An investigation of LH pulsatility in burned men by bioassay and radioimmunoassay

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LH pulsatility studies were performed in six burned patients by removing blood samples at 10 min intervals over a 6 h period. All samples were assayed for LH by bioassay (B-LH), LH by radioimmunoassay (I-LH) and testosterone. Mean serum testosterone concentrations of the burned patients were low (6.7 ± 1.6 nmol/l). I-LH levels were lower than B-LH in all samples. Frequency of bioactive or immunoreactive pulses as well as mean B-LH and I-LH concentrations were similar to previously published data from normal men examined in the same laboratory. The mean biological activity of LH (expressed as the ratio of B-LH to I-LH, the B:I ratio) was lower in burned subjects (1.9 ± 0.1) than previously reported in normal men. The B:I ratios of burned men were lower (p < 0.01) at pulse peaks than at nadirs (1.8 ± 0.1 vs 2.0 ± 0.1) and an increase in serum testosterone concentration did not follow an LH peak. Serum testosterone concentrations did not cross-correlate with B-LH or I-LH. This contrasts with the findings in normal subjects where the B:I ratios have been found to be higher at pulse peaks than at nadirs and an increase in serum testosterone concentration follows a pulse peak and serum testosterone cross-correlates with B-LH and I-LH. LH secreted in a pulse peak in normal men may contain a particularly biologically potent form of the molecule but this may not be the case in burned men.

A low serum testosterone concentration is a common finding in ill men. This has been recognized for many years in patients with chronic renal failure (1) and chronic liver disease (2), probably because sexual dysfunction is common in such patients. A similar abnormality has been noticed in many other conditions which are not usually associated with sexual difficulties, for example respiratory failure (3), surgery (4), myocardial infarction (5), head injury (6) and diabetic ketoacidosis (7). Burns injury is associated with an even more profound degree of suppression of serum testosterone concentrations with mean levels depressed to 19% of those of a normal control group (8) as compared with 65% in respiratory failure (3), 48% following surgery (4) and only 83% following myocardial infarction (5). In a recent study we found 12 of 19 burned men having serum testosterone concentrations within the female range (< 2 nmol/l) at some stage after burns injury (9).

Burned patients with low serum testosterone concentrations usually have immunoreactive LH levels (I-LH) within the normal range and similar to age-matched healthy men (8, 9, 10). The testes of burned men are not refractory to LH as HCG stimulates release of testosterone in a normal fashion (9). Thus the very low serum testosterone concentrations found in burned men cannot be explained on the basis of primary testicular failure or a classical hypogonadotrophic state. Neither are changes in binding proteins or prolactin of sufficient magnitude to account for these very low serum testosterone concentrations (9).

In normal man LH secretion is pulsatile (11). Patients with chronic renal failure and chronic liver disease have reduced or absent LH pulsatility (12, 13) and in our previous study we showed that some burned patients with low serum testosterone concentrations also had reduced I-LH pulsatility (9). Moreover, LH biological activity measured in single samples from these patients was reduced, a finding since confirmed by others (14). LH biological activity is known to be higher at a pulse peak than between pulses in normal men (15, 16) and thus the low LH biological activity of burned men might have been a reflection of their reduced LH pulsatility. In order to investigate this further, LH pulsatility studies were performed in six burned men. LH was measured by both bioassay and radioimmunoassay at each point through a pulse series.

Methods and subjects

Patients were studied between the second and fourth weeks after burn injury as our previous study had suggested that suppression of the hypothalamic-pituitary-testicular axis would be maximal at this stage (9). The characteristics of the burned men are described in Table 1. All patients had normal renal and hepatic function at the time of study. It was not possible to
exclude entirely the effects of drugs in this study as four patients (B1–B4) had received parenteral opiates for up to 24 h after admission. Analgesia thereafter was provided where necessary with a variety of oral analgesics (see Table 1).

Data from 11 normal men (age range, 20–40 years, mean age 27 years) are referred to for comparison purposes and were derived from Talbot et al. (16). All samples were, however, treated in the same manner and all assays were carried out in the same laboratory over the same nine-month period using the same quality controls.

Blood sampling

Pulsatility studies were carried out on six burned men using a 6 h sampling period between 14.00 and 22.00. An indwelling venous cannula was inserted in the antecubital fossa and kept patent with 0.5 ml heparin solution (10 000 U/l). Blood was withdrawn every 10 min for 6 h and transferred into Lithium Heparin tubes on ice and centrifuged with plasma being snap frozen and stored at –40°C until hormone assay.

