Reduced levels of circulating 7α-hydroxy-dehydroepiandrosterone in treated adolescent obese patients

Ludmila Máčová, M.Sc., Ph.D., Marie Bičíková, M.A., Hana Zamrazilová, M.A., Ph.D., Martin Hill, M.Eng., D.Sc., Hana Kazihnitková, M.Sc., Barbora Sedláčková, M.Sc. and Luboslav Stárka, Prof., M.D., M.A., D.Sc.

Institute of Endocrinology, Prague, Czech Republic

Running title: Reductive treatment and 7-hydroxy/ oxo derivatives of dehydroepiandrosterone

Correspondence:

Ludmila Máčová

Dept. of Steroids and Proteofactors

Institute of Endocrinology

Národní 8

116 94 Prague 1

Czech Republic

Phone: +420 724 090 856

Fax: +420 224 905 325

Email: lmacova@endo.cz

Abstract

Objective: Elevated levels of glucocorticoids lead to the development of obesity and metabolic syndrome. Local glucocorticoid levels are regulated through the enzyme 11β-hydroxysteroid dehydrogenase 1 (11β-HSD 1), an enzyme that regenerates active cortisol from inert cortisone. Increased expression of 11β-HSD 1 in adipose tissue promotes higher body mass index (BMI), insulin resistance, hypertension, and dyslipidemia. Human 11β-HSD 1 is also responsible for inter-conversion of 7-hydroxylate metabolites of dehydroepiandrosterone (7-OH-DHEA) to their 7-oxo-form. To better understanding the mechanism of the action, we focused on 7-OH- and 7-oxo-DHEA, and their circulating levels during the reductive treatment in adolescent obese patients.

Methods: We determined plasma levels of 7α-OH-DHEA, 7β-OH-DHEA, and 7-oxo-DHEA in 55 adolescent patients aged 13.04-15.67 years, BMI greater than 90th percentile. Samples were collected before and after one month of reductive therapy.

Results: Circulating levels of 7α-OH-DHEA decreased during the reductive therapy from 1.727 (1.614; 1.854, transformed mean with 95 % confidence interval) to 1.530 nmol/L (1.435; 1.637, $p < 0.05$) in girls and from 1.704 (1.583; 1.842) to 1.540 nmol/L (1.435; 1.659, $p < 0.05$) in boys. With regard to the level of 7-oxo-DHEA, a significant reduction from 1.132 (1.044; 1.231) to 0.918 nmol/L (0.844; 1.000, $p < 0.05$) was found after the treatment, but only in boys. No significant difference in 7β-OH-DHEA levels was observed.

Conclusions: Diminished levels of 7α-OH-DHEA indicate its possible effect on activity of 11β-HSD 1. Further studies are necessary to clarify whether competitive substrates for 11β-HSD 1 such as 7α-OH-DHEA could inhibit production of glucocorticoids and may be involved in metabolic processes leading to reduction of obesity.

Key words: 11β-hydroxysteroid dehydrogenase 1 (11β-HSD 1), obesity, body mass index (BMI), 7-hydroxy-dehydroepiandrosterone (7-OH-DHEA), 7-oxo-dehydroepiandrosterone (7-oxo-DHEA)

Introduction

Elevated levels of glucocorticoids are implicated in the development of obesity and fat distribution, as seen in patients with Cushing's syndrome (Rebuffe-Scrive *et al.* 1988). The action of glucocorticoids depends not only on the circulating concentrations regulated through hypothalamic-pituitary-adrenal axis, but also on the peripheral production in target tissue. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is crucial for the local reactivation of cortisol from inactive cortisone (Seckl and Walker 2001). 11β-HSD1 is expressed in various tissues of the human body, such as the liver, brain, placenta and adipose tissue (Ricketts *et al.* 1998), and its activity is tissue specific as seen in mice transgenic models (Lavery *et al.* 2012; Paterson *et al.* 2004) as well as in humans (Rask *et al.* 2001; Rask *et al.* 2002).

Obesity seems to be associated with increased 11β-HSD1 activity in adipose tissue in both rodent (Masuzaki *et al.* 2001) and human models (Kannisto *et al.* 2004; Rask *et al.* 2001). Reciprocally, in 11β-HSD1 knockout mice improvement in glucose tolerance and resistance to the development of the obesity was observed (Kotelevtsev *et al.* 1997). However, the available data are inconsistent (Tomlinson *et al.* 2002). These data suggest that reduction in enzymatic activity of 11β-HSD1 may provide an effective treatment option for the obesity and metabolic syndrome.

