Vitamin D Supplementation Changed Relationships, Not Levels of Metabolic-Hormonal Parameters in Autoimmune Thyroiditis

K. VONDRA¹, R. BÍLEK¹, P. MATUCHA¹, M. SALÁTOVÁ¹, M. VOSÁTKOVÁ¹,
L. STÁRKA¹, R. HAMPL¹

¹Institute of Endocrinology, Prague, Czech Republic

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Summary

In women with chronic autoimmune thyroiditis and vitamin D deficiency we have found reference levels of relevant metabolic-hormonal parameters except for parathormone and total calcium. Three months supplementation with vitamin D (4300 IU/day, cholecalciferol) did not lead to significant changes of investigated hormonal parameters, while the levels of parathormone and calcium reached normal levels. However, a correlation analysis revealed marked changes in mutual relations. First, an inverse correlation of vitamin D with parathormone, insulin secretion (C peptide, insulin) and its efficiency (HOMA IR) disappeared. Relationships of vitamin D to hepatic insulin resistance (insulin/C peptide), to DHEA (both negative), and to DHEAS/DHEA ratio (positive) were newly found. Second, a positive correlation of CRP with insulin secretion remained, while its relation to insulin efficiency (HOMA IR, insulin/C peptide) was newly observed. Analogical positive correlations appeared also among anti TPO and insulinemia, insulin/C peptide, HOMA IR, and anti Tg to C peptide. A relationship of the CRP with anti TPO became significant (+). Third, out of glucose metabolism parameters only insulin/C peptide and glycemia did not correlate with vitamin D during its deficiency, while after supplementation insulin/C peptide alone correlated positively with both DHEAS and DHEA, and negatively with vitamin D.

Key words

Vitamin D deficiency • Autoimmune thyroiditis • Supplementation of vitamin D

Corresponding author

K. Vondra, Institute of Endocrinology, Národní 8, 116 00 Prague 1, Czech Republic. E-mail: kvondra@endo.cz

Introduction

Cross sectional, as well as observation studies point to association of vitamin D deficiency with autoimmune thyroiditis (AIT) and its forms. Our own experience fully agrees with the literature data (Vondra *et al.* 2015).

On the other hand results of prospective studies on a direct effect of vitamin D supplementation in humans are very limited. It is caused above all by the fact that the reason for association between increased occurrence of vitamin D deficiency, not only in the AIT, but also in other autoimmune diseases, has not yet been explained, though a causal relationship is not accepted by most of authors. In the context of available experimental data we may speculate about the role of insufficient immunomodulatory effect of low 1,25-(OH)₂D₃ concentrations in AIT, for instance due to lowered activity of both macrophages and dendritic cells, resulting in increased autoantigen presentation, or increased formation of pro-inflammatory cytokines and cell destructive Th1 lymphocytes. Considering the importance of vitamin D supplementation for the course of the AIT, the data from several intervention studies should be mentioned pointing to its beneficial effect. In the Polish study administration of vitamin D to non-lactating women with postpartum thyroiditis led to a decrease of anti TPO titers and this effect was more pronounced in women with vitamin D deficiency (Krysiak *et al.* 2016). In another open randomized control study in India three-month supplementation of vitamin D to patients with AIT led to a decrease of anti TPO titre, this effect was significant primarily in patients with TSH levels below

10 mIU/l (Chaudhary *et al.* 2016).

In this work we attempted to gain more detailed information on a) the relationship of vitamin D deficiency to selected metabolic hormonal markers related to inflammation, insulin efficiency and bone metabolism in women with AIT, b) on the effects of vitamin D supplementation on relationships found during the deficiency.

Patients

Thirty women treated for a long time for chronic autoimmune thyroiditis (AIT) in the Institute of Endocrinology (Prague) were investigated for the levels of vitamin D, selected clinical and laboratory metabolic-hormonal parameters related to inflammation, glucoregulation, insulin efficacy and bone metabolism, before and after 3 months supplementation with vitamin D. All the patients were receiving a substitution therapy with thyroid hormones (levothyroxine). A selection criteria were at least one year lasting clinical and laboratory normal thyroid function, current vitamin D level below 50 nmol/l (<20 ng/ml), absence of diabetes mellitus of both types, autoimmune or other serious diseases. Investigation was carried out during winter months between 2016/2017. The study was approved by the Ethical Committee of the Institute of Endocrinology, Prague.

The detailed characteristics of the patient group before starting of vitamin D substitution and after deficiency adjustment are shown in Table 1.

Vitamin D was administered in a form of cholecalciferol (Vigantol gtts) for 3 months in a dose 30 drops twice a week, corresponding in average to 4300 IU/day. The vitamin D substitution did not lead to any complication for the whole period or to any side effects. Initial levels of 25(OH)D varied within the range 10-44 nmol/l, median 31 nmol/l, while corrected values after 3 months were within the range 65-136 nmol/l, median 57 nmol/l.

