Changes of BMI, steroid metabolome and psychopathology in patients with anorexia nervosa during hospitalization

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Abstract
Anorexia nervosa (AN) is associated with various alterations including the dysfunction of the HPA axis and consequently the hypercortisolemia and deficit in sex hormones but the comprehensive evaluation of changes in circulating steroids during the hospitalization of AN patients is lacking. We investigated the effect of realimentation of women with AN during hospitalization on 45 circulating steroids, the relationships between BMI, its change during hospitalization and physical activity, on one side and initial levels and their changes for two adipokines, circulating steroids, anorexia-specific (hunger, appetite and satiety), and anorexia non-specific symptoms (anxiety, depression fatigue, sleep, and body pain) on the other side. We included 33 women with anorexia who were hospitalized for 38 (35, 44) days (median with quartiles).

The increase of BMI from the initial value 15.2 (13.2, 16.6) kg/m² was 1.69 (1.37, 2.66) kg/m². The patients with more severe anorexia showed higher activity in 7β-, and 16α-hydroxylation of androgen precursors, which declined during hospitalization. Otherwise, the 7α-hydroxylation activity is higher in AN patients with less severe malnutrition and the ratio of 5-androstene-3β,7a,17β-triol to 5-androstene-3β,7β,17β-triol increased during the realimentation.

Our data allow to speculate that the intensive 7β-, and 16α- and possibly also the 7α-hydroxylation of C19 A³ steroids participate in the pathophysiology of anorexia by additional catabolism of substrates available for synthesis of active androgens and estrogens. However, the question remains whether the synthetic analogues of 7αβ- and 16α-hydroxy-steroids prevent the catabolism of the sex steroid precursors, or further activate the “energy wasting” mitochondrial thermogenic metabolism.

Keywords: Anorexia nervosa, Specific symptoms and physical activity, Depression, Refeeding, Steroid metabolome

1. Introduction

Anorexia nervosa (AN) is a psychiatric disorder associated with persistent food restriction and impaired body image. Prevalence of this disorder ranges between 0.2 and 4% in adolescents and young adults [1]. AN is associated with many endocrine alterations. The endocrine dysregulation involves a dysfunction of the hypothalamic-pituitary axis associated with hypercortisolemia, hypogonadotropic hypogonadism and impaired food intake. Another endocrine disorder associated with AN is the dysfunction of the HPA axis (hypothalamic-pituitary-adrenal axis) with the resulting hypercortisolemia, which is linked to energy wasting and mitochondrial thermogenic metabolism [2].

The interconversion of steroids is a complex process that is influenced by variations in the activity of 17α-hydroxylase, 17,20-lyase (17αOH and 17βOH), 21-hydroxylase, 11β-hydroxylase, 11β-hydroxysteroid dehydrogenase (11βHSD-1 and 11βHSD-2) and 3β-hydroxysteroid dehydrogenase (3βHSD) [3]. The enzymes responsible for the hydroxylation and dehydrogenation of steroids have a crucial role in the regulation of steroid synthesis and metabolism [4].

The 17α-hydroxylase is the rate-limiting enzyme in the synthesis of cortisol, aldosterone, androgens, and estrogens. In AN, 17α-hydroxylation of C19 androgens (17αOH C19) is increased, while 17β-hydroxylation (17βOH C19) is decreased [5].

The 17β-hydroxysteroid dehydrogenase type 1 (17βHSD-1) reduces 17β-hydroxysteroids to 17α-hydroxysteroids, whereas 17βHSD-2 converts 17α-hydroxysteroids to 17β-hydroxysteroids. The 17βHSD-1 is upregulated in AN, while the 17βHSD-2 is downregulated [6].

The 21-hydroxylase is responsible for the synthesis of cortisol and aldosterone. In AN, the 21-hydroxylase activity is increased, resulting in hypercortisolemia [7].

The 11β-hydroxylase is responsible for the synthesis of cortisol and aldosterone. In AN, the 11β-hydroxylase activity is decreased, resulting in hypocortisolism [8].

The 11β-hydroxysteroid dehydrogenase type 1 (11βHSD-1) reduces 11β-hydroxysteroids to 11α-hydroxysteroids, whereas 11βHSD-2 converts 11α-hydroxysteroids to 11β-hydroxysteroids. The 11βHSD-1 is downregulated in AN, while the 11βHSD-2 is upregulated [9].

The 3β-hydroxysteroid dehydrogenase is responsible for the synthesis of pregnenolone, progesterone, and androstenedione. In AN, the 3βHSD activity is decreased, resulting in lower levels of these hormones [10].

The interconversion of steroids is a complex process that is influenced by variations in the activity of 17α-hydroxylase, 17,20-lyase (17αOH and 17βOH), 21-hydroxylase, 11β-hydroxylase, 11β-hydroxysteroid dehydrogenase (11βHSD-1 and 11βHSD-2) and 3β-hydroxysteroid dehydrogenase (3βHSD) [11].

The enzymes responsible for the hydroxylation and dehydrogenation of steroids have a crucial role in the regulation of steroid synthesis and metabolism [12]. The interconversion of steroids is a complex process that is influenced by variations in the activity of 17α-hydroxylase, 17,20-lyase (17αOH and 17βOH), 21-hydroxylase, 11β-hydroxylase, 11β-hydroxysteroid dehydrogenase (11βHSD-1 and 11βHSD-2) and 3β-hydroxysteroid dehydrogenase (3βHSD) [13].

The enzymes responsible for the hydroxylation and dehydrogenation of steroids have a crucial role in the regulation of steroid synthesis and metabolism [14].
with estrogen and androgen deficit, growth hormone resistance, and a variety of further complications. Patients with AN have low levels of leptin (an anorexigenic adipokine) but elevated levels of ghrelin (an orexigenic gut peptide). These changes are mostly adaptive to activate energy reserves and save energy for vital body functions. Hypercortisolism in AN patients normalizes after weight recovery [2,3]. Nevertheless, the chronic undernutrition may induce numerous diseases including impaired skeletal integrity and neuropsychiatric disturbances [4]. Furthermore, hypercortisolism in patients with AN is associated with the severity of psychopathological symptoms independently of the body mass index (BMI) [5]. Some of the endocrine changes may persist even after recovery and contribute to predisposition to a relapse of AN [6]. Even if cortisol production and circadian rhythm are normal in AN subjects, its metabolic clearance is attenuated [7,8], which results in hypercortisolism with a lack of cortisol suppression by dexamethasone [9] due to hypersecretion of corticotropic-releasing hormone (CRH) in AN subjects [10].

