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The Effect of Physiologic Hyperinsulinemia during an Oral Glucose Tolerance Test on the Levels of Dehydroepiandrosterone (DHEA) and Its Sulfate (DHEAS) in Healthy Young Adults Born with Low and with Normal Birth Weight

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ß ¼ ¬¿½ð Several data support that adrenal hyperandrogenism affects women with low birth weight (LBW). We also found an association between serum dehydroepiandrosterone (DHEA) and fasting insulin levels. The aim of our study was to detect the acute effects of reactive hyperinsulinemia during oral glucose tolerance test (OGTT) on DHEA(S) levels in LBW men and women. Fifty three men and 47 women (of those, 37 men and 33 women were LBW) were enrolled. DHEA, DHEAS, and insulin levels were measured before and during OGTT. Cortisol was also measured. DHEA/cortisol ratio during OGTT was calculated to analyze the acute effect of hyperinsulinemia on DHEA levels. During OGTT, DHEA and cortisol levels decreased in each individual, independently of gender and birth weight. Serum DHEAS decreased to a minor (but significant) extent only in LBW women ($p<0.05$). The rate of DHEA/cortisol increased in both gender, independently of birth weight. The increase of the rate of DHEA/cortisol during OGTT was associated with maximal insulin response ($r = 0.45$, $p<0.05$) and with the $insulin_{AUC}$ ($r = 0.48$, $p<0.05$) in women. Our results suggest that reactive hyperinsulinemia during OGTT might activate the androgen pathway of adrenal cortex including DHEA production. Therefore acute hyperinsulinemia might counterbalance to some extent the diurnal decrease of DHEA during OGTT.

Key words: Cortisol, Dehydroepiandrosterone, Hyperinsulinemia, Insulin, Low birth weight

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PREVIOUS data support the presence of exaggerated adrenarche in adolescent girls born with low birth weight (LBW) [1–3]. In our recent study we have also demonstrated in young adult LBW women that adrenal hyperandrogenism also exists without overt clinical symptoms [4]. We also found higher fasting insulin levels and higher insulin response during an oral glucose tolerance test (OGTT).

The cause of functional adrenal hyperandrogenism is still unknown. As an explanation it has been supposed that such women have a mild form of 3-beta-hydroxysteroid-dehydrogenase deficiency or non-classic 21-hydroxylase deficiency. However, recent data do not support these hypotheses [5]. Instead, the role of increased insulin levels in the development of hyperandrogenism has emerged.

The causative role of hyperinsulinemia in the development of hyperandrogenism is supported by data obtained in patients with polycystic ovary syndrome (POS). Functional adrenal hyperandrogenism is a risk factor for POS characterized by functional ovarian hyperandrogenism [2]. Although adrenal androgen production increases estron production (estron-hypothesis), it has been also suggested, that in POS two elements of the enzyme complex P450c17alpha are

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dysregulated. In POS, the LH-induced androgen production of theca cells is increased, principally because of high activity of the enzymes 17-hydroxylase and 17,20-lyase [6, 7]. Insulin and insulin-like growth factors probably play a role in high activities, because they reportedly enhance LH-induced androgen production in theca cells. Clinical observations in POS that suggest a strong association between insulin and androgen levels also support the importance of hyperinsulinemia in hyperandrogenism [8].

In this study we assumed that, similarly to POS, acute reactive hyperinsulinemia might contribute to the increased production of adrenal androgens in LBW. To test this hypothesis we analyzed the impact of reactive hyperinsulinemia during OGTT on DHEA and DHEAS levels in all those individuals with or without LBW who had presented an association between fasting insulin levels and DHEA-levels in our previous study.

Subjects and methods

Í « ¾ ½=œBetween 1977 and 1979 418 infants born with low birth weight (900–2500 g) were admitted to the Neonatal Intensive Care Units of the Semmelweis Medical University. Of those 315 (172 females, 143 males) were discharged without apparent neurological, cardiovascular and renal complications. We have selected those (163 women / 117 men) of the expremature adults in whom medical records did not indicate maternal gestational hypertension and diabetes. Then we have sent a letter inviting them to participate in our study. Sixty women and forty-three men agreed. The exclusion criteria were the presence any chronic disease in the medical history, abnormal mental or physical development, previous or present use of oral contraceptive, irregular menstrual cycles, presence of hirsutism, thyroid dysfunction, Cushing syndrome, hyperprolactinaemia, and body mass index >30 kg/m^2. Standardised questionnaire, laboratory measurements and physical examination were used to exclude the presence of these conditions. Thirty-three subjects were excluded: 24 women because of oral contraceptive use and 3 women/6 men because of chronic disease. Female subjects were investigated in the early follicular phase of the menstrual cycle. Finally 33 LBW women and 37 LBW men were enrolled. Table 1 presents the clinical characteristics of the enrolled subjects.