Methods

Glucose was measured spectrophotometrically, using an enzymatic method with hexokinase (Cobas 6000, Roche, Mannheim, Germany). Total calcium was measured spectrophotometrically, using an NM-BAPTA method (5-nitro-5'-methyl-(1,2-bis(o-aminophenoxy)ethan-N,N,N',N'-tetraacetic acid) (Cobas 6000, Roche,

Mannheim, Germany).

Thyrotropin (TSH), free thyroxine fraction (fT4), free triiodothyronine (fT3), C peptide (Cp), insulin, parathyroid hormone (PTH) and vitamin D (25(OH)D total – 25OHD₂+25OHD₃) were measured by immuno-analytic ECLIA methods (Cobas 6000, Roche, Mannheim, Germany).

C reactive protein (CRP) was measured spectrophotometrically, using an immunoturbidimetric assay (Cobas 6000, Roche, Mannheim, Germany). Sex hormone binding globuline (SHBG) was measured by immunoradiometric assay (SHBG IRMA KIT) (Beckman Coulter Inc., Chaska, Minnesota, USA). Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) were measured by radioimmunoassay (RIA DHEA) (Beckman Coulter, Prague, Czech Republic).

Thyroid auto-antibodies (T-Ab) – antibodies to thyroid peroxidase (anti TPO) and antibodies to thyroglobulin (anti Tg) were measured using an ELISA method (Aesku.Diagnostics, Wendelsheim, Germany) on an Immunomat BASE (Serion Immunologics, Germany).

Statistics

Descriptive statistics was performed by Microsoft Excel. The effect of vitamin D supplementation on the investigated parameters was performed by paired signed rank test.

Correlation of initial and corrected data was performed by Spearman correlation analysis.

Results

Initial values as well as those after vitamin D supplementation of clinical and laboratory parameters, expressed as medians and 25th and 75th quartiles are shown in Table 1. For comparison, there are also reference values for analyzed parameters as used in the Institute of Endocrinology, Prague (see www.endo.cz). It is evident from Table 1 that initial data are within the range of reference values and no significant changes occurred after vitamin D supplementation as proven by paired signed rank test. Changes of parathormone (PTH) and total calcium were the only exception, where the supplementation led to their full correction.

The results of Spearman correlation analysis of selected parameters before and after correction of vitamin D deficiency and their significance are given in Tables 2 and 3.

Table 1. Baseline and control parameters after vitamin D supplementation.

Variable	Median (quartiles)		p-value	Reference interval
	Baseline parameters	Control parameters		
<i>Age (years)</i>	41 (31, 48)			
<i>BMI (kg/m²)</i>	24.8 (21.3, 28.5)	24.7 (21.7, 28.5)	n.s.	18.5-25.0
<i>TSH (mIU/l)</i>	2.80 (1.59, 3.83)	2.81 (1.62, 3.58)	n.s.	0.27-4.20
<i>fT4 (pmol/l)</i>	15.8 (14.2, 17.4)	16.8 (14.6, 17.8)	n.s.	12.0-22.0
<i>fT3 (pmol/l)</i>	4.52 (4.28, 4.82)	4.58 (4.32, 4.92)	n.s.	3.10-6.80
<i>anti TPO (IU/ml)</i>	178 (35.0, 371.0)	230 (32.0, 352.0)	n.s.	<40.0
<i>anti Tg (IU/ml)</i>	29.0 (6.9, 126.0)	31.0 (10.0, 90.0)	n.s.	<120.0
<i>CRP (mg/l)</i>	1.10 (0.45, 2.65)	1.05 (0.60, 2.27)	n.s.	0.0-5.0
<i>C peptide (nmol/l)</i>	0.65 (0.56, 0.80)	0.71 (0.56, 0.93)	n.s.	0.30-1.47
<i>Insulin (mIU/l)</i>	8.80 (6.75, 11.2)	8.9 (6.85, 12.4)	n.s.	2.60-24.90
<i>Glucose (mmol/l)</i>	5.10 (4.80, 5.30)	5.05 (4.80, 5.30)	n.s.	3.90-5.60
<i>HOMA IR</i>	1.9 (1.38, 2.64)	1.93 (1.51, 2.46)	n.s.	x-x
<i>PTH (ng/l)</i>	50.3 (41.9, 61.3)	43.9 (36.8, 52.0)	***	15.0-65.0
<i>Calcium total (mmol/l)</i>	2.40 (2.28, 2.43)	2.43 (2.40, 2.49)	**	2.15-2.55
<i>SHBG (nmol/l)</i>	50.4 (37.1, 93.3)	59.0 (31.0, 102.1)	n.s.	43.0-95.0
<i>DHEA (nmol/l)</i>	22.7 (17.3, 32.3)	19.9 (10.8, 29.6)	n.s.	4.3-61.0
<i>DHEAS (umol/l)</i>	3.54 (1.97, 4.66)	3.52 (2.04, 4.50)	n.s.	1.80-7.20
<i>Vitamin D (nmol/l)</i>	30.9 (22.5, 38.5)	86.4 (57.5, 98.0)	***	75.0-200.0

