61] versus 54.5 years [47; 60], p=0.063) but had significantly higher MELD scores (12 [8; 17] versus 9 [7; 11], $p<0.001$).

102 low testosterone patients were recruited for the study and were compared with a control group of 256 age and BMI matched men (Table 1). The median age of the liver cohort was 55 years [51, 60], the median BMI was 28.8kg/m^2 [26.0, 32.5] and 93% of men were Caucasian. The median MELD score was 14 [10, 17] and median Child Pugh score was 9 [7, 11]. 20 patients were classified as Child Pugh A, with 36 and 46 Child Pugh B and C patients respectively. 41% of patients had alcohol as a primary or significant cofactor and 29% of

patients had hepatitis C virus infection.

76 patients (75%) had current or recent ascites, with 35 of these requiring abdominal paracentesis within the preceding 3 months and 41 controlled on medications alone. 66 patients (65%) were taking spironolactone at the time of assessment. 68 patients had gynecomastia (67%) and 45 patients (44%) reported altered hair distribution. 54 patients (53%) had recent episodes of hepatic encephalopathy or were on medications to treat encephalopathy.

Hormone profile

Of the 102 patients recruited to the clinical trial, 95 patients had stored serum for hormone profile analysis by mass spectrometry with serum testosterone, DHT, DHEA, estradiol, estrone compared with the age-matched controls in table 1.

In the liver cohort, serum testosterone correlated positively with serum estradiol (correl +0.278, $p<0.001$) but not with serum estrone (correl -0.065, $p=0.349$). Testosterone demonstrated a strong correlation with DHT (correl +0.702, $p<0.001$).

Comparison of hormone profile to control cohort

Hormone levels in the liver cohort and control cohort are displayed in Table 1. Despite selection for low serum testosterone, men with cirrhosis had much higher circulating estrone as well as estradiol, as compared to controls (controls 133.8pmol/L [109.2, 165.9] and 84.6pmol/L [66.5, 100.5] respectively). See Figure 1a, b. The close relationship that is usually observed between serum estradiol and serum testosterone was distorted in the liver cohort, with much wider variation (but similar correlation) in estradiol levels for any given testosterone level (Figure 1b).

Hormone profile by Child Pugh Score

All measured androgens fell progressively with increasing severity of liver disease. Median serum estradiol levels were similar across all Child Pugh classes but serum estrone levels rose with increasing severity of liver disease. The ratio of estrogens to testosterone significantly increased with increasing Child Pugh class. In particular, the median estrone to testosterone ratio was over 10 fold higher in Child Pugh C patients as compared to Child Pugh A (413 vs 31.6mol/L, $p<0.001$) Serum SHBG was very elevated across all groups of men with cirrhosis but was less markedly elevated in Child Pugh C patients. See Table 2.

Relationship between sex hormone profile and MELD score

All androgens were inversely associated with severity of liver disease according to MELD score (Testosterone: correl -0.294, p<0.001, DHT: correl -0.225, p<0.001, DHEA: correl -0.313, p<0.001). Serum estrone levels progressively rose with increasing MELD score (correl +0.306, p<0.001), however there was no significant correlation between MELD score and serum estradiol (correl +0.033, p=0.640). SHBG demonstrated a weak inverse correlation with MELD (correl -0.142, p=0.393). See Table 3.

Relationship between sex hormone profile and haematological and biochemical parameters

There was a positive association between each of the androgens and haemoglobin concentration (testosterone correl +0.282, p<0.001; DHT correl +0.243, p<0.001, DHEA correl +0.189, p=0.007), and an inverse association between estrone and haemoglobin (correl -0.177, p=0.012). No similar relationships were seen for estradiol. SHBG correlated positively with haemoglobin (correl +0.159, p=0.039).

There was a positive correlation between circulating androgens and both serum sodium and albumin, a positive correlation between SHBG and sodium, and a negative correlation between serum estrone (but not serum estradiol) levels and both serum sodium and albumin. The MELD components serum bilirubin and INR inversely correlated with circulating androgen levels and positively correlated with serum estrone, but creatinine demonstrated no significant correlations. See Table 3. There was a positive correlation between androgen levels and prostate specific antigen (PSA); serum testosterone correl +0.285, p<0.001 DHT correl 0.282, p<0.001.