All samples were assayed in duplicate for LH by radioimmunoassay (I-LH). LH by bioassay (B-LH) and testosterone so that a mean coefficient of variation could be calculated for each set of samples from each patient. All samples from a subject were assayed in the same assay.

Pulse analysis

Pulse analysis was performed by a modification of the method of Santen and Bardin (17) which has been previously described (18). A pulse was detected if there were at least two points greater than the preceding nadir by at least three times the mean intra-assay coefficient of variation for the samples in that pulse profile. Each peak also had to have a descending limb. Results from each subject are expressed as the mean of all LH levels obtained, the number of pulses during each 6 h study period and the mean amplitude of each subject’s pulses. The amplitude was calculated by subtracting values from the preceding nadir from the mean of the two highest values constituting a peak, expressed both as a percentage and as an absolute increment.

Radioimmunoassays

Testosterone was measured in ether extracts of serum using a double antibody radioimmunoassay based on a rabbit anti-testosterone-3-0-carboxymethyl oxime-BSA serum and testosterone-3-(carboxymethyl)-histamine-125I (Amersham). The intra-assay and interassay coefficients of variation for the testosterone assay were 3.8% and 7.1% respectively.

Serum LH was measured using a conventional double antibody radioimmunoassay and was standardized using the First International Reference Preparation MRC 68/40. The LH antibody employed was raised in the rabbit against human pituitary LH (code 87/2, a gift from Professor WR Butt, University of Birmingham). The free α subunit cross-reactivity with the LH antisera was 0.1% (19). The LH tracer was obtained from the WHO matched Reagents Programme, Department of Chemical Pathology, Hammersmith Hospital. The interassay coefficient of variation was 6.1%. From imprecision profiles the intra-assay coefficient of variation of LH was 7.9, 5.1, 4.3, 4.5 and 4.9% at 2.3, 4.7, 9.0, 19 and 38 IU/l. The sensitivity of the LH radioimmunoassay was 0.78 IU/l. The assay performance, based on external quality controls from the National External Quality Assessment Scheme for gonadotrophins, was within the accepted limits of bias and variability (<9%). There was no evidence of non-parallelism with this assay over the concentration ranges presented in this study.

LH bioassay

The biological activity of LH was measured using a modification of the isolated interstitial cell bioassay (20). Two male mice were killed by cervical dislocation. Their testes were removed, decapsulated and placed in Dulbecco’s Modified Eagle’s Minimum Medium (DMEM) without bicarbonate and with HEPES (20 mmol/l) containing 4% donor calf serum. The testes were cut into small pieces using fine scissors and were dispersed by mechanical agitation for 10 min at 37°C in air. The resulting cell
suspension was filtered through nylon (100 µm mesh), collected by centrifugation (150 g, 5 min at 4°C), resuspended in DMEM with serum and allowed to incubate for 1 h at 37°C with gentle shaking. Cells were collected again by centrifugation, resuspended in medium at a concentration of 0.66 × 10³ viable cells per l. Routine cell viability is in excess of 90% as assessed by the trypan blue exclusion method. Three quality controls, graded doses of LH (First International Reference Preparation MRC 68/40) or dilutions of unknown plasma samples each at 50 µl, were dispensed into a 96 well tissue culture plate and treated with 50 µl of cell suspension.

After a 4 h incubation, culture plates were frozen until testosterone levels could be estimated in duplicate samples by standard radioimmunoassay. All samples from the same individual were assayed within one assay to eliminate interassay variation. The intra-assay coefficient of variation was <8% (N = 765) over the concentration range found in this study and in the study of normal men previously referred to (16). This error also encompassed that associated with the two dilutions (each assayed in duplicate) used for each plasma and thus provides good evidence of parallelism between dilution of plasma and standard curve. The interassay coefficient of variation for this assay was 10.9% and was derived from quality control data obtained from three pools of plasma (containing 4.5, 16 and 45 IU/l) assayed in the 17 assays used in the two studies compared in this paper. Human FSH, TSH, ACTH, GnRH, growth hormone, prolactin, oxytocin and antidiuretic hormone did not influence the bioassay method at levels likely to be found in biological samples. Parallelism in the dose response curve was observed between the plasma samples and the reference preparation. The sensitivity of this assay (defined as the minimum concentration of LH that was significantly different from control) was 1.9 U/l.