BMI – body mass index, TSH – thyrotrophic hormone, fT4 – free thyroxine fraction, fT3 – free triiodothyronine fraction, anti TPO – anti-thyroid peroxidase, anti Tg – anti-thyroglobulin, CRP – C-reactive protein, PTH – parathyroid hormone, HOMA IR – insulin resistance index, SHBG – sex hormone-binding globulin, DHEA – dehydroepiandrosterone, DHEAS – dehydroepiandrosterone sulfate. Mann-Whitney test was used for evaluation of between group differences: ** P<0.01, and *** P<0.001, n.s. – not significant.

A correlation analysis revealed marked changes in mutual relations after supplementation as follows:

a) An inverse correlation(s) of vitamin D with PTH, insulin secretion (C peptide, insulin) and its efficiency ((HOMA IR) after supplementation no more were proven. Newly found relationships of vitamin D to hepatic insulin resistance (insulin/C peptide), to DHEA (both negative), and to DHEAS/DHEA ratio (positive).

b) A positive correlation of CRP with insulin secretion remained, while newly recorded was its relation to insulin efficiency ((HOMA IR, insulin/C peptide). Analogical positive correlations appeared also among anti TPO and insulinemia, insulin/C peptide, HOMA IR, and anti Tg to C peptide. A relationship of the CRP with anti TPO became significant (+).

c) As parameters of glucose metabolism concern, only insulin/C peptide and glycemia did not correlate with vitamin D during its deficiency; while after supplementation insulin/C peptide alone correlated positively with both DHEAS and DHEA, and negatively with vitamin D.

d) Inversion relationships of insulin, HOMA IR and insulin/C peptide respectively to sex hormone-binding globulin remained unchanged after supplementation.

Discussion

In our work we attempted to gain more detailed information on the relationship of the vitamin D deficiency before and after its correction with selected metabolic-hormonal laboratory parameters related to inflammation, glucoregulation, adrenal androgens, and bone metabolism in women with most frequent autoimmune endocrinopathy, namely AIT. The results brought a number of new insights on relationships between the degree of vitamin D deficiency and investigated parameters. In the discussion we tried to comment the results in the context of recent, often scarce knowledge.

Table 2. Correlation analysis of selected baseline data (the first line – correlation coefficient, the second line – n, the third line – p-value).

