BIOCHEMICAL MARKERS OF BONE REMODELING CORRELATE NEGATIVELY WITH CIRCULATING TSH IN POSTMENOPAUSAL WOMEN

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Objective. To evaluate the interrelations between circulating TSH and bone metabolism in postmenopausal women.

Patients and Methods. In a total of 60 postmenopausal women serum level of several hormones (thyrotropin [TSH], free thyroxine [FT4], dehydroepiandrosterone sulfate [DHEAS], parathyroid hormone [PTH]), bone turnover markers (carboxyterminal propeptide of type I procollagen [PICP] and cross-linked telopeptide of type I collagen [ICTP]) as well as of other compounds such as IGF-I, sex hormone binding globulin (SHBG), 25-OH vitamin D_3 (25-OHD_3) and urinary free deoxypyridinoline (Dpd (2h)) concentrations were estimated. Bone mineral density (BMD) at the spine and BMD at the hip were measured by DXA method.

Results. Sperman´s correlation showed negative association between serum TSH and urinary Dpd ($p<0.021$) and borderline, but not significant negative correlation between TSH and ICTP ($p<0.064$). However, no correlation was found between TSH and serum PICP. In addition, no correlation was found between FT4 and such parameters of bone remodeling. Expected positive association between serum IGF-I and DHEAS ($p<0.000$), between body mass index (BMI) and serum DHEAS ($p<0.015$) and negative correlation between BMI and SHGB ($p<0.002$) were confirmed. Moreover, negative correlation was found between bone mineral density at the hip and serum SHBG levels ($p<0.000$) and positive correlation between BMD at the hip and DHEAS level ($p<0.003$). Additionally, 36.5 % variability in TSH levels and 30.5% variability in FT4 in our cohort shared with the factor TSH and bone remodeling (factor analysis).

Conclusion. This cross-sectional study suggested negative association between serum TSH and markers of bone resorption in postmenopausal women. It also confirmed the well known mutual interrelations between BMD at the hip and a number of hormonal indices. Although our results did not provide any evidence on the effect of serum TSH and/or SHBG and DHEAS on bone metabolism, they showed some predictive value of these parameters to bone health.

Key words: TSH- Bone remodeling – PICP – ICTP - Bone mineral density

So far we do not completely understand the role of systemic hormones in the coordination and balance of skeletal remodeling. Hyperthyroidism seems to have an adverse effect on bone. It is well known that long-term treatment with thyroxine, similarly as Graves disease itself result in an increase of bone turnover and might contribute to bone loss in both pre- and postmenopausal women (CUMMINGS et al. 1995; DEROSA et

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al. 1997;Greenspan and Greenspan 1999; Sijanovic et al. 2000; Kumeda et al. 2000). On the other hand, radioiodine treatment in subclinical hyperthyroidism resulting in normalization of serum thyroid stimulating hormone (TSH) prevented continuing bone loss (Faber et al. 1998). Apart from that it has been observed that even a minimal excess of thyroid hormones leading to borderline low TSH level correlated with increased remodeling markers (Krakauer and Kleereker-per 1992; Engler et al. 1999). Nevertheless, none of the above mentioned studies explained the effect of hyperthyroid state on bone.

The osteoporosis associated with hyperthyroidism is considered a consequence of thyroxine overproduction which explains the negative correlation between serum bone alkaline phosphatase, urine deoxypyridinoline or serum cross-linked telopeptide of type I collagen (ICTP) and free thyroxine (FT4) in hyperthyroid or euthyroid subjects (BenShlomo et al. 2001) and in patients with TSH secreting adenoma (Persany et al. 1997). For more then past 10 years it has been known that osteoblasts possess nuclear receptors for thyroid hormone and thus play an important role in mediating the thyroid hormone stimulation of osteoclastic resorption (Allain et al. 1992; Britto et al. 1994). On the other hand, direct inhibitory effect of TSH on bone formation and resorption mediated via TSH receptors in osteoblast and osteoclast precursors has been shown by Abe et al. (2003). Thus, the inhibition of circulating TSH caused by excessive thyroxine production in hyperthyroidism might be responsible for accelerated remodeling and bone loss in these subjects.

