

Steroid Hormones Related to 11 β -hydroxysteroid Dehydrogenase Type 1 in Treated Obesity

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Summary

The local concentration of glucocorticoids is intensively regulated by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD 1). Human 11 β -HSD 1 also reversibly catalyzes the inter-conversion of 7 α -hydroxy- and 7 β -hydroxy-dehydroepiandrosterone (DHEA) into 7-oxo-DHEA. The cohort of 282 obese adolescents, 154 girls (median age 15.31 years, range 14.17-16.68 years) and 128 boys (median age 14.95 years, range 13.87-16.16 years), BMI (Body Mass Index) >90th percentile was examined. In samples collected before and after one month of reductive diet therapy, circulating levels of steroids were analyzed by liquid chromatography-tandem mass spectrometry and radioimmunoassay methods. The model of the treatment efficacy prediction was calculated. A significant reduction in circulating levels of cortisone, E2 and increased levels of 7 β -hydroxy-DHEA after the reductive treatment was observed. Levels of cortisol, DHEA, DHT sustained without any significant change. The predictive Orthogonal Projections to Latent Structures (OPLS) model explained 20.1 % of variability of BMI, z-score change by the basal levels of 7 α -hydroxy-DHEA, DHEA, cortisol and E2 as the strongest predictors. Reduced levels of circulating cortisone and reduced ratios of oxygenated/reduced metabolites reflect increased reductase activity of 11 β -HSD 1 with reduced BMI, z-score. We hypothesize whether these changes can be attributed to the altered activity of 11 β -HSD 1 in the liver.

Key words

Steroids • Obesity • 11 β -hydroxysteroid dehydrogenase type 1 • Glucocorticoids • Dehydroepiandrosterone

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Introduction

According to the recent data of the World Obesity Federation, there are around 1.5 billion overweight adults and over 200 million overweight school-age children. This vast number of obese patients is now recognized as one of the most important public health problems.

It has been well documented, that steroid hormones, especially glucocorticoids (GC), are involved in maintaining energy balance. Its disruption may lead to obesity and metabolic syndrome, as seen in the condition of chronic stress (Rosmond 2005) and patients with Cushing's syndrome (Rebuffe-Scrive *et al.* 1988, Bista and Beck 2014). Indeed, in addition to effects on glucose metabolism (Schacke *et al.* 2002), GC increase serum levels of free fatty acids (Djurhuus *et al.* 2002), increase lipolysis (Tomlinson *et al.* 2007) and stimulate differentiation of adipocytes (Hauner *et al.* 1987). There has therefore been great interest in revealing the role of endogenous GC in the development of obesity.

The effect of GC depends not only on plasma levels, regulated through the hypothalamic-pituitary-adrenal axis, but also on intracellular levels, regulated by tissue specific enzymes. One of these enzymes, 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD 1) amplifies local GC action. This enzyme is responsible for the regeneration of cortisol from inert cortisone (Seckl and Walker 2001) and is present in various tissues including adipose tissue (Ricketts *et al.* 1998). Numerous papers report that the elevated expression and activity of adipose 11 β -HSD 1 is linked to obesity and metabolic syndrome in humans (Rask *et al.* 2001, 2002, Kannisto *et al.* 2004, Valsamakis *et al.* 2004) as well as animals

(Livingstone *et al.* 2000, Masuzaki *et al.* 2001). At the same time, Stimson *et al.* (2009) anticipate that a 10 kg/m² increase in BMI might elevate the whole-body adipose production of cortisol of ~12.7 nmol/min (accounting for ~15 kg increase in fat mass). Conversely, 11 β -HSD 1 knockout mice have demonstrated an improved lipid profile, resistance to stress and obesity-related hyperglycemia together with a reduced gluconeogenic response (Kotelevtsev *et al.* 1997, Morton *et al.* 2001). Nevertheless, the literature on the expression of 11 β -HSD 1 in human obesity is discordant. Some studies found no significant difference in expression of adipose 11 β -HSD 1 in obese vs. non-obese subjects (Tomlinson *et al.* 2002).

The specific inhibition of adipose 11 β -HSD 1 provides a promising tool in the treatment of obesity. The most recent data of clinical studies with several selective 11 β -HSD 1 inhibitors have shown moderate body weight reduction as well as improvement in insulin sensitivity and hyperglycemia (Rosenstock *et al.* 2010, Feig *et al.* 2011, Gibbs *et al.* 2011, Shah *et al.* 2011, Anagnostis *et al.* 2013, Heise *et al.* 2014). However, these compounds have limitations and none of them provides overall protection from obesity and metabolic syndrome.

It is of interest that some endogenous steroids act as modulators of 11 β -HSD 1 activity. The first of these are glucocorticoids employing 11 β -HSD 1 for their metabolism. They also enable the stimulation of 11 β -HSD 1 enzymatic activity as well as the expression of the enzyme (Hammami and Siiteri 1991, Bujalska *et al.* 2006, Zhu *et al.* 2010).