Serum estrone and creatinine were positively related (correl +0.142, p=0.043). There was an inverse association between serum estrone and platelet count (correl -0.223, p=0.002) that was not seen for androgens. None of the androgens correlated with platelet count or serum creatinine, and estradiol did not significantly correlate with any biochemical parameter.

Body composition and Bone Mineral Density

19.6% of men had osteoporosis at the lumbar spine as defined by a T score of -2.5 or below, and a further 21.6% were classified as osteopenic (T score -1.5 to -2.5). 10.8% met criteria for osteoporosis at the femoral neck, with a further 26.5% having osteopenia. Sarcopenia (as

defined by height adjusted muscle mass at the 4th lumbar vertebrae level of less than 52.4cm^2/m^2 (15)) was observed in 46.1% of men.

There was no significant association between androgen levels and muscle mass by CT or with serum creatinine. There was however an inverse association between estrone levels and lumbar spine bone mineral density (correl -0.145, p=0.038) and a positive association between DHEA and lumbar spine bone density (correl +0.153, p=0.029). No significant correlations were identified for other androgens.

Muscle strength, Physical activity and Quality of Life

Handgrip strength significantly correlated with serum testosterone (correl +0.242, p<0.001). A similar positive correlation was seen between DHT (correl +0.237, p<0.001) and DHEA (correl 0.22, p=0.002) and handgrip strength.

The Timed-Up-and-Go (TUG) score was inversely correlated with testosterone levels (correl -0.368, p<0.001), DHT (correl -0.337, p<0.001) and DHEA (correl -0.269, p<0.001) meaning mobility was better in men with higher androgen levels. In keeping with this, the physical activity component of the SF36 quality of life questionnaire also positively correlated with testosterone (correl +0.15, p=0.050). Quality of life and handgrip strength were lower in the cirrhotic cohort than controls (Table 1).

Serum estradiol was inversely correlated to the TUG score (correl -0.18, p=0.015). There was no correlation between either SHBG or serum estrone and any component of physical activity or quality of life. There were no significant correlations between any of the sex hormones with the mental health component of SF36 or sexual function as measured by the ADAM questionnaire.

Other Clinical features

Median serum testosterone levels were lowest in patients with severe hepatic encephalopathy (3.78nmol/L [1.27, 12.83] compared to those without (10.85 nmol/L [7.04, 17.96]), p=0.007. Median testosterone levels were also lowest in men with more hospital admissions (3.26nmol/L for multiple admissions, vs 7.27nmol/L for 1 or 2 admissions, vs 11.0nmol/L for zero, p=0.025). No such findings were seen for other androgens or the estrogens. Both total testosterone and DHT fell and estrone rose with increasing severity of ascites. No significant relationships were

observed for estradiol. See Table 4.

Gynecomastia and Loss of body hair

Serum testosterone levels were similar in patients with and without gynecomastia. There was also no significant difference between the ratio of estrogen to androgen levels or in SHBG levels.

Both testosterone (6.81nmol/L vs 11.3nmol/L, p=0.005) and DHT (77pmol/L vs 147pmol/L, p=0.002) levels were significantly lower in men with self-reported loss of body hair. By contrast, serum estradiol (but not estrone) levels were significantly lower in men with loss of body hair (149pmol/L versus 188pmol/L, p=0.017), meaning that these men had serum estradiol levels that were closer in value to the controls.

Spironolactone use

Serum testosterone was lower in the 66 men taking spironolactone than those who were not (6.73nmol/L versus 13.5nmol/L, p<0.001). Serum estrone was higher (1000pmol/L versus 573pmol/L, p =0.004) but serum estradiol unchanged (173nmol/L versus 166nmol/L, p = 0.547) in men taking spironolactone. Gynecomastia was more common among spironolactone users than non-users (76% vs 50%, p=0.016) and the odds ratio for developing gynecomastia was 3.1 [1.3, 7.5], p=0.01 for spironolactone users vs non-users. Although median MELD score was higher in spironolactone users than non-users (15 vs 9, p<0.001), MELD score was not associated with the development of gynecomastia (p=0.53).