The aim of this study was to analyze the relationship between serum TSH level and markers of bone remodeling and/or bone mineral density (BMD) in postmenopausal women in the relation with a number of metabolically active hormones.

Subjects and Methods

Subjects. The cross-sectional study was conducted in 60 postmenopausal female subjects of Caucasian origin, who were screened for osteoporotic risk evaluation. None of these had a history of early or late menarche or premature menopause (before 45 years of age). Six subjects were less than two years, but not less than 18 months after menopause. Women with unclear menopausal status were not included in the study. The prior menstrual history of normal and low BMD subjects was regular (11-13 cycles/year). The study group did not include any alcoholics, heavy smokers, women with psychosis or serious internal disease or clinically manifested endocrine disorders including primary hyperparathyroidism. In nine women, hypothyroidism was diagnosed biochemically (serum TSH >4.6 mU/l) and six women had subclinical hyperthyroidism (TSH <0.260 mU/l) due to suppressive treatment of goiter. Four women had biochemically documented secondary hyperparathyroidism due to vitamin D deficiency (PTH >65 ng/l). None of the women was markedly underweight or obese and they had normal calcium, caloric and/or protein intake. The daily life of the women was usual in physical activity. None of them had been treated with estrogen or calcitropic drugs including vitamin D. Informed consent was obtained from all patients and all procedures were approved by the Ethical Committee of the Institute of Endocrinology, Prague.

Protocol. Blood for the measurements of TSH, free thyroxine (FT4), IGF-I, dehydroepiandrosterone sulfate (DHEAS), sex hormone binding globulin (SHBG), intact parathyroid hormone (PTH), 25-OH vitamin D_3 (25-OHD_3), carboxyterminal propeptide of type I procollagen (PICP) and cross-linked telopeptide of type I collagen (ICTP) was collected in the morning after overnight fasting. Samples were stored at –80 °C until analyzed. A spot after fasting on the second morning urine/2 hours was collected from all women for the measurement of free deoxypyridinoline (Dpd (2h)) concentrations. The values were expressed in nmol/mmol of urinary creatinine.

Analytical methods. Serum TSH and FT4 were estimated by electrochemiluminescence method ECLIA using the Roche Elecsys 1010/2010 (Switzerland) and Modular analytics E170 immunoassay analyzer (Mannheim, Germany). Serum IGF-I was measured by immunoradiometric analysis using an IGF-I IRMA kit from Immunotech (France), DHEAS and SHBG were evaluated by RIA or immunoradiometric methods, respectively, using kits from Immunotech (Prague, Czech Republic). Serum PTH and 25-OHD_3 were assessed using Nichols Institute kits (USA). Serum ICTP and PICP levels were determined by RIA kits from Orion Diagnostica (Finland) (normal ranges 1.8-5.0 ug/l and 80-145 ug/l, respectively). Dpyd (2h) in urine was determined by Elisa kit (Metra Pyrilinks, USA) (normal values 3.0-7.4 nmol/mmol creatinine) and urinary creatinine was estimated photometrically using an automatic analyzer (Merck VitaLab-Eclipse).

