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ORIGINAL ARTICLE

Decreased dehydroepiandrosterone sulfate but normal insulin-like growth factor in Chronic Fatigue Syndrome (CFS): Relevance for the inflammatory response in CFS

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Abstract

There are a few reports that chronic fatigue syndrome (CFS) may be accompanied by changes in hormones, such as dehydroepiandrosterone (DHEA) and insulin-like growth factor (IGF1). This study examines the serum concentrations of DHEA-sulfate (DHEAS), IGF1 and IGF1 binding protein-3 (IGFBP3) in 20 patients with CFS and in 12 normal controls. The IGFBP3/IGF1 ratio was computed as an index for IGF1 availability. We found significantly lower serum DHEAS concentrations in CFS, but no significant differences either in IGF1 or the IGFBP3/IGF1 ratio between CFS patients and normal controls. The decrease in serum DHEAS was highly sensitive and specific for CFS. There were significant and positive correlations between serum DHEAS and serum zinc and the mitogen-induced expression of the CD69 molecule on CD3+CD8+ T cells (an indicator of early T cell activation). There was a significant and negative correlation between serum DHEAS and the increase in the serum alpha-2 protein fraction (an inflammatory marker). Serum IGF1, but not DHEAS, was significantly and inversely correlated to age. The results show that CFS is accompanied by lowered levels of DHEAS and that the latter may play a role in the immune (defect in the early activation of T cells) and the inflammatory pathophysiology of CFS.

Introduction

Chronic Fatigue Syndrome (CFS) is a condition characterized by a persistent debilitating fatigue lasting at least 6 months, muscle-related symptoms (muscle or joint pain), neuro-cognitive disorders (difficulties concentrating, disorders in short term memory), sleep disorders (awakenings and unrefreshing sleep), and inflammatory symptoms (sore throat, tender cervical and axillarys lymph modes, malaise and irritable bowel) [1].

There is now some evidence that neuro-immune disorders play a fundamental role in the pathophysiology of CFS [1]. Thus, CFS is accompanied by activation of the inflammatory response system with an impaired production of proinflammatory cytokines [2,3,4]; signs of inflammation with lowered levels of serum zinc [5] and increased levels of the serum alpha-2 protein faction [5]; decreased natural killer cell activity [6,7]; and defects in the early activation of T cells [8].

Moreover, hormonal disorders, such as decreased levels of dehydroepiandrosterone (DHEA) or DHEA-sulphate (DHEAS) and insulin-like growth factor (IgF1), have been proposed as other pathophysiological factors in CFS. Some [9], but not all [10,11], found significantly lower serum DHEA(S) in CFS patiens than in normal controls. DHEA and DHEAS are the major secretory products of the human adrenal gland and serve as precursors for androgenic / estrogenic steroids. DHEA and DHEA-S have multivarious functions associated with fatigue and energy [12], autoimmunity [13], mood [14], neurocognition [15], immunity [16], sleep [17] and skeletal muscles [18]. In addidion, studies have shown that DHEA may be helpful in the treatment of CFS [19].

Serum IGF1 levels are significantly lower in patients with CFS than in controls in some [20], but not all studies [21,22]. IGF1 is a hormone produced by the liver and target tissues with a molecular structure which is very similar to that of insulin. IGF1 production is stimulated by growth hormone and, therefore, the serum levels of IGF1 can be used as a screening test for growth hormone deficiency or excess. The main effect of IGF1 is promotion of cell growth and multiplication. Thus, IGF1 plays an important role in T cell activation [23], cytokine production [24], muscle cell function [25], and growth, development and protection of nerve cells [26,27]. Moreover, IGF1 regulates mood [28], sleep [29], and memory [30,31]. Given the known effects of both DHEA(S) and IGF1 on energy, mood, muscles, the immune system and cognition it is probable that decreases of both hormones could play a role in the pathophysiology of CFS.

The aim of the present study was to examine whether serum DHEAS and IGF1 are lower in CFS patients than in normal controls and whether immune disorders in CFS, such as the defect in early T cell activation and the activation of the inflammatory response system are related to the serum levels of DHEAS and IGF1.