The sex steroids were intensively studied to explain gender different metabolism and body fat distribution. Only a few studies have examined the effect of sex steroids on 11 β -HSD 1, but with controversial outcomes. Some studies have reported an inhibitory effect of 17 β -estradiol (E2) in adipose tissue on mRNA, enzyme expression and enzymatic activity of 11 β -HSD 1 (Hammami and Siiteri 1991, Gomez-Sanchez *et al.* 2003, Tagawa *et al.* 2009). Other authors (Andersson *et al.* 2010) observed the same effect in liver and visceral, but not in subcutaneous fat. These observations are in contrast with Dieudonné *et al.* (2006), who found E2 to be a strong up-regulator of 11 β -HSD 1 mRNA expression in women's preadipocytes. In addition, androgens exhibit a significant stimulation effect on the 11 β -HSD 1 activity and expression after the treatment with 5 α -dihydrotestosterone (DHT) (Dieudonné *et al.* 2006) and testosterone (Liu *et al.* 1998). Nevertheless, the

combined treatment of testosterone and cortisol did not increase the level of 11 β -HSD 1 as seen with each steroid alone (Zhu *et al.* 2010).

Finally, there is a group of dehydroepiandrosterone (DHEA) and its metabolites that has been reported to regulate enzymatic activity of 11 β -HSD 1. DHEA suppresses the expression and activity of 11 β -HSD 1 in adipose tissue, which contributes to the clarification of the anti-obesity effect of DHEA (Apostolova *et al.* 2005, Tagawa *et al.* 2011). 7-hydroxy metabolites of DHEA, found in our laboratory as early as 50 years ago (Starka *et al.* 1962, Starka and Hampl 1964), have been over the past decade suggested as substrates for 11 β -HSD 1 (Robinson *et al.* 2003). Indeed, the enzyme 11 β -HSD 1 reversibly catalyzes the conversion of 7 α -hydroxy- and 7 β -hydroxy-DHEA into 7-oxo-DHEA (Muller *et al.* 2006b, Zhou *et al.* 2012), and both 7-hydroxylated metabolites of DHEA competitively inhibit cortisol oxidation (Hennebert *et al.* 2007). Moreover, DHEA itself as well as its 7-hydroxylated derivatives perform anti-GC effects (Chmielewski *et al.* 2000), which may be explained by the favored production of 7-hydroxy-DHEA over that of active glucocorticoids (Muller *et al.* 2006a).

Despite the fact that the GC excess is associated with central obesity and insulin resistance, the relationship between circulating cortisol levels and abdominal obesity has been highly inconsistent (Abraham *et al.* 2013). In order to contribute to the revelation the role of 11 β -HSD 1 in obesity, we followed the alteration of circulating levels of steroids related to 11 β -HSD 1 in patients treated for obesity.

Materials and Methods

Subjects

The cohort for the present study was recruited from the Childhood Obesity Prevalence and Treatment project; the epidemiological and intervention study evaluated the prevalence and treatment of obesity in children from the Czech Republic. A group of 282 obese patients, 154 girls (median age 15.31 years, range 14.17-16.68 years) and 128 boys (median age 14.95 years, range 13.87-16.16 years), BMI >90th percentile for age and sex of the Czech reference (Kobzova *et al.* 2004), was examined. Exclusion criteria were endocrine disorders including diabetes and use of drugs affecting body weight (glucocorticoids, psychotropic drugs, contraceptives, etc.). The hydration status and plasma

protein of the subjects were normal.

The study protocol was approved by the Ethics Committee of the Institute of Endocrinology in Prague and was performed in accordance with the ethical standards of Helsinki Declaration II. and its later amendments. Written informed consent was obtained from all participants and their parents prior to their inclusion into the study.

All patients underwent reductive therapy that consisted of adjustment of energy intake and observance of physical activity. The weight management program was provided on an in-patient basis in a center specialized in weight management for children/adolescents within a period of 4 weeks and was strictly medically supervised (pediatricians, dietitians, physiotherapists, psychologists). Adolescents had aerobic exercise 3.5-4.0 h per day (e.g. jumping, jogging, ball games, fitness, swimming, dancing, and hiking 4-10 km/day). Screen time was limited to maximum of 1 h a day. The decreased daily energy intake (to 5-7 MJ/day) was individually modified according to age and BMI in order to ensure the recommended daily consumption of all essential nutrients and appropriate macronutrient balance. The intake of fat, protein and carbohydrates was aimed at 30 %, 15-20 % and 50-55 % of energy intake, resp. sugar intake was <5 % of total energy intake. For more details see (Hlavaty *et al.* 2010).

Anthropometric data

Anthropometry was performed after 12 h of fasting on subjects dressed in their underwear. Height (to the nearest 0.5 cm) was measured by a stadiometer, body weight (to the nearest 0.1 kg) by Tanita BC-418 MA (Tanita Corporation, Tokyo, Japan). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). Each BMI was converted to a BMI, z-score, which reflects the number of standard deviations that an individual subject deviates above or below the BMI mean matched with general Czech population (Kobzova *et al.* 2004) of the same gender and age.