Hormone Profile by Aetiology of Liver disease

In our cohort the MELD score was similar in those with and without alcohol excess (13 versus 15, p=0.302). There were no significant differences in serum sex steroids, SHBG, DHT, DHEA, estrogen to testosterone ratios or the presence of gynecomastia in patients with and without alcohol-related cirrhosis.

Discussion

This paper employs mass spectrometry steroid profiling to comprehensively evaluate sex hormone profile in men with cirrhosis and thus provides new insight into androgen and estrogen

277 status in men with cirrhosis. Despite being preselected on the basis of low testosterone (a
278 precursor of estradiol), these cirrhotic men have markedly increased estrogen levels compared to
279 controls. We report the novel finding that serum estrone rises with increasing severity of liver
280 disease and positively correlates with features of chronic liver disease. By contrast, serum
281 estradiol levels do not correlate with severity of liver disease or its clinical sequelae although
282 estradiol was elevated and the estradiol to testosterone ratio did rise with increasing severity of
283 liver disease. This data also demonstrate that not only are lower serum androgen levels
284 associated with increasing severity of liver disease, but they also correlate with specific clinical
285 and biochemical features in this cohort.

286 Estrone, due to its very low intrinsic potency as an estrogen, has traditionally been thought to be
287 a pro-estrogen of no clinical importance in men. In our cohort we observed higher estrone levels
288 (though missing statistical significance) in the presence of gynecomastia confirming a similar
289 association in cirrhosis reported previously (7). There have only been two previous
290 epidemiological studies assessing the role of serum estrone in men, neither in men with liver
291 disease. Consistent with our findings, in the Framingham Heart study, higher estrone levels were
292 also associated with adverse health outcomes including higher BMI and increased prevalence of
293 diabetes and cardiovascular disease (20). By contrast, in a recent series of studies of older
294 Australian men, low serum estrone levels were significantly associated with poorer health status
295 (21).

296 Our novel finding suggests that the significance of serum estrone in men should be re-examined
297 as a novel general health biomarker. Our data are consistent with the possibility that in men
298 with liver disease, serum estrone levels may correlate better with health outcomes than the more
299 bioactive estrogen, estradiol. This also focuses attention on the relevance of the steroidogenic
300 enzyme 17β hydroxysteroid dehydrogenase which converts estrone to estradiol and raises the
301 question of which tissue is its activity is disproportionately inhibited in chronic liver disease.

302 Serum testosterone, and DHT were positively associated with both haemoglobin and serum
303 sodium. Exogenous testosterone increases erythropoiesis, and polycythaemia is the most
304 frequent complication of testosterone therapy (22), thus this relationship could plausibly be
305 causal despite its origin in observational data. The association with sodium however is more
306 likely to reflect an increasing severity of portal hypertension in patients with hyponatremia.

Knowing that serum testosterone inversely correlates with severity of liver disease, the association may simply reflect falling testosterone in a sicker patient group. Our observational findings relating to clinical manifestations of liver failure were equally consistent for all measures of androgen status including serum testosterone, DHT and the pro-androgen DHEA.

Whether there is a causal relationship between serum androgens and clinical outcomes such as hospitalization, hepatic encephalopathy and ascites is unclear. Again, the lower testosterone may purely reflect a sicker patient group, but it is also possible that low testosterone may directly contribute to some somatic aspects of poor health. It is known that exogenous testosterone influences muscle mass and function (23), and although in our cohort androgens did not correlate with muscle mass, this study was underpowered to assess for such a link in the context of multiple contributing factors (24). In our cohort circulating testosterone significantly correlated with handgrip strength, timed-up-and-go, and physical activity questionnaires, which may be more prognostically important than muscle mass (25). Thus this study raises the proposition that low circulating testosterone may increase frailty and thus risk of hospitalisation.

