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ORIGINAL ARTICLE

Time course of gonadal hormone profiles in male patients with sepsis

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Abstract

Background: Low testosterone levels are frequently found in male patients with critical illness. The underlying mechanisms are not exactly known. More knowledge of the pathophysiology is needed before considering replacement therapy.

Methods: Observational pilot study to assess the changes in androgen metabolism in male patients with sepsis who require mechanical ventilation. Gonadal hormone levels and their steroid precursors were measured during the first week of mechanical ventilation.

Results: Mechanical ventilation in male patients with sepsis was associated with very low testosterone levels, markedly elevated serum oestradiol levels, and inappropriately low luteinising hormone and follicle-stimulating hormone levels. During the first week of mechanical ventilation oestradiol levels gradually decreased, whereas testosterone levels remained low. Precursor steroids such as high-density lipoprotein cholesterol and dehydroepiandrosterone were reduced whereas the androstenedione concentration was maintained within the high-normal range.

Conclusion: Sepsis in male patients on mechanical ventilation is associated with very low testosterone and markedly elevated oestradiol levels, caused by a combination of reduced gonadotropin secretion, reduced androgen precursor availability and increased aromatisation. The impact of hypotestosteronaemia on recovery from critical illness needs further study.

Introduction

Although the large majority of male patients staying on an intensive care unit (ICU) have markedly reduced serum testosterone levels, the clinical implications remain unknown.[1-13] Hypotestosteronaemia develops rapidly after major somatic events; testosterone levels decline within 40 minutes after the start of coronary artery bypass grafting and within six hours after endotoxin injection.[11,14] It is conceivable that a chronic lack of testosterone and its anabolic actions may affect recovery after major illness and could play a role in the pathogenesis of ICU-acquired weakness. If so, replacement therapy might be indicated. However, before considering such treatment there is a need to further elucidate the pathophysiology of hypotestosteronaemia in critically ill men.

Reduced gonadotropin secretion, altered steroidogenesis and increased aromatisation of androgens appear to play a role.[7,15,16] However, current knowledge of these processes is still limited. To date, most research has focused on the changes in hypothalamic-hypopituitary action induced by critical illness, whereas the study of gonadal hormone synthesis pathways has received little attention.[17] An overall view of the key changes in the complex gonadal hormone pathway is needed to help to decide whether an endocrine intervention might be a logical choice, and to determine the optimal type, timing, and duration of hormonal replacement therapy. To gather this information and to avoid the obscuring effects of heterogeneous study populations, we decided to study the gonadal hormone pathway in a strictly defined group of patients on the ICU at risk of developing ICU-acquired weakness: male patients with sepsis and respiratory failure requiring mechanical ventilation.

Materials and methods

Patients

This pilot study was performed in a medical-surgical ICU of a teaching hospital in the Netherlands. Consecutive male patients presenting with at least severe sepsis and respiratory failure, who required mechanical ventilation, were eligible. Sepsis was defined according to the 2016 American College of Chest Physicians/Society of Critical Care Medicine consensus conference criteria. Patients known with hypogonadism, and those using testosterone replacement or drugs that could affect the hypothalamic-pituitary-testicular axis were excluded. Written informed consent was obtained before inclusion, either from the patient or from his legal representative.

The study was conducted according to the guidelines of the hospital's ethics committee. Illness severity was assessed by the APACHE II (Acute Physiology and Chronic Health Evaluation) and SOFA (Sequential Organ Failure Assessment) scores.

Blood samples were drawn from an indwelling arterial catheter at 08.00 hrs on day 1 (the first day after intubation), day 3, and day 7. On day 1 the androgen-oestrogen synthesis pathway was examined extensively by measuring serum luteinising hormone (LH), follicle-stimulating hormone (FSH), prolactin, cholesterol fractions and their lipoproteins apolipoprotein (apo)A1 and apoB100, the steroid precursors 17-hydroxy-progesterone (17-OHP), dehydroepiandrosterone (DHEA), DHEA-sulphate (DHEAS), androstenedione, total testosterone, sex hormone binding globulin (SHBG), albumin, total oestradiol, adrenocorticotropic hormone (ACTH), and cortisol. On day 3 and 7 CRP, LH, FSH, total testosterone, SHBG, albumin, oestradiol, cortisol, apoA1, apoB100, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and high-density lipoprotein cholesterol (LDL-C) were measured for follow-up.

