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ORIGINAL ARTICLE

Decreased levels of testosterone and gonadotrophins in men with long-standing tetraplegia

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Study Design: Blood samples were frequently collected during a 24-h period from six tetraplegic men. The results were compared with those of eight able-bodied controls.
Objective: Previous studies have reported conflicting results regarding the plasma concentrations of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in tetraplegia. The objective of this study was to examine the pituitary–gonadal axis by determining the plasma concentrations and circadian variations of these hormones in men with long-standing tetraplegia.
Setting: Sunnaas Hospital, Norway.
Methods: The plasma concentrations of hormones were measured with standardized assays.
Results: All three hormones and free testosterone index were decreased in the tetraplegic subjects compared with the able-bodied controls ($P<0.05$). We also determined the morning levels of hormones with regulatory effects on testosterone, LH and FSH. Whereas plasma leptin was significantly higher in the tetraplegic group, no significant differences in the morning plasma values for insulin, SHBG, GH or IGF-1, or in the 24-h urine concentrations of cortisol were detected between the two groups. The plasma concentration of LH displayed a circadian variation ($P<0.05$) in the tetraplegic group, but not among the able-bodied. No circadian variation was noted for the plasma concentrations of testosterone and FSH in either group.
Conclusion: Our data indicate that, over time, tetraplegic male subjects might be at risk of developing hypogonadism.
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Keywords: circadian rhythm; gonadotrophins; sex hormone; spinal cord injury; tetraplegia

Introduction

Impaired sexual function and a large loss of muscle mass below the injury level are frequent findings in patients with long-standing cervical spinal cord injuries (SCIs). Furthermore, chronic SCI may alter the plasma concentrations of the sex hormone testosterone and the two gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Earlier studies have given conflicting results regarding the levels of these hormones in tetraplegic men. Thus, the blood levels were reported higher, lower or similar to those of able-bodied controls.[1–6]

In SCI, hormone blood levels seem also to be influenced by both the duration and the level of injury. For example, a previous study with repeated measurements of androgen hormones in paraplegic men showed the testosterone concentrations to be consistently below the normal values during the first 3 months after injury, before they returned towards those of able-bodied subjects.[3] Another study demonstrated that SCI subjects with T_8–T_{11} injuries had a higher number of hormonal abnormalities when compared to all other injury levels.[4]

It is not known whether the observed change in the plasma concentrations of testosterone is of importance regarding deficient fertility and impaired sexual function in SCI, and replacement therapy has been tried without convincing effects.[7]

To better understand the underlying mechanism of the gonadal–pituitary axis impairments in tetraplegic men, an evaluation of circadian rhythms is essential. Apparently only one study has previously been published examining possible circadian variations of testosterone and gonadotrophins in paraplegic SCI,[3] while the circadian rhythms in chronic tetraplegic men have, to the best of our knowledge, not been investigated in detail.

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The main objective of the present investigation was therefore to study how the plasma concentrations of testosterone LH and FSH vary during a 24-h period in a group of well-defined chronic tetraplegic subjects compared with an able-bodied control group.

Materials and methods

Study subjects

The study was approved by the Regional Committee on Research Ethics (Health Region East of Norway), and written consent was obtained from all study participants. We included six outpatient Caucasian men aged (mean ± s.d.) 36 ± 3.3 years with a body mass index (BMI) of 22.9 ± 0.8 kg m^{-2} and a long-standing SCI (C_5–C_7) initially caused by an acute trauma more than 5 years ago. These were compared with eight able-bodied Caucasian male control subjects aged 33 ± 1.5 years, and with a BMI of 24.5 ± 0.7 kg m^{-2}. The diagnosis of complete tetraplegia was based on internationally accepted criteria.[8] None of the included 14 subjects suffered from any chronic haematological, immunological, inflammatory, endocrinological or malignant disease. They did not use any medication regularly. A spasmolytic agent (Baclofen) was used infrequently among five of the six tetraplegic subjects, but not during the observation period. None of the tetraplegic men had children and five of the six reported decreased potency or impotence.

