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Immunoprotective Steroids and SHBG in Non-Treated Hypothyroidism and their Relationship to Autoimmune Thyroid Disorders

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Summary

Immunomodulatory steroids, dehydroepiandrosterone and its 7-hydroxylated metabolites and sex hormone-binding globulin (SHBG) were determined in sera of 88 women aged 18-75 years. The group consisted of 34 healthy women, 37 women with subclinical and 17 women with manifest hypothyroidism. In all subjects the laboratory parameters of thyroid function (thyrotropin, free thyroxine and triiodothyronine) and thyroid autoantibodies to thyroid peroxidase and thyroglobulin were determined. The aim was to find out 1) whether the above steroids and SHBG levels differ in individual groups according to thyroid status, 2) whether correlations exist among investigated steroids and thyroid laboratory parameters, and 3) whether the respective steroid and SHBG levels differ according to the presence of principal thyroid autoantibodies. With the exception of 7β-hydroxy-dehydroepindrosterone levels, which were decreased in patients with manifest hypothyroidism ($p<0.05$), no significant differences in steroid and SHBG levels among groups according to diagnosis were found. On the other hand, significantly decreased levels of all the immunomodulatory steroids studied were found in subjects with positive titres of thyroid autoantibodies. This finding was supported by a tight negative correlation among the above steroids and thyroid autoantibodies. In addition, these steroids correlated negatively with thyrotropin and positively with free thyroid hormones. The results point to a negative relationship between the above mentioned immunoprotective steroids and the extent of the autoimmune process in hypothyroidism.

Key words

Hypothyroidism • Autoimmunity • Dehydroepiandrosterone • 7-hydroxy-dehydroepiandrosterone • SHBG

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Introduction

Dehydroepiandrosterone (DHEA) and its 7-hydroxylated metabolites (7-OH-DHEA) are believed to act as locally active immunoprotective agents, attenuating some immunosuppressive effects of glucocorticoids (Šulcová *et al*. 2001, Morfin 2002, Muller *et al*. 2006). The involvement of sex hormone-binding globulin (SHBG) in the thyroid axis and its positive correlation with thyroid hormone levels due to stimulation of its synthesis in liver by thyroid hormones is well known and it was even suggested as an additional marker for monitoring the treatment of thyroid disorders (Brenta *et al*. 1999). Hypothyroidism, including its subclinical form, is an important issue today in regards to its early diagnostics and monitoring of adequate treatment (Vanderpump and Tunbridge 2002, Wilson and Curry 2005). In the search for additional laboratory markers which would enable us to refine the diagnostics of hypothyroidism, we have focused on the above mentioned immunomodulatory steroids and SHBG in relation to thyroid laboratory parameters reflecting thyroid gland function and the extent of the autoimmune process. The following aims were addressed: 1) to find out whether the above mentioned steroids and SHBG levels differ in individual groups according to thyroid status (manifest and subclinical hypothyroidism and healthy controls), 2) whether correlations exist among the investigated steroids and thyroid laboratory parameters, and 3) whether the respective steroid and SHBG levels differ according to the presence of thyroid autoantibodies.

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Materials and Methods

Subjects

The studied group consisted of 88 women from a broad age range (18-75 years). Out of them, 34 were healthy euthyroid women (18-70 years), 38 patients had subclinical hypothyroidism aged 18-83 years, diagnosed on the base of serum levels of thyrotropin (TSH) and free thyroid hormones (fT4, fT3), without overt endocrine disorders, and 17 women, aged 27-75 years, showed manifest hypothyroidism confirmed by ultrasonography. All subjects had not received prior treatment for thyroid or other endocrine disorders and were coming to the Institute of Endocrinology for the first time when participating in our study.

Blood samples were drawn from the cubital vein between 7-8 h a.m., obtained sera were analyzed for the hormones and other analytes listed below. All analyses were a part of routine laboratory testing for thyroid disorders.