Assays

LH, FSH, total testosterone, DHEAS, ACTH and prolactin were measured by solid-phase, two site chemiluminescent immunometric assay (CLIA) on an Immulite 2500 analyser (Siemens, Diagnostic Products Corporation Los Angeles, USA). Male reference ranges are: LH 1-8 IU/l, FSH 1-11 IU/l, total testosterone 9.0-30.0 nmol/l, DHEAS 2.2-15.2 µmol/l, ACTH <46 ng/l, and prolactin 53-360 mU/l. Oestradiol and cortisol were measured with an electrochemiluminescence immunoassay (ECLIA) on an E170 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Male reference ranges: oestradiol 40-160 pmol/l, cortisol 08.00 hrs 170-540 nmol/l, and 16.00 hrs 60-340 nmol/l. SHBG was measured by ECLIA on the E-module of a COBAS 6000 system (Roche Diagnostics GmbH, Mannheim, Germany). Male reference range: 14.5-48.4 nmol/l. Androstenedione was measured with a competitive radiometric immunoassay (Siemens, Diagnostic Products Corporation Los Angeles, USA). Male reference range: 2.8-10.5 nmol/l. DHEA was measured by radioimmunoassay after chromatographic purification of the samples (home-made assay). 17-OHP was measured by radioimmunoassay competition assay (Immunotech, Marseille, France, male reference range: 1.5-7.2 nmol/l. ApoA1 and apoB were measured with nephelometry on a BN ProSpec (Siemens Healthcare Diagnostics Products GmbH, reference range apoA1 male: 1.25-2.15 g/l, apoB male: 0.55-1.25 g/, apoB/apoA1 ratio male 0.30-0.90).

All other laboratory parameters (CRP, cholesterol, albumin, triglycerides) were measured by routine laboratory assays (Roche Diagnostics, Almere, the Netherlands).

Calculations

Calculation of free testosterone was based on the method of Vermeulen et al. (normal range 225-625 pmol/l), and free oestradiol was calculated by the method of Sodergard et al. (normal range: 1.1-4.7 pmol/l).[18,19] The total oestradiol / total testosterone ratio was used to assess aromatase activity (normal range 1.3-17.7 (x 10^{-3})).

Statistics

Data are shown as median values and ranges. A Mann-Whitney U test was used to compare median values. Correlations were calculated by Spearman's rank correlation coefficients. A p value <0.05 was considered statistically significant.

Results

Baseline characteristics of the 18 consecutive male patients included in the study are shown in *table 1*. Seven patients were admitted directly from the emergency department, whereas 11 patients were admitted from a clinical ward where they had been admitted for a median of three days (range 0-14). The median duration of illness was four days (range 1-21 days). The median APACHE II score was 21 (range 10-43). Thirteen patients were diagnosed with pneumosepsis, four had abdominal sepsis, and one had urosepsis. Five patients were excluded from further analysis due to incomplete follow-up, i.e. one patient died within the first week, three patients were discharged from the ICU to a general ward within one week and one patient was transferred to another hospital at day 4. Six of the remaining 13 patients with complete follow-up (46%) were extubated during the first week. None of the patients were on mechanical ventilation for more than three weeks. Prior to admission, none of the patients had used corticosteroids chronically. Two out of 18 patients received corticosteroids 2-3 days before inclusion, 6/18 patients received corticosteroids the day before inclusion, and 2/18 patients received corticosteroids on the day of inclusion. In four other patients, steroids were started after inclusion. Corticosteroids were continued in eight patients for a median of three days. All patients except one received morphine during

Table 1. Baseline characteristics of 18 male patients with severe sepsis and respiratory failure (n=18)

	Median	Range
Age (years)	72	43-84
Weight (kg)	72	59-100
APACHE II	21	10-43
SIRS	3	2-4
SOFA	7	4 -12

ICU scoring systems: APACHE II = acute physiology and chronic health evaluation II; SIRS = systemic inflammatory response syndrome; SOFA = sequential organ failure assessment score

their stay on the ICU, administered continuously by intravenous pump. All except one received norepinephrine at the time of inclusion, and two patients also received dobutamine. One patient received a single bolus of etomidate for rapid sequence induction. Five patients used statins before admission, and all were discontinued at admission.

Pituitary-gonadal axis at inclusion

The pathophysiology of hypotestosteronaemia was examined by evaluation of the pituitary-gonadal axis and a gonadal hormone precursor analysis on day 1 in 18 patients *(table 2)*. LH and FSH levels were in the low-normal range, i.e. inappropriately low for the severely reduced testosterone levels. Prolactin levels were within the normal range in 8/17 patients, and 9/17 patients had mild hyperprolactinaemia ranging from 428-1253 mU/l. In one patient we were unable to measure serum prolactin due to a lack of material. Median testosterone levels were markedly reduced. Total and free testosterone were below the lower limit of normal on day 1 in 17/18 patients. In contrast, median oestradiol levels were markedly increased. Sixteen out of 18 men had a serum oestradiol exceeding the upper normal level of 160 pmol/l.