Spironolactone use in our cohort was associated with significantly lower testosterone levels and higher estrone levels, as well as the presence of gynecomastia. However, its use was closely associated with increasing severity of liver disease due to the associated increasing prevalence of ascites. This increase in disease severity itself correlates with lower testosterone levels. It is therefore statistically very difficult to determine from this cohort whether or not the medication itself caused or was merely associated with reduced serum testosterone levels given the high prevalence of spironolactone use (69%). We do know however from prior research that spironolactone can reduce serum testosterone levels however the effect can vary with varying doses of drug (26). To better assess the clinical impact of spironolactone, sex hormone profile should ideally be performed pre and post-spironolactone.

Limitations of our study include recruiting only cirrhotic men with low testosterone levels, due to predefined trial eligibility criteria. If anything, this restricted range of testosterone values would have led to an underestimation of the associations with clinical parameters observed, and thus strengthens the validity of our findings. Although only 57% of screened men from a general hepatology setting met entry criteria, serum testosterone levels are almost universally low in pre-transplant cohorts of men with cirrhosis, and low testosterone is itself an independent poor

prognostic factor (5). Thus the findings reported here are expected to be pertinent for the sickest cohort of cirrhotic men, for whom significant associations may be of most clinical utility due to the increased need for new therapies and prognostic markers.

Another limitation, given the possibility that high estrogens may be related to porto-systemic shunts (27), was the lack of quantitative shunt data and hepato-portal venous pressure gradients. It is also important to note here that we are only measuring circulating hormone levels, and it remains possible that tissue levels may be different as estrone can be converted to the more biologically active estradiol within tissues (28).

Strengths of this study include the comprehensive assessment of androgen and estrogen status via a sex steroid profile with validated gold standard steroid mass spectrometry measurements. Furthermore we studied a large cohort of men with well-characterised liver cirrhosis, who had a careful standardized phenotypical characterisation, and included age and BMI matched men without chronic liver disease. Given that the significance of "free" testosterone remains controversial and equations such as Vermeulen's have not been specifically validated by direct comparison with dialysis-based laboratory methods in cirrhosis where there is elevated SHBG, we do not report data on calculated free testosterone.

In conclusion, this study represents the first comprehensive analysis of sex hormone profile in men with cirrhosis using mass spectrometry. We identified markedly elevated circulating estrogen levels in comparison to a control group despite low serum testosterone. Serum estrone levels rose with increasing severity of liver disease and were associated with adverse health outcomes and thus may represent a novel marker of ill health in this population. Low serum testosterone and DHT correlated with severity of liver disease and adverse health outcomes, including anaemia, hyponatremia, reduced handgrip strength and hospitalisation. Interventional studies are required to assess whether or not these associations are causal or purely reflect a poorer health status in this population.

References

1. Burnett-Bowie SA, McKay EA, Lee H, Leder BZ. Effects of aromatase inhibition on bone mineral density and bone turnover in older men with low testosterone levels. The Journal of clinical endocrinology and metabolism. 2009;94(12):4785-92. Epub 2009/10/13.

2. Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT, Wittert GA. Clinical review: Endogenous testosterone and mortality in men: a systematic review and meta-analysis. The Journal of clinical endocrinology and metabolism. 2011;96(10):3007-19. Epub 2011/08/06.

3. Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, et al. Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. The Journal of clinical endocrinology and metabolism. 1997;82(2):407-13. Epub 1997/02/01.

4. Meier C, Nguyen TV, Handelsman DJ, Schindler C, Kushnir MM, Rockwood AL, et al. Endogenous sex hormones and incident fracture risk in older men: the Dubbo Osteoporosis Epidemiology Study. Archives of internal medicine. 2008;168(1):47-54. Epub 2008/01/16.

5. Grossmann M, Hoermann R, Gani L, Chan I, Cheung A, Gow PJ, et al. Low testosterone levels as an independent predictor of mortality in men with chronic liver disease. Clinical endocrinology. 2012;77(2):323-8. Epub 2012/01/28.

6. Zietz B, Lock G, Plach B, Drobnik W, Grossmann J, Scholmerich J, et al. Dysfunction of the hypothalamic-pituitary-glandular axes and relation to Child-Pugh classification in male patients with alcoholic and virus-related cirrhosis. European journal of gastroenterology & hepatology. 2003;15(5):495-501. Epub 2003/04/19.