Laboratory tests

Thyroid parameters, serum thyroid stimulating hormone, thyrotropin, (TSH, normal range 0.27-4.30 mIU/l), free thyroxine (fT_4, normal range 12.0-22.0 pmol/l) and free trioiodothyronine (fT_3, normal range 2.80-7.10 pmol/l) were measured by ECLIA from Roche Diagnostics GmBH, Mannheim, Germany, using commercial Elecsys System 2010. The intra-assay- and inter-assay variability were 3.0, 2.0 and 2.0, and 7.2, 4.8 and 3.4 for TSH, fT_4, and fT_3, respectively. Anti thyroid peroxidase (AntiTPO) and anti thyroglobulin autoantibodies (AntiTg), physiological levels below 25 and 125 U/ml, respectively, were assessed by enzyme-linked immunosorbent assay (ELISA) (Aesku. Diagnostics, Wendelsheim, FRG). The intra-assay- and inter-assay coefficients of variation were 5.4 and 5.6, and 8.4, and 8.6, for AntiTPO and AntiTg, respectively. Dehydroepiandrosterone, its sulfate, and SHBG were measured by commercial radioimmunoassay and immunoradiometric kits from Immunotech (Czech Division, Marseille, France). Physiological levels of the latter analytes strongly depended on age and our data agreed with those reported by the manufacturer. 7β-Hydroxydehydroepiandrosterone (7β-OH-DHEA) and its 7α-hydroxyisomer (7α-OH-DHEA) were determined by the in-the-laboratory developed radioimmunoassays (Lapčík *et al*. 1999, Lapčík *et al*. 1998). The intra-assay-, and inter-assay variability, for DHEA, DHEAS, 7α-OH-DHEA, 7β-OH-DHEA, and SHBG were 7.2, 4.2, 7.1, 6.7, 6.1 %, and 11.0, 7.2, 10.6, 10.0, and 7.9 %, respectively.

Statistics

The differences among the groups were evaluated by analysis of covariance (ANCOVA) with age as a covariate, using the statistical software Statgraphics Plus version 7 (Manugistics Inc., Rockville, MA, USA). Spearman and Pearson correlation analyses were applied for evaluation of mutual relationships between laboratory data. P value less than 0.05 was considered to indicate significance.

Results

Table 1 shows the basic statistical data concerning thyroid hormone levels, two major thyroid autoantibodies, DHEA, its sulfate, both 7-hydroxylated DHEA metabolites and SHBG in three groups of women – healthy controls and untreated patients with subclinical- and manifest hypothyroidism. The significant differences from the control group at the 95 and 99 % levels are marked with asterisks. Besides expected differences in thyroid hormones and thyroid autoantibodies, the only significant difference ($P < 0.05$) in steroid levels was decreased 7β-OH-DHEA in women with manifest hypothyroidism. The decreased levels of SHBG in both hypothyroid groups were insignificant.

As demonstrated in Table 2, when all 88 subjects were divided into subgroups according to positivite and negativite autoantibody titres (AntiTPO and AntiTg negative, AntiTPO positive, both AntiTPO and AntiTg positive), three immunomodulatory steroids (DHEA, 7α- and 7β-OH-DHEA) showed significantly decreased levels when compared with controls. In healthy women, only 5 out of 34 (14.7 %) had positive titre of at least one autoantibody, while in patients with subclinical hypothyroidism there were 24 women (64.9 %), and in women with manifest hypothyroidism only out of seventeen one had negative titres of both autoantibodies. No differences among these subgroups were found in SHBG levels.

Table 1. Survey of thyroid hormone levels, thyroid auto-antibodies, selected immunomodulatory steroids and SHBG in healthy women and in patients with subclinical or manifested hypothyroidism.