Chemicals and reagents

Cortisol, cortisone and dehydroepiandrosterone (DHEA) were purchased from Koch-Light Laboratories LTD (Colnbrook, Great Britain), 7 α -OH-DHEA, 7 β -OH-DHEA, 7-oxo-DHEA, 16 α -OH-DHEA and D3-DHEA were from Steraloids (Newport, USA). D4-Cortisol was from CDN isotopes (Ponte-Claire, Canada).

2-hydrazinopyridine, ammonium formate, methyl tert-butyl ether and trifluoroacetic acid were from Sigma-Aldrich (St. Louis, USA). LC-MS grade methanol, water and diethyl ether were from Merck AG (Darmstadt, Germany). The physiological solution (0.9 % sodium chloride) was from B-Braun (Melsungen AG, Germany). [1,2,6,7-³H]Cortisol, specific radioactivity 3.04 TBq/mmol was from Amersham Biosciences, Inc. (Amersham, UK).

Hormonal analysis

Hormonal analysis was performed on blood samples collected from fasting patients between 7:00 and 9:00 a.m. Plasma samples were stored at -80 °C in plastic tubes until laboratory processing.

LC-MS/MS analysis was performed using an API 3200 (AB Sciex, Concord, Canada) triple stage quadrupole – mass spectrometer with electrospray ionization connected to the UHPLC Eksigent ultraLC 110 system (Redwood City, CA, USA). Chromatographic separation was carried out on a Kinetex C18 2.6 μ m (150 x 3.0 mm) column (Phenomenex, Torrance, CA, USA) with a corresponding security guard. The CSF levels of cortisol, cortisone, DHEA, 7 α -hydroxy-DHEA, 7 β -hydroxy-DHEA, 7-oxo-DHEA and 16 α -hydroxy-DHEA were measured by the method described elsewhere (Sosvorova *et al.* 2015).

DHT was assayed by the original radioimmunoassay (RIA) developed previously in the author's laboratory (Hampl *et al.* 1990). E2 was determined using the RIA kit (Orion Diagnostica, Espoo, Finland).

Statistical analysis

The differences (Δ) in values before and after the reducing treatment were calculated for each subject separately. The effects of gender and treatment on individual parameters were evaluated using ANOVA model with factors Gender, Age group and Gender \times Age group interaction. Least significant multiple comparisons followed the ANOVA model. To attain data symmetry and constant variance, the original data was transformed by Box-Cox transformations. Statistical software Statgraphics Centurion v. XV from Statpoint (Herndon, VA, USA) was used for the analysis. The relationships between change of BMI, z-score on the one hand and basal BMI, z-score and steroid levels and ratios on the other hand, were evaluated using multivariate regression with a reduction of dimensionality (model of orthogonal

projections to latent structures, OPLS). This approach allows for severe multicollinearity in the set of predictors to be coped with and also enabled the separation of variability shared between dependent variable and predictors from the variability shared within the matrix of predictors. As in the case of ANOVA testing, the original data was transformed to symmetry and homoscedasticity using the Box-Cox transformations.

Results

Significant decreases in BMI, z-score before and after treatment were found in all examined groups. The reductive therapy was the most effective in fourteen-year-old boys, whereas seventeen-year-old boys showed the smallest weight reduction after the reductive treatment (Fig. 1).

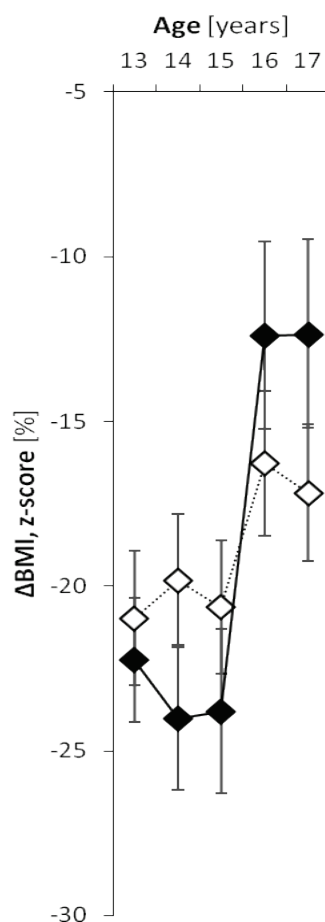


Fig. 1. The difference (Δ) of body mass index, z-score after the reductive treatment. The ANOVA model consisting of factors Gender and Age group and Gender \times Age group interaction followed by least significant difference multiple comparison was used. The symbols with error bars represent the group means with their 95 % confidence intervals. Black symbols mean males, white females. The significance of factors and interaction was as follows: Gender: $F=0$, $p=0.9675$; Age: $F=11.4$, $p<0.0001$; Gender \times Age: $F=3$, $p=0.0198$.