Material and methods

Subjects

Thirty-two subjects participated in the present study, 20 patients with CFS and 12 unrelated controls (staff or their family members). The CFS patients were admitted to the M-Care4U Outpatient Clinics, Lanaken and Hasselt, Belgium. The diagnosis of CFS was made by means of the Centers for Disease Control and Prevention (CDC) criteria [32], i.e. a) the patient must have a severe chronic fatigue of six months or longer, while there is no other known medical condition which can explain the fatigue; and b) the patient must have four or more of the following symptoms: substantial impairment in short-term memory or concentration, sore throat, tender lymph nodes, muscle pain, multi-joint pain without swelling or redness, headache of a new type, pattern or severity, unrefreshing sleep, and post-exertional malaise lasting more than 24 hours. The severity of CFS was measured by means of the Fibromyalgia and Chronic Fatigue Syndrome Rating Scale (the FibroFatigue sacle) [33,34]. The latter is a reliable and valid rating scale with 12 items measuring the following items: pain, muscular tension, fatigue, concentration difficulties, failing memory, irritability, sadness, sleep disturbances, autonomic disturbances, irritable bowel, headache, and subjective experience of infection [33].

In the present study we excluded subjects with life-time psychiatric DSM IV diagnoses, such as (bipolar) depression, anxiety disorders, schizophrenia, substance use disorders and organic mental disorders. We excluded subjects who ever had been treated with neuroleptics, anticonvulsants or mood stabilizers. None of the subjects had been taking antidepressants or benzodiazepines for at least 12 months. Not one of the subjects had ever been treated with DHEA, growth hormone or acclydine. All subjects were free of medical disorders and drugs known to be accompanied by alterations in endocrine and immune functions. All subjects had normal results for alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium, creatinine, electrolytes, thyroid stimulating hormone (TSH), total protein, and iron or transferrin saturation. Patients and controls gave written informed consent after the study protocol was fully explained. The study has been approved by the local ethical committee.

Methods

Fasting blood was sampled in all subjects for the determination of serum DHEAS, IGF1, IGFBP3, zinc, the serum alpha-2 protein fraction and the CD69 expression on the CD3+CD8+ T cells. DHEAS was measured with a RIA method (DSL DHEA-S RIA; DSL-3500). The intra-assay CV values were 9.4%, 7.8% and 6.3% at concentrations of 0.20, 1.87 and 5.93 ng/mL, respectively. The inter-assay CV values were 9.6%, 10.0% and 9.9% at concentrations of 0.21, 1.73 and 5.61 ng/mL, respectively. IGF-1 was measured with

an IRMA method (DSL extraction; coated tube IRMA; DSL 5600). The intra-assay CV values were: 3.4%, 3.0% and 1.5% at concentrations of 9.4, 55.4 and 263.6 ng/mL, respectively. The inter-assay CV values were 8.2%, 1.5% and 3.7% at concentrations of 10.4, 53.8 and 255.9 ng/mL, respectively. IGF-BP3 was measured by means of an IRMA method (DSL IGFBP-3 IRMA; DSL-6600). The intra-assay CV values were 1.8%, 3.2% and 3.9% at concentrations of 8270, 2750 en 740 ng/mL, respectively. The inter-assay CV values were 1.9%, 0.5% en 0.6% at concentrations of 7690, 2150 and 800 ng/mL, respectively. Serum zinc levels were determined by means of an atomic absorption method (PerkinElmer Analyst 200, Brussels, Belgium). The analytical inter-assay CV value of our assay was <10%. The serum alpha-2 protein fraction was measured by means of electrophoresis (SEBIA Benelux, Raketstraat, Brussels). The inter-assay CV value was < 2.0%. The CD69 expression on the CD3+CD8+ T cells after mitogen stimulation of whole blood was measured by means of the BD FastImmuneJ assay system. This method monitors the expression of the early activation marker, CD69, in whole blood after stimulation with mitogenic stimuli [8]. Blood samples for the CD69 assays were collected in lithium heparinized tubes. 200µl of whole blood was stimulated with pokeweed mitogen (PWM) 10 µg/ml (Sigma) at a final concentration of 5 µg/ml. Mitogen solutions were prepared in RPMI 1640 medium with L B glutamin (300 mg/ml, Gibko). All samples, including 200µl unstimulated blood were incubated 18 hours in humidified atmosphere at 37 EC with 5% CO2. Fifty microliters of stimulated and unstimulated samples were triple stained with the immunofluorescent mABs (FastImmune Becton Dickinson), specific for CD3, CD8, and CD69. All samples were proceeded on the multi Q-prep workstation (Coulter) and analyzed on a Coulter Epics XL flow cytometer using a tree color immunofluorescence staining protocol. The analysis determined the percent of cells expressing CD69 on the T suppressor cells (CD3+CD8+).