7. Green JR, Mowat NA, Fisher RA, Anderson DC. Plasma oestrogens in men with chronic liver disease. Gut. 1976;17(6):426-30. Epub 1976/06/01.

8. Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME. Challenges to the measurement of estradiol: an endocrine society position statement. The Journal of clinical endocrinology and metabolism. 2013;98(4):1376-87. Epub 2013/03/07.

9. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. The Journal of clinical endocrinology and metabolism. 2013;98(10):3971-3. Epub 2013/10/08.

10. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. The Journal of clinical endocrinology and metabolism. 1999;84(10):3666-72. Epub 1999/10/16.

11. Corvol P, Michaud A, Menard J, Freifeld M, Mahoudeau J. Antiandrogenic effect of spirolactones: mechanism of action. Endocrinology. 1975;97(1):52-8. Epub 1975/07/01.

12. Brazier JE, Harper R, Jones NM, O'Cathain A, Thomas KJ, Usherwood T, et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. Bmj. 1992;305(6846):160-4. Epub 1992/07/18.

13. Hagstromer M, Oja P, Sjostrom M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. Public health nutrition. 2006;9(6):755-62. Epub 2006/08/24.

14. Tancredi A, Reginster JY, Schleich F, Pire G, Maassen P, Luyckx F, et al. Interest of the androgen deficiency in aging males (ADAM) questionnaire for the identification of hypogonadism in elderly community-dwelling male volunteers. European journal of endocrinology / European Federation of Endocrine Societies. 2004;151(3):355-60. Epub 2004/09/15.

15. Montano-Loza AJ, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, et al. Muscle wasting is associated with mortality in patients with cirrhosis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2012;10(2):166-73, 73 e1. Epub 2011/09/07.

16. Harwood DT, Handelsman DJ. Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously measure androgens and estrogens in serum without derivatization. Clinica chimica acta; international journal of clinical chemistry. 2009;409(1-2):78-84. Epub 2009/09/15.

17. Martin SA, Haren MT, Middleton SM, Wittert GA, Members of the Florey Adelaide Male Ageing S. The Florey Adelaide Male Ageing Study (FAMAS): design, procedures & participants. BMC public health. 2007;7:126. Epub 2007/06/28.

18. Fellows I. A data analysis GUI for R. . Journal of Statistical Software. 2012;49(8):1-15.

19. core team R. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.

20. Jasuja GK, Travison TG, Davda M, Murabito JM, Basaria S, Zhang A, et al. Age trends in estradiol and estrone levels measured using liquid chromatography tandem mass spectrometry in community-dwelling men of the Framingham Heart Study. The journals of gerontology Series A, Biological sciences and medical sciences. 2013;68(6):733-40. Epub 2012/10/30.

21. Hsu B, Cumming RG, Waite LM, Blyth FM, Naganathan V, Le Couteur DG, et al. Longitudinal Relationships between Reproductive Hormones and Cognitive Decline in Older Men: The Concord Health and Ageing in Men Project. The Journal of clinical endocrinology and metabolism. 2015;100(6):2223-30. Epub 2015/04/14.

22. Maggio M, Snyder PJ, Ceda GP, Milaneschi Y, Luci M, Cattabiani C, et al. Is the haematopoietic effect of testosterone mediated by erythropoietin? The results of a clinical trial in older men. Andrology. 2013;1(1):24-8. Epub 2012/12/22.

23. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, et al. Testosterone dose-response relationships in healthy young men. American journal of physiology Endocrinology and metabolism. 2001;281(6):E1172-81. Epub 2001/11/10.

24. Sinclair M, Gow PJ, Grossmann M, Angus PW. Review article: sarcopenia in cirrhosis - aetiology, implications and potential therapeutic interventions. Alimentary pharmacology & therapeutics. 2016. Epub 2016/02/06.

25. Lai JC, Feng S, Terrault NA, Lizaola B, Hayssen H, Covinsky K. Frailty predicts waitlist mortality in liver transplant candidates. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2014;14(8):1870-9. Epub 2014/06/18.

