CLINICAL STUDY

Sex steroids and body composition in men with cystic fibrosis

E Leifke, M Friemert, M Heilmann¹, N Puvogel¹, C Smaczny², A von zur Mühlen and G Brabant

Abteilung Gastroenterologie, Hepatologie und Endokrinologie, ¹Abteilung Pneumologie der Medizinischen Hochschule Hannover, Carl-Neuberg-Str. 1, 30165 Hanover, Germany and ²Abteilung Pneumologie der Johann Wolfgang Goethe Universitäts-Klinik, Theodor-Stern-Kai 7, 60590 Frankfurt-on-Main, Germany

(Correspondence should be addressed to E Leifke; Email: leifke.eckhard@mh-hannover.de)

Abstract

Objective: Delayed sexual maturation and low body weight is common in cystic fibrosis (CF). Concomitant data on sex hormones and concomitant body composition are lacking in men with CF.

Design: Cross-sectional study.

Subjects and methods: Serum levels of testosterone, 17 β -oestradiol (E₂), 25-hydroxyvitamin D (25(OH)D), sex hormone-binding globulin (SHBG) and LH were measured by RIA and total and regional lean body mass (LBM), fat body mass (FBM), bone mineral content and bone mineral density (BMD) were assessed by dual-energy X-ray absorptiometry, in men with CF ($n = 40$; age 24.7 ± 5.4 years) and age-matched healthy controls ($n = 28$; age 25.7 ± 3.7). Only men without acute disease exacerbation or systemic glucocorticoid treatment were included.

Results: Mean levels of hormonal serum parameters differed significantly between healthy controls (testosterone = 20.2 ± 5.5 nmol/l; E₂ = 95.0 ± 20.2 pmol/l; 25(OH)D = 62.8 ± 28.3 nmol/l) and patients (testosterone = 15.9 ± 4.1 nmol/l; E₂ = 60.7 ± 19.4 pmol/l; 25(OH)D = 39.5 ± 17.8 nmol/l; $P < 0.001$) while no difference was found for SHBG or LH. Eleven (for E₂, 19 of 40, for 25(OH)D, 20 of 40) out of 40 patients had serum testosterone levels 2 s.d. below the mean of normal. Men with CF showed a relative shift from FBM to LBM and a different body fat distribution compared with healthy controls ($P < 0.01$). Testosterone was not correlated with weight, total or regional LBM or FBM, but significantly with BMD ($r = 0.32$; $P < 0.05$) independently from body height and 25(OH)D levels. E₂ was correlated with regional and total FBM ($r = 0.48$; $P < 0.05$). In a multiple regression analysis of the joint effect of testosterone and body components on E₂, a testosterone-independent effect was found for FBM.

Conclusions: CF patients with stable disease have moderately reduced serum testosterone levels. This might already imply detrimental effects on bone. The change in LBM of patients appears to have no direct association with sex hormone levels while low FBM might cause reduced net conversion of serum testosterone to E₂ with possible effects on FBM distribution.

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Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disorder among Caucasians with a frequency of 1 in 2000 live births (1). The disease is clinically characterised by chronic recurrent pulmonary infections, pancreatic insufficiency and cholestatic liver disease with variable manifestation. Life expectancy in these patients continues to increase into adult life due to advances in medical care and transplantation facilities (1–3). Thus, health problems secondary to the disease, such as loss of bone and muscle mass become increasingly important.

Body weight is of prognostic value in CF and the integrity of bone and muscle mass might be especially important for post-transplantation survival (2, 4, 5). It is not surprising that dramatic changes in body

weight and composition are observed in patients with endstage disease or under systemic glucocorticoid treatment (4, 6, 7). More subtle changes in body composition might, however, start early in the disease and might be associated with alterations of sex hormones, which are known to be important for bone mineral density (BMD) and muscle mass (8–10). Clinical signs of impaired gonadal function such as delayed puberty, osteopaenia and growth retardation are frequent findings in children with CF (11–13). Although BMD has been investigated in patients with CF in numerous studies there are only few and conflicting data on sex hormone levels in male adults with CF (14–20). Moreover, the relationship between circulating sex steroids, 25-hydroxyvitamin D (25(OH)D), weight and body composition has not been systematically analysed yet in men with CF disease.