		Thyroid parameters						Steroid parameters and SHBG				
		Age Years	**TSH mIU/l**	**fT_4 pmol/l**	**fT_3 pmol/l**	**AntiTPO IU/ml**	**AntiTg IU/ml**	**DHEAS µmol/l**	**DHEA nmol/l**	**7alpha nmol/l**	**7beta nmol/l**	**SHBG nmol/l**
Healthy controls	*mean*	45.8	1.909	15.0	4.68	20.3	71.4	3.14	13.4	0.87	1.38	60.3
	SD	13.8	0.701	1.53	0.84	43.6	335.9	1.61	8.46	0.56	0.71	35.9
	median	46	1.895	15.1	4.60	3.91	11.3	2.96	10.1	0.92	1.19	50.0
	min	18	0.580	12.1	3.38	0.0	0.0	0.58	4.40	0.19	0.42	27.6
	max	70	3.770	18.3	7.09	199.0	1998	7.24	38.9	2.36	3.08	146.0
	n	34	34	34	34	34	34	29	27	30	30	21
Subclinical hypothyroidism	*mean*	47.0	6.486**	13.2	4.52	318.7**	279.0	3.22	15.2	1.03	1.58	47.3
	SD	14.3	2.194	1.42	0.93	487.1	673.1	2.22	12.4	0.85	1.05	21.2
	median	51	5.430	12.6	4.57	160.0	31.9	1.95	10.1	0.71	1.20	42.6
	min	18	4.410	11.8	2.09	0.0	0.0	0.84	5.22	0.24	0.55	15.0
	max	69	14.420	17.1	5.95	2422.2	3230.2	10.16	58.4	3.73	4.56	97.0
	n	37	37	37	37	37	37	27	27	32	32	24
Manifested hypothyroidism	*mean*	54.3	45.703**	7.29**	3.41*	439.1**	958.4**	2.77	13.1	0.88	0.97*	48.0
	SD	15.0	26.477	3.44	1.26	216.8	1503	3.50	13.9	1.07	0.87	27.1
	median	55	35.400	7.80	3.65	406.8	250.0	1.15	7.49	0.48	0.56	43.0
	min	27	18.430	1.40	1.19	0.0	0.0	0.21	1.13	0.10	0.16	17.3
	max	75	100.000	11.9	4.96	837.0	5000	13.0	47.4	4.18	3.20	108.6
	n	17	17	17	17	16	16	15	17	17	17	16

Symbols * and ** denote differences significant from control group at 95 and 99% level.

Table 2. Thyroid hormone levels, thyroid auto-antibodies, selected immunomodulatory steroids and SHBG in all 89 women (healthy controls and patients with subclinical or manifested hypothyroidism), divided according to negative or positive levels of thyroid autoantibodies (AntiTPO above or equal to 25 IU/ml, AntiTg above or equal to 125 IU/ml).

		Thyroid parameters						Steroid parameters and SHBG				
		Age Years	**TSH mIU/l**	**fT_4 pmol/l**	**fT_3 pmol/l**	**AntiTPO IU/ml**	**AntiTg IU/ml**	**DHEAS µmol/l**	**DHEA nmol/l**	**7alpha nmol/l**	**7beta nmol/l**	**SHBG nmol/l**
Abs negative	*mean*	42.1	3.992	14.33	4.73	4.83	14.88	3.54	17.09	1.17	1.78	50.2
	SD	13.8	5.388	2.51	1.16	6.00	18.65	1.86	11.82	0.84	1.03	25.2
	median	41.5	2.415	14.40	4.67	2.87	11.26	3.19	13.17	1.05	1.61	49.6
	min	18.0	0.580	5.40	1.49	0.00	0.00	0.58	4.68	0.19	0.53	15.0
	max	70	35.400	20.10	8.16	24.08	100.36	10.16	58.44	3.73	4.56	146.0
	n	42	42	42	42	42	42	35	34	36	36	23
AbTPO positive	*mean*	53.5	19.536**	11.21**	4.12	439**	544**	2.46**	10.54**	0.74*	1.03*	55.2
	SD	13.2	23.507	3.62	1.08	426	943	2.49	9.24	0.72	0.65	31.4
	median	55.5	7.240	11.80	4.23	355	45.0	1.55	7.21	0.59	0.93	45.4
	min	25	1.640	1.40	1.19	29.0	0.0	0.21	1.13	0.10	0.16	17.3
	max	84	100.000	17.40	5.73	2422	3688.	13.04	47.40	4.18	3.20	146.0
	n	44	44	44	44	44	44	35	36	41	41	36
Abs positive	*mean*	55.8	25.098**	10.29**	4.05	617**	1363**	2.24**	10.68*	0.84**	1.04*	50.2
	SD	12.9	24.026	4.38	1.20	541	1097	3.13	11.55	0.96	0.71	24.1
	median	55.0	18.430	11.60	4.33	414	1139	1.49	7.13	0.53	0.93	43.8
	min	27	2.140	1.40	1.19	130	250	0.21	1.13	0.13	0.26	20.1
	max	76	80.640	16.00	5.41	2422	3688	13.04	47.40	4.18	3.20	101.0
	n	17	17	17	17	17	17	14	16	17	17	15

