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Effects of levothyroxine on bone mineral density, muscle force and bone turnover markers: A cohort study

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Die Promovendin ist Ärztin.

Meinen lieben Eltern

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1. Introduction

To date published literature has provided limited and inconsistent evidence for detrimental effects of levothyroxine (LT_4) administration for different thyroid disorders on bone mineral density (BMD) and bone metabolism. The impact of thyroid stimulating hormone (TSH) suppressive LT_4 treatment is of particular interest, especially in comparison with physiological dosage of LT_4 replacement therapy and given noticeably increasing incidence of thyroid carcinoma in the course of the last 15 years (Robert Koch-Institut, 2010). LT_4 suppressive medication is suggested to inhibit tumour recurrence and therefore prescribed as lifelong post-operative treatment for thyroid cancer patients who have nearly normal life expectancy today, whereas duration of LT_4 replacement therapy may vary by indication.

Mainly observational studies of descriptive (Florkowski CM, 1993; Gonzales DC, 1991; Görres G, 1996; Greenspan SL, 1991; Heijckmann AC, 2005; Jódar E, 2001; Mirzaei S, 1999) or mostly cross-sectional, analytical character (Baldini M, 2002; De Rosa G, 1995; Diamond T, 1991; Eftekhari M, 2008; Franklyn JA, 1992; Frusciante V, 1998; Giannini S, 1994; Marcocci C, 1994; Marcocci C, 1997; Nuzzo V, 1998; Paul TL, 1988; Reverter JL, 2005; Reverter JL, 2010; Ribot C, 1990; Sajjinanont T, 2005; Schneider DL, 1995; Schneider P, 1991; Stěpán JJ, 1992; Toivonen J, 1998) on the impact of LT_4 medication on bone currently exist. Results have been controversial, also with regard to the patient population and skeletal site most at risk, and with regard to dose-dependency of effects.

Cross-sectional studies evaluating prevalence do not imply a causal relationship between LT_4 administration and bone, and cannot account for all biases (Schneider R, 2003). Beside inappropriate study design, study quality has often been poor due to small sample size or heterogeneous patient cohorts with respect to (prior) metabolic thyroid state, underlying thyroid disease, or type of LT_4 treatment. Definitions of LT_4 suppressive or replacement therapy have been unclear restricting analysis of dose-response relationship. Including post-menopausal women has provoked the difficulty to separate detrimental effects

of oestrogen deficiency on skeletal integrity. Furthermore, previously used methods in measuring BMD have missed important information about bone structure (Cummings SR, 2002).

Longitudinal studies examining only pre-menopausal women or men, and including a healthy control population are still rare (Rosen HN, 1998; Müller CG, 1995; Pioli G, 1992; Jódar E, 1998; De Rosa G, 1997; McDermott MT, 1995) and therefore warranted.

Several narrative (Allain TJ, 1993; Kann P, 1997; Lauwers A, 1997; Mandel SJ, 1993; Ross DS, 1994; Williams JB, 1997) and systematic reviews (Greenspan SL, 1999; Heemstra KA, 2006; Murphy E, 2004; Quan ML, 2002; Schneider R, 2003) have tried to improve evidence base. The only two meta-analyses investigating the influence of exogenous thyroid hormone on bone were restricted to cross-sectional studies (Uzzan B, 1996) and to TSH suppressive LT_4 doses (Faber J, 1994).

In endogenous hyperthyroidism, bone remodelling activity is stimulated and increased resulting in irreversible net bone loss (Ziambaras K, 1998). Iatrogenic administration of exogenous thyroid hormone is assumed to be responsible for similar alterations (Lauwers A, 1997). By uncoupling the closely related osteoclast-osteoblast activities, bone resorption finally exceeds bone formation (Baran DT, 1994; Bassett JH, 2003; Lauwers A, 1997; Vestergaard P, 2003), which histomorphometrically manifests in both cortical and trabecular bone, but most marked in cortical bone (Allain TJ, 1993; Greenspan SL, 1999).

At least in adults, an intact remodelling course is essential for maintaining mechanical integrity and strength of bone (Bassett JH, 2003). In a dynamic process of bone turnover (Williams GR, 2009), micro-damages in bone material are repaired and bone architecture is remodelled to maximise its flexibility and resistance to load (Murphy E, 2004; Ziambaras K, 1998). No hormonal, paracrine or nutritional factors, but feedback mechanisms based on physical stresses and strains by appropriate muscle groups primarily condition and control constant adaption of bones (Hasegawa Y, 2000). Mechanical forces

acting on bone define its geometry and function. Thus, muscle strength is regarded as first-order determinant of biomechanical bone quality (Ferretti JL, 2000; Hasegawa Y, 2000; Hasegawa Y, 2001; Seeman E, 2003).

In terms of interactive dependence of the musculoskeletal system, alternative mechanisms may lead to harmful effects of LT_4 on bone. Former researchers proposed that bone loss was attributed to direct effects of thyroid hormone or TSH on bone cells or synthesized matrix indicating a metabolic bone disease (Allain TJ, 1993; Bassett JH, 2003; Heijckmann AC, 2005; Lauwers A, 1997; Moseklide L, 1990; Murphy E, 2004), i.e. afore mentioned remodelling disorder. However, following a close muscle-bone relationship bone loss may also be indirectly mediated by decreasing muscle strength. Although there is only sparse information about muscular dysfunction caused by LT_4 medication, detailed evidence of muscle weakness in endogenous hyperthyroidism already exists (Brennan MD, 2006; Dubois S, 2008; Gonçalves A, 2006; Klein I, 2000; Olson BR, 1991; Ramsay ID, 1966; Zürcher RM, 1989).

Peripheral quantitative computed tomography (pQCT) features some architectural and strength parameters useful to investigate a suggested model of some interactions such as the functional unit of bone and muscle. Bone material properties are better described by accurate non-invasive pQCT measurements of muscle features and cross-sectional bone strength than by BMD alone (Ferretti JL, 2000; Hasegawa Y, 2000). Also, in contrast to dual X-ray absorptiometry (DXA) the pQCT device refers to the three-dimensional world of bone (Cummings SR, 2002; Seeman E, 2003) implicating bone architecture and is therefore able to examine the distinct effects of LT_4 in trabecular and cortical bone separately (Ross DS, 1994; Greenspan SL, 1999; Horikoshi T, 1999).

As the interrelationship of bone architecture, bone strength, and muscle strength in the context of LT_4 therapy has been neglected in presently available publications, the objective of this longitudinal observational controlled study was to evaluate whether or not LT_4 suppressive or replacement treatment induced any or differing effects on BMD at peripheral or central skeletal sites, on bone

strength or maximum grip strength. Biochemical markers of thyroid and liver function as well as calcium and bone metabolism were also assessed to investigate their impact. Pre-menopausal women and men were enrolled as patients and respective controls.

Primary study endpoints were defined as annual absolute changes from baseline in BMD, bone strength and maximum grip strength, and secondary study endpoints as annual absolute changes from baseline in biochemical markers of calcium and bone metabolism.

The study was conceived and designed by Prof. Dr. Ch. Reiners, Prof. Dr. P. Schneider and Dr. R. Schneider. The study was conducted and data were acquired by Dr. R. Schneider at the outpatient centre of the Department of Nuclear Medicine of the University Hospital of Würzburg, Germany. Data evaluation, editing and statistical analysis as well as data presentation and interpretation in the context of the latest literature were performed by Mara Schneider.

2. Material and methods

2.1. Study participants

This prospective controlled study included a total number of 97 men and pre-menopausal women treated for either well-differentiated thyroid carcinoma or non-toxic goitre. Quantification of LT_4 exposure by degree of TSH suppression and stratification of patients by gender resulted in three patient subgroups (Figure 1).

Group 1 consisted of 28 men and group 2 of 46 pre-menopausal women on LT_4 suppressive therapy after near total thyroidectomy and ^{131}I remnant ablation for well-differentiated thyroid carcinoma. All cancer patients were free from metastases. Group 3 included 23 pre-menopausal women on LT_4 replacement therapy after strumectomy for non-toxic goitre. None of the goitre patients had a history of prior thyrotoxicosis.

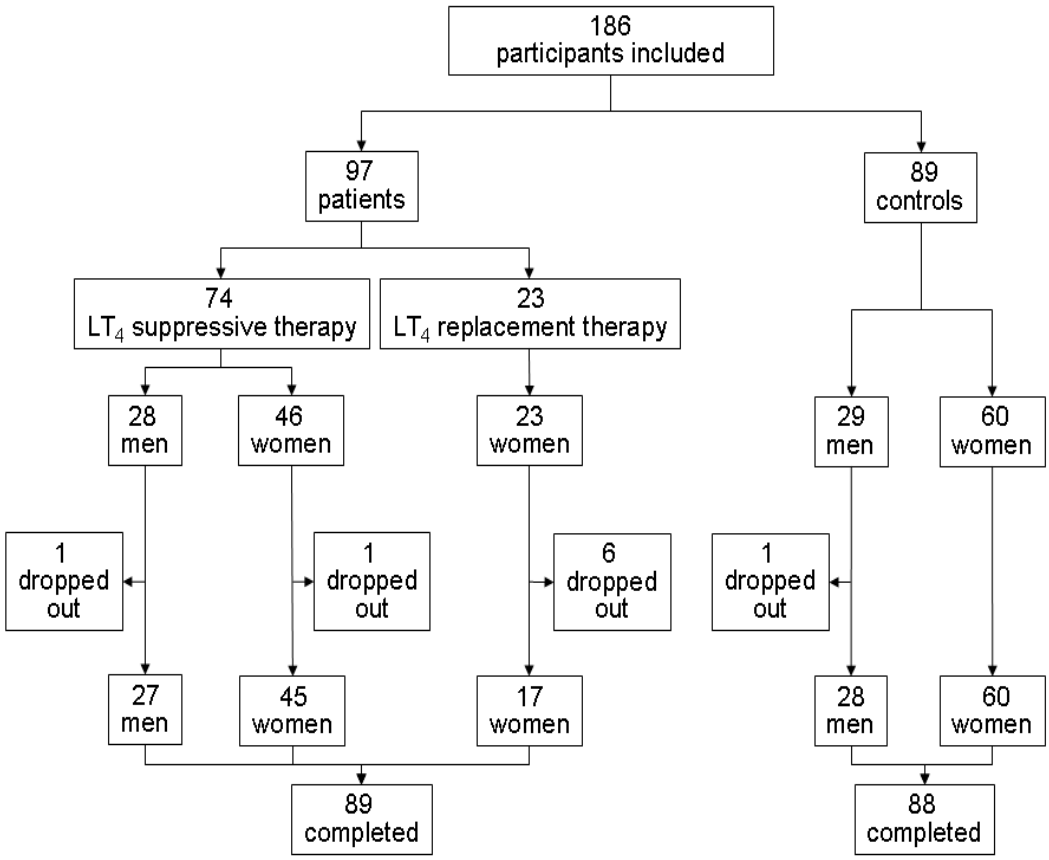

Figure 1. Study enrolment diagram

Throughout post-surgical follow-up the adequacy of the LT_4 administration was monitored by a third-generation TSH assay.