Besides numerous articles focused on the role of cortisol and further common steroids in the pathophysiology of AN [11–16], there are some studies investigating the steroid metabolism in AN more comprehensively. Wassif et al. [17] demonstrated changed urinary excretion of steroids reflecting changes of several steroid catabolizing enzymes as was the consistent shift of the balance between 5α- and 5β-reductase activities towards the 5α-reduced steroids at increased concentrations of both androgen and pregnan 5α/5β-reduced catabolites, a shift of the balance between tetrahydrocortisols and tetrahydrocortisone towards the latter steroid indicating a decrease in activity of type 1 11β-hydroxysteroid dehydrogenase HSD11B1 and a shift from 20-hydroxy- to 20-oxocortisol metabolites probably indicating decreased activity of type 1 aldoketoreductase of C1 subfamily (AKR1C1) after refeeding. The data from Wassif et al. [17] were consistent with the earlier results from others [18,19]. Like the hypercortisolism, all aforementioned indices tended to normalize during the treatment.

Steroidogenic enzymes are widely expressed in adipose tissues and may significantly influence body weight. Engel et al. [20] reported negative correlation of type 2 11β-hydroxysteroid dehydrogenase expression (HSD11B2, enzyme catalyzing the conversion of active cortisol to inactive cortisone) in subcutaneous adipose tissue with BMI. Other authors [21–23] showed that the ratio of type 3 17β-hydroxysteroid dehydrogenase (HSD17B3) mRNA (enzyme catalyzing the conversion of androstenedione to testosterone) to CYP19A1 mRNA (enzyme catalyzing conversion of androgens to estrogens) in intra-abdominal adipose tissue positively correlates with BMI [24]. The expression of type 3 aldoketoreductase of C1 subfamily (AKR1C3) functioning similarly as HSD17B3 may be even more relevant as this enzyme is substantially more expressed in adipose tissue than the HSD17B3. Furthermore, the conversion of potential androgen 5α-dihydrotestosterone to its inactive metabolite 5α-androstane-3α,17β-diol, which is catalyzed by type 2 aldoketoreductase of C1 subfamily (AKR1C2) takes place in adipose tissue of both genders and the rate of 5α-dihydrotestosterone conversion in omental fat positively correlates with BMI, fat cell size and visceral adipose tissue area [21–23]. Wassif et al. in the study on urinary steroid excretion, also documented that BMI change during hospitalization tightly correlates with 5α-/5β-tetrahydrocortisol ratio (r = 0.95, p < 0.001) [17].

Whereas the urinary steroid excretion in patients with AN (including changes during the treatment) was thoroughly investigated by several authors [17–19], the comprehensive evaluation of changes in circulating steroids (serum) during the realimentation of AN patients is still lacking. Therefore, we investigated the effect of hospitalization on steroid metabolome in the circulation of women with AN as well as the relationships between BMI, its changes during hospitalization on one side and initial levels and the changes in two adipokines, 45 circulating steroids and anorexia-specific (hunger, appetite, and satiety), and anorexia non-specific indices (anxiety, depression fatigue, sleep, and body pain) on the other side.

2. Materials and methods

2.1. Subjects

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the First Faculty of Medicine, Charles University in Prague. All participants signed informed consent prior to the study. Thirty-three patients with AN, both restrictive and purgative type (age: 22 (19, 25) years, BMI 15.2 (13.2, 16.6) kg/m², shown as median with quartiles) were recruited consecutively from the inpatients of Eating Disorders Unit at the Psychiatric Clinic of the First Faculty of Medicine, Charles University, Prague. AN patients were diagnosed according to the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, 1994 and all were clinically stable. Patients with eating disorders not otherwise specified, psychosis, or current abuse of psychoactive substances were excluded. The additional exclusion criteria were current analgesic medication, pregnancy, diabetes, or a neurologic illness. The duration of the hospitalization was 38 (35, 44) days (median with quartiles). Samples of blood were collected from the participants at the beginning and the end of the hospitalization from the cubital vein at 7.30 AM. All patients were investigated the 3rd day of hospitalization. Blood tests conducted before the initiation of the study confirmed normal values for blood count, fasting blood glucose, liver, and renal functions.

2.1.1. Refeeding program

Women with AN follow an intensive comprehensive in-patient program with individual and group psychotherapy. The refeeding program for the patients is individual and depend on the current BMI. The basic diet for the patients reaching over 19 kg/m² the diet 2500 kcal/day consists of 90 g of proteins, 80 g of fat, 320 g of saccharides. For the patients between 18 and 19 kg/m² the diet is supplemented for additional snacks (350 kcal, proteins 20 g, fat 12 g, saccharides 37 g) and for the patients under the 18 kg/m² the enriched additional meal is appended (814 kcal, proteins 38 g, fat 23 g, saccharides 109 g) as well as 1 × 200 mL of Fresubin from Fresenius Kabi Deutschland GmbH (Bad Homburg, Germany) (200 kcal, proteins 7.6 g, fat 6.8 g, saccharides 27.6 g). All patients in the program eat together under supervision. Patients are informed of their target weights and of their current weights.

2.1.2. Physical activity, behavioral parameters and blood pressure assessment

The mean physical activity of the patients was recorded in the same days as the blood samples were collected and it was recorded using a wrist-worn Actiwatch AWSC device (Cambridge Neurotechnology Ltd., UK) with one-minute sampling frequency, placed on their non-dominant hands for 24 h. In the present study, total movement volume was defined as any movement detected by the Actiwatch device and finally expressed as an average of activity counts over the specified period. The physical activity was considered as any non-sedentary movement or activity. On the same day, the patients recorded the subjective score of anorexia-specific (hunger, appetite, and satiety), and anorexia non-specific symptoms (anxiety, depression fatigue, sleep, and body pain) using the same device (Actiwatch). The parameters were expressed as self-evaluation scores according to the intensity from 0 to 10 and recorded in 2 h intervals from 8 A.M. to 8 P.M. at the beginning of the study and at the end. The final values for further statistical processing were the means across the investigated period. The systolic (BPs) and diastolic blood pressure were recorded at the admission and end of the treatment.