Subjects and methods

Subjects

Forty male adult patients with CF were investigated. Age-matched healthy volunteers recruited from the medical staff of our hospital served as controls. The diagnosis of CF was confirmed by a pathological sweat chloride test in all patients as well as by the appropriate clinical history. A homozygous $\Delta F508$ mutation of the CF transmembrane conductance regulator (CFTR) gene was documented in 29 of 39 patients (72%). Ten patients were either compound heterozygous ($\Delta F508$ and another CF-associated mutation) or homozygous for a non- $\Delta F508$ mutation. One patient had no genetic analysis. All CF patients received antibiotic prophylaxis against recurrent respiratory pseudomonas infection. The majority of patients had impaired pulmonary function (mean forced expiratory 1 s volume as per cent of predicted = $50 \pm 19\%$, ranging from 29 to 100%) and were on inhalative β_2 -mimetics on a regular basis. None of the patients included used concurrently or had a past history of long-term use (> 2 months) of systemic glucocorticoids. Two patients used the inhalative glucocorticoid budesonide. None had reached pulmonary end-stage disease, was listed for lung transplantation or was critically ill at the time of investigation. All patients suffered from exocrine pancreas insufficiency based on a pathological stool fat content, a reduced elastase stool concentration and chymotrypsin activity. Patients received multivitamin supplements providing 500 IU vitamin D daily and enteric coated microspheres of pancreatic enzymes. Furthermore, diabetes mellitus was documented in 12 of 40 patients (28%) with good metabolic control during the last 2–3 months (mean glycosylated haemoglobin in diabetic vs all patients: 6.2 ± 0.86 vs $5.5 \pm 0.75\%$). All patients were sexually mature. Reliable data on the history of puberty were not available.

Patients and volunteers signed informed consent and the study was approved by the local Ethic Committee of our hospital.

Assessment of body composition and BMD

Bone, muscle and body fat mass and total BMD were assessed using whole-body scans by dual-energy X-ray-absorptiometry (DEXA) (Lunar, USA) for both groups (CF = 40; controls = 28). Regions were positioned manually. The trunk was defined as followed: first, a horizontal line was placed across the shoulders to demarcate the head region; secondly vertical lines were drawn through the shoulder joints, defining the arms; thirdly diagonal lines were placed through the hip joints, defining the lower limbs distal to this; and finally the trunk was defined by excluding limb and head regions. Coefficients of variance were determined by repeated measurements and were 1% for total lean body mass (LBM), 2.2% for fat mass and 1.6 and 7% for the corresponding

measurements within the anatomical subregions, which are consistent with those reported in the literature (21–24). BMD measurement by DEXA – with a precision error of 2% – is well established and widely used for clinical and scientific purposes (25).

Relative body composition was expressed as the body component (lean body mass (LBM), bone mineral content (BMC), fat body mass (FBM)) as a per cent of total body weight. Fat distribution was determined by calculation of the limb (arms and legs) fat to trunk fat ratio.

Serum parameters

Blood was drawn (CF = 40; controls = 28) between 0800 and 1100 h. Serum levels of total testosterone, 17β -oestradiol (E_2), sex hormone-binding globulin (SHBG) (including all controls but only 35 CF patients due to shortage of serum for SHBG), 25(OH)D and luteinising hormone (LH) were determined in duplicates using commercially available RIAs: DSL (Sinsheim, Germany) for testosterone and E_2 , with maximal intra- and inter-assay variations and sensitivities of 8.1%, 9.1% and 0.28 nmol/l, and 3.8%, 3.9% and 2.08 pmol/l respectively; Nichols Institute (Diagnostics, Bad Nauheim, Germany) for 25(OH)D, with maximal intra- and inter-assay variations and sensitivity of 10.2%, 10.9% and 5.5 nmol/l, with 100% cross-reactivity with 25(OH)D, 25-hydroxyvitamin D2 and 24,25-dihydroxyvitamin D3; DSL for SHBG, with maximal intra- and inter assay variations and sensitivity of 3.4%, 10.3% and 3 nmol/l; Nichols Institute for LH, with maximal intra- and interassay variations and sensitivity of 4.4%, 5.9% and 0.1 mIU/ml. Additionally, normal levels (means ± 2 S.D.) of serum testosterone and E_2 were available from a large group of healthy young men ($n = 117$) (26) (see shaded area in Fig. 1).