One of the possibilities to locally reduce high concentrations of cortisol is specific inhibition of 11β-HSD1. Among the 11β-HSD1 inhibitors, there is a number of natural (Moore *et al.* 1999) and synthetic substances (Liu *et al.* 2011; Rosenstock *et al*. 2010). Interestingly, some natural steroid hormones such as 17β-estradiol (Tagawa *et al.* 2009) and dehydroepiandrosterone (DHEA) (Apostolova *et al.* 2005; Tagawa *et al.* 2011) modulate enzymatic activity of 11β-HSD1 in adipose

tissue and possess anti-obesity effect. Despite the positive effect on the treatment of obesity, there are limitations and side effects on the human organism of these compounds.

A completely novel insight into the metabolic processes of glucocorticoids enabled the discovery of the oxidoreductase activity of 11β-HSD1, which is responsible for the reversible oxidation not only of cortisol to cortisone but also of two endogenous DHEA metabolites, 7α-hydroxy-dehydroepiandrosterone (7α-OH-DHEA) and its 7β-hydroxyisomer (7β-OH-DHEA) to 7-oxo-DHEA (Muller *et al.* 2006b; Robinzon *et al.* 2003). 7-Hydroxydehydroepiandrosterone was in our laboratory found as early as 50 years ago to be an abundant metabolite of DHEA in normal human subjects (Starka and Hampl 1964; Starka *et al.* 1962).

7-Hydroxylated metabolites of DHEA represent competing substrates for 11β-HSD1 and may play a role in reduction of the enzymatic activity. Therefore, we focused on the metabolites of DHEA mentioned above as potential local competitors of the enzyme 11β-HSD1 (Muller *et al.* 2006a). The 7-hydroxylated DHEA metabolites are known for their anti-glucocorticoid (Chmielewski *et al.* 2000) and anti-dietary effects, but the mechanism of the action remains unclear. There are only few studies dealing with the effect of these 7-hydroxylated metabolites of DHEA on human energy (Sedlackova *et al.* 2012). Similarly in 7-oxo-DHEA, there is only one study focused on the effect of transdermal application of 7-oxo-DHEA on cholesterol and lipid metabolism (Sulcova *et al.* 2001). Despite the fact that lack supporting studies, some of these metabolites are the basis of products used for weight reduction (www.dietspotlight.com/lean-xtreme-review/). The real effectiveness of these supplements has not yet been investigated (Bicikova and Starka 2011).

For a better understanding of the action of the enzyme 11β-HSD1, we investigated circulating levels of DHEA 7-hydroxy/oxo-metabolites during the reductive treatment in juvenile patients. Here we try to contribute to the clarification of the role of these metabolites as new hormonal factors that influence obesity.

Subjects and Methods

Subjects

Subjects were recruited from epidemiological and intervention study in Czech adolescents, the COPAT (Childhood Obesity Prevalence and Treatment) project, aimed at monitoring the occurrence and treatment of childhood obesity among children in the Czech Republic. Plasma samples from 55 obese patients (30 girls and 25 boys) who underwent reductive therapy were included.

The reduction program was based on the adjustment of energy intake according to nutritional guidelines for each age group, reductions in dietary fat (< 30 % of energy intake) and simple carbohydrate intakes and regular food consumption (3 main meals and 2 snacks per day). Regular physical activity of moderate intensity lasting at least 4 hours per day and supervised by physiatrist or exercise physiologist was introduced into the weight management program which was also supported by cognitive behavior intervention (Hlavaty *et al.* 2010). The age of investigated participants ranged from 13.04 to 15.67 years, the average age was 13.4 ± 0.28 for girls and 14.9 ± 0.38 for boys. Their hydration status and plasma proteins were normal, the increased body mass was not at account of water retention.

Other inclusion criterion was BMI greater than the 90^{th} percentile relative to age and sex. The Czech references for BMI specified for sex and age were used to evaluate the weight status (Kobzova *et al.* 2004). Exclusion criteria were endocrinopathies including diabetes of the first type and using of drugs that can affect body weight (glucocorticoids, psychotropic drugs, etc.).

The presented study was approved by the Ethics Committee of the Institute of Endocrinology and was performed in accordance with the Helsinki Declaration. The written informed consent with COPAT project was obtained from all individuals and their parents. Only those participants were included in the study, who signed the agreement on the further use of data and biological material for research purposes of the Institute of Endocrinology.