Patients were matched for gender, age and body mass index (BMI) to 89 healthy volunteers with a negative history of thyroid disease as control subjects. Control group 1 consisted of 29 healthy men and control group 2 of 60 healthy pre-menopausal women.

Exclusion criteria for patients and controls were any diseases known to affect BMD, the musculoskeletal or nervous system and any medications known to interact with the musculoskeletal system. Smoking history and daily calcium intake were also assessed.

2.2. Study design

Patients were recruited consecutively within six months from the Department of Nuclear Medicine of the University Hospital of Würzburg, Germany. During the same time period, healthy volunteers from the local area of the City of Würzburg enrolled in the study protocol and were examined. Patients and controls were followed and studied for a mean time of 1.1±0.2 years. Of 186 participants entering the study 177 (i.e. 95%) completed it (Figure 1). Reasons for withdrawal included failure to follow-up, initiation of oestrogen administration, introduction of LT_4 therapy in healthy controls, moving away and death.

The ethics committee of the Faculty of Medicine of the University of Würzburg had no concerns and the study was also approved by the German Bundesamt für Strahlenschutz. Every participant gave written informed consent.

2.3. Methods

Measurements of BMD and grip strength as well as laboratory serum tests were performed within one day at baseline and follow-up appointment respectively.

2.3.1. Bone mineral density

Central areal BMD at the lumbar spine (L2-L4), at the left and right femoral neck as well as at the left and right total hip was assessed by DXA (GE Lunar Prodigy, Lunar Inc., Madison, WI, USA). Peripheral volumetric total and trabecular BMD at the non-dominant ultra-distal radius were measured by pQCT (XCT2000, Stratec GmbH Pforzheim, Germany). The methods are described in detail elsewhere (Cummings SR, 2002; Schneider P, 1999). Rigorous device quality control was ensured during the entire study period. The long-term precision of both devices was <0.1% standard deviation using calibration phantoms.

2.3.2. Bone strength

Polar stress strain index (SSI_p) at the ultra-distal radius was calculated by the software of the pQCT device. This index mathematically expresses the section modulus of bone material that reflects bending stresses acting on beams of cortical bone. Thus, it represents bone strength in terms of biomechanical bone quality (Hasegawa Y, 2001).

2.3.3. Grip strength

Maximum grip strength was determined at the non-dominant forearm by a hand-held dynamometer (Grip-D, Takei Scientific Instruments CO., LTD, Tokyo, Japan). Study subjects took part in this assessment without prior training. After adjustment of the device handle to the participant's grip with him or her sitting

and the forearm flexed, the measurement was repeated four times within three minutes. The average value of the two peak forces was considered as maximum grip strength. The measurement outcome was displayed as kilogram-force (kgf). This parameter indicates mechanical loading or stress exerted by defined muscle groups on the corresponding bone area and is assumed to best display prior and current status of musculoskeletal adaption of the forearm (Hasegawa Y, 2001).

2.3.4. Serum biochemical markers

Serum specimens were kept frozen at -20°C until analysis, except for those tested by routine laboratory methods.

2.3.4.1. Thyroid function

Serum free thyroxine (FT_4) (Immulite 2000 FT_4, DPC Biermann, BadNauheim, Germany), serum free triiodothyronine (FT_3) (Amerlex-MAB FT_3, Trinity Biotech plc, Wicklow, Ireland) and serum TSH (Immulite 2000 TSH, DPC Biermann, Bad Nauheim, Germany) were measured as biochemical markers of thyroid function. The detection limits were >1.8-44 pmol/l for FT_3, >2.6-77 pmol/l for FT_4, and, >0.01-75 mU/l for TSH. The intra- and inter-assay coefficients of variation were 4.4-7.5% and 4.8-9.0% for FT_4, 3.5-5.8% and 6.4-9.8% for FT_3, and 3.8-12.5% and 4.5-12.5% for TSH, respectively. Reference range was 11-23 pmol/l for FT_4, 3.4-7.6 pmol/l for FT_3, and 0.3-4.0 mU/l for TSH.

Follicular cells of the thyroid gland synthesize both FT_4 and FT_3. While predominantly FT_4 is released, only a small amount of the actually biologically active FT_3 is set free. Most of the circulating FT_3 results from mono-deiodination of FT_4 by extra-thyroidal tissue. In a precise negative feedback mechanism depending on serum FT_3 and FT_4 concentrations, production and secretion of TSH is regulated by the pituitary (Mandel SJ, 1993).

2.3.4.2. Liver function

Sex hormone binding globuline (SHBG) was measured by an immunoradiometric assay (SHBG IRMA, Orion Diagnostica, Espoo, Finland). Normal range was 15.5-114 nmol/l in women and 10.4-59.5 nmol/l in men.

SHBG is a serum marker reflecting thyroid hormone action on peripheral tissue level (Földes J, 1990). Being triggered int. al. by thyroid hormones (Pugeat M, 1996), liver cells produce SHBG and set it free into circulation (Faber J, 1990).

2.3.4.3. Calcium and bone metabolism

Serum total calcium (Ca) and serum phosphate (P) were evaluated by routine laboratory methods. The reference ranges were 2.0-2.7 mmol/l and 0.87-1.45 mmol/l, respectively.

Calcitonin (CT) was measured by a chemiluminescence immunoassay (CHEMI CALCITONIN kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Normal values ranged from 0-10 pg/ml in women and from 0-27 pg/ml in men. CT is synthesized by parafollicular C-cells of the thyroid gland and acts as bone anabolic hormone. By direct inhibition of bone resorption, CT lowers serum calcium concentration (McDermott MT, 1983; Heaney RP, 2003; Schneider P, 1991; Seibel MJ, 1997).

As its counter regulator of serum calcium homoeostasis, parathyroid hormone (PTH) was measured by a chemiluminescence immunoassay (CHEMI INTACT PTH kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) with reference values between 10-60 ng/l. Produced by the parathyroid glands, its secretion is mainly affected by decreasing serum concentrations of ionized calcium. PTH has a catabolic effect on bone by indirectly stimulating and increasing osteoclastic bone degradation. By the same way also calcium is released resulting in elevated blood levels (Heaney RP, 2003; Heijckmann AC, 2005; Seibel MJ, 1997; Talmage RV, 2000).

Serum alkaline phosphatase (AP), serum osteocalcin (OC) and C-terminal propeptide of type I procollagen (PICP) were measured as bone formation markers.

Serum AP was assessed by routine laboratory methods with a reference range of 55-170 U/l. Constituting a system of different isoenzymes, bone isoenzyme is the major contributor to serum concentration of AP. Activity of this isoenzyme reflects bone formation, as it is expressed as constitutive protein by osteoblasts (Ziambaras K, 1998).

Serum OC was determined by a radioisotopic assay (OSTEOCALCIN IRMA kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Normal range was 7-30 ng/ml. OC is the most abundant noncollagenous protein of bone matrix, which is synthesized predominantly by mature osteoblasts and incorporated into the extracellular matrix of bone. Approximately 10-25% of newly produced OC is released into circulation (Faber J, 1990; Ross DS, 1991; Ziambaras K, 1998).

PICP was measured by a radioimmunoassay (UniQ PICP RIA, Orion Diagnostica, Espoo, Finland) with a reference range of 70-230 µg/l. PICP is a cleavage product in the formation process of type I collagen fibrils, while type I collagen amounts to more than 90% of the organic matrix of bone. Osteoblasts synthesize the precursor molecule of type I collagen, type I procollagen. Before the procollagen molecules are assembled into fibrils, int. al. their C-terminal ends (also referred to as propeptides) are proteolytically removed and released into circulation (Miyakawa M, 1996; Seibel MJ, 1997; Ziambaras K, 1998).

As the only biochemical marker of bone resorption, type I collagen telopeptide (ICTP) was measured by a radioimmunoassay (UniQ ICTP RIA, Orion Diagnostica, Espoo, Finland). Normal range was 1.6-5.0 µg/l in women and 1.3-5.2 µg/l in men. During the process of bone degradation, the C-terminal telopeptide region of type I collagen is proteolytically cleaved and liberated into circulation as a breakdown product (Miller PD, 1999; Miyakawa M, 1996; Persani L, 1997; Seibel MJ, 1997).

2.4. Statistics

Statistical analyses were performed with STATISTICA® 10 (StatSoft Inc., Tulsa, OK, USA). Normal distribution of data was confirmed by Shapiro-Wilks' W test. As descriptive statistics means ± standard deviations (SD) were used.

For cross-sectional analysis at baseline, unpaired two-tailed (student's) t-test was applied. For longitudinal analysis, case-wise deletion of missing data was implemented due to losses during follow-up. Because of individual variable follow-up time periods, the rate of change from baseline was reported as annual change and given in absolute number. Within-group differences were tested by paired two-tailed (student's) t-test and between-group-differences by unpaired two-tailed (student's) t-test.