Statistical analysis

Variables were checked for normal distribution by the Kolmogorov–Smirnov one-sample test for goodness-of-fit. Variables showing a non-normal distribution were transformed logarithmically to achieve normal distribution before ANOVA, Student's *t*-test or Pearson's correlation were applied for differences between the means or bivariate relationship. In addition, multiple regression analysis was applied for joint effects of serum testosterone and body components on serum levels of E_2 . If not otherwise stated, all values are given as means $\pm$ S.D. All procedures were calculated using SPSS (SPSS Inc. 1999)

Results

Serum hormone parameters

Serum levels of testosterone, E_2 and 25(OH)D were significantly lower in patients than in controls while the

Figure 1 Serum levels of testosterone (T) and E_2 for CF patients and controls. The shaded area represents the normal range (means $\pm$ 2 s.d.) for each hormonal parameter as defined in Subjects and methods.

testosterone to E_2 ratio was slightly higher for CF patients than for controls and no differences between the means were found for serum LH and SHBG. The testosterone to SHBG ratio as an index of free serum testosterone showed also significant differences between the means of both groups. Data are summarised in Table 1.

There was a significant positive correlation between serum testosterone and E_2 ($r = 0.44$; $P < 0.01$), but not between serum testosterone and LH in patients with CF ($P = 0.48$). This was compatible with the lack of significant difference between mean serum levels of LH if patients were subgrouped according to their serum testosterone into patients with normal and those with subnormal levels (serum testosterone < 11 nmol/l).

Table 1 Means $\pm$ s.d. of serum Testosterone/ E_2 , LH and 25(OH)D and the Testosterone/ E_2 ratio in healthy controls and men with CF.

	Controls	Men with CF
Testosterone (nmol/l)	20.2 $\pm$ 5.5	15.9 $\pm$ 4.1**
Testosterone/SHBG	0.44 $\pm$ 0.11	0.29 $\pm$ 0.12 ^a **
E_2 (pmol/l)	95.0 $\pm$ 20.2	60.7 $\pm$ 19.4***
Testosterone/ E_2	213 $\pm$ 69	261 $\pm$ 110*
LH (IU/l)	2.8 $\pm$ 1.0	3.2 $\pm$ 1.2
SHBG (nmol/l)	46 $\pm$ 16	50 $\pm$ 19 ^a
25(OH)D (nmol/l)	62.8 $\pm$ 28.3	39.5 $\pm$ 17.8**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test; ^a $n = 35$.

As shown in Fig. 1, 11 of 40 CF patients (28%) had serum levels of testosterone 2 s.d. below the mean of healthy young men (< 11 nmol/l) with the lowest value at 6 nmol/l (26). For E_2 , 19 of 40 patients had serum concentrations lower than 2 s.d. (< 65 pmol/l) of the mean of age-matched normal values. For 25(OH)D, 20 of 40 patients had serum levels lower than 2 s.d. (< 38 nmol/l) of normal.

Body composition and BMD

Body weight and height were significantly different between patients and healthy controls. Ten of 40 patients had a body mass index (BMI) between 15 and 19 kg/m². Accordingly, total and regional body components (trunk, arms and legs) – except LBM of the trunk – and BMD were significantly lower in men with CF. The most prominent relative decrease was observed in FBM compared with controls. Differences between the means of BMD remained significant if controlled for body height ($P < 0.05$). Differences between body components were not significant if CF patients were subgrouped with respect to their serum testosterone into patients with subnormal ($n = 11$; testosterone < 11 nmol/l) and those with normal serum testosterone ($n = 29$). Only BMD showed a significant difference between the mean of both groups (1.0 ± 0.1 vs 1.13 ± 0.1 g/cm²; $P < 0.01$). In addition, the differences in mean BMD between patients and controls remained significant only if patients with normal serum testosterone (> 11 nmol) were included in the comparison ($P < 0.01$). Table 2 includes all anthropometric and body compositional parameters assessed in patients with CF and the controls.

Table 2 Anthropometric parameters, total and regional body components and total BMD ($\pm$ s.d.) of men with CF and controls.