Anthropometric measurements

Height was measured to the nearest 0.5 cm with a stadiometer. Body weight was measured with the subject dressed in underwear to the nearest 0.1 kg using BIA Tanita BC-418 MA scale (Tanita Corporation, Tokyo, Japan). The body mass index (BMI) was calculated as the weight (in kg) divided by the square of the height (in m^2). Each BMI value was standardized by conversion to a z-score (BMI-standard deviation score, BMI-SDS) with respect to age and gender. The BMI-SDS represents the number of standard deviation's an individual subject deviates from the mean BMI of the age and sex matched general Czech population (Kobzova *et al.* 2004).

Steroid analysis

Peripheral blood samples were collected between 7:00-9:00 a.m., after 12 hours of fasting. Plastic tubes with silicone coating were used, and plasma was stored at -80°C until analysis. 7-oxo and 7-hydroxy- metabolites of DHEA were determined by original RIA methods developed in the author's laboratory (Kazihnitkova *et al.* 2007; Lapcik *et al.* 1998; Lapcik *et al.* 1999). Intra- and inter-assay coefficients of variation did not exceed 10.2%, the detection limits for 7α-OH-, 7β-OH-, 7-oxo-DHEA were 1.06, 0.95 and 18 pg/tube, respectively. The cross reactivity of the antibodies with the structurally closest derivatives of DHEA was lower than 1.95%.

Statistical analysis

To evaluate the relationships between dependent and independent variables, we have used the repeated ANOVA model consisting of Subject factor, Gender (between-subject factor), Stage (within-subject) factor and Gender × Stage interaction followed by least significant difference (LSD) multiple comparisons. The original dependent variables were transformed by power transformations to attain a constant variance and symmetric distribution of the data and residuals (Meloun *et al.* 2000). Statistical software Statgraphic Centurion version XVI (Herndon, VA, USA) was used for calculations. The

homogeneity of the data and residual were checked as described elsewhere (Meloun *et al.* 2004; Meloun *et al.* 2002).

Results

In both girls and boys significant decrease of BMI-SDS after the treatment was found (Fig.1), which proved the success of the reductive therapy.

As seen in Fig.2, significant decrease in circulating levels of 7α-OH-DHEA after the reductive therapy was observed. Similarly, concentrations of 7-oxo-DHEA were reduced after the treatment, but this effect was statistically significant only in boys. Reductive therapy did not significantly influenced levels of 7β-OH-DHEA neither in girls nor boys.

Discussion

In the present study, we tested hypothesis that 7-hydroxy and 7-oxo-DHEA (as well as glucocorticoids) are implicated in development of obesity hence their circulating levels would change during the reductive therapy in obese patients. Our hypothesis was based on the fact that metabolites of DHEA hydroxylated in position 7 and glucocorticoids manifest opposite effect on basic physiological processes, such as immune response and human metabolism. While glucocorticoids suppresses autoimmune response, 7-hydroxylated derivatives of DHEA exhibit immune-protective effect (Morfin and Courchay 1994). However, the mechanism of action has not been fully elucidated. A link between the actions of these two groups of steroids may represent the enzyme 11β-HSD1, which on the one hand converts inert cortisone to biologically cortisol, and on the other hand converts the 7-hydroxylate metabolites of DHEA to their 7-oxo-form and vice versa. Therefore, these substrates for 11β-HSD1 may act as local competitive inhibitors.

We demonstrated that concentrations of 7α-OH-DHEA were significantly reduced when compared before and after the reductive therapy in juvenile obese patients. However, concentrations of

7β-OH-DHEA were independent on the treatment. Similar results in recent study (Sedlackova *et al.* 2012) were observed, where basal levels of 7α-OH-DHEA were higher in obese boys when compared with lean boys. In contrast to our findings authors did not observed the same effect in girls. This might be caused by limited number of subjects investigated in the cited study.

Biological consequences may arise from the amount of 7α- and 7β-epimers circulating in the body. The 7α - and 7 β-hydroxy-DHEA are each oxidized into 7-oxo-DHEA with quite dissimilar K(M) (70 and 9.5 microM, respectively) but at equivalent V(max) (Muller *et al.* 2006b). In contrast, the 11 β-HSD1-mediated reduction of 7-oxo-DHEA led to the production of both 7α- and 7β-hydroxy-DHEA with equivalent K(M) (1.1 microM) but with a 7β-hydroxy-DHEA production characterized by a significantly greater V(max). 11β-HSD1 is strictly NADPH-dependent. As NADPH is overwhelmingly present in various tissues, it displaces the 11β-HSD1 towards 7-oxo reduction. For the outcome of the ratio of 7α- and 7β-epimers the 11β-HSD1 K(M)s (Muller *et al.* 2006b) should be considered.