	anti TPO	anti Tg	CRP	Insulin/C p	HOMA IR	Glucose	C peptide	Insulin	Vitamin D	PTH	Calcium	DHEAS/ DHEA	DHEAS	DHEA	SHBG
anti TPO	1	0.300493	0.176174	0.294872	0.278125	0.144826	0.185095	0.278821	0.194882	-0.106185	0.216441	0.106185	0.218694	-0.022989	-0.073344
	28	28	27	27	27	27	27	27	28	28	27	28	28	28	28
anti Tg	-	0.120251	0.379393	0.135404	0.160113	0.471068	0.355334	0.159027	0.320334	0.590725	0.278206	0.590725	0.26354	0.907563	0.710707
	28	29	28	28	28	28	28	28	29	29	28	29	29	29	29
CRP	0.300493	1	0.012337	-0.308703	-0.212946	-0.179474	0.027112	-0.211088	0.317773	-0.036453	-0.07629	0.155665	0.334524	0.119704	0.184236
	28	29	28	28	28	28	28	28	29	29	28	29	29	29	29
Insulin/C p	0.120251	-	0.950317	0.109966	0.276607	0.360802	0.891072	0.280919	0.092987	0.851085	0.69961	0.420046	0.076108	0.536244	0.338706
	28	29	28	28	28	28	28	28	29	29	28	29	29	29	29
HOMA IR	0.176174	0.012337	1	0.249452	0.357917	0.125756	0.394268	0.403538	-0.056632	-0.115949	0.103368	0.419885	0.148616	-0.147034	-0.380659
	27	28	29	28	28	28	28	28	29	29	28	29	28	28	29
Glucose	0.379393	0.950317	-	0.200493	0.061475	0.523709	0.037885	0.033215	0.770447	0.549195	0.600669	0.023351	0.450398	0.446584	0.041634
	28	28	28	28	28	28	28	28	29	29	28	29	28	28	29
C peptide	0.294872	-0.308703	0.249452	1	0.715359	0.279048	0.28663	0.736972	-0.148805	-0.010345	0.060931	-0.095074	0.335021	0.271429	-0.193596
	27	28	28	29	29	29	29	29	29	29	28	29	28	29	29
DHEAS/DHEA	0.135404	0.109966	0.200493	-	0.000013	0.14267	0.131686	0.000005	0.441065	0.957525	0.758081	0.623723	0.081385	0.154362	0.314296
	28	28	28	28	28	28	28	28	29	29	28	29	28	28	28
PTH	0.278125	-0.212946	0.357917	0.715359	1	0.659918	0.819571	0.977575	-0.416903	-0.050006	0.15125	0.09681	0.23378	0.000739	-0.369504
	27	28	28	29	29	29	29	29	30	30	28	29	28	29	29
SHBG	0.160113	0.276607	0.061475	0.000013	-	0.000098	0.000098	0	0.024456	0.79671	0.442318	0.61737	0.231178	0.996964	0.048523
	28	28	28	28	28	28	28	28	29	29	28	29	28	28	28
DHEAS	0.144826	-0.179474	0.125756	0.279048	0.659918	0.485546	0.485546	0.530634	-0.357664	-0.174343	0.066832	-0.068651	-0.175656	-0.217065	-0.122485
	27	28	28	28	29	29	29	29	29	29	28	29	28	29	29
DHEA	0.471068	0.360802	0.523709	0.14267	0.000098	-	0.007584	0.003063	0.056791	0.365718	0.735442	0.72345	0.371276	0.258029	0.526749
	28	28	28	28	28	28	28	28	29	29	28	29	28	29	29
Calcium	0.185095	0.027112	0.394268	0.28663	0.819571	0.485546	1	0.82717	-0.410206	-0.039187	0.075117	0.24276	0.146535	-0.203574	-0.387677
	27	28	28	29	29	29	29	29	30	30	28	29	28	29	29
Insulin	0.35334	0.891072	0.037885	0.131686	0	0.007584	-	0	0.027094	0.840056	0.704022	0.204483	0.456834	0.28951	0.037716