Blood sampling

Upon arrival on the experimental day, which followed an overnight fast, the subjects had normal body temperatures, chemically normal urine tests and a serum C-reactive protein concentration below 4 mg 100 ml^{-1}. Routine clinical examinations revealed no abnormality. A cannula was inserted into an antecubital vein and secured. Blood samples were collected from the subjects in the supine position over a time span of 24 h while staying in the ward. A 4–5 ml of blood sample was collected into vacutainer tubes containing 0.5 ml of buffered citrate, at the following time points: 0700, 0800, 0815, 0830, 0845, 0900, 0915, 0930, 1000, 1030, 1130, 1200, 1300, 1330, 1400, 1500, 1600, 1700, 1800, 1830, 1900, 2300, 0200, 0400 and 0700 hours. Whereas samples from all 25 time points were included in the analyses of gonadotrophins, samples from 7 time points were available for testosterone measurements. The plasma concentrations of testosterone LH and FSH may be regulated at several levels, including the concentration of binding proteins and hormone regulators of growth and metabolic activity. To control for possible effects of such putative modifiers, we determined the morning levels of a panel of hormones with regulatory effects on the plasma concentrations of testosterone and the two gonadotrophins, namely, insulin, sex hormone-binding globulin (SHBG), GH, IGF-1, leptin and 24-h urine concentrations of cortisol. The citrate vacutainer tubes were centrifuged immediately (2000 *g*, 20 min at room temperature) to separate plasma, which was aliquoted, frozen and kept at −70 °C until further analyses. Following each blood sampling, the cannula was flushed with saline. In-between the blood samplings, subjects were performing low-activity tasks or resting during the day (0700–2200 hours) and sleeping at night (2200–0700 hours). The ambient temperature varied between 18 and 20 °C. They consumed a standardized three-meal diet (35 kcal kg^{-1}) with carbohydrates yielding 55% of the energy intake, fat 30% and protein 15%. They drank water during the 24 h study period.

Hormone assays

The blood samples were analysed for testosterone LH and FSH using commercial immunoassays at the Hormone Laboratory, Aker University Hospital, Oslo, Norway. Testosterone was determined with a radioimmunoassay (RIA) using a kit from Orion Diagnostica (Espoo, Finland) with a lower detection limit of 0.5 nmol l^{-1} and assay variability with coefficient of variation (CV) of 9–14%. LH (lower detection limit 0.6 IU l^{-1} and CV 5–6%), FSH (lower detection limit 1.0 IU l^{-1} and CV 4%) and GH (lower detection limit 0.1 mIU l^{-1} and CV 5–10%) were assayed by time-resolved fluoroimmunoassay (DELFIA; PerkinElmer, Turku, Finland). The plasma concentration of leptin (lower detection limit 0.5 μg l^{-1} and CV <10%) was measured with a RIA kit (Human Leptin RIA kit; Linco Research, St Charles, MO, USA). Insulin was measured with standard procedures and the interassay CV was less than 10%. SHBG (lower detection limit 2 nmol l^{-1} and CV 6–7%), cortisol (lower detection limit 28 nmol l^{-1} and CV 9–13%) and IGF-1 (lower detection limit 3.3 nmol l^{-1} and CV 8%) were assayed by immunoluminometric assays (Immulite 2000; Diagnostics Products, Los Angeles, CA, USA). Plasma-free testosterone index was calculated as the ratio of the plasma concentrations of testosterone to SHBG × 10.

Calculations and statistical analyses

Values are reported as means ± s.d.. In addition, we used the 24-h pattern of the individual plasma concentrations to calculate their respective areas-under-the-curve (AUC). Differences in AUC values between the two study groups were evaluated with *t*-tests. Circadian variations as well as differences in the plasma concentrations of the various hormones between the two study groups were evaluated with ANOVA with repeated measures. *P*-values <0.05 were considered to indicate statistical significance. The statistical analyses were performed with SPSS version 13.0 (Chicago, IL, USA) and the MedCalc Software (Mariakerke, Belgium).

Results

Decreased plasma levels of testosterone in tetraplegia

The AUC for the plasma concentrations of testosterone in the tetraplegic men was lower ($P<0.005$) than in the able-bodied group: 192.3 ± 36.8 nmol (l h)$^{-1}$, vs 304.8 ± 48.3 nmol (l h)$^{-1}$, respectively. Using ANOVA with repeated measurements we found no significant circadian variation in the plasma concentration of testosterone in either study groups (Figure 1).