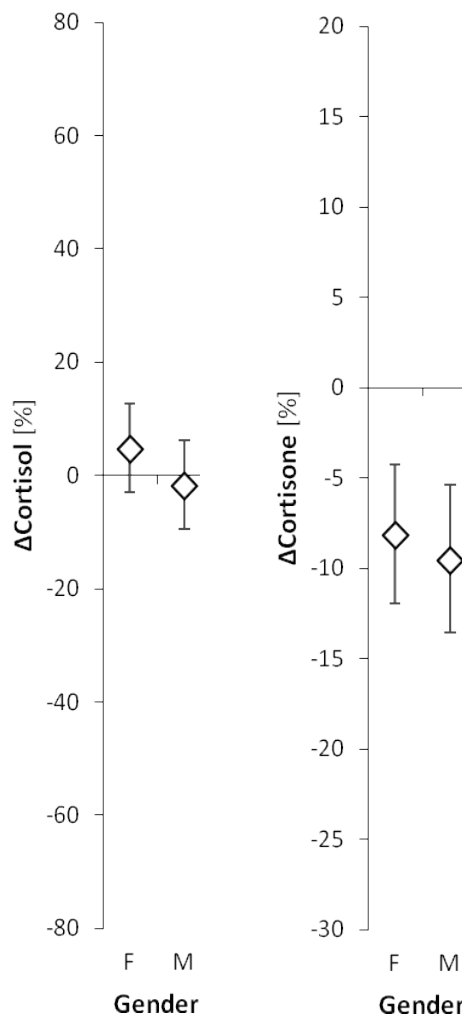


Fig. 2. The difference (Δ) of circulating cortisol and cortisone before and after the reductive treatment. The ANOVA model with factor Gender followed by least significant difference multiple comparison was used. The symbols with error bars represent the group means with their 95 % confidence intervals. F is used for females, M for males. The significance of factors and interaction for Δ cortisol was as follows: Gender: $F=0.1$, $p=0.7346$; Age: $F=1.1$, $p=0.3729$; Gender \times Age: $F=1.4$, $p=0.2335$ and for Δ cortisone it was: Gender: $F=0.7$, $p=0.4163$; Age: $F=0.9$, $p=0.4676$; Gender \times Age: $F=0.4$, $p=0.8447$.

Among glucocorticoids (Fig. 2), we found significant reduction in levels of cortisone after the reductive treatment, whereas levels of cortisol remained without significant change.

Circulating levels of selected sex steroid were significantly decreased in the case of E2, while DHT levels remained unchanged after the reduction treatment (Fig. 3).

Interestingly, while changes in concentration of DHEA did not reach statistical significance, levels of its metabolites did vary before and after the treatment (Fig. 4). We found increasing levels of 7β -hydroxy-DHEA, while the same tendency in the case of 7α -hydroxy-DHEA was observed only in girls.

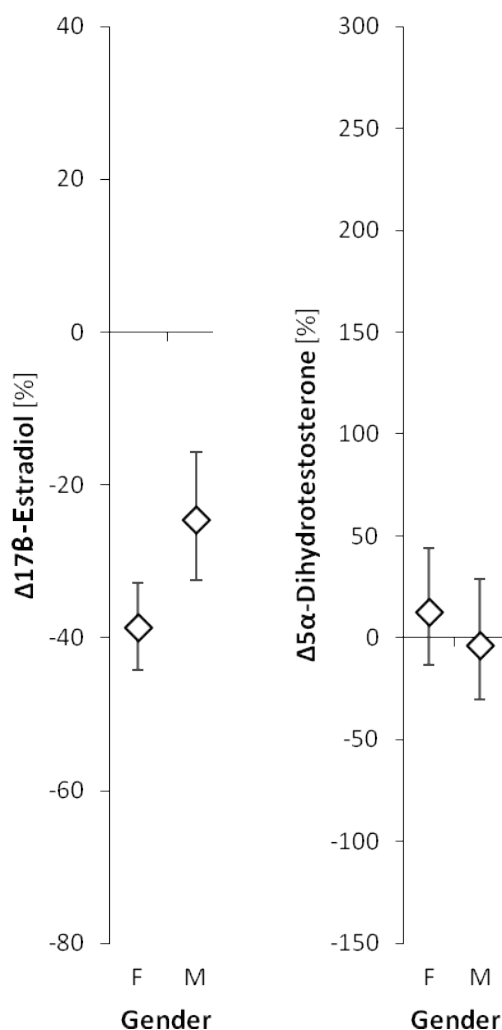


Fig. 3. The difference (Δ) of circulating 17 β -estradiol and 5 α -dihydrotestosterone before and after the reductive treatment. The ANOVA model with factor Gender group interaction followed by least significant difference multiple comparison was used. The symbols with error bars represent the group means with their 95 % confidence intervals. F is used for females, M for males. The significance of factors and interaction for $\Delta 17\beta$ -estradiol was as follows: Gender: $F=4.1$, $p=0.0451$; Age: $F=6.8$, $p<0.0001$; Gender \times Age: $F=2.3$, $p=0.0638$ and for $\Delta 5\alpha$ -dihydrotestosterone it was: and Gender: $F=0.3$, $p=0.5733$; Age: $F=2.1$, $p=0.0828$; Gender \times Age: $F=1$, $p=0.4268$, respectively.