Table 2. Blood levels of key products in the androgen and oestrogen synthesis pathways at day 1 (n=18)

	Median (range)	Reference range
CRP (mg/l)	152 (3-348)	0-9
ApoA1 (g/l)	0.48 (0.19-0.99)	0.94-1.78
ApoB100 (g/l)	0.51 (0.32-1.02)	0.63-1.33
HDL-cholesterol (mmol/l)	0.3 (0.1-2.1)	0.8 - 1.8
LDL-cholesterol (mmol/l)	1.1 (0.2-4.5)	3.5-4.5
ACTH (ng/l)	11 (5-52)	0-46
Cortisol (nmol/l)	935 (100-6130)	170-540
LH (U/l)	3 (0-10)	1-8
FSH (U/l)	2 (0-7)	1-11
Prolactin (mU/l)	418 (104-1253)	53-360
17-OH-progesterone (nmol/l)	1.1 (0.5-5.2)	1.8-10
DHEA (nmol/l)	0 (0-5)	15-45
DHEAS (µmol/l)	0.5 (0.4-3.4)	2.2-15.2
Androstenedione (nmol/l)	14.1 (1.2-33.0)	2.8-10.5
Total testosterone (nmol/l)	2 (1-9)	9-30
SHBG (nmol/l)	23.0 (7.8-50.0)	14.1-68.9
Albumin (g/l)	19 (12-27)	35-50
Free testosterone (pmol/l)	57 (10-290)	225-625
Oestradiol (pmol/l)	313 (97-1339)	40-160
Free oestradiol (pmol/l)	18.7 (7.8-65.3)	1.1-4.7

CRP = C reactive protein; ACTH = adrenocorticotropic hormone; LH = luteinising hormone; FSH = follicle stimulating hormone; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin

Table 3. One-week follow-up of gonadal hormone profiles in 13 patients; five out of 18 patients were excluded because of a follow-up of less than 7 days

	Reference range	Day 1 n=13	Day 3 n=13	Day 7 n=13
CRP (mg/l)	0-9	159 (3-348)	104 (21-313)	80 (13-301)
HDL-cholesterol (mmol/l)	0.8-1.8	0.3 (0.1-1.1)	0.3 (0.0-0.8)	0.6 (0.1-1.0)
LDL-cholesterol (mmol/l)	3.5-4.5	1.1 (0.2-4.5)	1.1 (0.2-3.6)	1.5 (0.5-2.5)
LH (U/l)	1-8	3 (1-8)	2 (1-6)	2 (1-11)
FSH (U/l)	1-11	2 (0-4)	1 (0-4)	1 (0-7)
Androstenedione (nmol/l)	2.8-10.5	17.2 (1.2-33.0)	8.3 (1.4-30.0)	6.7 (0.0-52.9)
Total testosterone (nmol/l)	9-30	2 (1-3)	1 (1-2)	1 (1-7)
Free testosterone (pmol/l)	225-625	61 (10-160)	26 (0-80)	32 (10-280)
Oestradiol (pmol/l)	40-160	356 (97-1339)	240 (53-884)	156 (74-533)
Free oestradiol (pmol/l)	1.1-4.7	21.2 (0.9-5.3)	12.6 (3.0-60.1)	7.4 (2.5-25.0)
Total oestradiol / Total testosterone ($x10^{-3}$)	1.3-17.7	223 (97-549)	131 (53-594)	129 (15-485)

CRP = C-reactive protein; LH = luteinising hormone; FSH = follicle-stimulating hormone

Steroid precursor analysis at inclusion

All cholesterol fractions and their apoproteins were markedly reduced *(table 3)*. HDL-C, was <0.25 mmol/l in seven patients. HDL-C levels were not correlated with 17-OHP, DHEA, androstenedione, total or free testosterone. Median levels of the androgen precursors 17-OHP and DHEA were decreased. In contrast, median androstenedione levels were increased. Twelve out of 18 patients had androstenedione levels exceeding the upper normal limit. Median total and free testosterone levels were markedly reduced, whereas oestradiol levels were elevated. Total oestradiol/testosterone levels were elevated in all patients. Androstenedione and total oestradiol levels were correlated (R^2=0.47, p<0.0001; *figure 3*). Androstenediol and oestrone levels were not measured.