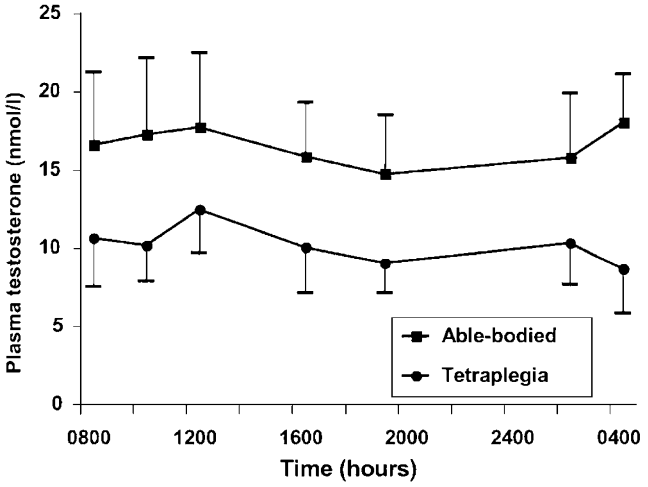

Figure 1 Twenty-four hour plasma concentrations of testosterone in tetraplegic subjects (circles) and in able-bodied controls (squares). Values are means ± s.d.

Decreased plasma levels and a circadian variation of LH in tetraplegia

Figure 2 shows individual time curves for the plasma concentrations of LH in the able-bodied (Figure 2a) and tetraplegic subjects (Figure 2b). The AUC for the plasma concentrations of LH in tetraplegia was decreased ($P<0.005$) when compared to the able-bodied group: $66.1 \pm 22.4\,\text{IU}\,(\text{l}\,\text{h})^{-1}$ vs $99.5 \pm 11.2\,\text{IU}\,(\text{l}\,\text{h})^{-1}$. Using ANOVA with repeated measures we found a circadian variation ($P<0.05$) in the plasma concentration of LH in the tetraplegic group, but not among the able-bodied group. Moreover, in both groups, clearly identifiable spiking of LH secretion was present.

Decreased FSH levels without circadian variation in tetraplegia

In Figure 3 we display individual time curves for FSH for the able-bodied (Figure 3a) and tetraplegic subjects (Figure 3b). The AUC for the plasma concentrations of FSH in tetraplegia was lower ($P<0.005$) than in the able-bodied group: $192.3 \pm 36.8\,\text{IU}\,(\text{l}\,\text{h})^{-1}$ vs $304.8 \pm 48.3\,\text{IU}\,(\text{l}\,\text{h})^{-1}$. Using ANOVA with repeated measures we found no circadian variation in the plasma concentration of FSH in either of the study groups, and no clear spiking-pattern was identified.

Increased morning leptin levels in tetraplegia

While the morning plasma leptin levels were significantly higher ($P<0.05$) in the tetraplegic group compared with the controls, we could not detect any significant differences in the morning values for the plasma concentrations of insulin, SHBG, GH or IGF-1, or in the 24-h urine concentrations of cortisol (Table 1). The plasma-free testosterone index was decreased ($P<0.05$) in tetraplegic compared with control subjects when assaying morning blood samples (Table 1).

Discussion

Our results show significantly lower mean values for the plasma-free testosterone index and for the plasma concentrations of testosterone, LH and FSH in the tetraplegic subjects when compared to the able-bodied controls. Moreover, a circadian variation of the plasma concentration of LH was detected in the tetraplegic group, but not among the able-bodied group. Neither the plasma concentrations of testosterone nor FSH showed any clear circadian variation in either group. The mean plasma concentrations of testosterone and both gonadotrophins were within the reference limits in the able-bodied controls.[9–11]

The major strengths of our study are the carefully controlled environment including standardized meals during the 24 h sampling period and well-defined study groups.

A potential limitation is the relatively small number of subjects in both groups. In addition, half-lives, clearance rates and metabolites of plasma testosterone, LH and FSH were not measured, and differences in these parameters between the two study groups might possibly change the absolute values and the 24-h profiles of the three hormones. Alterations in SHBG levels modify the values of plasma testosterone by changing the ratio of free-to-SHBG bound hormone; however, morning levels of SHBG did not vary between the two groups in our study, the plasma-free testosterone index did.

Our findings are at variance with other studies showing increased or unchanged plasma levels of testosterone, LH and FSH in tetraplegic men compared to healthy controls.[2,5,6] These discrepancies may be explained by several factors, including number of samples, different sampling frequencies,[9–11] and level, type and duration of injury. Importantly, the present study is apparently the first to employ frequent blood sampling in a group of well-defined and otherwise healthy tetraplegic patients. Our results are in agreement with yet other reports, indicating that these patients might be at risk of developing hypogonadism.[1,4]