We followed ratios of hormones representing products/precursors to evaluate the activity of the enzyme. The cortisone/cortisol ratio, associated with the activity of the 11 β -HSD 1, was decreased after the treatment, but with statistical significance only in girls. The 7-oxo-DHEA/7 α -hydroxy-DHEA ratio, also reflecting the activity of the 11 β -HSD 1, was significantly decreased in boys. Moreover, the amount of 7 α -hydroxylation expressed as the 7 α -hydroxy-DHEA/DHEA ratio was increased after the reductive therapy (Fig. 5).

Some of the measured parameters showed significance enabling treatment efficacy to be predicted. Using the OPLS method we explained 20.1 % of variability of BMI, z-score change as the dependent variable. The strongest predictor among the examined steroids was the basal levels of 7 α -hydroxy-DHEA, followed by basal levels of DHEA, cortisol and E2 (Fig. 6).

Discussion

Our study gives an overview of changes in steroid hormone levels related to the enzyme 11 β -HSD 1, which occur during reduction treatment. Unlike other studies, we simultaneously examined circulating levels of glucocorticoids, metabolites of DHEA and other steroids known for their ability to modulate the activity of the enzyme 11 β -HSD 1.

Consistent with the majority of studies reviewed elsewhere (Seimon *et al.* 2013), we did not observe changes in cortisol levels. As far we know there are no data on impact of reductive therapy on the levels of serum cortisone or ratio cortisone/cortisol in juvenile obese patients.

In accordance with our expectations, we observed reduced levels of E2 after the treatment. Our finding was consistent with other studies performed mostly on females (Boyar *et al.* 1988, Heber *et al.* 1991, Campbell *et al.* 2012) or girls (Dorgan *et al.* 2003). Surprisingly, we did not prove significant differences in levels of DHT in the treated subjects, despite the other study that showed an increase of circulating DHT with decreasing BMI in men (Dušková *et al.* 2013). This discrepancy may be the result of the different gender and age of the examined cohorts.

As we reported in our preliminary study (Macova *et al.* 2014), circulating 7-hydroxy- and 7-oxo-derivatives of DHEA change during the reductive treatment. In the present study, we observed similar results, although some parameters varied. First of all, these differences may be the result of the extension of recruited patients. Secondly, we used the novel advanced LC-MS/MS method, which is more sensitive and specific than immunoassays we used in previous study. Moreover, a different statistical approach was used to evaluate the measured data. Whereas in the previous study we calculated the absolute values of variables, in the present study the values were expressed as a percent of change measured after treatment.