One-week follow-up of testosterone and oestradiol levels

Thirteen patients completed the one-week gonadal hormone follow-up on the ICU. Their data are summarised in *table 3* and shown in *figure 2*. On the first day of mechanical ventilation, total and free testosterone levels were very low in most patients and this remained so during the next days. Most patients reached their nadir testosterone at day 3. Three patients demonstrated a substantial rise in total and free testosterone on day 7, but the median testosterone levels were not significantly different from the days before. Six patients were extubated during the course of the week. At day 7, total testosterone was reduced to a similar extent in patients who had been extubated as in those who were not (1.8 nmol/l vs. 1.1 nmol/l, p=0.45). In the majority of patients

total and free oestradiol levels were markedly increased at day 1, approaching levels commonly observed in healthy premenopausal women. During the course of the week total oestradiol decreased from 356 pmol/l at day 1 to 156 pmol/l at day 7, and free oestradiol followed a similar pattern *(figure 1)*. Total oestradiol levels did not differ between patients who had been extubated and those who remained on the ventilator (149 pmol/l vs. 169 pmol/l, p=0.37). The total oestradiol / total testosterone ratio, a marker of aromatase activity, was markedly increased at baseline (223 x 10^{-3}), rapidly decreased thereafter, but was still tenfold higher than the upper normal limit at day 7 (129 x 10^{-3}, normal range: 1.3-17.7 x 10^{-3}).

Discussion

This study describes the changes in gonadal hormones of severely septic male patients who require mechanical ventilation for respiratory failure. It mainly concerned patients who were weaned from the ventilator in a relatively short period of time. They had a characteristic profile of very low testosterone and very high oestradiol levels, which was already present on the first day after intubation. Testosterone levels remained low as long as patients were ventilator dependent, whereas oestradiol levels gradually declined. Previous reports have shown that chronically ventilated patients also suffer from hypotestosteronaemia.[12,13] The time course of oestradiol levels during chronic ventilation has not been documented previously.

Under normal circumstances, over 95% of testosterone production occurs in the testis, in a complex pathway stimulated by LH. The results of the present study indicate that the reduction in testosterone synthesis in the early phase of sepsis is multi-factorial. LH levels were inappropriately low for the degree of hypotestosteronaemia, and thus failed to stimulate testosterone synthesis. This suppression of hypothalamic-pituitary action is in line with previous reports.[17] It has been attributed to cytokine action, medication such as corticosteroids, opioids, and dopamine, and the markedly elevated oestradiol levels.[16]. Oestradiol exerts a strong negative feedback on LH/FSH secretion in men.[16]

Reduced LH secretion is not the only factor explaining the low testosterone levels. Our data indicate that hypotestosteronaemia is also related to changes in its synthesis pathway. Reduced substrate availability may have played a role in some patients. All cholesterol fractions and their apoproteins were markedly reduced *(table 3)*. HDL-C, the deliverer of the base material for steroid hormone production, was <0.25 mmol/l in seven patients.[20] However, the lack of correlations of HDL-C with 17-OHP, DHEA, androstenedione, and total or free testosterone suggest that factors other than reduced substrate availability are important. Reduced activity of some androgen generating enzymes might be another explanation. The testis can generate testosterone from DHEA after its conversion into either androstenediol or androstenedione *(figure 2)*. The low levels of both DHEA and testosterone, and the lack of correlation between DHEA and total or free testosterone, suggest that the DHEA- androstenediol-testosterone pathway activity is markedly reduced in critical illness, but definitive proof is lacking since measurements of androstenediol were not available. In contrast, androstenedione levels were elevated. Androstenedione can be generated from DHEA or from 17-OHP *(figure 2)*. As serum levels of both precursors were decreased, this suggests preferential and stimulated synthesis of androstenedione to an extent that it leads to partial depletion of its precursors. In addition, the combination of elevated androstenedione levels and low testosterone levels suggests a (partial) block in the conversion of androstenedione into testosterone, a conclusion that is supported by the lack of correlation between androstenedione and testosterone levels. Androstenedione seems to hold a key position in the altered gonadal hormone pathway of critical illness: 12 out of 18 patients had androstenedione levels exceeding the upper normal limit. The combination of low testosterone and high oestradiol levels indicates that androstenedione is shunted into the oestrogen pathway by conversion into oestrone, a process that requires high aromatase activity. The high total oestradiol/total testosterone ratio and the correlation between androstenedione and total oestradiol levels (R^2=0.47, p<0.0001; *figure 3*) support this hypothesis.