	28	28	28	28	28	28	28	28	29	29	28	29	28	28	28
Vitamin D	0.278821	-0.211088	0.403538	0.736972	0.977575	0.530634	0.82717	1	-0.394824	-0.013552	0.140308	0.110632	0.257667	0.02464	-0.400887
	27	28	28	28	29	29	29	29	29	29	28	29	28	29	29
DHEAS	0.159027	0.280919	0.033215	0.000005	0	0.003063	0	-	0.034036	0.944375	0.476387	0.56778	0.185581	0.899043	0.031146
	28	29	29	29	29	29	29	29	30	30	28	29	28	28	28
PTH	0.194882	0.317773	-0.056632	-0.148805	-0.416903	-0.357664	-0.410206	-0.394824	1	-0.481976	-0.122253	0.150645	0.077482	-0.095238	0.277926
	28	29	29	29	29	29	29	29	30	30	28	29	28	30	30
Calcium	0.320334	0.092987	0.770447	0.441065	0.024456	0.056791	0.027094	0.034036	-	0.006996	0.527538	0.426842	0.689524	0.616642	0.137
	-0.106185	-0.036453	-0.115949	-0.010345	-0.050006	-0.174343	-0.039187	-0.013552	-0.481976	1	-0.145204	-0.305451	-0.018229	0.349944	-0.23782
DHEAS/DHEA	0.216441	-0.07629	0.103368	0.060931	0.15125	0.066832	0.075117	0.140308	-0.122253	-0.145204	1	0.063959	0.215972	0.217065	-0.163231
	27	28	28	28	28	28	28	28	29	29	29	29	28	29	29
DHEAS	0.278206	0.69961	0.600669	0.758081	0.442318	0.735442	0.704022	0.476387	0.527538	0.452324	-	0.74169	0.269678	0.258029	0.397523
	28	28	28	28	28	28	28	28	29	29	28	29	28	28	28
DHEA	0.106185	0.155665	0.419885	-0.095074	0.09681	-0.068651	0.24276	0.110632	0.150645	-0.305451	0.063959	1	0.251755	-0.361513	-0.220912
	28	29	29	29	29	29	29	29	30	30	29	30	29	30	30
SHBG	0.590725	0.420046	0.023351	0.623723	0.61737	0.72345	0.204483	0.56778	0.426842	0.100708	0.74169	-	0.187689	0.049656	0.240742
	28	28	28	28	28	28	28	28	29	29	28	29	29	29	29
DHEAS	0.218694	0.334524	0.148616	0.335021	0.23378	-0.175656	0.146535	0.257667	0.077482	-0.018229	0.215972	0.251755	1	0.708215	-0.18352
	28	29	28	28	28	28	28	28	29	29	28	29	29	29	29
DHEA	0.26354	0.076108	0.450398	0.081385	0.231178	0.371276	0.456834	0.185581	0.689524	0.925224	0.269678	0.187689	-	0.000017	0.34062
	28	29	28	28	28	28	28	28	29	29	28	29	29	29	29
SHBG	-0.022989	0.119704	-0.147034	0.271429	0.000739	-0.217065	-0.203574	0.02464	-0.095238	0.349944	0.217065	-0.361513	0.708215	1	-0.095884
	28	29	28	28	28	28	28	28	29	29	28	29	29	30	30
DHEAS/DHEA	0.907563	0.536244	0.446584	0.154362	0.996964	0.258029	0.28951	0.899043	0.616642	0.057998	0.258029	0.049656	0.000017	-	0.614242
	28	29	28	28	28	28	28	28	29	29	28	29	29	30	30
SHBG	-0.073344	0.184236	-0.380659	-0.193596	-0.369504	-0.122485	-0.387677	-0.400887	0.277926	-0.23782	-0.163231	-0.220912	-0.18352	-0.095884	1
	28	29	29	29	29	29	29	29	30	30	29	30	29	30	30
SHBG	0.710707	0.338706	0.041634	0.314296	0.048523	0.526749	0.037716	0.031146	0.137	0.205694	0.397523	0.240742	0.34062	0.614242	-