2.2. Chemicals

Steroids were purchased from Steraloids (Newport, RI, USA), Sylon

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B from Supelco (Belleville, PA, USA), methoxyamine hydrochloride and trimethylchlororosilane (TMCS) for hydrolysis of steroids conjugates were from Sigma-Aldrich (St. Louis, MO, USA). Sylon BFT, methoxyamine hydrochloride and all other solvents and chemicals were from Merck (Darmstadt, Germany). All solvents were of HPLC grade.

2.3. Instruments and chromatographic conditions

A GCMS-TQ8040 system (Shimadzu, Kyoto, Japan) consisting of a gas chromatograph equipped with automatic flow control, an AOC-20 s autosampler and a triple quadrupole detector with adjustable electron voltage of 10–195 V was utilized. The analysis was conducted in Q3-SIM mode. A capillary column with a medium polarity RESTEK Rxi column (diameter 0.25 mm, length 15 m, film thickness 0.1 μm) was used for analyses. Electron-impact ionization with electron voltage fixed at 70 V and emission current set to 160 μA was used for the measurements. The temperatures of the injection port, ion source, and interface were maintained at 220, 300, and 310 °C, respectively. Analyses were carried out in splitless model with a constant linear velocity of the carrier gas (He) maintained at 60 cm/s. The septum purge flow was set at 3 mL/min. The samples were injected using high pressure mode applied at 200 kPa and maintained for 1 min. The detector voltage was set at 1.4 kV.

2.4. Analysis of neuropeptides

Total plasma ghrelin was determined by commercially available RIA kits (Linco Research, Inc., St. Charles, MO, USA). The intra- and inter-assay coefficient of variation for total ghrelin was 6.4% and 16.3%, respectively and the method sensitivity was 93 pg/mL. Plasma leptin was measured by a commercial RIA kit (Linco Research, St. Charles, MO, USA). Sensitivity, inter- and intra-assay variability were 0.05 ng/mL, 8.6% and 5.9%, respectively.

2.5. Steroid analysis

In total, the levels of 45 analytes were quantified in the circulation of volunteers. These analytes included a group of liver function tests, 28 unconjugated steroids and 17 steroid conjugates. The steroid metabolome in the maternal circulation included the levels of C21 and C19 Δ5 steroids, C21 and C19 Δ4 steroids, estrogens, C21 5α/β- reduced steroids, and C19 5α/β-reduced steroids. Most of the steroids were measured by GC-MS using our previously published method (for details, see [25–27]); however, cortisol levels were quantified by RIA kits (Immunotech, Marseille, France).

2.6. GC–MS analysis

Samples for GC–MS analysis were prepared as follows: unconjugated steroids were extracted from 1 mL of serum fluid with diethyl ether (3 mL). The diethyl ether extract was dried in a block heater at 37 °C. The lipids in the dry residue of the diethyl ether extract were separated by partitioning between a mixture of methanol–water 4:1 (1 mL) and pentane (1 mL). The pentane phase was discarded and the polar phase was dried in a vacuum centrifuge at 60 °C (5 h). The dry residue from the polar phase was derivatized in a vacuum centrifuge at 60 °C (2 h). The dry residues were reconstituted with 1 mL of chromatographic water and then further processed in the same way as the free steroids. In contrast to the sample preparation of free steroids, the dry residue after the second derivatization step was dissolved in 200 μL of isooctane instead of 20 μL of isooctane. Prior to further processing, the original samples and the polar phases after diethyl-ether extraction, which were used for the quantification of the steroid conjugates, were spiked with 17α-estradiol (as an internal standard) to attain concentrations of 1 and 10 ng/mL, respectively. The internal standard was recorded at the mass to charge ratios m/z = 231, 285, and 416.

2.7. Statistical analysis

Due to non-Gaussian data distribution and non-constant variance in most steroids, the original continuous variables were transformed by power transformations prior further processing to attain data symmetry and homoscedasticity [29]. The homogeneity and distribution of transformed data was checked as described elsewhere [30,31]. Statistical software Statgraphics Centurion 18 from Statpoint (The Plains, VA, USA) was used for Box-Cox transformations. The differences of changes during hospitalization (calculated as the situation at end of treatment - situation on the beginning of hospitalization) were evaluated by a robust Wilcoxon’s paired test using NCSS 12 statistical software from Number Cruncher Statistical System (Kaysville, UT, USA).

Pearson’s correlations after transformation of original data by Box-Cox transformations were used for evaluation of relationships between two continuous variables. The non-homogeneities were detected using Hotelling’s T² statistics and excluded. NCSS 12 statistical software was used for the correlation analysis. Relationships between steroid ratios and stage of the trial (initial vs. final) were evaluated using an ANOVA model consisting of subject factor explaining the interindividual variability and stage factor (initial vs. final). The ANOVA model was applied on the data after the Box-Cox transformations. Statistical software Statgraphics Centurion 18 was used for ANOVA testing.

The importance of individual predictors to discriminate between individual groups was evaluated using multivariate regression with a reduction of dimensionality known as orthogonal projections to latent structure (OPLS) for one predicted (dependent) variable in the model [32–34]. These methods are effective in coping with the problem of severe multicollinearity within the matrix of predictors (high inter-correlations) where the ordinary multiple regression fails to correctly evaluate such data as well as in the matrix of predicted variables (for details see Supplementary statistical information). It is obvious that predictors in our data set (steroids in metabolic pathways) are highly intercorrelated.

2.8. Terminology of steroid polar conjugates

Concerning the terminology of the steroid polar conjugates used here, the term steroid sulfate was used in the case of the dominance of 3α/β-monosulfate over other forms of steroid conjugates, while the term conjugated steroid was used in the case of comparable amounts of conjugate forms (sulfates, disulfates, and glucuronides). This terminology was based on relevant literature, with appropriate citations for each steroid as follows: pregnenolone sulfate [35,36], 20α-dihydroprogrenolone sulfate, 16α-hydroxy-DHEA sulfate [36,37], 5α-androstan-3β,17β-diol (androstenediol) sulfate [36,38], allopregnanolone sulfate [39], isopregnanolone sulfate [40], conjugated pregnanolone (sulfate + glucuronide [39]), epipregnanolone sulfate, conjugated 5α-androstane-3α,17β-diol (glucuronide + sulfate [38]), and conjugated 5α-androstane-3β,17β-diol (sulfate + glucuronide...
Table 1
Initial and final parameter values and their changes at end of treatment (n = 33). The effect of treatment was tested using a robust Wilcoxon’s paired test (parameters with significant changes (p < 0.05) are in bold). The results are shown as median with quartiles.