Frequencies of gonadotrophin blood level peaks can be used as marker of central hypothalamic-pituitary activity. A downregulation of activity is reflected in a reduced number of peaks of pituitary hormones and vice versa. Low gonadotrophins levels among the tetraplegic men in our study could be an indication of central downregulation. An even higher blood sample frequency, especially during the night, at equal time intervals is needed for a better evaluation of hypothalamus–pituitary function. Our sampling frequency did not permit formal quantitative evaluation of the number of circadian secretory spikes. Our data show circadian LH plasma variation only in the tetraplegic group. This finding could be explained by a larger inter-individual variation of the plasma concentration of LH among the able-bodied group, resulting in a lack of periodicity when the group is calculated as a whole. A circadian variation of LH plasma concentration among the tetraplegic group argues for a functioning pituitary; however, we cannot specify whether or not the detected variations follow a normal circadian pattern. Interestingly, a fall in LH plasma concentration among the tetraplegic group occurred during the night, which was not expected since normally, LH plasma concentrations rise during the night, as observed among the able-bodied group.

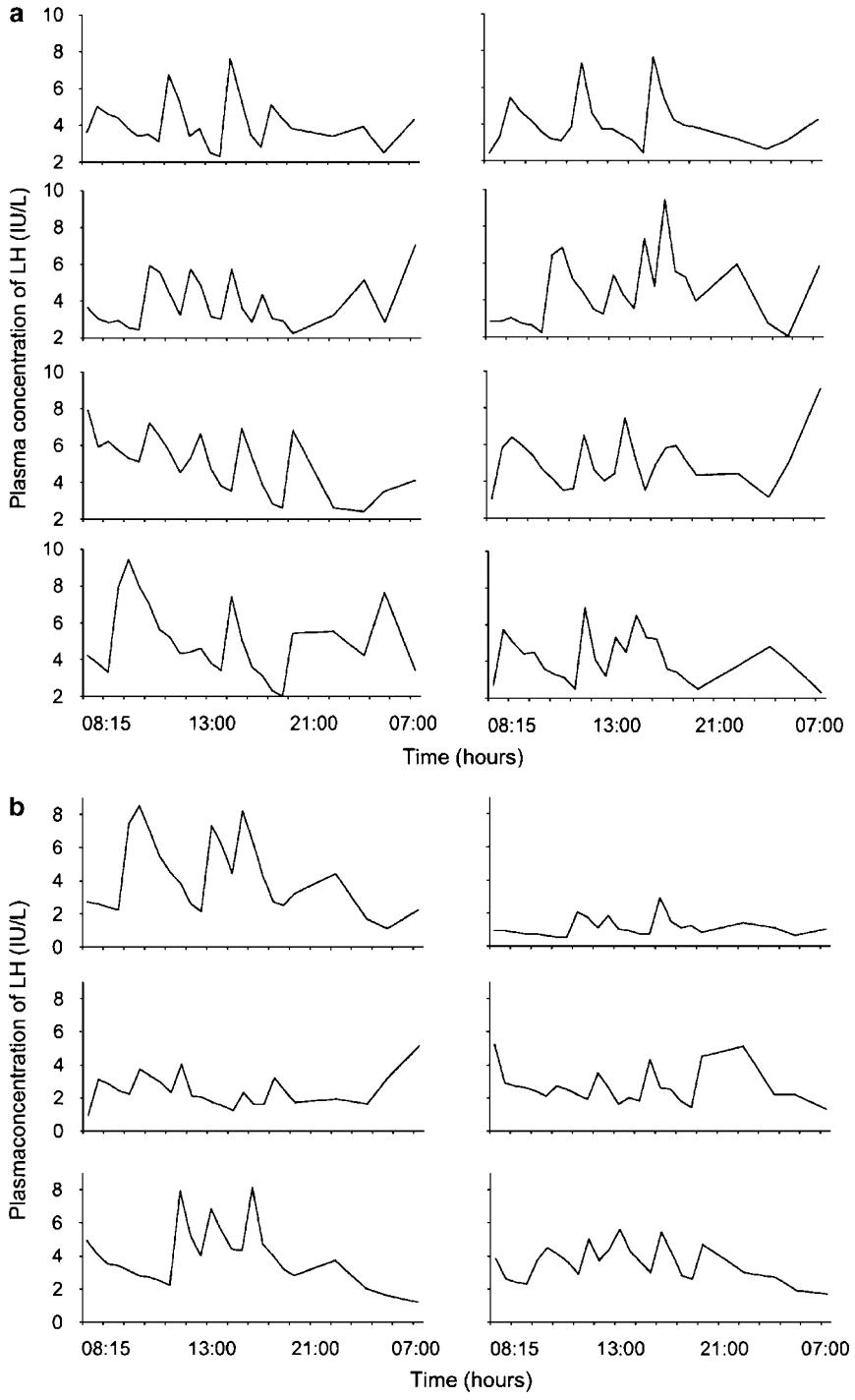

Figure 2 Individual time profiles for the plasma concentration of luteinizing hormone (LH) in the eight able-bodied (**a**) and six tetraplegic (**b**) subjects.