The biological quality of LH is usually assessed by expressing data as a ratio of LH as measured by bioassay to that measured by radioimmunoassay (B:LH ratio) (15. 21).

Data analysis

Data were analysed by the Wilcoxon rank test. Results are expressed as mean ± SEM.

To investigate the temporal relationship between LH and testosterone and between immunoreactive and bioactive LH, cross-correlation analyses were calculated at every realizable time lag (22).

Results (Table 2)

Mean serum testosterone concentrations of the burned patients were low (6.7 ± 1.6 nmol/l; normal range 11–37 nmol/l). Mean B-LH and I-LH levels were 11.9 ± 1.9 U/l and 6.3 ± 0.9 U/l respectively. I-LH levels were lower than B-LH in all samples.

In burned men there were 13 bioactive (mean 2.2/6 h) and 12 immunoreactive pulses (mean 2.0/6 h). One patient (B-I) showed no pulsatility. All immunoreactive pulses were synchronous with bioactive pulses. B-LH cross-correlated with I-LH with zero time lag in five of the six burned men (p < 0.01) (B-1, B-2, B-3, B-4, B-6). B:LH ratios of burned men were lower (p < 0.01) at pulse peaks than at the nadirs (1.8 ± 0.1 vs. 2.0 ± 0.1).

In only one of the six burned patients (B-5) did serum testosterone cross-correlate significantly (p < 0.01) with B-IH and I-IH with a delay of 40 and 20 min respectively.

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<th>Table 2. Mean hormone levels and B-LH/I-LH pulse characteristics in six burned men.</th>
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The mean hormone levels for each subject were calculated from the samples in that pulse profile.

Data for pulse characteristics are quoted as the median (range) and are calculated from the mean for each pulse series. Normal control data are derived from Talbot et al. (16).
Discussion

This study has examined both B-LH and I-LH through pulse series in burned men. Previous authors have only studied LH biological activity from single samples (9, 14). The low B:I ratios found in these studies might merely have been a reflection of their reduced LH pulsatility as LH B:I ratios are known to be greater at a pulse peak than between pulses (15, 16). The low B:I ratio previously described in burned men has been confirmed but has been shown not to be due to a reduction in LH pulse frequency.

We have used for comparison purposes the data of Talbot et al. (16) summarized in Table 2. These were collected in an identical fashion, assayed in the same laboratory within the same nine-month period and with the same quality controls. Serum testosterone concentrations were significantly lower in our burned patients (p < 0.01) but there was no significant difference in B-I, H-I, I-I or any of the pulse characteristics measured. In normal men B:I ratios were higher at pulse peaks than at nadirs (2.9 ± 0.2 vs 2.2 ± 0.1). In contrast B:I ratios of burned men were lower (p < 0.01) at pulse peaks than at nadirs (1.8 ± 0.1 vs 2.0 ± 0.1). In normal men there was a clear relationship between levels of B-IH-I-LH and serum testosterone with significant cross-correlation in all 11 men studied (p < 0.01) with a mean delay of 50 min in both cases. In contrast B-LH and I-LH concentrations cross-correlated significantly with serum testosterone concentrations in only one burned man.

It should be mentioned that because of potential problems in the measurement of low levels of LH by RIA, the interpretation of changes in B:I ratios has been questioned (23, 24), largely on the grounds of the specificity of the RIA giving rise to inaccurate estimates of the ratio. Although the problem of non-specific interference in radioimmunoassays is well known (25), this problem extends also to isotopic and non-isotopic immunometric assays (26). Indeed, of the nine UK laboratories which routinely employed the Pharmacia Delfia LH assay and that are involved in the UK EQAS for peptide hormones, four detected levels of LH between 0.5 and 1.0 IU/l in immunoaffinity-stripped LH free plasma (26). Clearly the problems encountered during the measurement of the glycoprotein hormones are complex (23) and the diagnostic relevance of any such method needs careful evaluation. Fortunately the radioimmunoassay level of LH detected in the plasma samples assayed in the present study were > 3 IU/l and any difficulties arising out of non-specificity at low levels of hormone (i.e. < 1.5 IU/l) would be minimized.

In contrast to our previous study (9) there was no significant difference in immunoreactive LH pulsatility between burned patients and normals. In both studies there was a heterogeneous pattern in burned patients with one or two patients in each study having absent pulsatility, a finding not found in normal subjects. The study described in this paper was carried out rather later (mean 19, range 10–25 days) after burn injury than the earlier study (mean 10, range 7–14 days). The timing of LH pulsatility investigations had not appeared critical as our earlier study had demonstrated suppression of serum testosterone concentrations for at least five weeks after burns injury.