Simple linear correlations (Pearson r) were assessed between primary study endpoints, i.e. annual absolute changes from baseline in peripheral volumetric or central areal BMD, SSI_p and maximum grip strength, and thyroid function tests or treatment characteristics at follow-up. Multiple regression analysis employed primary study endpoints as dependent variables. According to the created models, parameters of thyroid function and treatment characteristics at follow-up, as well as annual absolute changes from baseline in peripheral volumetric or central areal BMD, SSI_p or maximum grip strength served as independent variables, while gender, age and BMI were confounders. Independent and confounding variables were identified as predictors by significant positive or negative inter-variable correlations, and standardized (b^*) as well as un-standardized (b) regression coefficients, standard errors, and statistical significance were calculated for each model.

If the confidence intervals did not include zero, the difference was regarded as borderline statistically significant at $\alpha=0.05$ and as statistically significant at $\alpha=0.01$. Assuming in-vivo reproducibility of 2% (actually it was <1%) for all bone densitometric parameters, biometry estimated a minimum of 23 study subjects per group to determine a change of 2% in outcomes at a significance level of 1%.

3. Results

3.1. Cross-sectional analysis

3.1.1. Baseline demographics and thyroid function of patients and controls and treatment characteristics of patients

Table 1 and table 2 summarise baseline demographics and thyroid function of patients and controls, and treatment characteristics of patients, respectively. Patients and controls were well matched for gender, age, and BMI.

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All n=97	All n=89	Men n=28	Women n=46	Women n=23	Men n=29	Women n=60
Age [y]	40.4 (7.6)	41.5 (6.4)	40.8 (8.0)	39.2 (7.7)	42.4 (6.7)	41.6 (6.7)	41.5 (6.3)
Height [cm]	171 (8)	169 (9)	181 (6)	167 (6)	168 (5)*	181 (5)	165 (5)
Weight [kg]	74.7 (14.5)*	70.8 (13.4)	87.1 (13.1)	70.3 (11.7)*	68.4 (12.4)	84.8 (12.4)	65.7 (9.7)
BMI [kg/m^2]	25.4 (4.3)	24.5 (3.4)	26.7 (3.8)	25.3 (4.4)	24.2 (4.8)	26.0 (3.6)	24.0 (3.3)
FT_4 [pmol/l] (11-23)	25.4 (5.8)***	16.4 (2.6)	27.1 (4.7)***	27.3 (5.5)***###	19.5 (3.5)***	17.1 (3.5)	16.1 (2.1)
FT_3 [pmol/l] (3.4-7.6)	5.6 (0.9)***	4.8 (0.5)	5.7 (0.6)***	6.0 (1.0)***###	4.9 (0.6)	5.1 (0.4)	4.7 (0.5)
TSH [mU/l] (0.3-4.0)	0.29 (0.83)***	0.88 (0.48)	0.04 (0.07)***	0.05 (0.2)***###	1.09 (1.5)	0.84 (0.39)	0.89 (0.5)

Table 1. Baseline demographics and thyroid function, mean (SD)

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p <0.05

Level of significance between female cancer and female goitre patients: ###p<0.001 ##p<0.01 #p<0.05

For pooled patients, average daily LT_4 dose was 167±51 µg/day and average daily body weight (BW)-adjusted LT_4 dose 2.3±0.6 µg/kgBW/day yielding normal mean serum FT_3 concentration, but mean FT_4 level above and mean TSH level below reference range. Mean treatment duration of 7.2±6.8 years resulted in a mean cumulative dose of 388±342 mg and in a mean cumulative BW-adjusted dose of 5.40±4.87 mg/kgBW.

In the total population of controls, parameters of thyroid function were all in normal range and significantly different from patients (p<0.001 for all).

	Patients	Subgroups		
		Cancer		**Goitre**
	All n=97	Men n=28	Women n=46	Women n=23
Daily dose [µg/day]	167 (51)	204 (40)	175 (35)*****	105 (31)
Daily BW-adjusted daily dose [µg/kgBW/day]	2.3 (0.6)	2.4 (0.5)	2.5 (0.5)***	1.6 (0.5)
Cumulative dose [mg]	388 (342)	435 (386)	300 (300)******	505 (335)
Cumulative weight-adjusted dose [mg/kgBW]	5.40 (4.87)	5.07 (4.36)	4.47 (4.57)**	7.64 (5.46)
Time since surgery [y]	7.2 (6.8)	5.9 (5.1)	4.9 (5.2)******	13.3 (8.1)

Table 2. Baseline treatment characteristics, mean (SD)

Level of significance between female cancer and female goitre patients: ***p<0.001 **p<0.01 *p<0.05

Level of significance women and men: ***p<0.001 **p<0.01 *p<0.05

Thyroid function tests varied according to distinct treatment concepts for the underlying thyroid disease.

Female and male cancer patients had suppressed mean TSH levels of 0.05±0.2 mU/l and 0.04±0.07 mU/l, respectively. In both subgroups, average serum FT_3 concentrations were found within reference range, but with mean FT_4 levels above the upper limit. All markers of thyroid function differed significantly from respective controls (p<0.001 for all). Gender-related differences arose only with regard to treatment characteristics. Due to higher mean daily LT_4 dose and longer mean therapy length (p<0.01 for both), male cancer patients received higher mean cumulative LT_4 dose (p<0.001).

Female goitre patients had normal thyroid function, while higher mean serum FT_4 concentration differed significantly from female controls (p<0.001).

Comparing LT_4 suppressive versus replacement administration, higher average daily as well as daily BW-adjusted LT_4 doses resulted in higher mean serum

FT_4 and FT_3 concentrations as well as in suppressed mean TSH level ($p<0.001$ for all). The shorter mean treatment duration of 4.9 versus 13.3 years ($p<0.001$), though, led to lower average cumulative and cumulative BW-adjusted LT_4 doses ($p<0.01$ for both).

3.1.2. Baseline densitometric parameters and muscle strength

No significant differences were detected in normal mean absolute values of peripheral volumetric and central areal BMD, SSI_p and maximum grip strength neither between patients and controls nor within the patient population irrespective of treatment characteristics (Table 3 and 4).

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All n=97	All n=89	Men n=28	Women n=46	Women n=23	Men n=29	Women n=60
Total radius [mg/cm^3] (350±65/390±58)[§]	365 (63)	370 (76)	393 (61)	354 (67)*	354 (45)	416 (78)	355 (69)***
Trabecular radius [mg/cm^3] (160±43/205±65)[§]	151 (42)	146 (43)	178 (44)	137 (39)***	144 (28)	180 (41)	134 (37)***
Lumbar spine L2-L4 [g/cm^2] (1.18±0.12/1.22±0.12)[§]	1.269 (0.15)	1.240 (0.13)	1.253 (0.18)	1.268 (0.14)	1.291 (0.15)	1.226 (0.13)	1.245 (0.13)
Left femoral neck [g/cm^2] (0.96±0.12/1.04±0.13)[§]	1.029 (0.13)	1.017 (0.11)	1.055 (0.13)	1.009 (0.13)	1.036 (0.13)	1.015 (0.10)	1.017 (0.12)
Right femoral neck [g/cm^2] (0.96±0.12/1.04±0.13)[§]	1.025 (0.13)	1.012 (0.11)	1.03 (0.13)	1.012 (0.12)	1.037 (0.14)	1.015 (0.10)	1.011 (0.11)
Left total hip [g/cm^2] (0.99±0.12/1.06±0.13)[§]	1.061 (0.13)	1.053 (0.12)	1.099 (0.14)	1.039 (0.11)	1.055 (0.13)	1.085 (0.10)	1.041 (0.12)
Right total hip [g/cm^2] (0.99±0.12/1.06±0.13)[§]	1.059 (0.12)	1.047 (0.11)	1.087 (0.13)	1.045 (0.11)	1.051 (0.14)	1.079 (0.10)	1.035 (0.11)

Table 3. Baseline bone mineral density, mean (SD)

Level of significance between women and men: ***p<0.001 **p<0.01 *p<0.05

[§] (Reference range: women / men)

When looking at genders separately, men showed higher mean absolute values of peripheral volumetric BMD, SSI_p and maximum grip strength in patients and controls equally.

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All n=97	All n=89	Men n=28	Women n=46	Women n=23	Men n=29	Women n=60
Polar stress strain index [mm^2]	391 (130)	361 (108)	547 (117)	326 (71)***	331 (57)	495 (103)	312 (57)***
Maximum grip strength [kgf]	30.8 (9.7)	31.3 (9.6)	43.5 (6.8)	25.0 (4.1)***	26.8 (4.4)	44.9 (6.1)	26.4 (4.6)***

Table 4. Baseline bone strength and maximum grip strength, mean (SD)
Level of significance between women and men: ***p<0.001 **p<0.01 *p<0.05

3.1.3. Baseline biochemical markers of liver function, calcium and bone metabolism

Table 5 shows similar normal mean serum SHBG concentrations for all patient and control subgroups as well as both genders.

Regarding calcitropic hormones, just detectable average serum CT levels differed from respective controls in pooled as well as in stratified patients ($p<0.05$ to $p<0.001$). Mean serum PTH concentrations were within reference range and did not vary between groups except for borderline significantly lower average PTH level in female cancer patients as compared to female controls ($p<0.05$). Average serum Ca and P concentrations were completely unaffected.

With respect to bone turnover markers, in the total population of patients significantly higher mean serum concentrations of OC ($p<0.05$) and ICTP ($p<0.001$) were found than in pooled controls. When looking at patient subgroups separately, only female cancer patients showed significantly different mean serum concentrations from respective controls with higher mean absolute values for AP, OC ($p<0.01$ for both) and ICTP ($p<0.001$). In women on LT_4

suppressive therapy, mean ICTP blood level was significantly higher than in women on LT_4 replacement therapy ($p<0.01$).

Gender-related differences appeared only in controls with significantly lower serum CT, AP, OC and PICP levels in the female as compared to the male controls ($p<0.001$). In patients, average serum concentrations of calcium and bone metabolism markers were comparable regardless of gender except for borderline significantly lower mean serum PICP level in women on LT_4 suppressive treatment.