Symbols * and ** denote differences significant from control group at 95 and 99% level.

Table 3. Correlation matrix of thyroid hormone parameters, thyroid autoantibodies and selected immunomodulatory steroid in all 88 women (healthy controls and patients with subclinical or manifested hypothyroidism). Above the diagonal (upper right part): Pearson´s correlations, below the diagonal (lower left part): Spearman´s correlations. Each cell from above represents the correlation coefficient (r), number of correlated pairs (n) and significance (*P* value) and. Significant correlations are in **bold**.

	TSH	**fT_4**	**fT_3**	**AntiTPO**	**AntiTg**	**DHEAS**	**DHEA**	**7alpha**	**7beta**
		-0.838	-0.338	**0.740**	0.269	-0.323	**-0.389**	-0.082	**-0.358**
TSH		**31**	31	**31**	31	31	**31**	31	**31**
		0.000	0.063	**0.000**	0.143	0.077	**0.031**	0.662	**0.048**
	-0.838		**0.454**	**-0.565**	-0.025	0.230	0.321	-0.034	0.228
fT4	**46**		**31**	**31**	31	31	31	31	31
	0.000		**0.010**	**0.001**	0.893	0.213	0.078	0.858	0.218
	-0.364	**0.498**		**-0.414**	-0.044	**0.364**	**0.356**	0.164	**0.421**
fT_3	**46**	**46**		**31**	31	**31**	**31**	31	**31**
	0.015	**0.001**		**0.021**	0.813	**0.044**	**0.050**	0.378	**0.018**
	0.722	**-0.630**	**-0.351**		**0.515**	-0.339	**-0.407**	-0.253	**-0.436**
AntiTPO	**46**	**46**	**46**		**31**	31	**31**	31	**31**
	0.000	**0.000**	**0.019**		**0.003**	0.063	**0.023**	0.171	**0.014**
	0.336	-0.205	-0.112	**0.606**		-0.009	-0.008	-0.094	-0.030
AntiTg	**46**	46	46	**46**		31	31	31	31
	0.024	0.170	0.452	**0.000**		0.964	0.967	0.614	0.875
	-0.396	**0.414**	**0.382**	**-0.369**	-0.065		**0.876**	**0.469**	**0.688**
DHEAS	**46**	**46**	**46**	**46**	46		**31**	**31**	**31**
	0.008	**0.006**	**0.011**	**0.013**	0.662		**0.000**	**0.008**	**0.000**
	-0.418	**0.440**	**0.341**	**-0.435**	-0.123	**0.888**		**0.440**	**0.690**
DHEA	**46**	**46**	**46**	**46**	46	**46**		**31**	**31**
	0.005	**0.003**	**0.022**	**0.004**	0.411	**0.000**		**0.013**	**0.000**
	-0.249	0.278	0.240	**-0.390**	-0.168	**0.651**	**0.670**		**0.841**
7alpha	46	46	46	**46**	46	**46**	**46**		**31**
	0.095	0.062	0.108	**0.009**	0.260	**0.000**	**0.000**		**0.000**
	-0.427	**0.449**	**0.385**	**-0.511**	-0.108	**0.784**	**0.781**	**0.876**	
7beta	**46**	**46**	**46**	**46**	46	**46**	**46**	**46**	
	0.004	**0.003**	**0.010**	**0.001**	0.470	**0.000**	**0.000**	**0.000**	