LBW subjects were divided into small for gestational age (SGA) group (15 women, 17 men) and appropriate for gestational age (AGA) group (18 women, 20 men) according to gestational age and birth weight and the 5% percentile at a given gestational age as a cut-off value.

Control subjects were selected also from the university registry randomly. The inclusion criteria were:

Ì ¿¾» ïò Clinical characteristics of the enrolled subjects.
Abbreviations: SGA: small for gestational age; AGA: appropriate for gestational age; LBW: low birth weight

	WOMEN			
	SGA	AGA	All LBW subject	Control
number	15	18	33	14
age (year)	19.9 ± 0.7	20.2 ± 0.6	20.1 ± 0.6	20.5 ± 0.8
body mass index (kg/m2)	22.2 ± 5.2	21.5 ± 4.5	21.9 ± 4.8	21.3 ± 2.5
birth weight	1724 ± 386	1853 ± 474	1795 ± 435	3157 ± 379
gestational age	34.5 ± 2.2	29.6 ± 2.4	32.0 ± 3.4	39.0 ± 1.0
age of menarche	12.8 ± 1.2	12.8 ± 1.3	12.8 ± 1.3	12.5 ± 0.8
	MEN			
	SGA	AGA	All LBW subject	Control
number	17	20	37	16
age (year)	20.0 ± 0.8	20.8 ± 0.9	20.5 ± 0.9	20.2 ± 0.9
body mass index (kg/m^2)	22.8 ± 3.7	21.4 ± 2.8	22.0 ± 2.9	22.0 ± 2.9
birth weight	1632 ± 226	2002 ± 326	1832 ± 337	3384 ± 357
gestational age	34.8 ± 1.0	33.6 ± 2.0	34.0 ± 1.8	39.7 ± 1.2

singleton subjects born full-term (≥38 weeks) with a birth weight above 2500 g. The exclusion criteria were same as applied to LBW subjects. Thirty control subjects (14 women and 16 men) entered into the study.

The proportion of smokers was similar in LBW and control subjects (8/70 vs. 3/30). All of the participants had finished the secondary school. The study protocol was approved by the local university ethical committee and written informed consent was obtained from the participants.

Í ¬¤½§ °®±¯½±æ After an overnight fasting an oral glucose tolerance test (OGTT) was performed with 75 g glucose at 0800 am. Plasma glucose, DHEA, and DHEAS, insulin and cortisol levels were measured before glucose administration and at the 30th, 60th, 90th and 120th minutes of OGTT. As a possible indirect indicator of ACTH-independent DHEA production, DHEA/cortisol ratios before and after glucose administration were calculated to analyze the acute effect of hyperinsulinemia on DHEA production.

Ô¿¾±®¿ ¬±§³ »¿¬«®»³ »² ¬æ Commercially available kits and a Hitachi 704 automatic analyzer were used for determination of glucose concentrations.

Cortisol (normal reference range: 0.20–0.60 μmol/L), DHEA (normal reference range:6.2–40.3 nmol/L for men and 6.2–36.2 for women) and DHEAS (normal reference range: 4.0–12.0 μmol/L for men and 2.7–9.0 for women) levels were determined by direct radioimmunoassays (RIA). For RIA, highly specific antisera prepared in our laboratory were used. Intra- and interassay variations of cortisol, DHEA and DHEAS and were 6.5% and 10.6%, 7.2% and 11.3%, 7.8% and 10.%, respectively.

Serum insulin levels were measured by commercially available Abbott kits on an IMX analyser. The healthy reference is <15 μIU/ml for fasting insulin and is <40 μIU/ml for insulin during the first 60 min of OGTT.

Í ¶¬¬-¬½´ ¿²¿§-·-Hormonal levels before and during OGTT did not differ between SGA and AGA subjects, therefore their results were amalgamated into a common LBW group.

Insulin concentrations (μIU/ml) were log-transformed to achieve normal distribution. The alteration of hormone and blood glucose levels during OGTT are expressed as per cent values of baseline levels. The insulin response during OGTT is characterized by the area under curve (AUC_{ins}). Basal DHEA/cortisol ratios and the ratios of lowest DHEA/lowest cortisol during OGTT were calculated to analyze the acute effect of hyperinsulinemia on the shift between androgen and cortisol pathways in the adrenal cortex.