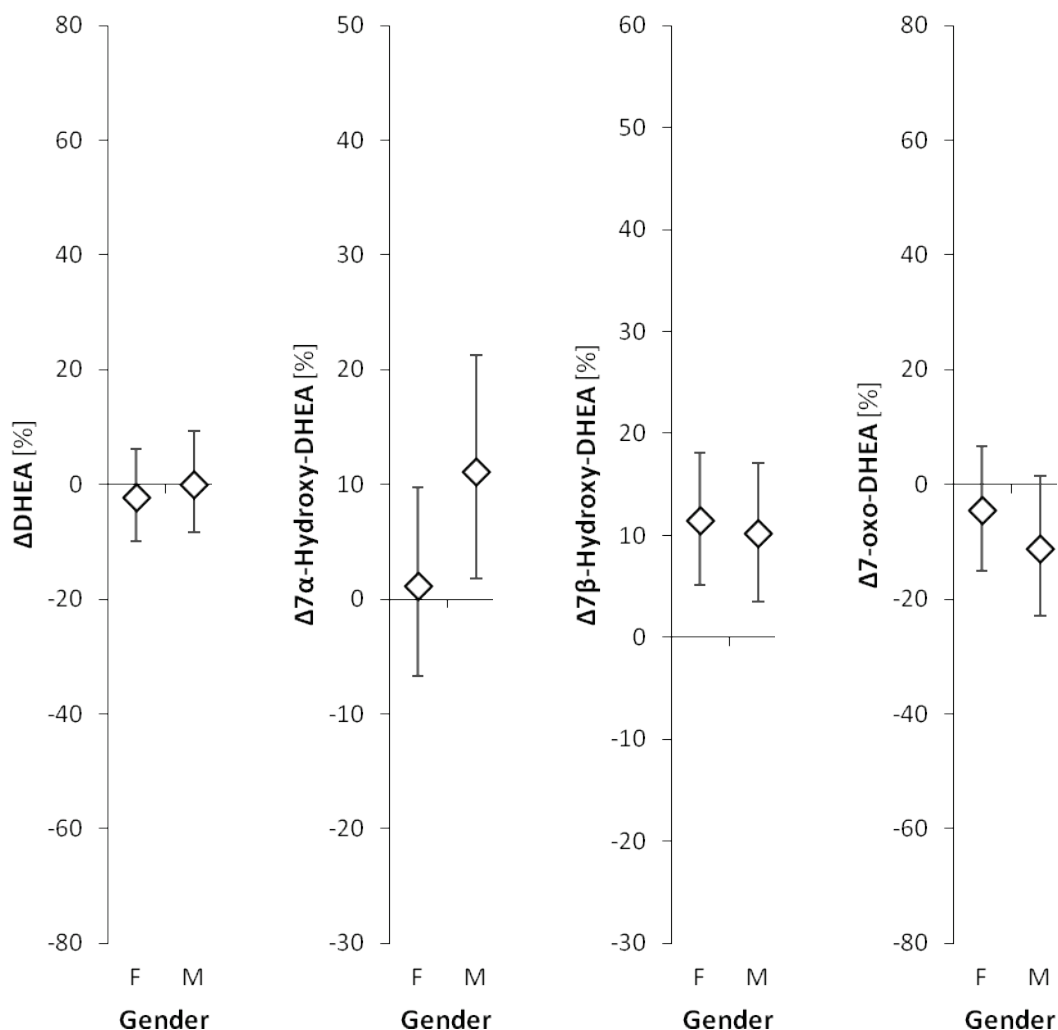


Fig. 4. The difference (Δ) of circulating dehydroepiandrosterone (DHEA), 7 α -OH-DHEA, 7 β -OH-DHEA and 7-oxo-DHEA before and after the reductive treatment. The ANOVA model with factor Gender group interaction followed by least significant difference multiple comparison was used. The symbols with error bars represent the group means with their 95 % confidence intervals. F is used for females, M for males. The significance of factors and interaction for Δ DHEA was as follows: Gender: $F=0.1$, $p=0.7884$; Age: $F=0.2$, $p=0.9391$; Gender \times Age: $F=1.2$, $p=0.3073$, for 7 α -hydroxy-DHEA it was Gender: $F=1.2$, $p=0.2759$; Age: $F=0.2$, $p=0.9538$; Gender \times Age: $F=0.5$, $p=0.738$, for 7 β -hydroxy-DHEA it was Gender: $F=0$, $p=0.8474$; Age: $F=0.3$, $p=0.8641$; Gender \times Age: $F=1.7$, $p=0.1617$, and for 7-oxo-DHEA it was Gender: $F=0.3$, $p=0.5733$; Age: $F=2.1$, $p=0.0828$; Gender \times Age: $F=1$, $p=0.4268$, respectively.

In the present study, we observed significantly reduced 7-oxo/7 α -hydroxy-DHEA ratio in boys and increased proportion of 7 α -hydroxylation expressed as increased 7 α -hydroxy-DHEA/DHEA ratio in the examined cohort. Both observations support the hypothesis on DHEA metabolites involvement in metabolic processes and maintaining energy balance of the human body. Indeed, 7-oxo-DHEA has been reported as an “ergosteroid” with a thermoregulatory effect, which induces shift from oxidative metabolism towards increased heat production (Lardy *et al.* 1995) and, after transdermal administration, improves hormonal and lipid parameters (Sulcova *et al.* 2001). Although the significance of DHEA derivatives on human metabolism

has not been fully elucidated, some drugs based on these compounds have already appeared on the market as anti-obesity medication (www.dietspotlight.com/lean-xtreme-review/).

According to our OPLS model, some of the examined parameters had significant power for BMI, z-score % change prediction. The amount of explained variability (20.1 %) is significant when taking into account that the model does not include parameters typically examined in obese patients (parameters of glucose and lipid metabolism or anthropometric variables such as waist circumference, body fat mass, skinfolds size, etc.).