Duplicate measurements were used to form mean values. The inter-assay coefficients of variation were as fol-

Table 1: Summary statistics of the anthropometric characteristics, hormones and markers of bone remodeling in postmenopausal women

	n	Mean	SD	Median	Quartiles		Minimum	Maximum
					Lower	Upper		
Age(years)	60	62.3	8.5	62.0	54.0	70.0	49.0	80.0
BMI (kg/m^2)	60	25.8	3.1	25.5	23.8	27.0	20.1	33.7
IGF-I (mg/l)	60	152	63	137	115	201	35	291
DPD (nmol/mmol creat)	60	6.53	2.16	6.50	5.00	7.75	2.30	15.30
PICP (mg/l)	60	122	39	123	91	146	35	222
DHEAS (nmol/l)	60	2.53	1.53	2.30	1.35	3.25	0.32	6.70
ICTP (mg/l)	60	2.94	0.94	2.82	2.24	3.51	1.13	5.82
PTH (g/l)	60	33.26	15.98	31.50	21.90	39.10	10.80	86.40
SHBG (nmol/l)	60	63.9	28.7	61.2	41.0	79.9	17.5	142.0
BMD at the hip (g/cm^2)	60	0.799	0.124	0.770	0.720	0.850	0.610	1.120
BMD at the spine (g/cm^2)	60	0.848	0.168	0.825	0.720	0.920	0.550	1.320
FT4 (nmol/l)	60	16.891	3.101	16.520	15.171	17.862	9.774	27.000
TSH (mIU/l)	60	2.98	4.64	1.89	0.96	3.67	0.00	33.80
25-OH-D_3 (ng/ml)	47	23.33	35.46	14.00	10.00	21.30	2.90	200.00

lows: TSH (3.2 %), FT4 (3.0 %), IGF-I (12.0 %), DHEAS (6.6 %), SHBG (8.0 %), PTH (10.6 %), 25-OHD_3 (9.9 %), PICP (9.0 %), ICTP (3.5 %), Dpyd (10.0 %).

The BMD (g/cm^2) of the lumbar spine (anteroposterior L1-L4) (BMDA) and BMD at the hip (BMDP) were measured by dual-energy X-ray absorptiometry (DXA, Hologic 'QDR-2000', USA). The precision of the method was 2.0 %.

Statistical evaluation. Besides a number of approaches that have been taken to manage large multivariable data sets, factor analysis (FA) reduces the number of original variables by finding less number of new latent variables that are linear combinations of the original variables. Such method is an efficient tool for revealing the inherent structure of the relations between the variables. In contrast to multiple regression, it is truly multi-dimensional. It evaluates the relationships between generally independent (orthogonal) so called "variates" or factors that are computed to obtain the maximum correlation between them and the corresponding variables. The factors (after appropriate rotation) could be commonly interpreted from the biological viewpoint. They are mostly more informative than the original indicator variables. Moreover, from the blur of great number of more or less correlated variables one would obtain considerably less number of easily interpretable "variates" (factors) enabling to evaluate the contribution of each original variable to the common feature expressed by the factor. Generally, one variable could contribute to more than one factor and the measure of contribution of the variable could be estimated using such approach.

To find out which characteristics of bone quality are associated with thyroid markers the data were processed using a correlation analysis (see Table 2) followed by factor analysis (see Table 3). From a total of 13 variables the 2 factors were extracted using a Generalized Least Squares Method. The factors underwent VARIMAX rotation with Kaiser normalization to enable their clear interpretation. Due to non-Gaussian data distribution, heteroscedasticity, and presence of severe outliers, the original data were treated by power transformations in individual variables before the further processing. The transformation parameters were found with use of the normal probability plot reaching minimum mean square error of the residuals for the optimum values. The univariate outliers detected by the normal probability plot were excluded from the computations of the transformation parameters. Statistical software Statgraphics Plus version 5.1 from Manugistics (Rockville, MA, USA) was used for searching the best transformation. Statistical software SPSS version 11.0 from SPSS, Inc. (Chicago, IL, USA) was used for the calculation of Spearman´s correlations and for the factor analysis. To detect the multivariate outliers, F-distributed Mahalanobis distance was used. Respecting the masking effect of severe outliers the outlier-searching procedure was applied repeatedly to identify all non-homogeneities. Statistical software NCSS 2002 was used for the detection of multivariate outliers. From the total num-

Table 2: Correlation matrix of anthropometric characteristics, indices of bone remodeling, bone density, hormonal parameters, IGF-1 and thyroid hormones (Spearman's correlations) in 60 postmenopausal women. BMDA…BMD at the spine, BMDP…BMD at the hip.