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All n=97	All n=89	Men n=28	Women n=46	Women n=23	Men n=29	Women n=60
SHBG [nmol/l] (15.5-114/10.4-59.5)§	52 (48)	61 (58)	26 (12)	63 (56)	62 (46)	23 (9)	75 (62)
CT [pg/ml] (0-10/0-27)§	0.10 (0.24)***	1.57 (2.37)	0.06 (0.10)***	0.12 (0.30)**	0.12 (0.25)*	3.84 (2.93)	0.75 (1.4)***
PTH [ng/l] (10-60)	40.6 (17.5)	44.8 (15.4)	42.5 (18.6)	37.8 (17.1)*	43.6 (16.8)	51.0 (25.0)	43.5 (13.9)
Ca [mmol/l] (2.0-2.7)	2.39 (0.15)	2.41 (0.14)	2.42 (0.15)	2.36 (0.17)	2.40 (0.09)	2.43 (0.10)	2.40 (0.15)
P [mmol/l] (0.87-1.45)	1.09 (0.18)	1.10 (0.18)	1.04 (0.20)	1.11 (0.19)	1.12 (0.14)	1.12 (0.21)	1.10 (0.17)
AP [U/l] (55-170)	111 (30)	104 (30)	119 (25)	112 (33)**	98 (24)	126 (34)	96 (24)***
OC [ng/ml] (7-30)	17.3 (7.3)*	15.3 (6.6)	20.0 (6.7)	16.9 (8.1)**	14.8 (5.1)	20.4 (8.4)	13.5 (4.6)***
PICP [µg/l] (70-230)	119 (32)	129 (39)	134 (39)	113 (30)*	115 (21)	151 (41)	120 (35)***
ICTP [µg/l] (1.6-5.0/1.3-5.2)§	3.7 (1.2)***	3.2 (0.8)	3.7 (1.3)	4.0 (1.3)*****	3.1 (0.8)	3.2 (0.7)	3.1 (0.8)

Table 5. Baseline biochemical markers, mean (SD)

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

Level of significance between female cancer and female goitre patients: ***p<0.001 **p<0.01 *p<0.05

Level of significance between women and men: ***p<0.001 **p<0.01 *p<0.05

§ (Reference range: women / men)

3.2. Longitudinal analysis

3.2.1. Annual absolute changes in demographics and thyroid function of participants and treatment characteristics of patients

Table 6 demonstrates that demographics remained approximately constant after a standardised year of follow-up resulting in patients and controls still well matched. In pooled controls, small but significant changes in mean FT_3 and TSH levels were found. Thyroid function in patients did not alter compared to baseline measurements.

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All n=89	All n=88	Men n=27	Women n=45	Women n=17	Men n=28	Women n=60
Weight [kg]	+0.8 (3.3)*	+0.24 (1.9)	+1.1 (3.2)	+0.52 (3.4)	+0.86 (3.0)	-0.04 (2.5)	+0.34 (1.6)
BMI [kg/m^2]	+0.3 (1.1)*	+0.09 (0.64)	+0.34 (1.0)	+0.18 (1.2)	+0.28 (1.1)	-0.01 (0.75)	+0.12 (0.56)
FT_4 [pmol/l] (11-23)	-0.61 (4.21)	-0.28 (2.59)	-0.44 (2.76)	-0.92 (5.17)	-0.03 (3.27)	+0.31 (3.21)	-0.16 (2.33)
FT_3 [pmol/l] (3.4-7.6)	-0.10 (1.02)	-0.15 (0.58)**	+0.13 (0.86)	-0.33 (1.18)	+0.15 (0.57)	-0.22 (0.54)*	-0.13 (0.59)
TSH [mU/l] (0.3-4.0)	+0.06 (0.86)	+0.23 (0.48)***	+0.01 (0.08)	+0.11 (0.45)	±0 (1.86)	+0.11 (0.35)	+0.28 (0.52)***

Table 6. Annual absolute changes from baseline in demographics and thyroid function, mean (SD)
Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Therapy regimens, i.e. average daily and daily BW-adjusted LT_4 doses, were not modified (Table 7). Mean cumulative LT_4 dose, cumulative BW-adjusted LT_4 dose, and treatment duration increased significantly with time ($p<0.001$ for all, Table 7).

	Patients	Subgroups		
		Cancer		**Goitre**
	All n=89	Men n=27	Women n=45	Women n=17
Daily dose [µg/day]	1.4 (17.8)	+2.8 (8.0)	-4.4 (20.8)	0 (19.8)
Daily weight-adjusted dose [µg/kgBW/day]	-0.05 (0.24)	-0.001 (0.11)	-0.08 (0.28)	-0.03 (0.30)
Cumulative dose [mg]	+0.20 (0.07)***	+0.24 (0.07)***	+0.0.19 (0.05)***	+0.13 (0.06)***
Cumulative weight-adjusted dose [mg/kgBW]	+0.002 (0.0009)***	+0.003 (0.0008)***	+0.003 (0.0009)***	+0.002 (0.0008)***

Table 7. Annual absolute changes from baseline in treatment characteristics, mean (SD)

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

3.2.2. Annual absolute changes in densitometric parameters and muscle strength

Basically, mean peripheral and central BMD did not change throughout patient and control groups notwithstanding gender (Table 8). In pooled patients, a borderline significant inverse trend was detected at the ultra-distal radius. Average total BMD decreased, while mean trabecular BMD concurrently increased ($p<0.05$ for both).

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All n=89	All N=88	Men n=27	Women n=45	Women n=17	Men n=28	Women n=60
Total radius [mg/cm^3] (350±65/390±58)§	-5.1 (21)*	-0.23 (15)	-4.1 (22)	-7.0 (22)****	-1.4 (13)	-0.58 (14)	-0.09 (15)
Trabecular radius [mg/cm^3] (160±43/205±65)§	+1.34 (0.59)*	+0.23 (5.17)	+1.62 (5.85)	+1.61 (6.58)	+0.20 (3.76)	-0.92 (5.60)	+0.66 (4.97)
Lumbar spine L2-L4 [g/cm^2] (1.18±0.12/1.22±0.12)§	+0.001 (0.035)	-0.005 (0.027)	+0.007 (0.047)	-0.005 (0.030)	+0.004 (0.025)	±0 (0.026)	-0.007 (0.028)*
Left femoral neck [g/cm^2] (0.96±0.12/1.04±0.13)§	-0.003 (0.032)	-0.001 (0.033)	-0.008 (0.036)	-0.005 (0.032)	+0.006 (0.028)	-0.002 (0.024)	±0 (0.036)
Right femoral neck [g/cm^2] (0.96±0.12/1.04±0.13)§	-0.002 (0.030)	-0.003 (0.027)	-0.003 (0.033)	+0.001 (0.029)	-0.009 (0.026)	+0.001 (0.027)	-0.004 (0.027)
Left total hip [g/cm^2] (0.99±0.12/1.06±0.13)§	±0 (0.021)	+0.001 (0.022)	-0.002 (0.024)	+0.001 (0.018)	+0.003 (0.026)	-0.001 (0.018)	+0.002 (0.023)
Right total hip [g/cm^2] (0.99±0.12/1.06±0.13)§	-0.001 (0.021)	±0 (0.020)	-0.001 (0.023)	±0 (0.022)	-0.002 (0.014)	-0.002 (0.020)	±0 (0.020)

Table 8. Annual absolute changes from baseline in bone mineral density, mean (SD)

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

§ (Reference range: women / men)

Being the only exceptional case the significant decrease of mean total radius BMD by 7.0±22 mg/cm^3 in female cancer patients ($p<0.001$) was greater than in female controls ($p<0.05$, Table 8 and Figure 2a).

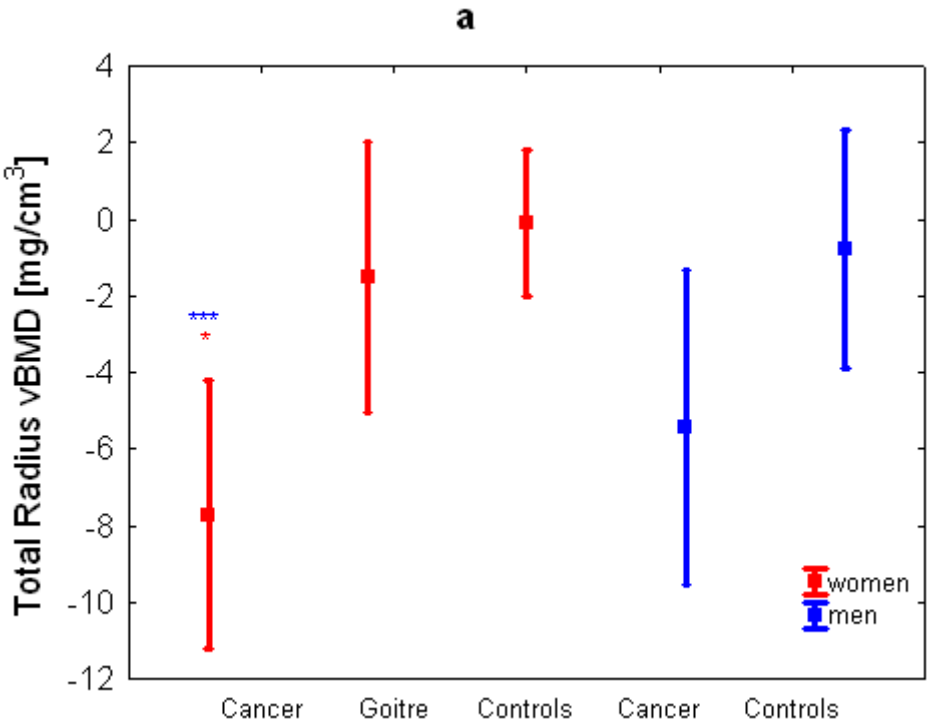

Figure 2a. Annual absolute changes from baseline in total radius volumetric BMD, mean ± SE

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

In patients and controls declining average total BMD at the ultra-distal radius (Table 8) was reflected in non- or marginally significant decreases of mean SSI_p (Table 9) aside from female goitre patients. Men on LT_4 suppressive treatment showed slightly greater decrements in bone strength than women ($p<0.05$, Table 9).