Finally, the data from all women were mutually correlated. Table 3 shows the correlation matrix for thyroid hormone parameters, thyroid autoantibodies and immunomodulatory steroids in all 88 women. Besides the expected correlations among thyroid parameters on one side, and DHEA, its sulfate and its 7-hydroxylated metabolites on the other, the following significant correlations were found between thyroid parameters and investigated steroids: DHEAS, DHEA and 7β-OH-DHEA negatively correlated with TSH, in the case of the latter two steroids when using both statistical approaches. On the other hand all of these steroids correlated positively with free thyroid hormones, as fT_3 concerns, using both statistical methods. DHEA, 7β-OH-DHEA, DHEAS and also 7α-OH-DHEA levels negatively correlated with autoantibodies to thyroid peroxidase (AntiTPO), the latter two steroids only when using Sperman´s analysis.

Discussion

With respect to the autoimmune origin of most of the thyroid disorders, this study is the first attempt to establish a relationship between thyroid laboratory parameters, including major thyroid autoantibodies and DHEA and its 7-hydroxylated metabolites, believed to act as immunoprotective agents (Šulcová *et al*. 2001, Morfin 2002, Muller *et al.* 2006). Recently we have demonstrated that administration of 7-oxo-DHEA, one of the candidates of steroid replacement therapy to healthy male volunteers influenced temporarily actual levels of thyroid hormones (Hampl *et al.* 2006). In addition, SHBG levels were investigated due to its involvement in

thyroid axis (Brenta *et al*. 1999). The studied groups consisted either of healthy women or women with subclinical- or manifest hypothyroidism, confirmed clinically.

Comparison of the above steroid levels in both patient´s groups and healthy subjects revealed only decreased levels of one of the 7-hydroxylated DHEA metabolites, 7β-OH-DHEA in patients with manifest hypothyroidism. We found decreased levels of 7-hydroxylated metabolites in our preliminary study of patients with Hashimoto thyreoiditis (Hampl *et al*. 1999) and our new data confirms this early finding. Surprisingly, the decreased level of SHBG in hypothyroid groups was insignificant. This finding, however, is consistent with our earlier report addressing the diagnostic value of SHBG determination in patients with various thyroid disorders. The serum levels of this protein were significantly increased in patients with hyperthyroidism, including its subclinical form, but the decrease of this protein's levels in hypothyroid subjects was insignificant (Hampl *et al*. 2003)

On the other hand, all of the immunoprotective steroid levels were significantly lower in subjects with positive titres of major thyroid autoantibodies, the occurrence of which was markedly increased in hypothyroidism. This finding was further supported by the tight negative correlation of DHEA and its 7-hydroxylated metabolites with AbTPO levels. This is also in agreement with a negative correlation of these steroids with TSH, along with their positive correlation with both free thyroid hormones.

The negative association of autoimmune thyroid disorders with immunoprotective DHEA metabolites has not yet been reported. In connection with this, it is of interest to note a recent report which showed a protective effect of DHEA and its 7α-hydroxylated metabolite against another autoimmune disorder, an experimentally induced colitis, despite the fact that the mechanism of the latter effect may be different from that which is probably operating in thyroid autoimmune disorders (Pélissier *et al*. 2006).

In conclusion, our data clearly demonstrates, for the first time, a negative relationship between the levels of the above mentioned immunoprotective steroids and the extent of the autoimmune process, at least in hypothyroidism.

Conflict of Interest

There is no conflict of interest.

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