	BMDA	BMDP	DHEAS	BMI	IGF-1	Age	SHBG	FT4	ICTP	PICP	DPD	TSH	PTH
BMDA		**0.636**[c]	*0.163*	**0.335**[b]	*0.100*	*-0.131*	*-0.203*	*-0.033*	*0.199*	*-0.009*	*0.078*	*0.039*	**-0.264**[a]
BMDP	**0.629**[c]		**0.383**[b]	**0.347**[b]	**0.258**[a]	**-0.388**[b]	**-0.452**[c]	*-0.201*	*-0.089*	*-0.054*	*-0.112*	*0.186*	*-0.126*
DHEAS	*-0.139*	*0.211*		**0.313**[a]	**0.465**[c]	**-0.393**[b]	**-0.363**[b]	*0.070*	*0.104*	*0.056*	*-0.119*	*-0.094*	*-0.006*
BMI	*0.212*	*0.024*	*0.227*		*-0.016*	*-0.117*	**-0.484**[c]	*-0.146*	*0.171*	*-0.117*	*-0.021*	*-0.010*	*0.092*
IGF-1	*0.111*	*-0.032*	**0.322**[a]	**-0.343**[a]		**-0.452**[c]	**-0.262**[a]	*-0.179*	*0.083*	*0.016*	**-0.274**[a]	*0.056*	*0.050*
Age	*0.147*	*-0.247*	*-0.206*	*-0.052*	**-0.347**[a]		*0.179*	*0.193*	*0.140*	**0.268**[a]	*0.112*	*0.024*	*0.174*
SHBG	*0.121*	*-0.232*	*-0.111*	**-0.427**[b]	*-0.182*	*-0.111*		*0.251*	*0.068*	*-0.017*	*0.184*	*-0.184*	*-0.088*
FT4	*0.063*	*-0.079*	*0.274*	*-0.097*	*-0.125*	*0.233*	*0.116*		*0.087*	*0.139*	*0.109*	**-0.519**[c]	**-0.282**[a]
ICTP	*0.207*	*-0.231*	*0.090*	**0.283**[a]	*0.246*	*0.160*	*0.158*	*-0.112*		**0.351**[b]	**0.311**[a]	*-0.241*	*-0.079*
PICP	*-0.052*	*0.117*	*0.083*	*-0.250*	*0.021*	*0.227*	*-0.151*	*0.077*	**0.317**[a]		*0.229*	*-0.135*	*0.128*
DPD	*0.129*	*-0.023*	*-0.019*	*-0.102*	**-0.310**[a]	*-0.124*	*0.036*	*-0.082*	*0.236*	*0.130*		**-0.298**[a]	*0.023*
TSH	*0.048*	*0.107*	*-0.011*	*-0.143*	*-0.055*	*0.173*	*-0.061*	**-0.471**[c]	*-0.097*	*-0.052*	*-0.243*		*0.174*
PTH	*-0.237*	*-0.019*	*0.058*	*0.226*	*0.177*	*0.249*	*0.059*	*-0.255*	*-0.180*	*0.155*	*0.142*	*0.055*	

Simple pair correlations and partial correlations (with adjustment to constant levels of all variables in the correlation matrix except the pair under study) are above and below the diagonal, respectively.
a…p<0.05, b…p<0.01, c…p<0.001

Table 3: Factor analysisa of the relations between anthropometric characteristics, hormones and markers of bone remodeling in 60b postmenopausal women