| Variable                  | Basal value       | Final value       | Treatment effect | p-value* |
|---------------------------|-------------------|-------------------|------------------|----------|
| Age [years]               | 22 (19, 25)       | –                 | –                | –        |
| BMI [kg/m²]               | 15 (13, 17)       | 17 (16, 18)       | 1.7 (1.4, 2.7)   | 0.000001 |
| Leptin [ng/mL]            | 2.3 (1.1, 3.9)    | 4.1 (1.5, 9.3)    | 1.4 (0.29, 3)    | 0.000307 |
| Pregnenolone sulfate      | 51 (37, 78)       | 72 (47, 110)      | 9.3 (~2.4, 25)   | 0.033481 |
| 20α-Dihydropregnenolone sulfate [nM] | 410 (210, 660) | 550 (280, 830) | 50 (~14, 170) | 0.039898 |
| 7α-Hydroxy-DHEA [nM]      | 0.83 (0.62, 1.1)  | 0.77 (0.57, 0.99) | –0.047 (~0.31, 0.058) | 0.066369 |
| 7β-Hydroxy-DHEA [nM]      | 0.61 (0.42, 0.89) | 0.48 (0.4, 0.62)  | –0.086 (~0.27, 0.017) | 0.007761 |
| 16α-Hydroxy-DHEA [nM]     | 0.16 (0.11, 0.2)  | 0.12 (0.088, 0.17) | –0.023 (~0.058, 0.01) | 0.018346 |
| Androstenediol sulfate [nM] | 280 (160, 540) | 200 (150, 480) | –0.30 (~4.19) | 0.076908 |
| 5-Androstene-3β,7β,17β-diol [nM] | 0.11 (0.08, 0.17) | 0.091 (0.061, 0.14) | –0.01 (~0.053, 0.0092) | 0.034996 |
| Allopregnanolone sulfate [nM] | 3.1 (1.7, 4.3) | 3.2 (1.4, 6.1) | 0.61 (~0.6, 1.6) | 0.066039 |
| Epipregnanolone (3β,5β-THP) [nM] | 0.051 (0.025, 0.069) | 0.035 (0.024, 0.055) | –0.016 (~0.032, 0.0059) | 0.045371 |
| Epipregnanolone (3β,5β-THA) [nM] | 0.047 (0.022, 0.062) | 0.022 (0.014, 0.034) | –0.014 (~0.034, ~0.0016) | 0.066069 |
| Epipregnanolone sulfate [nM] | 17 (5.5, 47) | 13 (6.5, 34) | –0.06 (~11, 2.2) | 0.066288 |
| 5α-Androstan-3α,17β-diol [nM] | 0.078 (0.03, 0.16) | 0.052 (0.041, 0.088) | –0.017 (~0.068, 0.016) | 0.063133 |

* Differences after refueling were evaluated using a robust Wilcoxon’s test. Only changes with p-value < 0.1 are shown.

3. Results

3.1. Values of parameters before treatment and their changes at end of the treatment

The values of parameters before treatment and their changes at end of the treatment are summarized in Table 1. Although body mass index (BMI) values at end of treatment significantly increased during the treatment, there were no significant changes in behavioral parameters. From the laboratory indices, leptin levels significantly increased, while ghrelin levels did not significantly change.

From the steroids, the levels of pregnenolone sulfate significantly increased during the treatment. Similarly, the levels of its 20α-hydroxy metabolite 20α-dihydopregnenolone sulfate significantly increased during the treatment. Alternatively, 7β-hydroxy-DHEA levels decreased at the end of treatment. Similarly, the levels of 5-androstene-3β,7β,17β-triol, decreased as well as the concentrations of epipregnanolone (3β,5β-THP) and epipregnanolone (3β,5β-THA). The remaining steroid levels did not significantly change as did the behavioral parameters such as physical activity, hunger, appetite, satiety, fatigue, sleep, and body pain.

In respect of increased steroids at the beginning of hospitalization, BMI positively correlated with age (r = 0.443, p = 0.014, n = 30) and leptin (r = 0.718, p < 0.001, n = 31) but negatively with ghrelin (r = –0.458, p = 0.008, n = 31) and age (r = –0.418, p = 0.019, n = 31). Several steroids, such as 16α-hydroxyprogrenolone (r = 0.368, p = 0.035, n = 33), DHEA (r = 0.383, p = 0.037, n = 30), 7α-hydroxy-DHEA (r = 0.419, p = 0.024, n = 29), androstenediol (r = 0.490, p = 0.006, n = 30), 5α-androstan-3β,7β,17β-triol (r = 0.568, p = 0.001, n = 31), androstenedione-3β,7β,17β-triol (r = 0.385, p = 0.027, n = 33), estrone while the analogous ratio (conjugated allopregnanolone + conjugated isopregnanolone)/progesterone reflecting activity of 5α-reductase isoforms (SRD5As) for C21 steroids did not significantly change. The analogous ratios for the 19 steroids reached significance for neither ARKD1 nor SRD5As.

The ratio of androstenediol sulfate to DHEA sulfate, reflecting the balance between reductive ARKD1 on one side and oxidative AKR1C3 on one side and oxidative HSD17B2 on the other side, significantly decreased during hospitalization (Fig. 2F).

Due to decrease of 7αβ-hydroxy-metabolites of 19Δ5 steroids, we also followed the product related to precursor ratios reflecting the concomitant activities of androgen-catabolizing enzymes CYP7B1, CYP3A4 and CYP3A7 catalyzing 7αβ- and 16α-hydroxylation of the androgens.

The 7αβ-hydroxy-DHEA/DHEA ratio reflecting the activity of CYP7B1 enzyme primarily catalyzing 7αβ-hydroxylation of 19Δ5 steroids showed a borderline decline (Fig. 3A). Concerning the 5α-androstan-3β,7α,17β-triol/androstenediol ratio reflecting the activity of the same enzyme, we observed no change during hospitalization. The 7αβ-hydroxylation of both DHEA and androstenediol, which is catalyzed by CYP3A4, CYP3A7, and CYP7B1 enzymes, showed more pronounced decrease as documented by consistently decreased 7β-hydroxy-DHEA/DHEA (Fig. 3B), 5α-androstan-3β,7β,17β-triol/androstenediol (Fig. 3C), and 16α-hydroxy-DHEA/DHEA (Fig. 3D) ratios during hospitalization.