26. Pentikainen PJ, Pentikainen LA, Huffman DH, Azarnoff DL. The effect of spironolactone on plasma levels and excretion of testosterone and oestrogens in the urine in males. (A preliminary report). The Journal of international medical research. 1974;2(6):439-43. Epub 1974/01/01.

27. Farrell GC, Koltai A, Murray M. Source of raised serum estrogens in male rats with portal bypass. The Journal of clinical investigation. 1988;81(1):221-8. Epub 1988/01/01.

28. Labrie F. All sex steroids are made intracellularly in peripheral tissues by the mechanisms of intracrinology after menopause. The Journal of steroid biochemistry and molecular biology. 2015;145:133-8. Epub 2014/06/14.

Tables

Table 1. Hormone profile of liver cohort compared to control cohort

	Cirrhotic cohort (n=95)	Controls (n=256)	p value
Age (years)	55 [51, 60]	55 [48, 62]	0.89
BMI (kg/m^2)	28.8 [26.0, 32.5]	28.3 [25.6, 31.2]	0.43
T (nmol/L)	9.2 [3.1, 14.42]	15.5 [12.5, 19.4]	p<0.001
DHT (nmol/L)	0.65 [0.31, 1.14]	1.5 [1.2, 2.0]	p<0.001
Estrone (pmol/L)	869.1 [576.9, 1331.4]	133.8 [109.2, 165.9]	p<0.001
Estradiol (pmol/L)	166.7 [125.6, 227.2]	84.6 [66.5, 100.5]	p<0.001
SHBG (nmol/L)	86.5 [83, 118]	34.6 [28, 44.4]	p<0.001

Handgrip strength	33.0 [27.2, 40.0]	45.3 [41.9, 51.7]	p<0.001
SF36 physical component	28.1 [23.6, 39.3]	52.6 [46.2, 56.2]	p<0.001
SF36 mental component	39.25 [30.5, 49.1]	56.2 [49.5, 59.7]	p<0.001

467 Legend: T: total testosterone, DHT: dihydrotestosterone, SHBG: sex hormone binding globulin,
468 SF36: short form survey. Data expressed as median (IQR)

469

470 Table 2. Sex hormone profile by Child Pugh score

	Child Pugh A	Child Pugh B	Child Pugh C	p value*
T nmol/L	14.5 [10.4, 18.9]	11.0 [6.68, 18.0]	3.22 [0.95, 8.98]	p<0.001
DHT nmol/L	0.96 [0.48, 1.12]	1.03 [0.57, 1.58]	0.37 [0.00, 0.71]	p<0.001
DHEA nmol/L	3.25 [2.24, 4.47]	3.29 [1.61, 4.98]	1.63 [0.90, 2.63]	p=0.001
Estrone pmol/L	492 [275, 655]	915 [600, 1345]	1108 [802, 1599]	p<0.001
Estradiol pmol/L	148 [102, 221]	179 [135, 234]	166 [126, 218]	p=0.654

Estrone/T ratio	31.6 [21.5, 61.5]	75.6 [49.1, 126]	413 [128, 1419]	p<0.001
Estradiol/T ratio	10.9 [9.2, 13.7]	15.5 [11.8, 22.7]	50.9 [21.3, 181]	p<0.001
SHBG nmol/L	91.0 [37.8, 110]	97.0 [70.8, 126]	72.5 [47.5, 109]	p=0.071

Legend: T: total testosterone, DHT: dihydrotestosterone, DHEA: dehydroepiandrostenedione, SHBG: sex hormone binding globulin. Data presented as median (IQR). *3 way comparison

Table 3. Associations between sex hormone profile and haematology and biochemistry