Table 3. Correlation analysis of selected control data (the first line – correlation coefficient, the second line – n , the third line – p -value).

	anti TPO	anti Tg	CRP	Insulin/C p	HOMA IR	Glucose	C peptide	Insulin	Vitamin D	PTH	Calcium	DHEAS/DHEA	DHEAS	DHEA	SHBG
anti TPO	1 24 24	0.276522 24 0.190861	0.447736 24 0.028237	0.430435 24 0.035763	0.418261 24 0.041952	0.313359 24 0.135952	0.303809 24 0.148944	0.431927 24 0.035057	0.180513 24 0.39862	-0.370435 24 0.074768	-0.31265 24 0.136887	0.046087 24 0.830672	0.210046 24 0.324563	0.150435 24 0.482902	-0.145217 24 0.498379
anti Tg	0.276522 24 0.190861	1 24 -	-0.0527 24 0.806793	-0.067826 24 0.752835	0.286957 24 0.173974	0.283723 24 0.179092	0.41741 24 0.042414	0.245324 24 0.247909	0.177034 24 0.407925	-0.214783 24 0.313517	-0.183758 24 0.390052	-0.043478 24 0.840133	0.188302 24 0.378231	0.128696 24 0.548959	0.148696 24 0.488034
CRP	0.447736 24 0.028237	-0.0527 24 0.806793	1 30 -	0.462877 30 0.010003	0.410703 30 0.024168	-0.124539 30 0.512015	0.310799 30 0.094589	0.50067 30 0.004834	-0.275647 30 0.140383	-0.117739 30 0.535493	-0.340328 30 0.065736	0.037235 30 0.845121	0.224329 30 0.233358	0.174359 30 0.356783	-0.216946 30 0.249506
Insulin/C p	0.430435 24 0.035763	-0.067826 24 0.752835	-0.067826 24 0.752835	1 30 -	0.024168 30 0.000039	0.512015 30 0.22666	0.094589 30 0.254675	0.004834 30 0.000081	0.140383 30 0.009001	0.535493 30 0.604328	0.065736 30 0.067428	-0.139043 30 0.463691	0.384692 30 0.035812	0.426919 30 0.01863	-0.370857 30 0.043639
HOMA IR	0.418261 24 0.041952	0.286957 24 0.173974	0.410703 30 0.024168	0.677419 30 0.000039	1 30 -	0.289572 30 0.120633	0.839715 30 0	0.973514 30 0	-0.256342 30 0.171513	-0.044944 30 0.813567	-0.160152 30 0.397893	0.036263 30 0.849121	0.209367 30 0.266833	0.189321 30 0.316347	-0.413571 30 0.023101
Glucose	0.313359 24 0.135952	0.283723 24 0.179092	-0.124539 30 0.512015	0.22666 30 0.228408	0.289572 30 0.120633	1 30 -	0.048555 30 0.798882	0.128446 30 0	0.042397 30 0.823965	0.151942 30 0.422827	-0.270382 30 0.148432	-0.087898 30 0.644171	-0.048416 30 0.799444	0.09459 30 0.619051	0.037925 30 0.842286
C peptide	0.303809 24 0.148944	0.41741 24 0.042414	0.310799 30 0.094589	0.254675 30 0.174413	0.839715 30 0	0.048555 30 0.798882	1 30 -	0.866815 30 0	-0.036406 30 0.84853	-0.141156 30 0.456861	0.060626 30 0.750298	0.111086 30 0.558948	0.089948 30 0.636434	0.025601 30 0.893176	-0.375334 30 0.040969
Insulin	0.431927 24 0.035057	0.245324 24 0.247909	0.50067 30 0.004834	0.656577 30 0.000081	0.973514 30 0	0.128446 30 0.498759	0.866815 30 0	1 30 -	-0.230187 30 0.221055	-0.068225 30 0.720177	-0.132479 30 0.485258	0.067216 30 0.724155	0.21803 30 0.24709	0.168484 30 0.373466	-0.469842 30 0.0088
Vitamin D	0.180513 24 0.39862	0.177034 24 0.407925	-0.275647 30 0.140383	-0.468625 30 0.009001	-0.256342 30 0.171513	0.042397 30 0.823965	-0.036406 30 0.84853	-0.230187 30 0.221055	1 30 -	-0.240792 30 0.199916	0.006238 30 0.612114	0.422786 30 0.926547	-0.279737 30 0.488049	-0.432132 30 0.134353	0.197819 30 0.294703
PTH	-0.370435 24 0.074768	-0.214783 24 0.313517	-0.117739 30 0.535493	0.098565 30 0.604328	-0.044944 30 0.813567	0.151942 30 0.422827	-0.141156 30 0.456861	-0.068225 30 0.720177	-0.240792 30 0.199916	1 30 -	-0.096458 30 0.612114	0.017577 30 0.926547	0.423676 30 0.019645	0.374903 30 0.04122	0.113695 30 0.549697
Calcium	-0.31265 24 0.136887	-0.183758 24 0.390052	-0.340328 30 0.065736	-0.338345 30 0.067428	-0.160152 30 0.397893	-0.270382 30 0.148432	0.060626 30 0.750298	-0.132479 30 0.485258	0.006238 30 0.973901	-0.096458 30 0.612114	1 30 -	-0.131641 30 0.488049	-0.223992 30 0.234079	-0.14456 30 0.445966	0.058358 30 0.75936
DHEAS/DHEA	0.046087 24 0.830672	-0.043478 24 0.840133	0.037235 30 0.845121	-0.139043 30 0.463691	0.036263 30 0.849121	-0.087898 30 0.644171	0.111086 30 0.558948	0.067216 30 0.724155	0.422786 30 0.926547	0.017577 30 0.926547	-0.131641 30 0.488049	1 30 -	0.030482 30 0.872962	-0.625362 30 0.00022	0.299221 30 0.108205
DHEAS	0.210046 24 0.324563	0.188302 24 0.378231	0.224329 30 0.233358	0.384692 30 0.035812	0.209367 30 0.266833	-0.048416 30 0.799444	0.089948 30 0.636434	0.21803 30 0.485258	-0.279737 30 0.926547	0.423676 30 0.926547	-0.223992 30 0.488049	0.030482 30 0.872962	1 30 -	0.695962 30 0.00022	-0.022027 30 0.108205
DHEA	0.150435 24 0.482902	0.128696 24 0.548959	0.174359 30 0.356783	0.426919 30 0.01863	0.189321 30 0.316347	0.09459 30 0.619051	0.025601 30 0.893176	0.168484 30 0.373466	-0.432132 30 0.017091	0.374903 30 0.04122	-0.14456 30 0.445966	-0.625362 30 0.00022	0.695962 30 0.00022	1 30 -	-0.267186 30 0.153478
SHBG	-0.145217 24 0.498379	0.148696 24 0.488034	-0.216946 30 0.249506	-0.370857 30 0.043639	-0.413571 30 0.023101	0.037925 30 0.842286	-0.375334 30 0.040969	-0.469842 30 0.0088	0.197819 30 0.294703	0.113695 30 0.549697	0.058358 30 0.75936	0.299221 30 0.108205	-0.022027 30 0.908022	-0.267186 30 0.153478	1 30 -

Baseline levels of thyroid hormones in investigated women laid in the normal range and thus at adequate substitution with thyroid hormones were not influenced by the vitamin D deficiency. The corrected values under the same thyroid substitution remained in the middle of the reference range. The correlation analysis during vitamin D deficiency revealed a positive relationship between the fT4/fT3 ratio and vitamin D level ($r=0.4359$, $p=0.016$). We may speculate about a compensatory adaptation on a deep vitamin D deficiency. This is supported by the disappearance of the correlation between fT4/fT3 and vitamin D after its correction.