Impairments of the androgen axis are likely to be induced at multiple levels. SCI most likely disrupts afferent signalling to the hypothalamus from the somatic nerves below the level of injury leading to a disruption of the hormonal secretion pattern. Interestingly, Lee *et al.*[12] have found an unsuspected brain-testicular circuit that interferes with the Leydig's cell function independently of the pituitary. If such a pathway is disrupted in SCI, impairments of the androgen axis might be the result. In addition, various forms of stress are also known to alter the secretions of cortisol, GH and gonadotrophins in SCI. However, we could not detect any significant changes in the morning values of cortisol or GH between the tetraplegic and the able-bodied groups. Reportedly, chronic illness may cause temporary hypogonadism with elevated gonadotrophins, low testosterone and higher peripheral conversion of androgens to

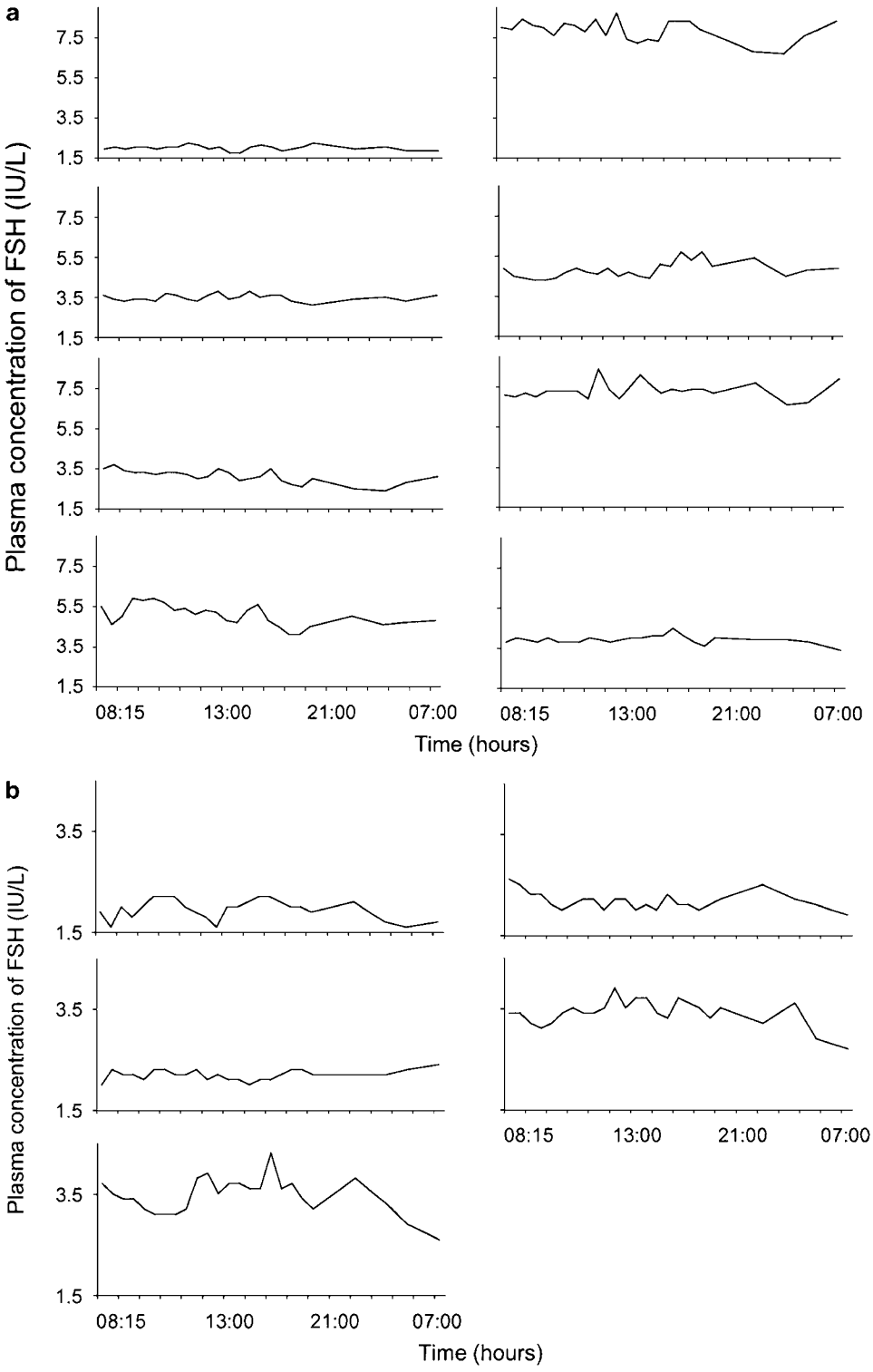

Figure 3 Individual time profiles for the plasma concentration of follicle-stimulating hormone (FSH) in the eight able-bodied (**a**) and five tetraplegic (**b**) subjects. Measurements from tetraplegic subject no. 4 were not available.

estrogens.[13] However, a pituitary hypofunction resulting from such hypogonadism is unlikely to have occurred in our tetraplegic subjects as they did no have any comorbidity and their SCI was caused by acute trauma several years ago.

Changes in BMI and/or body fat percentage could modify secretion patterns of gonadotrophins. For example, in healthy, overweight men BMI was negatively correlated with both serum-free testosterone concentrations and LH secretory burst frequency.[14] This apparent association is

Table 1 Binding protein and hormone regulators of testosterone, LH and FSH

Variable	*Tetraplegia (n = 6)*	*Able-bodied (n = 8)*
Leptin ($ng\,l^{-1}$)	$86.2 \pm 29.4^{*}$	23.9 ± 7.0
Insulin ($pmol\,l^{-1}$)	63.3 ± 4.3	69.5 ± 3.9
SHBG ($nmol\,l^{-1}$)	29.6 ± 6.4	25.1 ± 5.7
Urine cortisol ($nmol\,l^{-1}$ per 24 h)	223 ± 168	265 ± 172
GH ($mIU\,l^{-1}$)	0.5 ± 1	0.7 ± 3
IGF-1 ($nmol\,l^{-1}$)	27.8 ± 4.9	24.5 ± 5.9
Free testosterone index	$3.8 \pm 0.3^{*}$	5.1 ± 0.6

Values are means ± s.d.. The plasma hormone concentrations were determined in samples collected in the morning (0700 hours). A 24-h sampling of urine was used to measure cortisol. Free testosterone index was calculated as the ratio between the plasma concentrations of testosterone and SHBG × 10. $^{*}P < 0.05$.