Statistics

Relationships between variables were assessed by means of Pearson's product moment correlations or by means of automatic multiple regression analysis. The diagnostic performance of the hormones for CFS was checked by means of ROC (receiver operating characteristics) analysis with computation of the area under the ROC curve (AUC), sensitivity, specificity and predictive value of a positive test result (PV+) and with κ statistics [35]. Group mean differences were examined by means of analysis of variance (ANOVA) or covariance (ANCOVA). The significance was set at α=0.05 (two tailed).

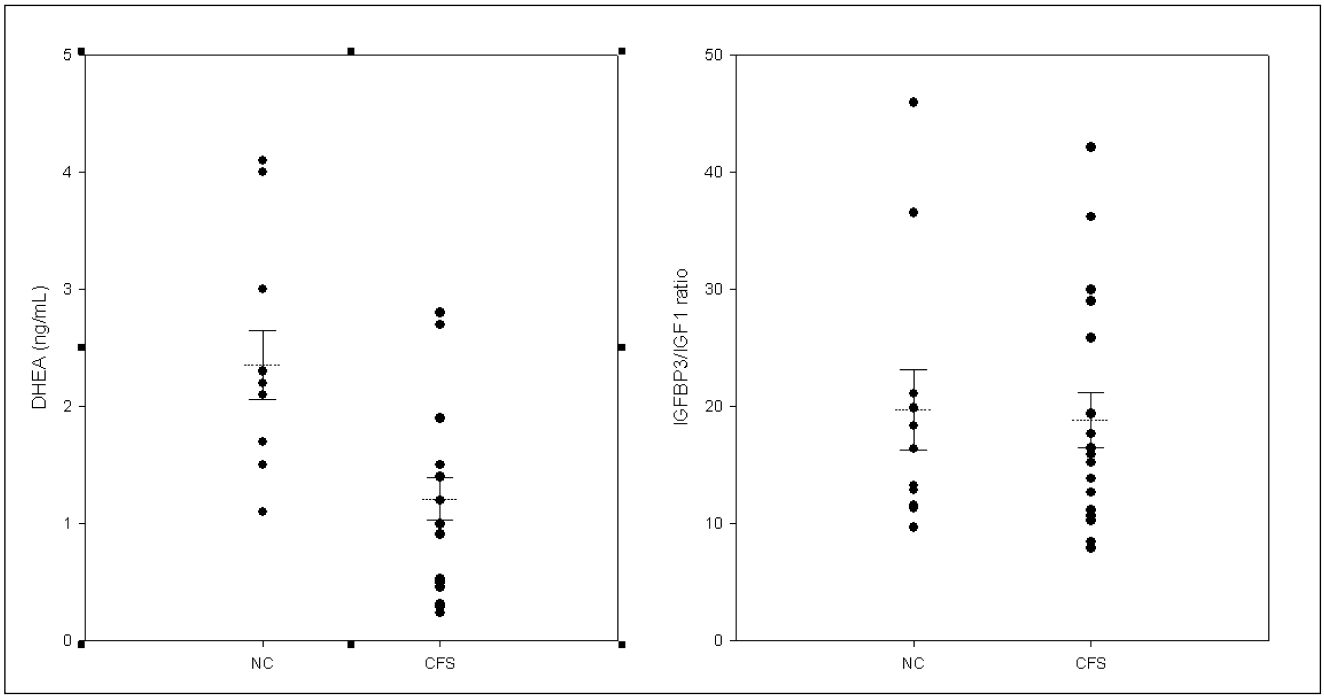
Results

Figure 1 shows the distributions of DHEAS and IGF1/IGFBP3 in CFS patients and in normal controls. ANOVA revealed significantly lower DHEAS in CFS patients than in normal controls (F=10.5, df=1/29, p=0.003). Covarying for age and gender did not change these results (results of ANCOVA: F=8.0, df=1/27, p=0.008). There were no significant differences in serum DHEAS between males and females (F=0.01, df=1/26, p=0.9). There were no significant correlations between serum DHEAS and age (r=–0.03, p=0.9). ROC analysis performed on serum DHEAS showed that the AUC was 80.4%. Using a cut-off value of DHEAS ≤1.50 ng/mL, we found a significant discrimination of CFS patients from normal controls (κ=0.55, t=3.71, p=0.001) with a sensitivity of 70.4%, specificity=90.9%; and PV+=93%.

ANOVA showed no significant differences in the IGFBP3/ IGF1 ratio between both study groups (F=0.1, df=1/32, p=0.8). ANOVA showed no significant differences in serum IGF1 (F=0.02, df=1/32, p=0.9) between CFS patients (mean ± SD= 300 ±136 ng/mL) and normal controls (mean ± SD=315 ±148 ng/mL). Covarying for age and sex did not change the above results. ANOVA showed no significant differences in IGFBP3 (F=1.6, df=1/33, p=0.2) between CFS patients (mean ±SD=4515 ±613 ng/mL) and normal controls (mean ±SD=4824 ± 806 ng/mL). There were no significant differences in IGF1 (F=0.9, df=1/30, p=0.6) and the IGFBP3/IGF1 ratio (F=1.0, df=1/30, p=0.3) between males and females. There were significant correlations between age and the IGF1BP3/IgF1 ratio (r=0.43, p=0.009) and between age and IGF1 (r=–0.47, p=0.006), but not between age and IGFBP3 (r=–0.15, p=0.6). ROC analysis showed that the AUC for the IGFBP3/IGF1 ratio was only 48.8%.