	Controls	Men with CF
<i>n</i>	28	40
Age (years)	25.7 $\pm$ 3.7	24.7 $\pm$ 5.4
Weight (kg)	78.8 $\pm$ 10.4***	61.4 $\pm$ 7.4
Height (m)	1.82 $\pm$ 5.0***	174 $\pm$ 8.0
BMI (kg/m ²)	23.7 $\pm$ 1.9***	20.1 $\pm$ 2.3
BMC (kg)	3.36 $\pm$ 0.14***	2.56 $\pm$ 0.39
Trunk	1.06 $\pm$ 0.14***	0.82 $\pm$ 0.15
Arms	0.44 $\pm$ 0.06***	0.32 $\pm$ 0.06
Legs	1.33 $\pm$ 0.23***	0.96 $\pm$ 0.17
LBM (kg)	59.59 $\pm$ 5.21***	50.69 $\pm$ 6.98
Trunk	27.78 $\pm$ 2.60	26.61 $\pm$ 3.88
Arms	7.04 $\pm$ 0.95***	5.08 $\pm$ 0.94
Legs	20.74 $\pm$ 2.13***	14.97 $\pm$ 2.72
FBM (kg)	15.80 $\pm$ 7.6***	8.15 $\pm$ 4.6
Trunk	7.93 $\pm$ 4.50***	4.4 $\pm$ 2.70
Arms	1.83 $\pm$ 1.14***	0.66 $\pm$ 0.53
Legs	5.14 $\pm$ 2.08***	2.44 $\pm$ 1.35
BMD (g/cm ²)	1.3 $\pm$ 0.1***	1.1 $\pm$ 0.1

*** $P < 0.01$, Student's *t*-test.

In order to compare the relative body composition between patients and healthy men – which eliminates also the impact of body size – the body components (BMC, LBM, FBM) were expressed as per cent of total body weight. The relative body composition for each group is shown in Fig. 2. Per cent FBM of total body weight decreased significantly in patients compared with controls (CF: $12.9 \pm 0.94\%$ vs controls: $20.2 \pm 1.29\%$ (means $\pm$ S.E.M.); $P < 0.01$) while per cent LBM increased accordingly (CF: $82.8 \pm 0.9\%$ vs controls: $75.6 \pm 1.23\%$ (means $\pm$ S.E.M.); $P < 0.01$). Mean per cent BMC was not different between both groups (CF: $4.1 \pm 0.08\%$ vs controls: $4.3 \pm 0.09\%$ (means $\pm$ S.E.M.)).

FBM showed also a different body distribution in patients compared with healthy controls. The limb (arms plus legs) to trunk ratio of fat mass was significantly lower in CF ($0.75 \pm 0.03\%$) than in controls ($0.96 \pm 0.05\%$; $P < 0.01$) (Fig. 3). This was also true if the arm to trunk and leg to trunk ratio were calculated separately (not shown).

Correlation analysis between serum hormone parameters and body composition

Significant correlation could be demonstrated for E_2 with total and regional FBM and for testosterone with BMD in patients with CF. The correlation found between BMD and testosterone was not dependent on height or serum levels of 25(OH)D. Coefficients of bivariate correlation between sex hormones and body components and BMD are summarised in Table 3. No correlation was found between 25(OH)D with either sex hormone serum levels (E_2 : $r = 0.19$, $P = 0.22$; testosterone: $r = 0.12$, $P = 0.44$), and body components

Figure 2 Body composition of men with CF and healthy controls. Total BMC, FBM and LBM as per cent of total body weight for each group. * $P < 0.05$; n.s. = not significant.

Figure 3 The ratio of limb (arms plus legs) to trunk fat mass for men with CF and healthy controls. Values are presented as box plots; ** $P < 0.01$.

(LBM: $r = 0.15$, $P = 0.3$; FBM: $r = 0.29$, $P = 0.09$; BMC: $r = 0.03$, $P = 0.86$) or BMD ($r = 0.01$, $P = 0.9$).

To determine the joint effect of serum testosterone and body components on serum E_2 levels all subjects

Table 3 Coefficients of bivariate correlations between Testosterone and E_2 with total and regional body compartments (LBM, FBM, BMC) and BMD controlled for 25(OH)D serum levels in CF patients.