consistent with an earlier notion that relative hypogonadism may accompany obesity. In addition, obesity is often associated with hyperleptinemia, and some studies have suggested that leptin levels might be a better indicator of obesity in SCI than BMI,[15] possible due to increased fat/muscle mass ratio. Leptin is necessary for normal gonadal function. Hypothalamic amenorrhoea in women can be treated with leptin, and children with leptin deficiency do not reach puberty before they are treated with leptin. Our tetraplegic men had higher body fat mass, as measured by dual energy X-ray absorptiometry (DEXA) scan and higher plasma levels of leptin compared to able-bodied men,[16] despite apparently similar BMI values, which is in agreement with previous studies.[17,18] Moreover, DEXA might be more suitable for detecting fat mass in able-bodied group than in tetraplegic group, as in contrast to able-bodied group, fat accumulates within muscle fibres in tetraplegia.[19] Higher body fat mass, intramuscularly fat accumulation and autonomic deficits in tetraplegia could all result in disturbed secretion of gonadotrophins, as well as higher leptin levels. Behre *et al*.[20] noted an association between morning serum levels of leptin and testosterone in both hypogonadal and healthy men, which was not confirmed in the present study. The very high leptin levels among the tetraplegic men, the number of study participants and the use of AUC values rather than one single morning value might explain this discrepancy. Although we could not detect any significant correlation between the AUC for testosterone and the AUC for leptin among the tetraplegic men (data not shown), we speculate that hyperleptinemia in tetraplegic subjects may induce leptin resistance in gonadotropic cells producing testosterone, FSH and LH.

In conclusion, both testosterone, LH and FSH displayed markedly lower plasma concentrations in tetraplegic subjects when compared to their able-bodied controls. Furthermore, tetraplegic subjects displayed circadian variations of the plasma concentrations of LH. Our data indicate that tetraplegic subjects are at risk of developing hypogonadism. Further studies are needed to fully understand the mechanisms leading to the affected gonadal–pituitary axis in spinal cord injured men.

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