There was no significant correlation between serum DHEAS and the severity of illness (r=0.01, p=0.98). In order to examine the symptoms profiles of the hormones we computed the regressions of DHEAS, IgFBP3, IgF1 and their ratio on the 12 symptoms of the FibroFatigue scale (automatic step up method with an F-to-enter of p=0.05). We found no significant correlations between any of the FibroFatigue scale items and serum DHEAS. Up to 33.4% of the variance in IGF1 could be explained (F=4.5, df=2/18, p=0.02) by the regression on age (F= 4.7, p=0.04, negatively loaded) and muscular tension (F=6.3, p=0.02, positively loaded). Up to 33.6% of the variance in serum IGFBP3 could be explained by the regression on concentration difficulties (F=4.5, p=0.04, negatively loaded) and sadness (F=5.6, p=0.03, positively loaded). We found that 38.2% of the variance in the IGFBP3/IgF1 ratio was explained by the regression on age (F=6.0, p= 0.01, positively loaded) and muscular tension (F=7.6, p=0.01, negatively loaded).

There was a significant and positive correlation between serum DHEAS and serum zinc (r=0.50, p=0.01). There was a significant and negative correlation between serum DHEAS and the alpha-2 protein faction (r=–0.53, p=0.003). There was a significant and positive correlation between serum DHEAS and the PWM-induced expression of the CD69 molecule on CD3+CD8+ T cells (r=0.65, p=0.02). There were no



significant correlations beween the IGFBP3/IGF1 ratio and serum zinc (r=0.18, p=0.6), the alpha-2 protein faction (r=–17, p=0.6), and the PWMBinduced CD69+ expression on CD3+CD8+ T cells (r=–0.21, p=0.5). Considering the possible effects of age (by means of computing the semipartial correlation coefficients) did not change any of the above results.

Discussion

The main findings of this study are that serum DHEAS is significantly lower in patients with CFS than in normal controls and that serum DHEAS is signficully related to various immune and inflammatory markers. IGF1 or the IGFBP3/IGF1 ratio, on the other hand, were not significulty related to the diagnosis CFS, but to some of its symptoms.