Concerning the interconversion between 7αβ-hydroxy-metabolites of the aforementioned steroids on one hand and their 7β-hydroxy-metabolites on the other hand, which is catalyzed by type 1 11β-hydroxysteroid dehydrogenase (HSD11B1), we observed a slight shift from the 7αβ-hydroxy- to the 7αβ-hydroxy-metabolites at the end of treatment as documented by an insignificant decrease for the 7αβ-hydroxy-DHEA/7αβ-hydroxy-DHEA ratio (data not shown) but the significant one for the 5α-androstan-3β,7α,17β-triol/5α-androstan-3β,7β,17β-triol ratio (Fig. 3E).

3.2. Body mass index

3.2.1. Initial values

At the beginning of the hospitalization, BMI positively correlated with BPs (r = 0.443, p = 0.014, n = 30) and leptin (r = 0.718, p < 0.001, n = 31) but negatively with ghrelin (r = –0.458, p = 0.008, n = 31) and age (r = –0.418, p = 0.019, n = 31). Several steroids, such as 16α-hydroxypregrenolone (r = 0.368, p = 0.035, n = 33), DHEA (r = 0.383, p = 0.037, n = 30), 7α-hydroxy-DHEA (r = 0.419, p = 0.024, n = 29), androstenediol (r = 0.490, p = 0.006, n = 30), 5α-androstan-3β,7β,17β-triol (r = 0.568, p = 0.001, n = 31), 5α-androstan-3β,7β,17β-triol (r = 0.385, p = 0.027, n = 33), estrene

[38])
sulfate ($r = 0.510, p = 0.006, n = 28$), estradiol ($r = 0.506, p = 0.004, n = 30$), androstenedione ($r = 0.519, p = 0.003, n = 30$), and epiandrosterone ($r = 0.437, p = 0.016, n = 30$) positively correlated with BMI.

OPLS analysis of the basal data found significant positive correlations of BMI with leptin, $16\alpha$-hydroxypregnenolone, androstenediol, $5\text{-androstene-3\beta,7\alpha,17\beta}$-triol, and progesterone, and a negative one with age (Table 2).

The model of ordinary multiple regression (MR) found the significance only for leptin and $5\text{-androstene-3\beta,7\alpha,17\beta}$-triol. These relationships kept significance even after adjustment to constant values of the remaining relevant parameters in the model and therefore their contribution to the model was at least partly independent of the remaining relevant parameters (Table 2).

### 3.2.2. Change during hospitalization

Concerning the relationships between the change of BMI at the end of treatment ($\Delta$BMI) and parameters at the beginning of the trial (basal parameters), $\Delta$BMI negatively correlated with basal BMI ($r = -0.665, p < 0.001, n = 33$), BPs ($r = -0.372, p = 0.033, n = 33$), physical activity ($r = -0.467, p = 0.007, n = 32$), anxiety score ($r = -0.416, p = 0.018, n = 32$), leptin ($r = -0.492, p = 0.004, n = 34$), $5\text{-androstene-3\beta,7\alpha,17\beta}$-triol ($r = -0.402, p = 0.025, n = 31$), and androstenedione ($r = -0.375, p = 0.035, n = 32$).

When investigating the relationships between the $\Delta$BMI and changes of parameters at the end of treatment ($\Delta$), we found only a slight but still significant positive correlations with an increase in body pain score ($r = 0.361, p = 0.042, n = 32$) and in the levels of conjugated pregnanolone ($r = 0.567, p = 0.002, n = 28$).

OPLS analysis of the basal data and changes at the end of treatment found that the $\Delta$BMI positively correlated with basal isopregnanolone $\Delta$Body pain score, $\Delta$Sleep score, $\Delta$Pregnenolone sulfate, $\Delta$Androstenedione, $\Delta$Allopregnanolone, $\Delta$5opregnanolone sulfate, and $\Delta$Androsterone and negatively correlated with basal values of BMI, satiety score, fatigue score, and leptin and with $\Delta$Appetite score, $\Delta16\alpha$-Hydroxypregnenolone, $\Delta7\beta$-Hydroxy-DHEA, and $\Delta5$-Androstene-3\beta,7\beta,17\beta-triol. The parameters Age, basal androsterone and basal conjugated $5\alpha$-androstane-3\beta,17\beta-diol were relevant to improve predictivity of the model (as indicated by significant VIP statistics) but did not reach significance in either OPLS or MR models (Table 3).

The MR model found a similar pattern of significance as in the case of the corresponding the OPLS model but in contrast to the OPLS model, it detected a significant negative correlation for $\Delta$Appetite score and an absent significance for $\Delta$Androsterone (Table 3).

### 4. Discussion

#### 4.1. The endocrine effects of refeeding

From the adipokines, we observed a significant increase only for leptin but an insignificant change in ghrelin, which is in line with some reports [41,42]. While leptin and insulin have stimulatory effects, ghrelin is inhibitory for GnRH secretion [43]. Leptin levels generally normalize with the recovery of body weight [44].

Numerous circulating steroids on the beginning of the hospitalization positively correlated with BMI (see section 2.1), which pointed to
more critical disruption of steroidogenesis in more severe anorexia. We found a significant increase of pregnenolone sulfate and 20α-dihydroprogrenenolone sulfate, the steroids in early steps of the steroidogenic pathway and significant drop in 7β-hydroxy-metabolites of C19 Δ5 steroids 7β-hydroxy-DHEA, and 5α-androstene-3β,7β,17β-triol [45,46] (Table 1), which are known as immunostimulatory steroids on one hand but anti-inflammatory and immunoprotective agents (suppressing autoimmunity) on the other hand [47].