During vitamin D deficiency we observed a typical increase of parathormone levels, which in about one fourth of patients exceeded the upper limit of the reference range (65.0 ng/l). Elevated values corresponded to secondary hyperparathyroidism as a result of insufficient saturation with vitamin D. It was verified by a full correction of parathormone levels after vitamin D adjustment and could be further supported by a proven inverse relationship between vitamin D and parathormone, which disappeared after supplementation. Basal values of total calcium laid within the reference range, their changes after correction of vitamin D deficiency were small though significant.

Cross matched studies dealing with association of vitamin D with inflammatory markers brought inconsistent results (Liefwaard *et al.* 2015, Azizieh *et al.* 2016). This is true about studies on both healthy subjects as well as on patients with some inflammatory diseases (Kruit and Zanen 2016). Generally, it seems that an inverse correlation of vitamin D to CRP is more pronounced in severe inflammatory diseases (Cannell *et al.* 2015, Kruit and Zanen 2016). There are many factors provided in the literature responsible for heterogeneity in the response of inflammatory markers to vitamin D supplementation: most frequently it is basal 25(OH)D₃ levels, but also initial value of inflammatory markers, the dose of supplemented vitamin D, its type (cholecalciferol or ergocalciferol) and the duration of vitamin D therapy. In these studies importance of higher basal values of inflammatory markers is emphasized, while in case of low values a further decrease after vitamin D levels correction is not probable.

Our findings on women under a long-term therapy with thyroxine substitution for AIT are in accordance with conclusions of the above cited studies. Low baseline values of CRP as well as T-Ab titres did

not further decrease after correction of vitamin D deficiency, in agreement with experience of already mentioned authors. It was probably the reason that a decrease of an anti TPO titre did not occur, as described by Krysiak *et al.* (2016) or Chaudhary *et al.* (2016). Mutual positive correlations of CRP values with T-Ab titres, recently mentioned in the literature (Taubner *et al.* 2014), could be proven here only in vitamin D-supplemented women, namely a positive correlation between CRP and anti TPO. A question arises whether a long-term administration of thyroid hormones to our investigated women participates at low values of CRP, T-Ab and their relationship to vitamin D levels. These relationships have not yet been systematically studied; only Krysiak *et al.* (2017) in a very recent study describes a decrease of thyroid autoimmunity by administration of vitamin D in levothyroxine-treated women with AIT and normal levels of vitamin D.

Sporadic literary data concerning the relationship of DHEA/S to AIT describe low levels of DHEA/S in T-Ab positive females, with an inverse relationship to anti TPO as well as to anti Tg (Ott *et al.* 2014). In our group the levels of DHEA/S (taking into account patient's age) laid in the middle of the reference range before as well as after correction of vitamin D deficiency.

In the state of vitamin D deficiency we have found only one significant positive correlation between CRP and the ratio DHEAS/DHEA reflecting the activity of steroids sulfotransferase enzyme. The meaning of this relationship may help explain experimental findings of Solerte *et al.* (2005). These authors have proven an association of dysregulation of natural killer (NK) cell cytotoxicity and NK-produced cytokines with thyroid autoimmunity, even during thyroxine therapy. In addition they have shown that a functional defect of NK cells can be reversed *in vitro* by incubation of NK cells with a dose-dependent treatment with DHEAS.

In the light of these experimental results the elevated values of the DHEAS/DHEA ratio at vitamin D deficient state associated with increasing levels of CRP could be considered a protection from higher activity and progression of the inflammation. This idea is supported by the fact that the correlation between DHEAS/DHEA to CRP disappeared after correction of vitamin D deficiency. On the other hand there were correlations of adrenal androgens (DHEA, DHEAS) to vitamin D, the same as described in healthy women. Zhao *et al.* (2017) described in a multiethnic cross sectional study with

2929 women an inversion relationship of low vitamin D levels to DHEA, independent on the lifestyle and adiposity. An analogical correlation was found in our women with AIT after correction of vitamin D deficiency, an inverse relationship between vitamin D and DHEA was also manifested by a positive correlation with DHEAS/DHEA ratio. These relationships may be considered a consequence of DHEA sulfotransferase (SULT2A1) stimulation by vitamin D supplementation. Such an interpretation comes out of works emphasizing an important role of vitamin D for stimulation of endogenous DHEA sulfotransferase expression, especially in liver and intestine (Echchgadda *et al.* 2004).