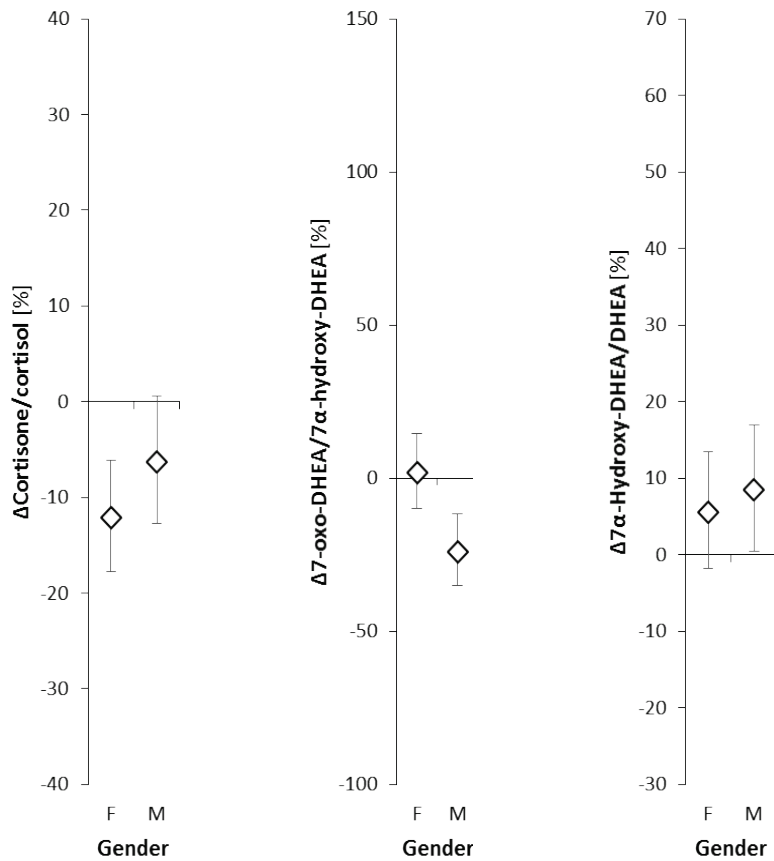


Fig. 5. The difference (Δ) of steroid ratios reflecting the activity of 11β-hydroxysteroid dehydrogenase type 1 and before and after the reductive treatment. The ANOVA model with factor Gender group interaction followed by least significant difference multiple comparison was used. The symbols with error bars represent the group means with their 95 % confidence intervals. F is used for females, M for males. The significance of factors and interaction for Δ (Cortisone/cortisol) was Gender: $F=0.9$, $p=0.3538$; Age: $F=1.5$, $p=0.2002$; Gender \times Age: $F=1.2$, $p=0.3367$; for Δ (7-oxo-DHEA/7α-hydroxy-DHEA) it was Gender: $F=4.6$, $p=0.0346$; Age: $F=3.4$, $p=0.0116$; Gender \times Age: $F=2.5$, $p=0.044$, and for Δ (7α-hydroxy-DHEA/DHEA) it was Gender: $F=0.1$, $p=0.7269$; Age: $F=0.6$, $p=0.6344$; Gender \times Age: $F=2.1$, $p=0.0807$, respectively.

Variable	Predictive component		
	Component loading	t-statistics	R ²
Age	0.409	11.44	0.655 **
BMI, z-score, basal	0.299	7.01	0.480 **
7α-Hydroxy-DHEA, basal	0.444	9.96	0.712 **
Cortisol, basal	0.331	6.92	0.531 **
Estradiol, basal	0.251	4.72	0.402 **
DHEA, basal	0.405	10.77	0.648 **
7β-Hydroxy-DHEA/7α-Hydroxy-DHEA, basal	-0.394	-10.17	-0.631 **
Cortisone/*cortisol, basal	-0.382	-6.98	-0.613 **
7β-Hydroxy-DHEA, %change	-0.178	-2.48	-0.284 *
(matrix Y) BMI, z-score, %change	1.000	4.86	0.448 **
Explained variability	20.1% (16% after cross-validation)		

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

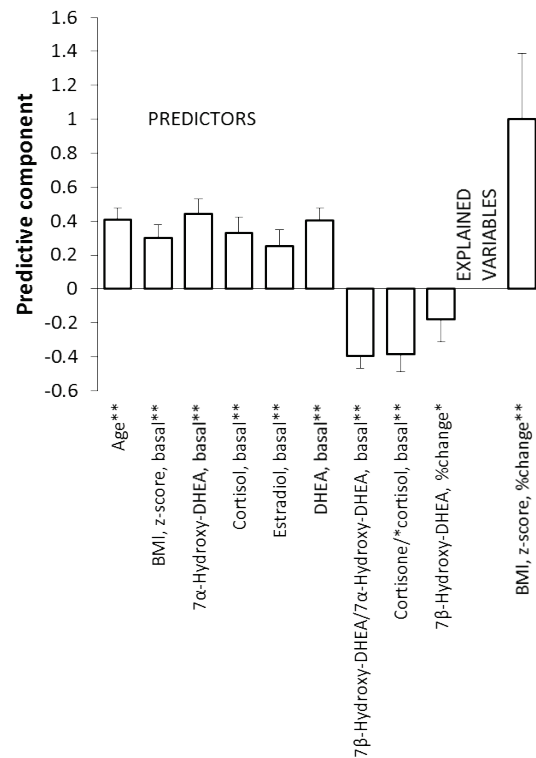


Fig. 6. Relationship between BMI, z-score, % change (matrix Y) and circulating steroids (matrix X), as evaluated by OPLS model (for details see Statistical analysis). T-statistic is a ratio of component loading and statistical error. BMI, body mass index; DHEA, dehydroepiandrosterone.

Our results demonstrate diminished ratios of circulating steroids towards reduced metabolites (cortisol, 7-hydroxy-DHEA) after the weight reduction therapy. However, the interpretation of the result is quite intricate with regard to 11 β -HSD 1. Primarily, the enzyme activity of 11 β -HSD 1 differs between tissues and, subsequently, the contribution of GC by various tissues into the circulation is different. In obese patients there has been suggested a mechanism of impaired reactivation of cortisone to cortisol by liver 11 β -HSD 1, which has been balanced by increased activity of 11 β -HSD 1 in adipose tissue (Rask *et al.* 2001).