Furthermore, the interconversion between the 7α-hydroxy, 7-αo xo-, and 7β-hydroxy-metabolites is catalyzed by the same enzyme (HSD11B1), which catalyzes the conversion of biologically inactive cortisone to active glucocorticoid cortisol. Therefore, there may be a competition for HSD11B1 between 7-oxygenated androgens and glucocorticoids [46]. There is also another mechanism providing the anti-inflammatory effect, which is based on catabolism of C19 Δ5 steroids (serving as substrate for the synthesis of bioactive androgens and autoimmunity-inducing estradiol) via 7α- and 16α-hydroxylation [47–52]. Moreover, estrogens may stimulate their own catabolism via the CYP7B1 metabolic pathway [53]. In spite of low levels and high clearance of 7α/β-hydroxy-steroids, the 7-hydroxylation metabolism appears to be of physiological importance. Our data demonstrate that refeeding in AN patients is associated with a significant drop in 7β-hydroxylation of the C19 Δ5 steroids (CYP3A4, CYP3A7, and CYP7B1 enzymes). We also found a shift from the 7β- to 7α-hydroxy-metabolites (Fig. 3E) in spite of declined 7α-hydroxylation of DHEA (Fig. 3A). The corresponding HSD11B1 enzyme catalyzing interconversion between 7-oxygenated C19 Δ5 steroids is active in adipose tissues [54–58]. Wassif et al. [17] reported a drop in HSD11B1 activity after refeeding, which is in line with our data.

The CYP7B1 gene providing the7α-hydroxylation is expressed in various tissues [59] but to our knowledge, CYP7B1 expression has not yet been investigated in human adipose tissues. Nevertheless, the 7α-hydroxylation of DHEA operates in differentiated 3T3-L1 adipocytes [60]. Our group previously reported that 7α-hydroxy-DHEA exert anti-obesity effects [61]. Taken together, these data allow to speculate that adipose tissue may be a site for 7α-hydroxylation of C19 Δ5 steroids. Various 7-oxygenated androgens stimulate enhanced formation of liver energy-producing enzymes [62]. Therefore, the excess of 7α/β-hydroxy-steroids in anorexic women may induce undesirable energy “wasting”. Alternatively, the expression of 7α/β-hydroxylation enzymes in adipose tissue may prevent from excessive energy storage in obese subjects. Elevated levels of the aforementioned substances on the beginning of the present study may be associated with undesirable energy expenditure in AN, which, however, declines after refeeding.

Based on the significant increase in the levels of sulfated pregnenolone and 20α-dihydroprogrenenolone during hospitalization (Table 1), which is in line with data of Udahne et al. reporting significantly suppressed pregnenolone formation in human adrenocortical H295R cells grown under starvation condition [63], we also estimated the changes in related steps of steroid metabolism and found that refeeding mitigates the lyase step of CYP17A1 (Fig. 2A,B), which accords with data from others [63–65]. Most probably, the starving organism tries to maintain the level of DHEA as high as possible as the gene for DHEA synthesis from cholesterol is highly upregulated while other genes involved in the production of other steroid hormones are downregulated. By the way, this data may also indicate a connection between starvation response and an anti-aging process [64].

In contrast to Wassif et al. [17] reporting a shift from urinary hydroxy to 20-oxo-metabolites catalyzed by AKR1C1, we observed the reverse shift from of pregnenolone to 20α-dihydropregneneolone (Fig. 2D). The AKR1C1 is a pluripotent aldo-ketoreductase primarily inactivating progesterone (20-oxoreductase activity), but at the same time synthesizing testosterone from androstenedione (17-oxoreductase activity) and inactivating dihydrotestosterone by its conversion to 5α-
androstan-3α,17β-diol (3-oxoreductase activity) [66]. AKR1C1 is relatively highly expressed in adipose tissue and 20-oxoreductase activity can be easily detected [67,68]. Therefore, the increase of 20α-dihydroprogrenolone/pregnenolone ratio during hospitalization of AN patients may be associated with increase of adipose tissues. Our data also indicate increased conversion of progesterone to its conjugated 5β-reduced metabolites (catalyzed by AKR1D1 enzyme) during the hospitalization (Fig. 2E), in accord with the data of Wassif et al. [17].

The balance between activities of reductive HSD17B isoforms and AKR1Cs on one side, and of oxidative HSD17Bs on the other side, affects the balance between active hormones and their inactive metabolites. We recorded decreased ratio of androstenediol sulfate to DHEA sulfate (Fig. 2F), which may reflect a shift from reductive AKR1C3 to the oxidative HSD17B2. In general, we recorded an increase in steroids the formation of which is catalyzed by enzymes of aldoketoreductase group such as AKR1C1, AKR1C3, and AKR1D1 (Fig. 2 D-F) but decrease of those formed by oxidative HSD17B2 (Figures D, F). This data is in line with reports of Bauer et al. [64]. At the same time, both aldoketoreductases and HSD17B2 are expressed in human adipose tissue [21–23,65].

Further significant change was increased sulfated/unsulfated steroid ratio for pregnenolone (Fig. 2C), which may indicate either increasing sulfotransferase SULT2A1 activity and/or declining steroid sulfatase (STS) activity in early stages of steroid metabolism. Both DHEA sulfatase and estrone sulfatase activities as well as STS mRNA protein and organic anion transporters for sulfate transport (OATP-B,
Table 3
Relationships between changes of BMI after treatment (ΔBMI) and other relevant parameters as evaluated by OPLS and multiple regression models (for details see Statistical analysis).

| Variable | OPLS | Multiple regression |
|----------|------|---------------------|
|          | Component loading | t-statistics | R² | Regression coefficient | t-statistics |
| Relevant predictors (matrix X) | | | | | |
| Age | 0.153 | 1.61 | 0.262 | 0.057 | 0.86 |
| Leptin | −0.371 | −4.07 | −0.633** | −0.249 | −4.44** |
| BMI | −0.424 | −5.11 | −0.722** | −0.059 | −1.29 |
| Satiety | −0.187 | −5.09 | −0.319** | −0.077 | −1.52 |
| Fatigue | −0.265 | −2.82 | −0.452* | −0.143 | −2.37* |
| Androstenedione | −0.250 | −2.01 | −0.426* | −0.072 | −1.51 |
| Isoeugenol | 0.195 | 2.49 | 0.332** | 0.162 | 2.63* |
| Androsterone | −0.114 | −1.45 | −0.194 | −0.016 | −0.38 |
| Conjugated 5α-Androsterone-3β,17β-diol | −0.096 | −1.08 | −0.163 | −0.104 | −1.40 |
| ΔAppetite | −0.155 | −1.52 | −0.264 | −0.085 | −2.28* |
| ΔBody pain | 0.310 | 3.02 | 0.528** | 0.173 | 4.73** |
| ΔSleep | 0.231 | 2.11 | 0.395* | 0.082 | 2.98* |
| ΔPregnenolone sulfate | 0.192 | 2.62 | 0.328* | 0.112 | 1.60 |
| Δ16α-Hydroxyprogrenolone | −0.155 | −2.09 | −0.264* | −0.129 | −4.30** |
| Δ17β-Hydroxy-DHEA | −0.189 | −3.87 | −0.322** | −0.107 | −2.92* |
| ΔS-Androsterone-3β,7β,17β-triol | −0.201 | −2.31 | −0.342* | −0.097 | −4.58** |
| ΔAndrostenedione | 0.094 | 1.91 | 0.160** | 0.014 | 0.39 |
| ΔAllopregnanolone | 0.057 | 1.94 | 0.096* | −0.026 | −0.70 |
| ΔIsopregnanolone sulfate | 0.341 | 2.07 | 0.581* | 0.240 | 2.30* |
| ΔAndrosterone | 0.111 | 2.00 | 0.189* | 0.022 | 0.40 |