Different patterns of LH and testosterone secretion were found in burned patients. A single patient (B-1) had no LH peaks and showed no evidence of testosterone secretory episodes. Four patients (B-2, B-3, B-4, B-6) had LH peaks but showed no evidence of testosterone secretory episodes. It is possible that there is a more consistent depression of LH pulsatility in the early days after injury with recovery occurring in a variable manner. This could only be clarified by sequential pulsatility studies which would not be compatible with patient care. More consistent findings might have been obtained if patients had been sampled within one week of injury but again considerations of patient care often require these studies to be delayed.

Burns injury leads to a wide variety of metabolic changes such as weight loss and intense catabolic activity which might be expected to have an adverse effect on pituitary-testicular function. However, a low serum testosterone concentration is a common feature of many acute and chronic illnesses in which such metabolic changes are not invariably found. It appears likely that this testosterone/LH coupling is not specific to burns injury but more a non-specific consequence of illness. We were unable to exclude the effects of exogenous opiates on our patients as they had all received various preparations at differing strengths. High dose intravenous diamorphine given for ten days has been shown to have a modest testosterone lowering effect (27). It seems unlikely that the doses of routine analgesics used by our patients could have led to the abnormalities detected although we cannot exclude some effect on the hypothalamic-pituitary testicular axis.

Pulse profiles of burned men differed from normal in two ways. The biological activity expressed as the B:I ratio was lower at the pulse peak than at the nadir. The reverse is true in normals (15, 16). LH pulses were usually followed by surges of testosterone in normal men but this happened only in a single burned man. This lack of a relationship between LH and testosterone secretion was demonstrated by the failure to cross-correlate LH and testosterone levels in burned men in contrast to the consistent cross-correlation found in normal men. These differences might be explained by a number of mechanisms. The testes of burned men might be refractory to stimulation by an LH secretory episode; unlikely in view of our previous finding that most burned men exhibit the expected rise in serum testosterone concentration following hCG, although this is a pharmacological rather than a physiological stimulus (9). An inhibitor of LH action might be present in the serum of burned men but one would expect to find non-parallelism and there was no evidence of this in the assay. The explanation that we
favour is that the LH secreted at the pulse peak is qualitatively abnormal and is unable to stimulate testosterone secretion. It is possible that the low LH B:I ratios might be a consequence of low serum testosterone concentrations rather than a cause, but men with primary testicular failure seem to have high LH B:I ratios returning to normal with testosterone replacement therapy suggesting that this is unlikely to be the case (28).

What mechanisms might exist to account for an alteration in biological activity of LH? This glycoprotein in man is synthesized and secreted in several distinct forms each with different biological activity (29, 30). This heterogeneity is not caused by changes in the amino acid sequence of the α or β chains but to variations in the nature of the carbohydrate moieties of the hormone (31). The presence of these sugars may be important for activity of polypeptide hormones. Deglycosylation of α subunits prior to recombination of the α and β subunits resulted in a glycoprotein with increased receptor binding but with no response from the target cell (32). Matzuk et al. (33), using mutant cell lines secreting hCG dimers with different oligosaccharides attached, found that dimers lacking oligosaccharides on the α chain had reduced biological activity and indeed some forms of hCG acted as a competitive inhibitor binding to the hCG receptor but blocking production of cAMP by native hCG.

LH of increased biological activity is secreted at a pulse peak in normal men and in men with hypogonadotrophic hypogonadism whose LH pulsatility is restored by GnRH therapy (15, 34). Low but not pharmacological doses of exogenously administered GnRH stimulate release of biologically rich LH (34). In vitro GnRH stimulates subunit glycosylation in pituitary cells from castrated rats (35). Thus in vivo and in vitro evidence exists to suggest that GnRH may alter glycosylation of LH: release of LH of high biological activity may thus depend on normal GnRH pulse generator activity.

A possible course of events might be as follows: soon after burns injury the LH pulse generator is switched off; intermittent GnRH release is mandatory for release of biologically active LH and possibly also for its synthesis; the LH secreted whilst capable of detection by radioimmunoassay may have altered glycosylation and is not able to stimulate testosterone secretion; when the pulse generator begins to recover, release of LH of lower biological activity may continue until biologically richer LH becomes available. This study may have been performed while the pulse generator was in the recovery phase but was not yet able to promote release of biologically rich LH.

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