	MELD	Na	Bilirubin	INR	Albumin
T					
Correlation	0.294*	+0.344*	-0.248*	-0.254*	+0.15*
p value	<0.001	<0.001	<0.001	<0.001	0.035
DHT					
Correlation	-0.225*	0.270*	-0.182*	-0.195*	0.133*
p value	<0.001	<0.001	0.010	0.008	0.065
DHEA					
Correlation	-0.313*	0.200*	-0.268*	-0.249*	0.263*

p value	<0.001	0.005	<0.001	<0.001	<0.001
E1					
Correlation	+0.306*	-0.208*	0.317*	0.309*	-0.274*
p value	<0.001	0.004	<0.001	<0.001	<0.001
E2					
Correlation	*0.033*	*0.076*	*0.050*	*0.063*	*-0.091*
p value	*0.6400*	*0.289*	*0.471*	*0.384*	*0.202*
SHBG					
Correlation	-0.141*	+0.161*	*-0.077*	-0.199*	*+0.088*
p value	0.039	0.020	*0.257*	0.005	*0.202*

Legend: T: total testosterone, DHT: dihydrotestosterone, E1: estrone, E2: estradiol, LH: luteinising hormone, Hb: haemoglobin, Na: sodium, INR: international normalised ratio, *statistically significant. *Italics:* not statistically significant

Table 4. Sex hormones and severity of clinical ascites

Ascites	T (nmol/L)	DHT (nmol/L)	DHEA (nmol/L)	Estrone (pmol/L)
None	14.18 [10.9, 18.9]	0.98 [0.50, 1.57]	4.33 [2.18, 6.17]	492 [286, 736]

Mild, salt modification	8.79	0.65	2.64	923
	[3.91, 14.2]	[0.36, 1.41]	[1.07, 4.64]	[789, 1465]
Moderate, diuretics req	9.28	0.72	2.64	1008
	[5.03, 14.9]	[0.47, 1.24]	[1.31, 3.48]	[808, 1327]
Required paracentesis	2.94	0.33	2.12	1065
	[0.89, 8.17]	[0.003, 0.79]	[1.36, 2.95]	[788, 1660]
	p<0.001	p=0.001	p=0.054	p<0.001

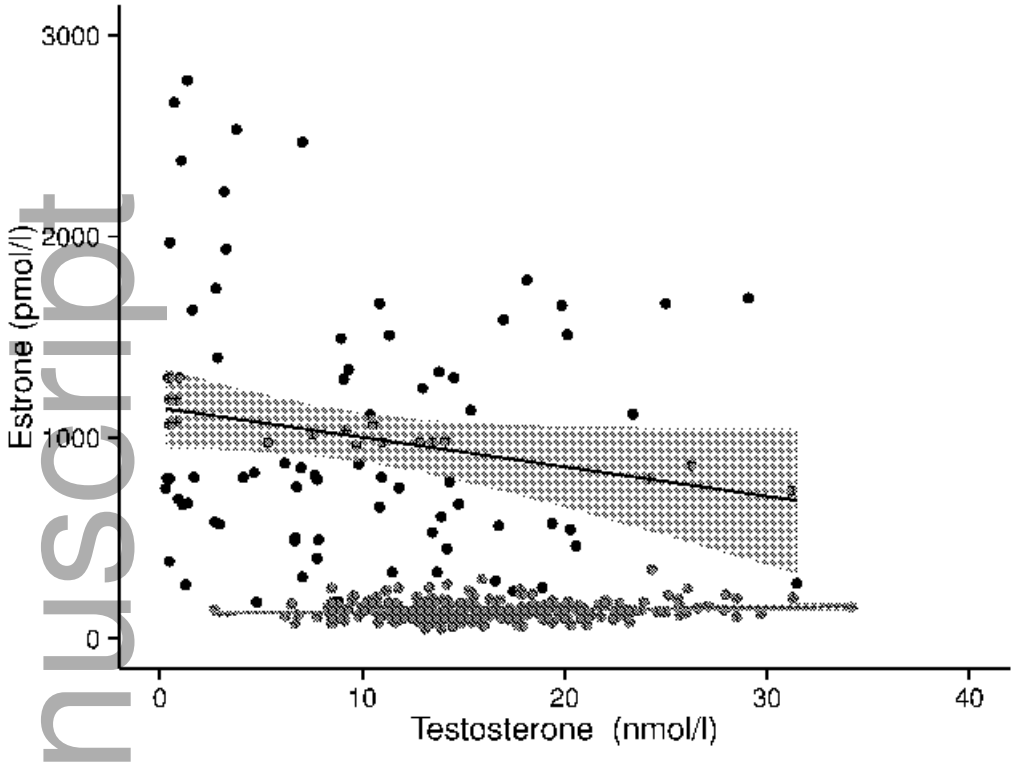
Legend: T: total testosterone. Data presented as median (IQR). p value refers to overall change