(matrix Y)

ΔBMI | 1.000 | 11.72 | 0.871** |

Explained variability 75.8% (63% after a cross-validation)

* R Component loadings expressed as correlation coefficients with predictive component, *p < 0.05, **p < 0.01.

OATPD and OATP-E were detected in adipose tissues [69,70].

To conclude, the aforementioned steroid ratios in the circulation as well as the levels of steroids showing a significant increase at end of hospitalization may be potentially employed as complementary markers of hormonal recovery in AN patients.

4.2. Relationships between physical activity, BMI, its change during hospitalization, behavioral indices, and laboratory parameters

Besides the positive correlation with leptin and body pain score, the initial BMI positively correlated with one of C19 Δ⁵ precursors of bioactive androgens androstenediol, its immunoprotective metabolites (5-androstene-3β,7α,17β-triol and 5-androstene-3β,7β,17β-triol), C21 Δ⁴ steroid 16α-hydroxyprogrenolone, and C21 Δ⁴ steroid progesterone and negatively correlated with age (Table 2). In addition to the relevant predictors of initial BMI, which were included the OPLS model, the Pearson's correlations between initial BMI and parameters absent in the OPLS model reached significance for some of them. We found significantly positive correlations even for BPs, DHEA, 7α-hydroxy-DHEA, estrone sulfate, estradiol, androsterone, and epiaandrosterone and negative ones for ghrelin and age. The OPLS and correlation analysis indicate that the patients with higher BMI and less severe AN have more active non-corticoid steroidogenesis and lower levels of ghrelin as their position is closer to the situation after refeeding. It was well documented that leptin levels are closely associated with BMI [71]. In our data, the OPLS model found several relevant predictors for the initial BMI.

In the OPLS model, we found positive correlations with increase in body pain (score and increase in sleep score but negative ones with initial scores of satiety and fatigue. A negative correlation between leptin level, anxiety, and activity at admission and observed increase in BMI during hospitalization indicate that patients with initially higher BMI and leptin are more anxious, and anxiety may be compensated with higher physical activity, and consequently negatively influence weight gain, which is in line with others [72]. A positive correlation between changes in BMI and body pain may reflect greater dissatisfaction with one's body after weight gain during hospitalization, which is in accordance with our previous data [73]. In other studies, the thermal pain threshold was higher in AN than in healthy women and correlated negatively with the level of DHEA and positively with cortisol/DHEA(S) ratio [74,75]. Although cortisol alone did not correlate with thermal pain, its balance with DHEA(S) brings significant information about the ability to cope with stress, which can be used as a marker of improvement of interoceptive awareness.

The increase of BMI in the OPLS model (Table 3) showed positive correlations with increases of C21 steroids (pregnenolone sulfate, allopregnanolone, isopregnanolone sulfate), C19 steroids (androstene-dione and androsterone) on one side but with drop of 7α- and 16α-hydroxy-metabolites on the other side. In other words, the increase of BMI is positively associated with restoration of non-corticoid steroidogenesis and, at the same time, with reduced catabolism of steroids via 7α- and 16α-hydroxylation of C19 Δ⁴ steroids, which means that the levels of some steroids inducing energy “wasting” via activation of thermogenic mitochondrial enzymes decline after refeeding. Moreover, the activities of enzymes catalyzing adrenal androgens and preventing their conversion to active sex hormones fall as well. This data point to an additional mechanism counteracting the synthesis of sex hormones in women with AN. The potential antiandrogenic effect of the 7αβ- and 16α-hydroxylation enzymes allows to speculate whether their inhibition might counterbalance the lack of sex hormones in AN patients, for instance via substrate inhibition of these enzymes by stable synthetic 7αβ- and 16α-hydroxy-steroid derivatives [47,49,76] but the question remains whether these derivatives activate the thermogenic mitochondrial enzymes like their endogenous analogues.

5. Conclusions

The main findings of the study consist of the importance of androgen-catabolizing 7β-, 16α- and 7α-hydroxylation steps. Our data indicate that the 7β-, and 16α-hydroxylation activities are higher in AN patients with less severe malnutrition and the ratio of 5-androstene-3β,7α,17β-triol to 5-androstene-3β,7β,17β-triol have increasing trend
during the realimentation. Our data allow to speculate that the intensive 7α-, and 16α- and perhaps also the 7α-hydroxylation of androgen precursors participate in the pathophysiology of anorexia by additional catabolism of substrates available for synthesis of active androgens and estrogens. The question also remains whether the synthetic analogues of 7αβ-, and 16α-hydroxy-steroids may prevent the detrimental catabolism of sex steroid precursors, or further activate the undesirable “energy wasting” mitochondrial thermogenic metabolism. This question should be a subject of further research. Although some features in the results from analyses in the circulation and urine are similar, these matrices are not interchangeable and their pathophysiologic and diagnostic importance may differ and need further studies confirmation.