Variable		Factor loadings[c]		Shared variability of individual variables with the factors[d]	
		Bone mass factor (1)	Factor of TSH and bone remodeling (2)	Bone mass factor (1)	Factor of TSH and bone remodeling (2)
1	BMD at the hip	**0.770**	-0.095	**59.3%**	0.9%
2	DHEAS	**0.603**	*0.086*	**36.3%**	0.7%
3	BMD at the spine	**0.583**	0.232	**34.0%**	5.4%
4	BMI	**0.522**	*0.048*	**27.2%**	0.2%
5	IGF-I	**0.470**	*-0.167*	**22.1%**	2.8%
6	AGE	**-0.505**	*0.184*	**25.5%**	3.4%
7	SHBG	**-0.572**	*0.210*	**32.8%**	4.4%
8	FT4	*-0.190*	**0.553**	3.6%	**30.5%**
9	ICTP	*0.097*	**0.533**	0.9%	**28.4%**
10	DPD	*-0.141*	**0.453**	2.0%	**20.5%**
11	PICP	*-0.061*	**0.357**	0.4%	**12.8%**
12	TSH	*0.070*	**-0.604**	0.5%	**36.5%**
13	PTH	*-0.116*	*-0.260*	1.3%	6.7%
Eigenvalue		2.55	1.44		
% of Variance		38.0	21.5		
Cumulative %		38.0	59.5		

[a)]Factor analysis (FA) was applied with use of principal factor method for factor extraction followed by VARIMAX factor rotation. Spearman's correlation matrix was used as a source data for performing FA.
[b)]One multivariate outlier was detected using F-distributed Mahalanobis distance and excluded from the analysis.
[c)]Factor loadings (correlation coefficients of the variables with the factors) in bold highlight the variables sharing more than 10% of the variability with the individual factors.
[d)]Number of factors was determined from the shape of the plot of eigenvalues (scree plot).

ber of patients (n=61), one subject identified as multivariate outlier was excluded from the further processing. To check the distributional symmetry in multivariate data set after power transformations, the multivariate fractile-fractile plot and symmetry plot were completed using the statistical software QC-Expert from Trilobyte (Pardubice, Czech Republic).

Bartlett's sphericity test ($p<0.001$) was used to test whether the use of factor analysis was effective. To determine the optimum number of factors, Kaiser's rule (factors with Eigenvalues>1) in combination with evaluation of the shape of the plot of Eigenvalues was applied. The Kaplan-Meyer-Olkin measure of sampling adequacy was used to test whether the number of subjects is sufficient for building the model of FA. The aforementioned tests were carried out using the statistical software SPSS.

Results

Means of biochemical and anthropometric characteristics are shown in Table 1. The correlation matrix (Table 2) indicated negative relationships between TSH and the marker of bone remodeling - Dpd(2h) ($p< 0.021$). Borderline, but not significant correlation was found between TSH and serum ICTP ($p< 0.064$). However, serum FT4 levels showed no correlation with parameters of bone remodeling. The other group of well correlating variables represented the indices of bone density, BMI, and hormonal parameters as serum DHEAS, IGF-I and SHBG levels. From the aforementioned groups the age dependence was more obvious in the latter one. BMD at the hip (BMDP) negatively correlated with serum SHBG levels ($p< 0.000$) and positively with serum DHEAS levels ($p<0.003$). Even as one could get the first notion from the correlation matrix, its straightforward interpretation could be erratic.

The matrix of factor loadings after VARIMAX rotation showed two independent interpretable factors (Table 3). The first of them explaining 38 % of total variability positively correlated with the bone density, BMI and hormonal indices as IGF-I and DHEAS and negatively with age and SHBG levels. The second one explaining about 21.5 % of the total variability positively correlated with the indices of bone remodeling and FT4 and showed a strong negative relationship with TSH levels. Additionally, 36.5 % variability in TSH levels and 30.5 % variability in FT4 in our cohort shared with the factor TSH and bone remodeling. On the other hand, 36.3 % variability in DHEAS, 22.1 % variability in IGF-I and 32.8 % variability in SHBG are shared with the bone mass factor.