Secondly, the enzyme 11 β -HSD 1 is bidirectional. Although reductase activity predominates *in vivo*, converting cortisol to cortisone, under certain conditions, the enzyme switches towards dehydrogenase activity, which has been described, for example, in the process of adipocytes maturation (Bujalska *et al.* 2002). The directionality of 11 β -HSD 1 is driven by pyridine nucleotide cofactor NADP⁺/NADPH redox potential, derived from hexose-6-phosphate dehydrogenase and co-localized with 11 β -HSD 1 in endoplasmic reticulum lumen (White *et al.* 2007). NADPH favors reductase activity of 11 β -HSD 1, whereas the predominance of NADP⁺ switches towards dehydrogenase activity (Zhou *et al.* 2012).

Moreover, there are other enzymes that regulate GC levels. The second type of isozyme 11 β -HSD (11 β -HSD 2) is expressed mainly in mineralocorticoid target tissues such as the kidney, colon and salivary glands (Shimojo *et al.* 1997). It converts cortisol to inactive cortisone in order to decrease local glucocorticoid levels, and thus protects mineralocorticoid receptors from cortisol activation. However, we do not expect that the changes described in our study were caused by changed activity of 11 β -HSD 2, as similar levels of 11 β -HSD 2 with different BMI were observed (Stewart *et al.* 1999).

The mechanism of GC regulation also involved 5 α -reductase, the enzyme responsible for the irreversible inactivation of cortisol leading towards waste metabolites excreted in the urine. Studies have reported the enhanced inactivation of cortisol by 5 α -reductase to be associated with obesity (Andrew *et al.* 1998) and, similarly, decreased activity of the enzyme after the weight loss was observed (Tomlinson *et al.* 2008). The same enzyme has the ability to metabolize testosterone to DHT. In our study, we did not examine levels of testosterone due to the generally

accepted statement that testosterone is usually reduced in male obesity (Alvarez-Castro *et al.* 2011, De Maddalena *et al.* 2012). If we assume increased levels of testosterone (substrate) associated with lower BMI, our observation with no significant change of circulating DHT (product) is consistent with the hypothesis of reduced 5 α -reductase activity after the weight loss (Tomlinson *et al.* 2008).

It is likely that, due to the complexity of the mechanism regulating GC levels, even subtle changes in circulating steroids may induce dramatic changes on the whole-body level. Changes of steroid concentrations toward reduced forms that occur during the reduction treatment and that are presented in our study might be explained by following mechanism: In obese patients, local cortisol production increases, due to the increased activity of 11 β -HSD 1 in adipose tissue. Although this cortisol production (obtained mostly from the subcutaneous adipose tissue) was estimated at approximately 10 % of the whole body activity (Stimson *et al.* 2009), such an increase is enough to trigger mechanisms leading to compensation. The mechanism involves the reduction of 11 β -HSD 1 in the liver and increase the activity of 5 α -reductase, both observed in obesity (Andrew *et al.* 1998, Rask *et al.* 2001). Moreover, the cortisol production rate by the liver has been estimated to be at least equivalent to that of the adrenal gland in a healthy population (Stimson and Walker 2013). The changes in GC metabolism that arise from hepatic 11 β -HSD 1 reduction predict lower plasma levels, which could explain the compensatory increase in the activity of hypothalamic-pituitary-adrenal axis found in obese patients (Pasquali *et al.* 1996).

Therefore, if calorie restriction and fat loss causes reduction in cortisol production in adipose tissue, it may be compensated by increased reductase activity of 11 β -HSD 1 in the liver to maintain cortisol levels balanced. Considering the prevailing contribution of hepatic 11 β -HSD 1 to extra-adrenal cortisol regeneration, we speculate that the changes in circulating cortisone levels we observed after the reductive treatment may be due to the increased activity of hepatic 11 β -HSD 1. Follow-up studies regarding hepatic activity of 11 β -HSD 1 are necessary to confirm our suspicion.

In summary, we have found reduced circulating levels of cortisone and reduced ratios of oxygenated/reduced metabolites after the reductive treatment in

juvenile obese patients. Moreover, DHEA and its 7-hydroxylated metabolites showed significance to the prediction of reductive treatment efficacy. Our findings support a role of 11 β -HSD 1 as well as derivatives of DHEA in the control of human metabolism. However, further studies are needed to determine whether the observed changes can be attributed to the altered activity of the liver 11 β -HSD 1.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

11 β -HSD 1, 11 β -hydroxysteroid dehydrogenase type 1; BMI, body mass index; DHEA, dehydroepiandrosterone; DHT, 5 α -dihydrotestosterone; E2, 17 β -estradiol; GC, glucocorticoids; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OPLS, orthogonal projections to latent structures; RIA, radioimmunoassay.

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