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All n=89	All n=88	Men n=27	Women n=45	Women n=17	Men n=28	Women n=60
Polar stress strain index [mm^2]	-11 (39)*	-2.2 (19)	-27.0 (62)*	-5.5 (21)*	+1.0 (13)	-5.9 (23)	-0.8 (17)
Maximum grip strength [kgf]	-0.5 (3.0)	+0.4 (2.9)	-0.87 (4.05)	-0.03 (2.32)	-1.2 (2.7)**	-0.31 (4.1)	+0.60 (2.3)*

Table 9. Annual absolute changes from baseline in bone strength and maximum grip strength, mean (SD)

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

Level of significance between women and men: ***p<0.001 **p<0.01 *p<0.05

Non-significant losses in average maximum grip strength were assessed in all patient subgroups, while lower mean grip strength in female goitre patients (p<0.01) differed significantly from a diminutive increase of average muscle strength (p<0.05) in female controls (Table 9 and Figure 2b).

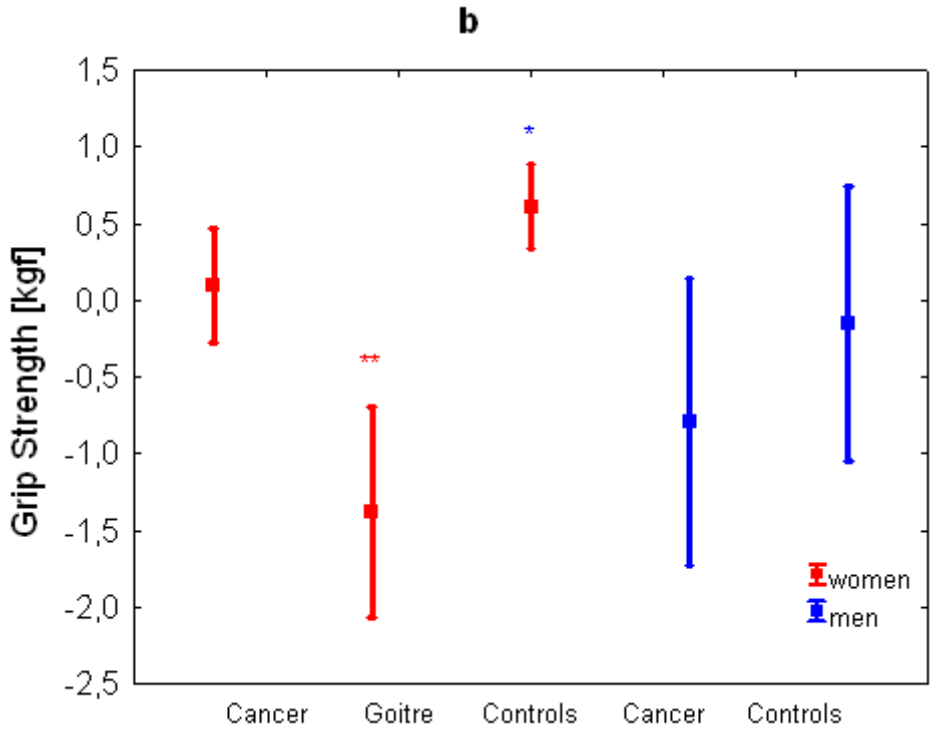

Figure 2b. Annual absolute changes from baseline in maximum grip strength, mean ± SE

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

3.2.3. Annual absolute changes in biochemical markers of calcium and bone metabolism

Referring to parameters of calcium metabolism (Table 10), in the total patient population only small increases in the hormonal regulator CT were determined ($p<0.05$) and differed significantly ($p<0.01$) from higher increases in pooled controls ($p<0.001$). Mean CT level rose slightly in female cancer patients ($p<0.01$) differing significantly from an increase by 1.07 pg/ml in female controls ($p<0.001$). In female goitre patients, only a small significant increase in mean serum CT concentration was assessed ($p<0.05$). Average serum Ca and P concentrations approximately levelled off in patient and control subgroups with no significant between-group differences. By trend, pooled patients showed increased and higher mean serum P concentration than controls ($p<0.05$ for both).

	Total population		Subgroups				
	Patients	**Controls**	**Cancer**		**Goitre**	**Controls**	
	All N=89	All n=88	Men n=27	Women n=45	Women n=17	Men n=28	Women n=60
CT [pg/ml] (0-10/0-27)§	+0.29 (1.14)***	+1.11 (2.52)***	+0.36 (1.79)	+0.21 (0.78)**	+0.41 (0.63)*	+1.22 (3.95)	+1.07 (1.74)***
Ca [mmol/l] (2.0-2.7)	-0.005 (0.118)	-0.001 (0.147)	-0.025 (0.109)	+0.002 (0.135)	+0.009 (0.072)	-0.021 (0.120)	+0.007 (0.157)
P [mmol/l] (0.87-1.45)	+0.04 (0.16)**	-0.02 (0.16)	+0.01 (0.16)	+0.05 (0.16)	+0.06 (0.14)	-0.05 (0.17)	-0.01 (0.16)
AP [U/l] (55-170)	+1.7 (19.4)	+0.3 (13.4)	+5.3 (15.6)	-1.6 (22.9)	+4.9 (12.5)	+0.31 (12.2)	+0.26 (14.0)
OC [ng/ml] (7-30)	-1.2 (5.4)*	-0.62 (4.62)	-1.3 (4.82)	-1.87 (6.17)	+0.61 (3.84)	-0.59 (6.76)	-0.64 (3.56)
PICP [µg/l] (70-230)	-31 (23)***	-35 (27)***	-34 (22)***	-30 (26)***	-32 (16)***	-39 (19)***	-34 (30)***
ICTP [µg/l] (1.6-5.0/1.3-5.2)§	-0.91 (0.96)**	-0.63 (0.61)**	-0.63 (0.73)**	-1.24 (0.94)****	-0.3 (0.91)**	-0.5 (0.75)**	-0.57 (0.64)**

Table 10. Annual absolute changes from baseline in biochemical markers, mean (SD)

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

§ (Reference range: women / men)

Distinct pattern of changes arose in single biochemical markers of bone turnover. In contrast to nearly unaltered average serum AP and OC levels, a significant decrease in mean PICP ranging from 30-34 µg/l was measured in all patient subgroups, but was similar to control subgroups ($p<0.001$ for all, Table 10 and Figure 2c).

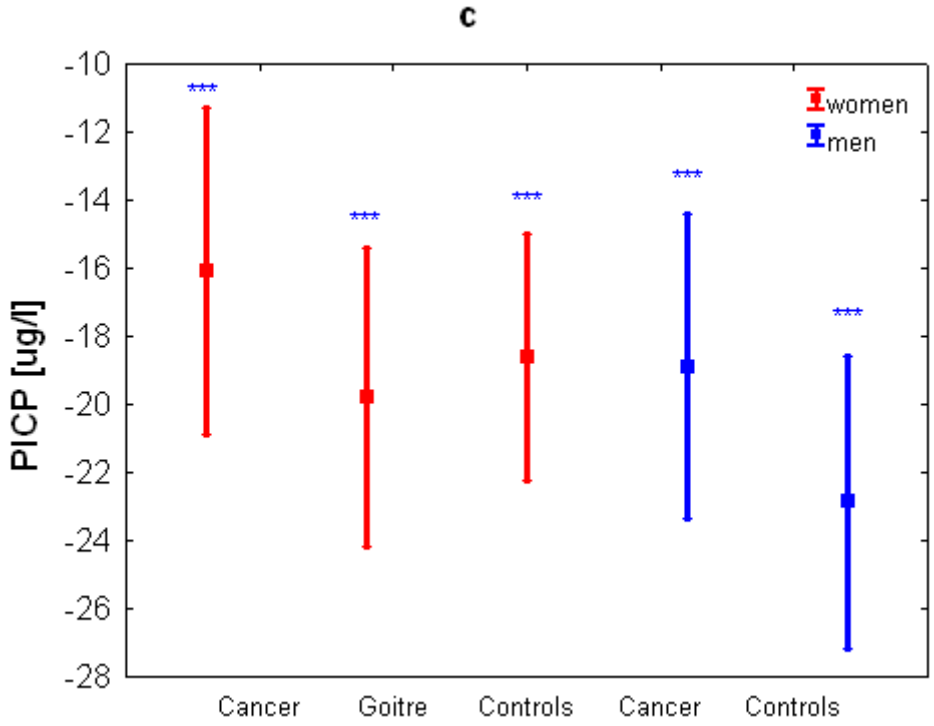

Figure 2c. Annual absolute changes from baseline in PICP, mean ± SE

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

Mean serum concentration of the bone resorption marker ICTP likewise declined in patients and controls by 0.3-1.24 µg/l ($p<0.01$ for all, Table 10 and Figure 2d). Only in female cancer patients, the decrease of ICTP was significantly greater than in female controls ($p<0.01$).

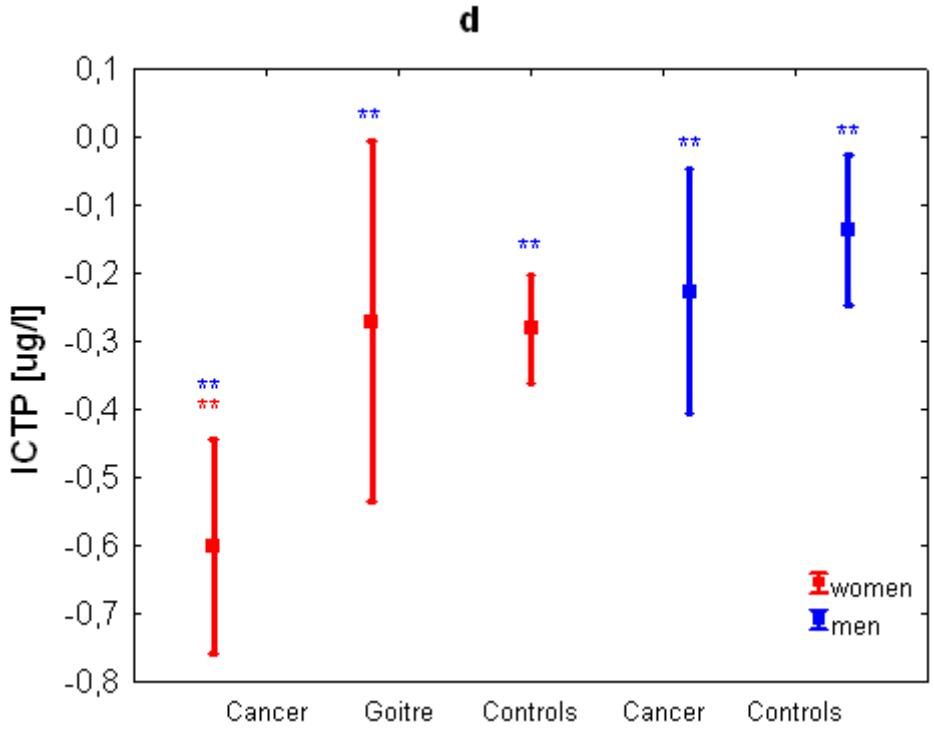

Figure 2d. Annual absolute changes from baseline in ICTP, mean ± SE

Level of significance within-group: ***p<0.001 **p<0.01 *p<0.05

Level of significance between patients and controls: ***p<0.001 **p<0.01 *p<0.05

No dose- or gender-related changes were observed in any measurement of biochemical markers.