Discussion

The results of present study show a significant negative relationship between circulating TSH and indices of bone remodeling in postmenopausal women. Exactly 36.5 % variability in TSH is shared with the factor TSH and bone remodeling in these subjects. Bone metabolism is directly influenced by a number of hormones including thyroxine (Cummings et al. 1995; Greenspan and Greenspan 1999; Bassett et al. 2008) and PTH (Pantazi et al. 2000). However, only 30.5 % of serum FT4 levels and 6.7 % of PTH values are shared with TSH and bone remodeling factor in our study. Thus, remarkably stronger association between bone remodeling parameters and circulating TSH than that between remodeling markers and FT4 or PTH level outlines the hypothesis on direct association between TSH levels and bone turnover.

Direct osteotropic effect of TSH has been recently supported by studies analyzing the influence of recombinant TSH on the skeleton. Thus, small doses of TSH injected intermittently to rodents displayed a powerful antiresorptive effect and prevented the lost bone after ovariectomy (Sampath et al. 2007; Sun et al. 2008). These experimental data correspond to the results of clinical study, in which the low serum TSH observed in thyroidectomized patients on L-thyroxine therapy was associated with a decrease of serum RANKL levels (Martini et al. 2008). Moreover, short-term stimulation with recombinant TSH induced acute decrease in serum CrossLaps in postmenopausal, but not in premenopausal, thyroidectomized women with low BMD. Thus antiresorptive effect of TSH seems to depend on the topical bone turnover in treated subjects (Mazziotti et al. 2005).

The stronger association of TSH versus FT4 levels with biochemical indices of bone remodeling may reflect the fact that TSH is more sensitive marker of prevailing status of thyroid hormone activity than FT4 levels. However, functional TSH receptors are expressed by several tissues besides the thyroid gland (Davies et al. 2002). The presence of such receptors in the skeleton explains the critical role of TSH on bone remodeling independent of thyroxine (Abe et al. 2003). The arrest of osteoclast differentiation due to the suppression of the pathway downstream of RANKL such as NF-κB and the MAPKs (JNK, ERK and p38) are prob-

ably responsible for TSH induced inhibition of bone remodeling (Wei et al. 2001; Karsenty and Wagner 2002).

Commonly known positive associations among BMD and BMI and/or DHEAS and SHBG levels (van Hemert et al. 1989) were confirmed in the present study. We failed, however, to find any association between BMD at the hip and/or at the spine and serum TSH or FT4. Similar negative results have been also obtained in children (Tumer et al. 1999) and in early postmenopausal women (BenShlomo et al. 2001). This phenomenon may be explained by involvement of further mechanisms with a stronger effect than TSH in postmenopausal women.

Our study has some limitations. First, serum prehormone FT4 (3,5,3´,5´-L-tetraiodothyronine), but not active T3 (3,5,3´-L-triiodothyronine) levels were measured. Nevertheless, a majority of circulating T3 is generated by the iodothyronine deiodase enzymes in the tissues converting T4 to T3 by monodeiodination. Thus, circulating T3 may not correspond to intracellular activity of the hormone in target tissues. Second, as to analysis of the relationship between thyroid hormones and BMD, the sample size is relatively small. Based on our data a trial should include more than 260 probands to detect a significant correlation between TSH and BMD at the spine with the statistical power 0.8.

In conclusion, this study demonstrates the strong negative association between the circulating TSH and the bone remodeling parameters in postmenopausal women, which is in agreement with similar data obtained by Guo et al. (1997). Although our observation does not provide any evidence of cause and effect of TSH levels in bone metabolism, it strengthens the hypothesis by Novack (2003), Abe et al. (2007) and Davies et al. (2002) that TSH itself has some role in the regulation of bone remodeling in postmenopausal women, most probably via functional TSH receptors. Moreover, the links between serum TSH and markers of bone resorption, similarly as between serum SHBG or DHEAS and BMD at the hip, suggest a possible importance of these hormones to bone health. Further investigation on the use of recombinant TSH in therapy of osteoporosis in selected postmenopausal women is needed.

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