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References

[1] F.R. Smink, D. van Hoeken, H.W. Hoek, Epidemiology, course, and outcome of eating disorders, Curr. Opin. Psychiatry 26 (2013) 543–548.
[2] U.F. Bailar, V.H. Kaye, A review of neuropeptide and neuroendocrine dysregulation in anorexia and bulimia nervosa, Curr. Drug Targets CNS Neurol. Disord. 2 (2003) 53–59.
[3] R. Balsch, et al., Association between neuroendocrinological parameters and learning and memory functions in adolescent anorexia nervosa before and after weight recovery, J. Neural Transm. (Vienna) 118 (2011) 963–968.
[4] M. Schor, K.K. Miller, The endocrine manifestations of anorexia nervosa: mechanisms and management, Nat. Rev. Endocrinol. 13 (2017) 174–186.
[5] E.A. Lawson, et al., Appetite-regulating hormones cortisol and peptide YY are associated with disordered eating psychopathology, independent of body mass index, Eur. J. Endocrinol. 164 (2011) 253–261.
[6] E.A. Lawson, A. Klibanski, Endocrine abnormalities in anorexia nervosa, Nat. Clin. Pract. Endocrinol. Metab. 4 (2008) 407–414.
[7] R.M. Boyar, et al., Cortisol secretion and metabolism in anorexia nervosa, N. Engl. J. Med. 296 (1977) 190–193.
[8] B.T. Walsh, et al., The production rate of cortisol declines during recovery from anorexia nervosa, J. Clin. Endocrinol. Metab. 53 (1981) 203–205.
[9] J. Lincic, M.I. Wong, P.W. Gold, The hypothalamic-pituitary-adrenal axis in anorexia nervosa, Psychiatry Res. 62 (1996) 75–83.
[10] M. Hotta, et al., The responses of plasma adrenocorticotropin and cortisol to corticotropin-releasing hormone (CRH) and cerebrospinal fluid immunoactive CRH in anorexia nervosa patients, J. Clin. Endocrinol. Metab. 62 (1986) 319–324.
[11] P. Monteleone, et al., Impaired reduction of enhanced levels of dehydroepiandrosterone by oral dexamethasone in anorexia nervosa, Psychoneuroendocrinology 31 (2006) 537–542.
[12] B. Wild, et al., Temporal relationships between awakening cortisol and psychosocial variables in inpatients with anorexia nervosa - a time series approach, Int. J. Psychophysiol. 102 (2016) 25–32.
[13] R.C. Casper, R.T. Chatterton Jr., J.M. Davis, Altersation in serum cortisol and its biological and diagnostic importance may differ and need further studies confirmation.
of gender, obesity, and fat localization, Obesity (Silver Spring) 15 (2007) 1954–1960.

[57] R. Desbriere, et al., 11beta-hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients, Obesity (Silver Spring) 14 (2006) 794–798.

[58] M.J. Lee, et al., Depot-specific regulation of the conversion of cortisone to cortisol in human adipose tissue, Obesity (Silver Spring) 16 (2008) 1178–1185.

[59] K. Rose, et al., Neurosteroid hydroxylase CYP7B: vivid reporter activity in dentate gyrus of gene-targeted mice and abolition of a widespread pathway of steroid and oxysterol hydroxylation, J. Biol. Chem. 276 (2001) 23937–23944.

[60] A. Marwah, et al., Redox reactions of dehydroepiandrosterone and its metabolites in differentiating 3T3-L1 adipocytes: a liquid chromatographic-mass spectrometric study, Arch. Biochem. Biophys. 456 (2006) 1–7.

[61] J. Sulcova, et al., Effects of transdermal application of DHEA on the levels of steroids, gonadotropins and lipids in men, Physiol. Res. 49 (2000) 685–693.

[62] H. Lardy, I.I. Ergosteroids, et al., Biologically active metabolites and synthetic derivatives of dehydroepiandrosterone, Steroids 63 (1998) 158–165.

[63] S.S. Udhane, et al., Retinoic acid receptor beta and angiopoietin-like protein 1 are involved in the regulation of human androgen biosynthesis, Sci. Rep. 5 (2015) 10132.

[64] M. Bauer, et al., Starvation response in mouse liver shows strong correlation with life-span-prolonging processes, Physiol. Genomics 17 (2004) 230–244.

[65] M. Fouad Mansour, et al., Oxidative activity of 17beta-hydroxysteroid dehydrogenase on testosterone in male abdominal adipose tissues and cellular localization of 17beta-HSD type 2, Mol. Cell. Endocrinol. 414 (2015) 168–176.

[66] T.M. Penning, et al., Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones, Biochem. J. 351 (2000) 67–77.

[67] Y. Zhang, et al., Progesterone metabolism in adipose cells, Mol. Cell. Endocrinol. 298 (2009) 76–83.

[68] S. Blanchette, et al., Expression and activity of 20alpha-hydroxysteroid dehydrogenase (AKR1C1) in abdominal subcutaneous and omental adipose tissue in women, J. Clin. Endocrinol. Metab. 90 (2005) 264–270.

[69] C. Martel, et al., Widespread tissue distribution of steroid sulfatase, 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase (3 beta-HSD), 17 beta-HSD 5 alpha-reductase and aromatase activities in the rhesus monkey, Mol. Cell. Endocrinol. 104 (1994) 163–111.

[70] L. Dalla Valle, et al., Tissue-specific transcriptional initiation and activity of steroid sulfatase complementing dehydroepiandrosterone sulfite uptake and intracrine steroid activations in human adipose tissue, J. Endocrinol. 190 (2006) 129–139.

[71] A. Strengel, et al., Leptin and physical activity in adult patients with anorexia nervosa: failure to demonstrate a simple linear association, Nutrients (2017) 9.

[72] K. Holtkamp, J. Hebebrand, B. Herpertz-Dahlmann, The contribution of anxiety and food restriction on physical activity levels in acute anorexia nervosa, Int. J. Eat. Disord. 36 (2004) 163–171.

[73] A. Yamamotova, et al., Dissatisfaction with own body makes patients with eating disorders more sensitive to pain, J. Pain Res. 10 (2017) 1667–1675.

[74] H. Paperno, A. Yamamotova, R. Uher, Elevated pain threshold in eating disorders: physiological and psychological factors, J. Psychiatr. Res. 39 (2005) 431–438.

[75] A. Yamamotova, V. Knoch, H. Papezoa, Role of dehydroepiandrosterone and cortisol in nociceptive sensitivity to thermal pain in anorexia nervosa and healthy women, Neuro Endocrinol. Lett. 33 (2012) 401–405.

[76] F. Nicoletti, et al., 17alpha-Ethynyl-androst-5-ene-3beta,7beta,17beta-triol (HE3286) is neuroprotective and reduces motor impairment and neuroinflammation in a murine MPTP model of Parkinson’s disease, Parkinsons Dis. 2012 (2012) 969418.