3.3. Simple linear correlations and multiple regression analysis

Simple linear correlations and multiple regression analysis evaluated the interrelationship between primary study endpoints, i.e. annual absolute changes from baseline in peripheral volumetric or central areal BMD, SSI_p and maximum grip strength, and thyroid function tests or treatment characteristics at follow-up in patients.

In pooled patients, a weak positive correlation was found between the minimal decrease in BMD at the right femoral neck and daily LT_4 dose ($r=+0.254$; $p<0.05$), but was not reproducible in more complex models of multiple regression analysis.

When looking at patient subgroups separately, in men on LT_4 suppressive therapy slightly decreased BMD at the left femoral neck was related positively to daily LT_4 dose ($r=+0.464$; $p<0.05$). Furthermore, the marginally reduced BMD at the left femoral neck and also at the right total hip correlated negatively with FT_3 ($r=-0.453$ and $r=-0.478$, respectively; $p<0.05$) and was predicted with $R^2=49\%$ ($p<0.08$) and $R^2=39\%$ ($p<0.25$), respectively, in non-significant models. Of the confounding and independent variables mean age, BMI, FT_4, FT_3, TSH, daily LT_4 dose, cumulative LT_4 dose and decreased maximum grip strength, only FT_3 showed significant negative partial correlations of $b^*=-0.66$ ($p<0.007$) and $b^*=-0.62$ ($p<0.017$), respectively. Enclosing the reduction of SSI_p in both models resulted in minimal increased predictability and similar partial correlation coefficients of FT_3 at slightly elevated levels of significance. Besides reduced BMD, a diminutive decline of maximum grip strength was related positively to FT_3 in male cancer patients ($r=+0.403$; $p<0.05$). For this change in muscle strength, predictability was 51% in a non-significant model ($p<0.17$). Mean age, BMI, FT_4, FT_3, TSH, daily LT_4 dose, cumulative LT_4 dose and inverse trends of total and trabecular radius BMD were used as confounders and independent variables, whereas only FT_3 was significantly involved ($b^*=+0.71$; $p<0.01$). With non-significant alterations in BMD at the ultra-distal radius being substituted for borderline significantly decreased SSI_p, the model lost predictability ($R^2=29\%$; $p<0.53$) and did not show any significant partial correlations.

In female cancer patients, a positive correlation was detected between minimal decreased BMD at the left femoral neck and daily BW-adjusted LT_4 dose ($r=+0.329$; $p<0.05$). A borderline significant model ($p<0.02$) employing mean age, FT_4, FT_3, TSH, daily BW-adjusted LT_4 dose, cumulative BW-adjusted LT_4 dose, reduced maximum grip strength and SSI_p as confounding and independent variables explained 40% of the afore mentioned change in BMD with a significant positive partial correlation of $b^*=+0.53$ ($p<0.001$) for SSI_p only. Positive correlations between a slightly increased BMD at the right and left total hip, and FT_3 ($r=+0.355$ and $r=+0.396$, respectively; $p<0.05$) did not appear to be significant determinants with respect to other independent variables.

Referring to female goitre patients, positive correlations were seen between marginally decreased BMD at the right total hip and treatment duration or cumulative LT_4 dose ($r=+0.550$ and $r=+0.523$, respectively; $p<0.05$). This change in BMD was predicted with $R^2=69\%$ ($p<0.20$) in a non-significant model. Mean age, FT_4, FT_3, TSH, daily BW-adjusted LT_4 dose, cumulative BW-adjusted LT_4 dose and decreased maximum grip strength served as confounders and independent variables, while only muscle strength appeared with a borderline significant negative partial correlation coefficient ($b^*=-0.78$; $p<0.04$). Adding the increased SSI_p to this model slightly improved predictability, but resulted in overall non-significant partial correlation coefficients of confounding and independent variables. Slightly reduced maximum grip strength was related positively to daily BW-adjusted LT_4 dose ($r=+0.535$; $p<0.05$). Non-significant models including mean age, FT_4, FT_3, TSH, daily BW-adjusted LT_4 dose, cumulative BW-adjusted LT_4 dose and changes in BMD at the ultra-distal radius or increased SSI_p explained this change in muscle strength with R^2 ranging from 55% to 59% ($p<0.59$ and $p<0.26$, respectively), but without any significant partial correlation coefficients.

4. Discussion

4.1. Material and methods

This controlled study of prospective observational design was the first to include reasonable numbers of men on LT_4 suppressive treatment and pre-menopausal women on LT_4 suppressive as well as on LT_4 replacement therapy. The objective was to evaluate potential effects and dose-response relationship of LT_4 administration on bone in the context of interactive dependence of the musculoskeletal system.

Stratified patient subgroups were homogenous with respect to gender or menopausal state as well as with regard to prior metabolic thyroid state, underlying thyroid disease, previous treatment and type of LT_4 therapy regimen due to appropriately and closely monitored thyroid function throughout post-operative treatment time. Also strict definition of TSH suppressive versus physiological thyroid hormone substitution therapy ensured suppressed TSH level in cancer patients and TSH level within normal range in goitre patients.

By use of pQCT and grip strength measurements, potential effects of LT_4 on volumetric cortical and trabecular BMD were evaluated within a complex model of a close muscle-bone interrelationship including structural, geometrical and biomechanical properties of bone material at the ultra-distal radius. Bones are three-dimensional masterpieces of biomechanical engineering meeting contradictory tissue properties of stiffness and flexibility, strength and lightness by their material composition and structural design (Seeman E, 2003). As bone material is exquisitely sensitive to minimal effective strains derived from contractions of regional muscles or to the lack of such strains (Seeman E, 2003), bone architecture combining structural geometry, strength and function of bone is primarily a result of mechanical loading acting on bone with muscle strength as first-order determinant of biomechanical bone quality (Ferretti JL, 2000; Frost HM, 1995; Hasegawa Y, 2000; Hasegawa Y, 2001). Geometrical parameters of the cortical shell such as cortical thickness, cortical area, and cross-sectional second moment of inertia are closely interrelated with the

bending or the torsional strength of intact bones (Horikoshi T, 1999, Louis O, 1995; Roldán EJ, 2009).

4.2. Primary study endpoints

No evidence of adverse LT_4 impact on primary study endpoints, i.e. annual absolute changes from baseline in BMD, bone strength and maximum grip strength, was found except for small detrimental effects at the peripheral skeleton.

A general trend of inversely affected total and trabecular BMD at the ultra-distal radius, and of decreased bone strength was observed, while only women on LT_4 suppressive treatment showed a highly significant reduction of total BMD at the ultra-distal radius. Decreased total BMD at the ultra-distal radius was attributable to cortical BMD loss, which was not balanced by the measured increase of trabecular BMD indicating elevated endocortical trabecularisation. Histomorphometrical analyses (Mosekilde L, 1990; Roldán EJ, 2009; Vestergaard P, 2003) and clinical assessments of areal BMD at skeletal sites with distinct proportions of cortical and trabecular bone (Allain TJ, 1993; Faber J, 1994; Greenspan SL, 1999; Pantazi H, 2000; Ross DS, 1994; Uzzan B, 1996) showed more marked impairment of cortical than trabecular bone due to LT_4 administration. Thinning and porosity of the cortical shell occurred basically due to endocortical resorption. Trabecular perforation might also appear, yet trabecular bone might be able to compensate harmful impact more rapidly and completely because of its higher surface to volume ratio and bone turnover rate.

As BMD is assumed to be the primary determinant of bone strength (Faulkner KG, 2000) reduced SSI_p at the ultra-distal radius might partially be explained by the decrease of total BMD at the same skeletal site.

Although affected by a highly significant reduction of BMD at the ultra-distal radius, physical stresses and strains of unchanged, intact muscle pull and compression acting on bone seemed to prevent from significant decline of bone

strength in women on LT_4 suppressive treatment. Even decreasing bone mass and architectural decay might be antagonized and thus bone strength might be sustained by fashioning and refashioning its shape and position in space (Seeman E, 2003). Also in women receiving LT_4 replacement therapy, mechanical forces derived from appropriate muscle groups might still be large enough to keep existing BMD and bone strength despite the finding of significantly reduced grip strength in comparison to female controls.

However, pre-menopausal women receiving LT_4 suppressive treatment might be at risk of accelerated loss of peripheral BMD, as suggested by the trend of greater decrease of total BMD at the ultra-distal radius when compared to female controls. The model of muscle-bone interactions does not provide any plausible explanation and further investigation is therefore warranted.

The study revealed no dose-response relationship in pre-menopausal women. One explanation for this might be that at the end of the follow-up period, average cumulative BW-adjusted dose of female goitre patients had almost reached the level of suppressive LT_4 dosage in women, accompanied by increased mean FT_3 level. The highest FT_3 level was still found in female cancer patients. Another explanation might be that sufficient oestrogen production in pre-menopausal women closed the gap to potentially more deleterious effects of LT_4 suppressive treatment, as oestrogen is known to inhibit bone remodelling process at the endosteal surface and thus to inhibit possible bone loss.