Changes of analytes during OGTT within groups were calculated by one-sample t-tests, while intergroup differences were analyzed by ANOVA. The association of parameters was tested by linear regression. The level of significance was set at $p<0.05$.

Results

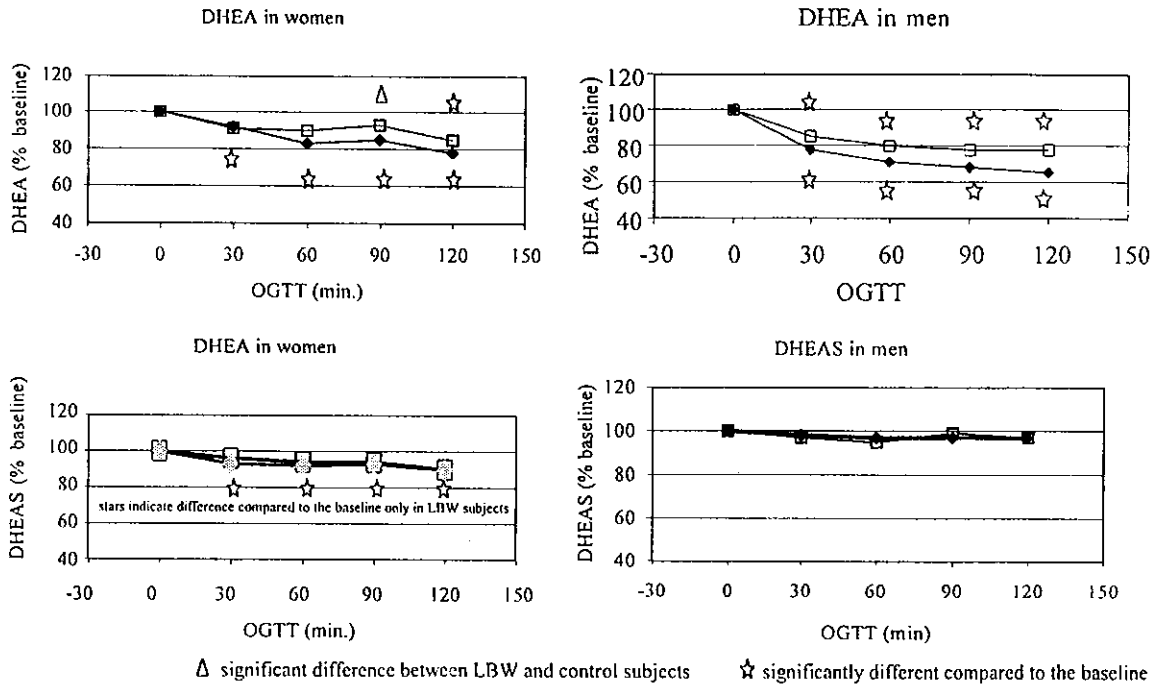
Fig. 1 presents the percent changes of DHEA and DHEAS levels under OGTT in LBW and in control individuals.

In men, DHEA levels decreased already by the 30th min of OGTT, independently of birth weight. Then lower than baseline levels were measured at each time-point. The lowest DHEA levels were measured in the 120th min in LBW men and in the 90th min in control men. Although decrease of DHEA-levels of LBW men exceeded those of control men each time-point, the difference between the two groups was never significant. DHEAS levels were unchanged during OGTT in men.

In LBW women, DHEA-levels also decreased by the 30th min of OGTT, then continuously decreasing, lower than baseline DHEA levels were measured at each time-point. In control women, DHEA levels were significantly lower than baseline values only in the 120th min of OGTT. In LBW women, DHEAS levels were lower than baseline values from the 30th min, but the maximal decrease of DHEAS was only 9%. In control women DHEAS did not change significantly during OGTT.

Table 2 presents the maximal alteration of blood glucose, insulin and cortisol levels and their alteration during OGTT. Basal DHEA/cortisol ratio and the ratio of lowest DHEA/lowest cortisol during OGTT are also presented.

Basal blood glucose, insulin and cortisol values, and the maximal increase of blood glucose and insulin levels during OGTT were independent of birth weight and gender. The extent of decrease of cortisol during OGTT was independent of birth weight. However, it was moderately higher in men than in women. Basal DHEA/cortisol levels did not differ in men and in women, in LBW and in control groups. The ratios of



Ú· ¹ò¹ò Serum DHEA and DHEAS levels during oral glucose tolerance test (OGTT) in subjects born with low birth weight (◆) or with normal birth weight (□).