In addition, we found subtle gender related differences in treatment effect on circulating levels of 7-oxo-DHEA. This may be due to gender specific regulation of the 11β-HSD1 (Low *et al.* 1994) or due to different levels of other steroids that affect enzymatic activity of 11β-HSD1, e.g. 17β-estradiol (Tagawa *et al.* 2009) or androgens.

There is no doubt that the 7-hydroxy- and 7-oxo- metabolites of DHEA are in some way involved in metabolic processes and maintain energy balance of the human body. Certain drugs based on these compounds appeared on the market as anti-obesity medication (www.dietspotlight.com/lean-xtreme-review/) . Moreover, 7-oxo-DHEA has been presented as an “ergosteroid” (Lardy *et al.* 1995) with thermoregulatory effect. This effect lies in reduction of efficiency and shift from oxidative metabolism towards increased heat production. Furthermore, other study reported that administration of gel with 7-oxo-DHEA improves hormonal and lipid parameters in humans (Sulcova *et al.* 2001).

Taken together, reported facts support the theory about the local impact of 7-hydroxy/7-oxo-derivatives of DHEA on glucocorticoid metabolism, known as an anti-glucocorticoid paradigm (Morfin 2002; Muller *et al.* 2006a; Muller *et al.* 2006b). Indeed, non-conjugated DHEA (precursor of 7-hydroxy-DHEA) was recently observed as a non-transcriptional inhibitor of 11β-HSD1 in adipocytes which explained anti-obesity effect of DHEA (Tagawa *et al.* 2011). It is likely that a similar mechanism operates in the case of 7-hydroxy-metabolites of DHEA. However, further studies in this field are needed to discovery exact mechanism, by which are 7-hydroxy/7-oxo-DHEA involved in the processes of incidence and development of obesity. Similarly, future study including cortisol and cortisone determination and hormonal analysis in the fat tissue could bring a better and more informative documentation of the role of 11β-HSD1 and the 7-oxygenated derivatives of dehydroepiandrosterone.

In conclusion, we have found diminished levels of circulating 7α-OH-DHEA after the reductive therapy in juvenile obese patients. Based on our findings, we hypothesize that 7α-OH-DHEA may be involved in metabolic processes leading to reduction of obesity.

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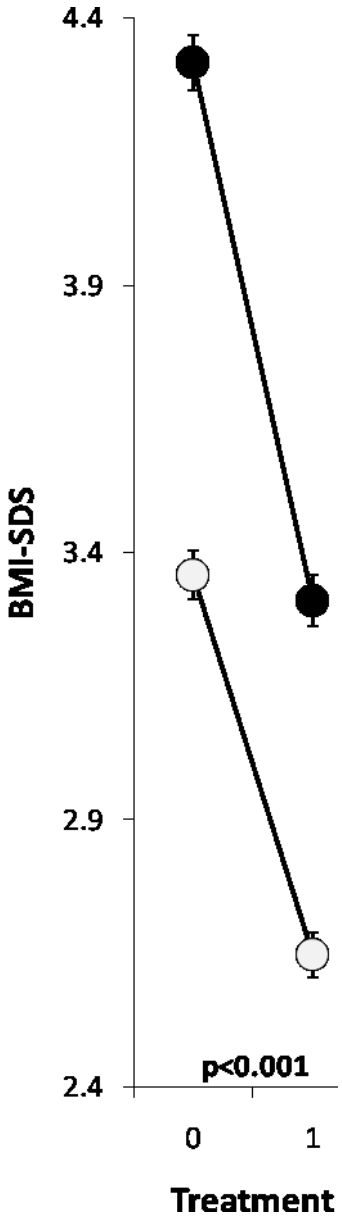

Fig. 1.Effect of reductive treatment on the body mass index- standard deviation score (BMI-SDS). The repeated ANOVA model followed by least significant difference (LSD) multiple comparisons was used. Black spots mean males; white spots females. On the axis Treatment, 0 means before and 1 after the treatment.