Considering only slight, non-significant reduction of peripheral total BMD and muscle strength, the trend of even greater decline of bone strength in male cancer patients as compared to female cancer patients was unexpected. This was also surprising taking into account some gender-related differences of bone remodelling in mature skeleton. The greater capability of periosteal apposition in men might result in less net bone loss, as the amount of resorbed endocortical bone is similar, irrespective of gender. Limited periosteal apposition might partially maintain the cross-sectional area of bone in the face of enhanced endocortical resorption due to LT_4 administration distributing otherwise

increasing compressive loads more widely and preserving resistance to bending, which is considered equal to bone strength (Seeman E, 2003). Factors of skeletal biomechanics such as bone mass, size, shape, architecture and strength are determined by net bone formation or resorption at the periosteal and endosteal (intracortical, endocortical and trabecular) surfaces where cellular activity of the mineralised skeleton takes place (Seeman E, 2003). Even though one explanation might be that inverse cellular activity on periosteal and endosteal surfaces of bone does not necessarily result in some change in BMD, but in bone geometry and strength, further evaluation is demanded.

BMD at central skeletal sites seemed not to be impaired and to be less affected than BMD at the ultra-distal radius probably due to a weight-bearing effect (Horikoshi T, 1999) defined by continuous mechanical stimuli on bone, which might lead to net bone formation of the remodelling process. Measuring integral BMD, i.e. the overlapping of cortical and trabecular BMD due to a two-dimensional projection, the method of DXA allows estimation only, but not an exact definition of LT_4 effects in different bone types at central skeletal sites. The weight-bearing effect on the axial skeleton might minimize the increase or even antagonize the onset of detrimental LT_4 action on cortical bone. As to some degree observed at the appendicular skeleton, a positive net result of stimulated bone turnover in trabecular compartments might completely compensate for harmful impact on cortical bone and result in stable integral BMD at central skeletal sites (Faulkner KG, 2000; Horikoshi T, 1999).

In this clinical setting, no harmful effects of LT_4 administration on muscle strength represented by maximum grip strength at the non-dominant forearm were observed.

Outcomes of multiple regression analysis confirmed the lack of deleterious LT_4 impact on primary study endpoints. The only significant moderate positive correlations between treatment characteristics and some non-significant changes in central BMD and maximum grip strength did not appear to be significant determinants with respect to other independent variables. In particular, small detrimental effects of LT_4 administration revealed at the

appendicular skeleton could not be explained by treatment characteristics properly. Rather blood levels of the biologically active hormone FT_3 that reflect tissue exposure more closely than bare treatment characteristics were partially correlated to primary study endpoints and showed significant partial correlation coefficients in few complex models of multiple regression analysis.

4.3. Secondary study endpoints

While BMD and its related factors are constant parameters reflecting primarily morphology of the functional unit of bone and muscle, biochemical markers of calcium and bone metabolism were considered complementary dynamic factors reflecting ongoing bone remodelling process. However, due to continuously and physiologically changing blood levels, it might be challenging to draw conclusions from some sporadic assessments of these serum markers.

Balanced calcium homoeostasis as well as stable or not increased but rather significantly decreased blood levels of bone turnover parameters confirmed the clinical finding of unaffected BMD except for small detrimental effects on the peripheral skeleton under LT_4 suppressive medication. As was assumed by some investigators, this might be considered as the biochemical evidence for the lack of high turnover bone loss due to LT_4 administration (De Rosa G, 1997; Diamond L, 1991; Karner I, 2005; Lecomte P, 1995; McDermott MT, 1995; Stěpán JJ, 1992; Toivonen J, 1998).

However, the distinct pattern of changes in bone turnover markers needs to be clarified. In contrast to both parameters, serum AP and OC, the (highly) significant decreases of serum PICP and ICTP might be explained by greater specifity for bone tissue. As reductions of PICP and ICTP blood levels were analogous in each subgroup, respectively, despite different changes in BMD, sensitivity of these parameters might be questioned. Biochemical markers of bone turnover might not be as useful as expected in the evaluation and interpretation of BMD changes, as no additional essential or clarifying

information was provided. Critical interpretation of these parameters is therefore demanded at least in this kind of setting.

4.4. Longitudinal studies

Six controlled studies of prospective longitudinal design investigating potential LT_4 effects on bone in patients affected by benign or malignant thyroid disorders were extracted from currently available publications and considered comparable to this study to the greatest possible extent (De Rosa G, 1997; Jódar E, 1998; McDermott MT, 1995; Müller CG, 1995; Pioli G, 1992; Rosen HN, 1998). Major selection criteria also included enrolment of pre-menopausal women or men and a healthy reference population, particularly free from any thyroid or musculoskeletal disease. Only one study enclosed male patients (Rosen HN, 1998).

As parameters of bone and muscle strength were neglected by these studies, only BMD changes assessed at various skeletal sites remained comparable to primary endpoints of this study. Merely one study followed serum markers of calcium and bone metabolism over the course of the study (De Rosa G, 1997).

Two studies only (Jódar E, 1998; Rosen HN, 1998) that recruited int. al. pre-menopausal women treated for well-differentiated thyroid carcinoma measured BMD at the ultra-distal radius - yet by DXA - and did not reveal any deleterious, but rather slightly beneficial LT_4 impact on that peripheral skeletal site. For direct comparison, in female cancer patients, this study showed a highly significant decrease of total BMD at the ultra-distal radius (pQCT) by 7.00±22 mg/cm³ per year ($p<0.001$), which also reached borderline significance when compared to female controls ($p<0.05$). The analogous outcomes of Jódar et al. and Rosen et al. seemed to contrast findings of this study at first glance, which might rather be due to small sample size, shorter mean treatment duration and partially not totally suppressed, but low normal mean TSH level. The latter constraint of both studies made the lack of detrimental LT_4 effects on BMD at the ultra-distal radius in pre-menopausal thyroid cancer patients more

comparable to the very same finding of this study, though in pre-menopausal goitre patients on LT_4 replacement therapy.

The absent impairment of BMD at the axial skeleton linked to LT_4 suppressive and replacement therapy in pre-menopausal women which was demonstrated by this study was partly contrary to four studies (De Rosa G, 1997; Jódar E, 1998; McDermott MT, 1995; Pioli G, 1992).

One study followed a limited number of 19 pre-menopausal and previously untreated women affected by non-toxic goitre for a time period of twelve months after initiation of LT_4 therapy (De Rosa G, 1997). The treatment regimen of goitre patients enrolled by De Rosa et al. differed from this study with respect to the lack of prior thyroidectomy and with respect to LT_4 administration aimed at TSH suppression. In all patients, TSH was suppressed within three months after the beginning of LT_4 therapy and remained suppressed throughout the study. Within-group comparison assessed a marked BMD loss of 1.7% per year ($p<0.05$) at the femoral neck, though it was not significantly different from respective controls (DXA; in vivo coefficient of variation by 0.8%). To compare, this study measured a non-significant mean annual percentage BMD reduction of 0.15% at the same skeletal site. BMD at other central skeletal sites such as the lumbar spine, trochanter and Ward's triangle was not impaired in De Rosa's study. The small detrimental LT_4 impact on BMD at the femoral neck might partially be attributed to a so-called catch-up effect of bone resorption. BMD assessment shortly after the onset of LT_4 treatment might not reflect a steady state, as many new remodelling units remained in the resorptive phase at that time (De Rosa G, 1997). By contrast, after longer mean treatment duration of 4.9 years, bone metabolism might reach a balanced state of bone turnover adapted to LT_4 administration resulting in levelled off BMD measurement outcomes as observed by this study in patients on LT_4 suppressive as well as replacement therapy.

This hypothesis of unbalanced bone metabolism during the early stage of LT_4 medication might also be reflected and confirmed biochemically. De Rosa et al. determined an early (from the three month-reassessment onwards) and

progressive significant increase of serum AP and OC ($p<0.05$) during the initial twelve months of LT_4 suppressive therapy in pre-menopausal goitre patients, whereas in this study pre-menopausal cancer as well as goitre patients on long-term LT_4 suppressive or replacement treatment did not show any significant changes in blood levels of the very same bone turnover markers.

Jódar et al. also examined only a limited number of 14 pre-menopausal women on long-term LT_4 treatment (mean treatment duration of 5.5 years) for well-differentiated thyroid cancer. The research group also assessed a significant decrease of BMD at the femoral neck by 1.6% per year ($p<0.05$) significantly different from respective controls ($p<0.05$) as the only central skeletal site affected besides lumbar spine and Ward's triangle. Again, this study revealed a mean annual percentage BMD decrease of merely 0.15% at the discussed skeletal site. The slight BMD impairment at the femoral neck, though, was considered to be of even minor statistical significance, as the DXA in vivo coefficient of variation by 1-2% differed only marginally from the measured BMD reduction, which was additionally aggravated by insufficient statistical power of a small sample size. Clinical relevance was also questioned due to the lack of increasing prevalence of fractures shown by epidemiological studies. In comparison with treatment characteristics of pre-menopausal cancer patients included by this study, slightly higher average daily BW-adjusted LT_4 dose (2.79 µg/kg per day), longer mean treatment duration (65 months) and therefore higher average cumulative LT_4 dose (351 mg) might contribute to a more marked BMD reduction at the femoral neck, although mean TSH serum concentration was found to be not totally suppressed but rather in its lower normal range (0.61 µU/ml; reference range values: 0.5-5.0 µU/ml).

At study entry, the research group determined few parameters of calcium and bone metabolism, which were – nevertheless - not re-evaluated over the course of the study. Serum Ca, P, AP and OC were found to be within normal range and comparable to biochemical baseline measurement outcomes of this study.

Two studies enrolling patients with various thyroid disorders or differing degrees of TSH suppressive treatment also detected some detrimental LT_4 effects on BMD at the central skeleton (McDermott MT, 1995; Pioli G, 1992).