Figure 1a. Estrone versus testosterone levels in cirrhotic cohort and control cohort

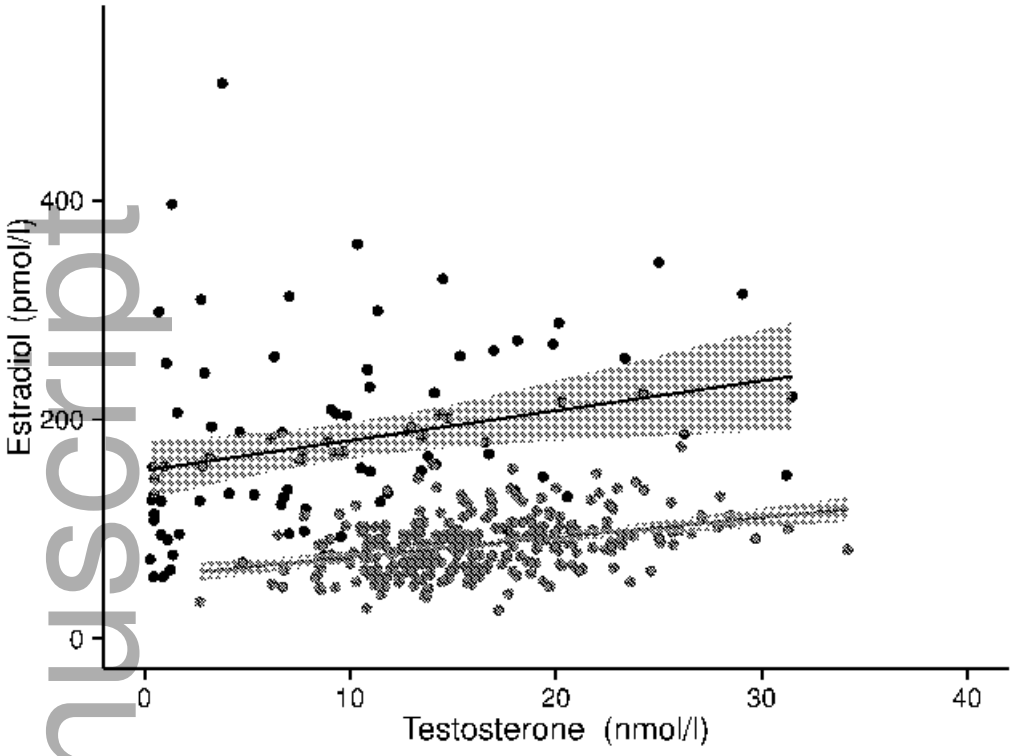
Legend: black represents cirrhotic men, grey represents controls. The regression lines depicted in this and the other figures are indicative only, and we relied on non-parametric correlations, as described in Text.

Figure 1b. Estradiol versus testosterone levels in cirrhotic cohort and control cohort

Legend: black represents cirrhotic men, grey represents controls.



liv_13122_f1a.tif



liv_13122_f1b.tif

Author/s:
Sinclair, M; Gow, PJ; Angus, PW; Hoermann, R; Handelsman, DJ; Wittert, G; Martin, S; Grossmann, M

Title:
High circulating oestrone and low testosterone correlate with adverse clinical outcomes in men with advanced liver disease

Date:
2016-11-01

Citation:
Sinclair, M., Gow, P. J., Angus, P. W., Hoermann, R., Handelsman, D. J., Wittert, G., Martin, S. & Grossmann, M. (2016). High circulating oestrone and low testosterone correlate with adverse clinical outcomes in men with advanced liver disease. LIVER INTERNATIONAL, 36 (11), pp.1619-1627. https://doi.org/10.1111/liv.13122.