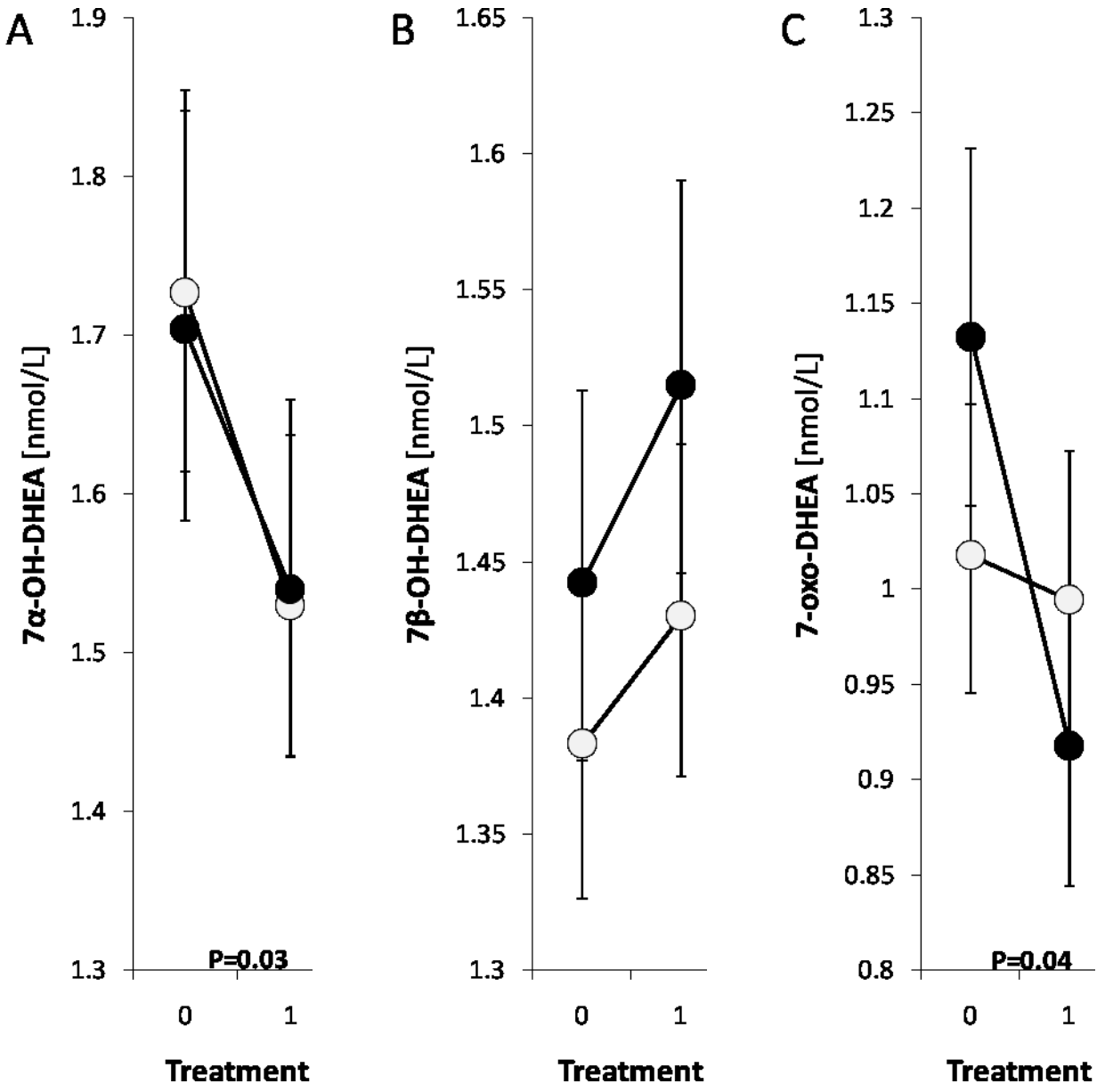

Fig.2. Effect of reductive treatment on circulating levels of 7α-hydroxy-dehydroepiandrosterone (7α-OH-DHEA), 7β-hydroxy-dehydroepiandrosterone (7β-OH-DHEA) and 7-oxo-dehydroepiandrosterone (7-oxo-DHEA). The repeated ANOVA model followed by least significant difference (LSD) multiple comparisons was used for data evaluation. Black spots are for males; white spots for females. On the axis Treatment, 0means before and 1 after the treatment.