It is currently not exactly known which steroid production site contributes most to the increased androstenedione levels. Although it is generally attributed to a combination of enhanced adrenal and testicular production, animal studies suggest that androstenedione can also be generated in adipose tissue.[21] It remains to be shown whether cytokines or other substances liberated by sepsis can activate this process.

The high oestradiol levels are attributed to a preferential conversion of androstenedione into oestrogens, due to increased aromatase activity, a process that mainly occurs in adipocytes and that is known to be stimulated by cytokines.[15,21,22] Increased adipocyte aromatase activity has indeed been observed in patients after coronary artery bypass grafting.[15] High oestradiol levels in the order of magnitude observed in these ICU patients exert a potent negative feedback on LH/FSH secretion and thus reduce the stimulus for testosterone synthesis.[23] However, it has recently been shown that low-dose endotoxin challenge produced a decline in serum testosterone without a concomitant change in LH or FSH, suggesting that endotoxin-driven inflammation can also impair Leydig cell function directly.[14]

Our findings and those of others indicate that oestrogen synthesis is strongly stimulated at the cost of androgen production, and that androstenedione holds a key position in the acute phase

of sepsis in male patients.[16,24] It is not clear whether this response is coincidental or whether it represents an adequate, beneficial adaptive host response, irrespective of gender. The latter interpretation is supported by studies in animals showing better outcomes in rat models with the lowest testosterone and the highest oestradiol levels.[16,22] Depressed immune systems and higher mortality rates have been observed in critically ill male animals with low oestradiol levels as well as in oestrogen depleted female animals who had depressed immune systems and demonstrated higher mortality rates.[11,22] In humans, however, elevated oestradiol levels in critically ill male as well as female patients are associated with worse outcome and survival in most studies.[15,22,25,26] This suggests that oestradiol reflects severity of illness; however, it does not tell us whether high oestradiol levels are beneficial for survival or not. In view of the anti-inflammatory, antioxidant and immunosuppressive properties of oestrogen, it has been postulated that high oestrogen levels may serve to reduce the damaging effects of an overwhelming inflammatory response.[27] It has also been suggested that maintaining high oestrogen levels during the acute phase of sepsis and prevention of a decline during the early recovery period might be beneficial; however, to date positive effects of oestrogen treatment have only been shown in animal resuscitation models, not in humans.[27-29]

Whether, when, and how either low oestrogen and/or testosterone levels in male patients on the ICU should be corrected is currently not known. The present study has shown that the gradual decline in oestradiol that occurred during the early recovery period was not associated with a normalisation of the LH-testosterone axis, and this ongoing lack of an anabolic hormone in a recovery period may increase the risk of wasting. The results of our study on steroidogenesis suggest that prevention of a premature decline in oestrogens might be achieved in several ways, i.e. by supplementation of DHEA, androstenedione, testosterone, or directly by oestradiol therapy. However, in view of the partial blocks in the testosterone pathways, normalisation of testosterone levels is unlikely to occur with supplementation of the precursors DHEA or androstenedione, but will require testosterone treatment.

The main limitations of the present study are the small number of patients, the fact that not all steps in the androgen-oestrogen pathways have been evaluated, and that the site of origin of measured androgen precursors was not established. Therefore, several conclusions remain hypothetical and lack ultimate proof, and will require further research. Another limitation is the fact that 11 patients were already admitted to the hospital, although their testosterone levels are not significantly lower, and the finding that a number of patients used drugs that could interfere with the results such as corticosteroids. Furthermore, as mass spectrometry was not available at the time of this study, all steroid measurements were performed with radioimmunoassays. The former technique is now preferred because of increased accuracy and specificity. However, a recent study measuring gonadal steroids by mass spectrometry in patients with burn injuries indicates that the general pattern of steroid hormone response is the same as detected by radioimmunoassays.[30] Finally, it remains to be shown that the changes in the gonadal steroid pathway detected in this strictly defined group also occur in other types of critically illness in male patients.

Conclusion

Hyperoestrogenic hypotestosteronaemia is a characteristic and frequent finding in the acute phase of severe sepsis in male patients on mechanical ventilation in the ICU. It is attributed to reduced hypothalamic/pituitary activity, blocks in androgen production and a shunting of androgens to oestrogens as a result of increased aromatisation. It remains to be shown whether raising oestradiol and/or testosterone levels in the recovery phase of sepsis can favourably affect outcome in male patients with critical illness.

Disclosures

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