Cross sectional studies report on association of lower vitamin D levels with insulinoreistance, accompanying various metabolic states such as diabetes mellitus type 2 (DM2), metabolic syndrome, PCOS and other. This association has been found even in healthy subjects, as shown e.g. in a large Canadian study (Badawi *et al.* 2014). As the relationships among AIT, glucoregulation or insulinoreistance and vitamin D levels are concerned, we have not found more detailed information in the available literature. Data exist only on the relationship between AIT and insulinoreistance, without any information about vitamin D levels. In the follow up of glycemia, insulinemia or HOMA IR the authors did not find differences between AIT and controls (Biyikli *et al.* 2014, Mazaheri *et al.* 2014). However, in patients with AIT with high anti TPO titres (more than 1000 IU/ml), higher basal insulinemia was found (Mazaheri *et al.* 2014).

The performed correlation analysis showed that even in women with AIT significant relationships could be proven, especially under deep vitamin D deficiency. Significant inverse relationships were found between the degree of vitamin D deficiency and markers of insulin secretion (insulin, C peptide) as well as with its efficacy (HOMA IR).

For explanation of this inverse relationship an association of vitamin D deficiency with low grade inflammation is also emphasized. A really marked positive correlation between levels of C peptide and insulin was found to an inflammation marker – CRP.

To illustrate the pathological impact of a positive relationship between CRP (according to some authors the best inflammatory marker, Kao 2006) with C peptide and insulin in our vitamin D deficient women with AIT, an inverse relationship of CRP to sex hormone binding globulin (SHBG) levels should be mentioned here. SHBG

is believed an important marker of insulinoreistance, its low levels as well as its decreased gene expression are associated with hyperinsulinemia, and after insulinoreistance compensation its levels increase (Winters *et al.* 2014). It fully agrees with our results showing an inverse negative relationship between SHBG and markers of glucose metabolism (HOMA IR, C peptide and insulin levels) found under vitamin D deficiency.

After correction of vitamin D deficiency the relationships between vitamin D and parameters of glucose metabolism disappeared, instead of that an inverse relationship of vitamin D to hepatic insulin resistance (insulin/C peptide) appeared, pointing to its decrease at more pronounced vitamin D supplementation. This relationship corresponds to conclusion of Leung (2016), according to which adequate vitamin D status may modulate hepatic insulin resistance in DM2.

As may be seen from Table 3, as compared with the situation before correction of vitamin D deficiency, new significant relationships appeared between parameters of glucose metabolism and inflammatory markers. Besides already described “deficient” correlation of CRP to insulin secretion the correlation analysis revealed also the relationship to markers of insulin resistance (HOMA IR as well as to hepatic insulin resistance (insulin/C peptide). Of a particular importance we consider newly appearing analogical positive correlations with the marker of thyroid autoimmunity – anti TPO. In addition let us mention a positive relationship of another marker of thyroid autoimmunity – anti Tg to C peptide.

Supplementation with vitamin D also emphasized the difference of relationships of hepatic insulin resistance (insulin/C peptide) from other parameters of glucose metabolism. In Table 3 we may see that under vitamin D deficient state the only insulin/C peptide ratio together with glycemia did not correlate with vitamin D. After supplementation this differences were even more pronounced: out of all parameters of glucose metabolism, only insulin/C peptide correlated positively with adrenal androgens (DHEAS as well as with DHEA), and was the only one negatively correlated with vitamin D. Liver is a key organ of metabolic homeostasis, regulating glucose uptake-storage-production, and it is the key target for insulin action. Hepatic insulin resistance leads to dysregulated glucose metabolism, resulting in hyperglycemia and disturbed interaction with lipogenesis, proteosynthesis and many

other hepatic functions (Leung 2016, Bechmann *et al.* 2012, Wallace *et al.* 2016). These processes in hepatocytes are an object of complicated regulations, studied on molecular-biological level. Our results stress importance of interaction of adrenal androgens with vitamin D in these regulations, especially in influencing hepatic insulin resistance and are an impulse for further clinical and experimental research.

Conclusions

In conclusion we may summarize: a correlation analysis proved remarkable differences in mutual relationships among vitamin D levels, inflammatory markers, insulin secretion and its efficacy, levels of adrenal androgens and SHBG, under vitamin D deficiency and after its supplementation.

The results opened a number of questions, especially about the relationship between vitamin D and inflammation. Primarily, new mutual relationships

appeared after vitamin D supplementation, namely between CRP and a marker of thyroid autoimmunity – anti TPO, and the same correlations of both markers to total and hepatic insulin resistance and also to insulin secretion, indicating that increasing 25(OH)D levels may also be related to proinflammatory states (Mellenthin *et al.* 2014). A correlation analysis also pointed to a changing role of adrenal androgens in relation to vitamin D, especially in relation to hepatic insulin resistance. The results in the context of existing knowledge may stimulate a discussion concerning vitamin D deficiency in other diseases as well.

Conflict of Interest

There is no conflict of interest.

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