	Testosterone	E_2
LBM, total body (kg)	0.08	-0.14
Arms (kg)	0.06	0.01
Legs (kg)	0.11	-0.12
Trunk (kg)	0.03	-0.21
FBM, total body (kg)	0.06	0.48*
Arms (kg)	0.02	0.43*
Legs (kg)	0.09	0.30
Trunk (kg)	0.01	0.52*
BMC, total body (kg)	0.24	0.12
Arms (kg)	0.12	0.06
Legs (kg)	0.17	0.15
Trunk (kg)	0.29	0.14
BMD, total body (g/cm^2)	0.32*	0.19
Arms (g/cm^2)	0.25	0.11
Legs (g/cm^2)	0.28	0.21
Trunk (g/cm^2)	0.33*	0.23

* $P < 0.05$, Student's *t*-test.

Table 4 Multiple regression analysis of the effect of serum Testosterone, FBM, LBM and BMC on serum levels of E_2 in all subjects ($n = 68$).

E_2	Predictors	Beta
	Testosterone	0.49*
	FBM	0.31*
<i>r</i>	0.6	—

* $P < 0.05$; LBM and BMC were excluded by multiple regression analysis ($P = 0.16$).

– CF patients and controls – were included in a multiple regression analysis. As shown in Table 4 only FBM showed a testosterone-independent effect on serum levels of E_2 in men.

Discussion

The present study shows that low normal serum levels of testosterone and E_2 are common in men with CF. Our findings are not due to differences in SHBG concentrations between patients and controls. Furthermore, possible effects of glucocorticoids can be ruled out in our study, because CF patients under systemic glucocorticoid treatment were not included.

Delayed puberty and an impaired gonadotrophin response to exogenous LH-releasing hormone have been reported in boys with CF (11, 13). For male adults, hypogonadism has been described in some but not all studies (14–19). These discrepant findings might be partly due to the clinical heterogeneity of the disease. In the present study most patients (72%) had serum testosterone concentrations within the lower normal range of age-matched healthy controls. Twenty-eight per cent of the patients were 2 S.D. (< 11 nmol/l) below the mean of age-matched healthy subjects but still above pre-pubertal values and these compare well with levels found in healthy elderly men (8, 26). In our study, the determination of LH serum levels was not helpful for the evaluation of the androgen status, as they exhibit no significant difference between patients and healthy men or between CF patients with normal and subnormal serum levels. Normal serum levels of LH in the presence of low serum testosterone suggest, however, a disturbed regulatory axis. Hypothalamic–pituitary dysregulation has been demonstrated in critical and acute illness and become clinically obvious if delayed puberty or amenorrhoea is present (11, 27). A combined hypothalamic–pituitary–gonadal defect in male adults with low but not pre-pubertally low serum testosterone – as in our study cohort – might be more difficult to assess. Information on LH pulsatility and/or bioactivity is not available in CF and disturbances at this level are not identifiable by single blood hormone measurements. There is increasing debate that cytokines – apart from stress- and medication-induced alterations of the gonadotrophic axis – might contribute to the normogonadotrophic hypotestosteronaemia found in the context of illness (27–29). This might well be transformable to CF, in which chronic and recurrent pulmonary infection is commonly present. Moreover, CFTR appears to be functionally active in gonadotrophin hormone-releasing-hormone-expressing hypothalamic cells, which may also help to explain the manifestation of hypogonadism in CF (30).

Low BMI and small body size are common in CF (11, 31). Accordingly, we demonstrate a reduction of all body

components and regions using DEXA. Considering the relative composition of body components a shift from FBM to LBM was observed in men with CF while BMC as a per cent of total body weight remained constant compared with healthy controls. Thus, the data show that reduction in weight is dominated by a decrease in FBM, which agrees with previous findings (32–34). Moreover, the distribution of body fat was different between men with CF and controls. CF in male adults appears to be associated with either a preferential loss of peripheral fat or a better preservation of central fat mass. This finding is consistent with the low serum levels of E_2 found in these patients. Sex hormones have an important impact on body fat distribution and there is evidence, at least for women, that a fall in E_2 – as observed during the menopause – is associated with a more central body fat distribution (35, 36). However, conclusions should be drawn with caution on the basis of the present findings, since the DEXA technique does not allow the metabolically important differentiation between visceral and subcutaneous fat mass.