Our findings that DHEAS is significally reduced in CFS patients is in agreement with a previous report that Japanese patients with CFS had a serum DHEAS deficiency [9]. Scott et al. [36] found lower DHEAS and DHEA in patients with CFS. De Becker et al. [10] found decreased DHEA responses to exogenous administration of ACTH intravenously. Scott et al. [37] reported that the DHEA/cortisol ratio decreased in response to ACTH in healthy controls, but not in CFS patients. Claere et al. [11], on the other hand, reported no significant differences in serum DHEAS between CFS patients and normal controls, whilst the DHEA levels were even higher in CFS. It is interesting to note that also in other disorders which show a high degree of comorbidity with CFS, e.g. fibromyalgia, serum DHEAS is often very low [38].

As explained in the Introduction, DHEAS and DHEA have a number of important functions which are relevant to CFS. Thus, DHEA(S) plays an important role in energy [12], the skeletal muscles [18], cognition and memory [15], mood [14] and sleep [17]. Based on the above literature it may be hypothesized that lowered DHEA may play a role in the occurrence of some CFS symptoms, such as fatigue, sleep disorders, muscle weakness, unrefreshing sleep, depressed mood, concentration difficulties and disorders in short term memory. In the present study however, no significant correlations could be established between the severity of those symptoms and serum DHEAS.

Another major finding of the present study is that – in CFS – there is a tight connection between serum DHEAS and markers of cellular immunity and inflammation. Thus, we found that serum DHEAS shows significant correlations with serum zinc, the alpha-2 protein fraction as measured by means of electrophoresis and the PWM-induced CD69 expression on CD3+CD8+ T cells. As explained in the Introduction, DHEA(S) modulates the immune system and plays a role in immune activation and inflammation. For example, DHEA may improve cellular immunity and has immunoenhancing effects [39]. DHEAS enhances natural killer cell cytotoxicity [16] and has a protective role in restoring T- and B-cell functions [40]. In addition, DHEA(S) displays anti-inflammatory effects: DHEA may inhibit the production of proinflammatory cytokines, such as tumor necrosis factorBalpha (TNF) and interleukinB6 (IL-6) [41]. The antiinflammatory effects of DHEA are in part mediated through inhibition of Nuclear Factor κ β (NFκβ) [42]. DHEA restores defects in pKC signal transduction pathways [43]. Therefore, it may be hypothesised that lowered levels of DHEAS in CFS may have jeopardized T cell-mediated immunity, which in turn may lead to defects in early T cell activation (acquisition of CD69 expression

on T cells), which involves defects in pKC-related mechanisms [8].

On the other hand, the relationships established between serum DHEAS and the inflammatory markers of CFS may also be explained by the effects of inflammation on serum DHEAS. Thus, in chronic inflammatory disorders serum levels of DHEAS are lowered [44]. Inflammation is indeed accompanied by significant TNF- and IL-1-induced decreases in the mRNA of Sult2A1 (sulfotransferase), the enzyme which plays an important role in DHEA sulfation [45]. Moreover, lowered serum DHEAS could also play a role in the increased oxidative stress and increased damage to oxidative stress in CFS [1]. Indeed, DHEAS has potent antioxidative capacities, which play a role in the inhibition of NFκβ [46].

As previous authors, we could not find that serum IgF1 and the IGFBP3/IGF1 ratio is lowered in CFS [21,22]. Berwaerts et al. [20], on the other hand, found serum IGF1 to be significally lower in CFS patiens than in normal controls. Nevertheless, we found correlations between IGFBP3, IGF1 and their ratio and the severity of muscular tension, concentration disorders and sadness. This may suggest that changes in IGF1 availability could play a role in some specific symptoms of CFS. As explained in the Introduction, IGF1 has important activities in muscle cell function [25], cell growth, development and protection of nerve cells [26,27] and mood regulation [28].

In conclusion, the present study shows that DHEAS is significantly lowered in CFS and suggests that DHEAS may play a role in the immuneBinflammatory pathophysiology of CFS. IGF1 probably does not play a major role in CFS.

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