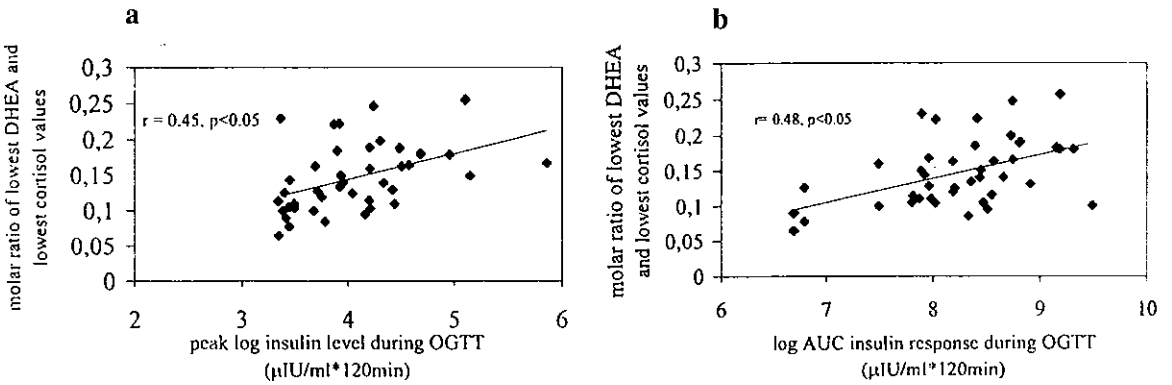
Ì¿¼» îò Blood glucose, insulin, cortisol levels and dehydroepiandrosterone (DHEA)/cortisol ratio before and during an oral glucose tolerance test (OGTT) in healthy young women and men born with low birth weight (LBW) or with normal birth weight (control).

*significantly ($p<0.05$) different compared to the control

⁺significantly different ($p<0.01$) compared to the baseline value

†significantly different ($p<0.01$) compared to the baseline value, evaluated men and women together.

	LBW women	Control women	LBW men	Control men
number	33	14	37	16
baseline blood glucose (mmol/l)	4.5 ± 0.5	4.7 ± 0.8	4.6 ± 0.5	4.7 ± 0.5
highest blood glucose during OGTT/ baseline blood glucose	1.7 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	1.5 ± 0.3
baseline log insulin	2.15 ± 0.55	2.11 ± 0.36	1.84 ± 0.54	1.82 ± 0.30
highest log insulin during OGTT/ baseline log insulin	2.00 ± 0.70	1.91 ± 0.32	1.75 ± 0.52	1.91 ± 0.37
baseline DHEA (nmol/l)	33.6 ± 13.1*	23.6 ± 8.7	29.1 ± 9.7	31.2 ± 8.0
minimal DHEA during OGTT (nmol/l)	23.7 ± 9.9	19.8 ± 6.0	18.6 ± 7.5	22.0 ± 6.6
baseline cortisol (μmol/l)	0.25 ± 0.07*	0.20 ± 0.07	0.26 ± 0.07*	0.22 ± 0.06
minimal cortisol during OGTT (μmol/l)	0.14 ± 0.05	0.13 ± 0.06	0.12 ± 0.04	0.12 ± 0.04
lowest cortisol during OGTT/ baseline log insulin	0.61 ± 0.16	0.64 ± 0.27	0.52 ± 0.11	0.57 ± 0.28
baseline DHEA/cortisol ratio	0.137 ± 0.053	0.124 ± 0.063	0.117 ± 0.038	0.134 ± 0.023
lowest DHEA during OGTT/ lowest cortisol during OGTT	0.157 ± 0.057⁺	0.145 ± 0.035⁺	0.164 ± 0.081⁺	0.192 ± 0.085⁺



Ú· ¹ò î ò The association between molar ratio of lowest dehydroepiandrosterone (DHEA) and cortisol values with peak insulin levels (a) and insulin response (b) during an oral glucose tolerance test (OGTT) among studied female subjects.

lowest DHEA/lowest cortisol levels during OGTT were significantly higher than those obtained before OGTT either in LBW ($p = 0.01$) or in control ($p<0.01$) individuals.

Serum cortisol and DHEA levels were correlated both in men and in women ($r = 0.42$, $p<0.01$, $r = 0.50$, $p<0.01$, respectively). However, basal cortisol and DHEAS levels were not correlated. The change of blood glucose levels was independent of the change of DHEA during OGTT.

In women, the lowest DHEA/lowest cortisol during OGTT correlated with maximal insulin response during OGTT ($r = 0.45$, $p<0.05$) and with AUC_{ins} ($r = 0.48$, $p<0.05$) (Fig. 2). In men, this correlation was not present.