Serum levels of E_2 correlated significantly with total and regional fat mass in our CF patients. The existence of a testosterone-independent effect of FBM on serum levels of E_2 was also confirmed in a multiple regression analysis of the joint effect of serum testosterone and all three body components – LBM, FBM and BMC – on circulating E_2 . Androgens are converted to oestrogens (37). Low serum levels of testosterone are expected to reduce serum levels of E_2 , as confirmed by our results. The increase in the testosterone to E_2 ratio from controls to patients, however, indicates that the reduction in serum levels of E_2 is not explained by a reduction in serum testosterone alone. Adipose cells are thought to be the main source of serum E_2 in men, although aromatase activity has been described in many different tissues (38, 39). It is, therefore, reasonable to assume that low E_2 serum levels, together with the increase in the testosterone to E_2 ratio, reflect reduced net conversion of testosterone to E_2 due to the marked reduction in FBM found in CF. This is well compatible with data on serum levels of E_2 in the elderly, which scarcely reflect the age-related decrease in serum testosterone in men, most likely due to an increase in fat mass with age (26, 40).

It is a matter of debate whether DEXA measurements underestimate BMD in CF because these patients are usually of small body size (41). Differences between BMD of healthy age-matched controls remained significant after adjustment for height. This finding supports the concept that reduced – true volumetric – bone density contributes to the low peak bone mass commonly found in young men with CF (20, 42–44).

The importance of adequate serum testosterone levels for acquisition and maintenance of bone and muscle mass has been generally accepted and body compositional changes in men under testosterone treatment have been described by several studies (10, 45, 46).

For CF patients no associations between serum testosterone levels and LBM or weight were observed, whereas only a weak but significant relationship was found for testosterone and BMD. LBM represents a mixture of different tissues, but in the absence of oedema, muscle mass might almost exclusively contribute to LBM of arms and legs – in contrast to the trunk (47). However, no correlation between LBM of different body regions and testosterone was found. It appears that the testosterone deficiency in male CF patients was too moderate to induce gross effects on muscle mass – at least identifiable on the basis of cross-sectional data – while BMD might already be affected. This finding is compatible with a previous study reporting that the manifestation of delayed puberty in girls with CF was independent of their nutritional status (48). Furthermore, it supports the view that different threshold levels – although undefined yet – of circulating serum testosterone for target effects on bone and muscle might exist (45, 49).

The strength of the correlation between serum testosterone and BMD was similar to that described in population-based studies in elderly men. Some studies found a stronger correlation for E_2 with BMD than for serum testosterone (49). Furthermore, there is substantial evidence that E_2 plays an essential role for the male skeleton (49, 50). This role, however, is paracrine rather than endocrine in nature (37). Serum E_2 might not necessarily reflect local concentrations and oestrogenic activity at target sites. Therefore, the lack of significant correlation between serum E_2 and BMD in our CF study cohort should not be overinterpreted. A clinical relevance for the association between sex hormones and BMD cannot be concluded nor excluded from cross-sectional data alone. This is especially true in CF, in which several factors apart from hypogonadism, such as high energy turnover, chronic infections, malnutrition, vitamin D deficiency, hyperparathyroidism and medication, might contribute importantly and to a variable extent to body compositional changes and low bone mass (17, 42–44). Exocrine pancreas insufficiency was present in all patients and vitamin D deficiency and low BMI – as indicators of malnutrition and possible undertreatment – were also confirmed in our study cohort (51–54). In addition, patients with serum testosterone within the normal range had still lower BMD than healthy controls, supporting the relevance of testosterone-independent factors for the pathogenesis of low BMD in the context of CF. However, the correlations we found for serum testosterone with BMD and for E_2 with body fat were not dependent on vitamin D serum levels. Furthermore, the mean BMD was also different between CF patients with subnormal and those with normal serum testosterone.

In conclusion, circulating levels of testosterone in men with stable CF disease are moderately reduced, to the extent found in healthy elderly men. This might already imply significant effects on bone, while

no associations with LBM were observed, supporting the concept of target-dependent threshold levels for testosterone effects. Marked reduction in FBM, common in male patients with CF, might cause reduced net conversion of circulating androgens to E_2 , which in turn might affect body fat distribution.

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