Discussion

Data are controversial about the effect of acute hyperinsulinemia on adrenal androgens. Experimentally induced supraphysiological hyperinsulinemia leads to a rapid decrease of DHEAS in women [9]. Several groups also observed a 30–40% decrease in DHEA and DHEAS at high-physiological insulin levels either in subjects with normal or in subjects with increased androgen levels [10–13]. However, more recent data obtained in reactive hyperinsulinemia during OGTT indicated that the extent of decrease of DHEA(S) levels is lower than it had been suggested in previous observation [14, 15]. Furthermore, another study found unchanged DHEA and DHEAS levels during OGTT [16].

Evaluating these results there are several questions that arise. Since DHEA production is primarily regulated by ACTH, it is reasonable to suggest that the diurnal change of ACTH is responsible for DHEA production as suggested by the decreasing cortisol levels during OGTT. To eliminate the disturbing effect of diurnal ACTH change most of the studies investigated DHEAS instead of DHEA, since ACTH influences DHEAS production to a lesser extent.

The 30–40% acute decrease of DHEAS in early studies is probably independent of the alteration of ACTH because the half-life of DHEAS is 9 hours. Rather, data suggest that hyperinsulinemia enhances the metabolic clearance of DHEAS, and that this mechanism might be responsible for the rapid decrease of DHEAS [17]. In fact, other studies that investigated the alteration of DHEAS under reactive physiological hyperinsulinemia could not demonstrate such a great decrease in DHEAS as those carried out under supraphysiological hyperinsulinemia [15, 16]. The hypothesis, that the decreasing effect on DHEAS of hyperinsulinemia is significant only in individuals with the highest insulin response, is further supported by our results. A slight decrease of DHEAS during OGTT was detected only in LBW women, but this change might be attributable to the physiological diurnal rhythm associated with ACTH production.

Our results indicate that hyperinsulinemia induced by OGTT in each individual coexists with a significant decrease of serum DHEA levels. However, DHEA/cortisol ratio increased during OGTT and this increase was associated with the maximal insulin response and $insulin_{AUC}$ during OGTT. This finding indicates that

while DHEA and cortisol levels decrease during OGTT as a result of the diurnal rhythm of ACTH, there can be an ACTH-independent increase of DHEA production presumably via increased activity of 17,20-lyase induced by the physiological hyperinsulinemia during OGTT.

Several clinical and experimental data support the view that acute physiological hyperinsulinemia might induce DHEA(S) production. Moghetti »¬¿ õreported that, after experimentally induced hyperinsulinemia, ACTH-stimulation caused a relative impairment of 17,20-lyase activity, but DHEA levels increased [18]. Martikainen »¬¿ õ found a positive association between fasting insulin and the levels of androgens in venous blood of adrenals [19]. Others reported an association between fasting insulin and DHEA/testosterone ratio [20]. In our previous study we also found an association between fasting insulin and DHEA levels [4].

The explanation for the contradictory effects of insulin on the levels of adrenal androgens is probably due to the different study design. Studies indicating the decreasing effect of insulin on the levels of adrenal androgens were carried out under supraphysiological or high-normal insulin levels. ›Zietz »¬¿ ´. also observed lower DHEA/cortisol rates in type2 diabetic men compared to healthy people [21]. Suzuki »¬¿ õ found that insulin resistance is the most important independent determinant of low DHEAS levels [22]. To the contrary, studies suggesting the inducing effect of insulin on the production of adrenal androgens were carried out under physiological insulin levels.

The bimodal, dose-dependent correlation between insulin and DHEAS levels is further supported by data indicating that insulin levels above 40 µIU/ml increase the metabolic clearance of DHEAS, while insulin levels under 40 µIU/ml enhances DHEAS production [23]. The dose-dependent bimodal effect of insulin on DHEA-production might also contribute to the age-dependent decrease of DHEA and DHEAS. In aging people hyperinsulinemia develops progressively due to insulin resistance and to the decrease of insulin clearence. Basal insulin levels and insulin response during OGTT are 50–100% higher in aged people than in young individuals [24]. The insulin levels in aged people are in that range that is associated with low DHEAS levels.

In our present study the maximal mean insulin response during OGTT did not exceed 40 µIU/ml. They were in that range that is associated with the induction of DHEA(S) production. Therefore based on our results we cannot decide if supraphysiological insulin levels induce DHEA(S) metabolism in young LBW adults. Since the risk of insulin resistance and type 2 diabetes in later life is especially high in this population, follow-up studies done in middle-aged and aged LBW people are needed to elucidate the bimodal effect of insulin.

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