Pioli et al. studied 14 pre-menopausal women who had undergone thyroidectomy either for non-toxic goitre (n=6) or well-differentiated thyroid carcinoma (n=8) at the beginning of consecutive LT_4 suppressive treatment. TSH suppression was achieved within four months in all patients and was maintained throughout the study. For the whole patient population, significant bone loss at the lumbar spine by 2.6% per year ($p<0.01$) differing significantly from respective controls ($p<0.001$) was demonstrated using the method of DPA (in vivo coefficient of variation of 3.5%). As treatment characteristics did not vary according to diagnosis, results were not presented separately for cancer and goitre patients. By contrast, this study assessed non-significant mean annual percentage BMD changes at the lumbar spine of -0.5% and +0.4% for female thyroid cancer and goitre patients, respectively. Again, this controversial finding might partially be attributed to limited statistical power of the small sample size combined with wide interindividual variability of BMD measurement outcomes. The DPA in vivo coefficient of variation of 3.5% being only marginally different from the assessed BMD reduction might provoke additional difficulty in statistical evaluation. Afore mentioned catch-up effect of bone resorption might be a physiological explanation for the decrease of BMD at the only axial skeletal site measured.

Two patient subgroups of malignant (n=24) and benign thyroid disorders (n=44), respectively, were stratified by the degree of TSH suppression, and followed in the study of McDermott et al. The mean annual absolute rates of bone loss at the lumbar spine and femoral neck assessed by DPA (in vivo coefficient of variation of 1-3%) in both patient subgroups were greater than those in controls, respectively, while patients on higher LT_4 dosage even showed significantly greater BMD reduction at the femoral neck ($p<0.001$ for all). However, McDermott's study lacked statistical evaluation of within-group comparison of BMD changes. In addition, direct comparison to this study is severely limited, as a different method of BMD measurement was applied and results were not

reported according to menopausal state, although a substantial proportion of post-menopausal women was enrolled in both stratified patient subgroups. Detrimental effects of oestrogen deficiency on BMD in post-menopausal women might be a strong confounder. Another major weakness was the long recruitment time of about eleven years.

In accordance with results of this study, two research groups assessed no BMD reductions at central skeletal sites in pre-menopausal women (Müller CG, 1995; Rosen HN, 1998).

The study of Müller et al. demonstrated that LT_4 administration did not induce adverse effects on BMD at the lumbar spine and femoral neck (DXA) in both cancer as well as goitre patients. Seven women treated for thyroid cancer and 14 women affected by non-toxic goitre of mixed menopausal state were studied for a mean of 1.5 years. The authors excluded influence of menopausal state on BMD before follow-up was started. All women received long-term LT_4 therapy (mean treatment duration ranged from 10.0 to 12.4 years), aimed at totally suppressed TSH level in thyroid cancer patients only.

Rosen et al. undertook a randomized, placebo-controlled trial to investigate potential benefits on BMD and bone metabolism from cyclic intravenous pamidronate (APD) administration in thyroid cancer patients on LT_4 medication. The patient subgroup on placebo consisted of 17 pre-, two post-menopausal women and eight men thyroidectomized for well-differentiated thyroid cancer on LT_4 treatment of at least six month-duration. The majority of patients showed TSH levels below the lower reference limit. No evidence of harmful LT_4 effects on BMD at the lumbar spine, femoral neck and total hip (DXA) was shown in female patients, while results did not differ significantly from respective healthy controls either. BMD at the trochanter, another central skeletal site measured, was not impaired either.

Rosen's APD trial also confirmed the absence of any deleterious LT_4 impact on BMD at the peripheral and central skeleton in men. The research group assessed no significant BMD changes at the same skeletal sites, i.e. at the

ultra-distal radius, lumbar spine, femoral neck and total hip. Yet results of longitudinal BMD assessments were not presented separately for men, but for the whole LT_4/placebo patient subgroup also including afore mentioned female patients. Furthermore, direct comparison to this study might be restricted due to smaller sample size, partly shorter mean LT_4 treatment duration and incompletely suppressed serum TSH concentration.

Rosen et al. also performed biochemical assessments of serum OC and PTH levels in the LT_4/placebo patient subgroup at baseline only. Measurement outcomes were not reported according to gender, however, blood levels of those parameters were found to be within reference range not differing significantly from the respective healthy controls and in accordance with baseline findings in all three patient subgroups of this study.

Additional seven studies of prospective longitudinal design examining potential LT_4 effects on bone in pre-menopausal or male patients treated for well-differentiated thyroid carcinoma or non-toxic goitre were extracted from currently available publications, but considered to be less comparable to this study. Two studies were of descriptive character only (Karner I, 2005; Mazopakis EE, 2006), while another five studies included a matched control population but not free from any thyroid disorder (Appetecchia M, 2005; Brenta G, 2003; Duncan WE, 1994; Knudsen N, 1998; Wesche MF, 2001). Only one of these studies recruited men (Karner I, 2005).

Again, BMD change rate was determined as the only primary study endpoint, respectively, not taking into account parameters of bone and muscle strength. Mostly newer studies (Appetecchia M, 2005; Brenta G, 2003; Mazopakis EE, 2006; Knudsen N, 1998; Wesche MF, 2001) also evaluated serum markers of calcium and bone metabolism during respective follow-up time period.

As none of these studies assessed BMD at the ultra-distal radius, the results of this study remained incomparable.

For central skeletal sites, conflicting results were found in two studies (Mazopakis EE, 2006; Wesche MF, 2001). Both studies followed-up a group of pre-menopausal patients, differing in sample size and underlying thyroid disease, during the initial two years of LT_4 suppressive treatment aimed at totally suppressed TSH levels.

One study (Mazopakis EE, 2006), which recruited 26 pre-menopausal women receiving LT_4 for well-differentiated thyroid carcinoma after near-total thyroidectomy and ^{131}I remnant ablation, found a significant BMD reduction at the femoral neck (DXA) by 7.5% at the end of the follow-up period ($p<0.001$). Other central skeletal sites such as the femoral trochanter and Ward's triangle were also significantly impaired.

In a randomized trial, Wesche et al. compared LT_4 suppressive therapy with the application of radioactive iodine (^{131}I) in the treatment of sporadic non-toxic goitre. In the patient subgroup of 18 pre-menopausal, non-thyroidectomized women medicated with LT_4 for this benign thyroid disorder, the study assessed significantly decreased BMD at the lumbar spine (DXA) by 3.6% ($p<0.001$) after two years of follow-up. This BMD reduction differed significantly from unchanged BMD at the lumbar spine in non-toxic goitre patients treated with ^{131}I ($p=0.001$). BMD at the femoral neck and trochanter also declined, although not significantly.

To compare secondary study endpoints, i.e. annual absolute changes from baseline in biochemical markers of calcium and bone metabolism, the two research groups reported partly contrary results. In agreement with this study, Mazopakis et al. assessed no significant changes in serum Ca, P and AP concentrations in pre-menopausal cancer patients. As has already been demonstrated by one study that examined pre-menopausal goitre patients during the initial twelve months of LT_4 suppressive medication (De Rosa G, 1997), Wesche et al. also determined a significant increase of the serum bone turnover markers AP and OC also differing significantly from the patient subgroup that received radioactive iodine, while serum Ca levelled off.

By contrast, absent detrimental LT_4 effects on BMD at the axial skeleton were supported by five studies.

Three research groups (Appetecchia M, 2005; Brenta G, 2003; Knudsen N, 1998) studied various numbers of pre-menopausal and previously untreated patients affected by non-toxic goitre, respectively, at the beginning of LT_4 therapy mostly aimed at reduced (i.e. below the lower limit of reference range), but not totally suppressed TSH. Control groups of these studies consisted of female goitre patients who either were untreated (Appetecchia M, 2005; Knudsen N, 1998) or received triiodothyroacetic acid instead of LT_4 (Brenta G, 2003).

Appetecchia et al. followed 40 pre-menopausal women for three years and did not find any BMD reduction at the lumbar spine (DXA) without any difference from the respective pre-menopausal control population. Brenta et al. enrolled 17 women not stratified by menopausal state for a follow-up time of eleven months. Knudsen et al. measured BMD changes (DPA) in 14 pre-menopausal women after a six month-withdrawal from a six month-LT_4 treatment trial. The latter both studies evaluated no impairment of BMD at the lumbar spine as well as femoral neck.

Yet, in biochemics, the three studies produced conflicting results. After respective follow-up time period, two of those research groups (Appetecchia M, 2005; Brenta G, 2003) reported unchanged and normal blood levels of calcium and bone metabolism markers not significantly different from respective control groups. Brenta et al. measured serum OC, while Appetecchia et al. additionally assessed serum Ca, AP and PTH. In Knudsen's study, serum concentrations of Ca, OC, AP and PICP increased significantly during the six months of LT_4 administration, which was significantly different from untreated controls and returned to basic levels after six months of withdrawal.

Furthermore, two studies (Duncan WE, 1994; Karner I, 2005) demonstrated unaffected BMD at the lumbar spine and femoral neck for differing mixed patient cohorts.

Karner et al. examined 19 pre-menopausal as well as nine male patients who were on long-term (mean treatment duration of at least 8.1±6.0 years) LT_4 administration of total TSH suppression for well-differentiated thyroid cancer. The study assessed the very same range of calcium and bone metabolism parameters, but merely at study entry. Irrespective of gender, all measured serum markers were within normal range reproducing baseline results of this study.

In Duncan's study, pre- and post-menopausal women on long-term LT_4 therapy for various malignant and benign thyroid disorders were included. However, results of thyroid function tests, treatment characteristics and BMD assessments were not stratified by menopausal state, underlying thyroid disease and therapy regimen, but by measuring site only.

4.5. Cross-sectional studies

Cross-sectional studies of either descriptive character only (Florkowski CM, 1993; Gonzales DC, 1991; Görres G, 1996; Greenspan SL, 1991; Heijckmann AC, 2005; Jódar E, 2001; Mirzaei S, 1999) or controlled design (Baldini M, 2002; De Rosa G, 1995; Diamond T, 1991; Eftekhari M, 2008; Franklyn JA, 1992; Frusciante V, 1998; Giannini S, 1994; Marcocci C, 1994; Marcocci C, 1997; Nuzzo V, 1998; Paul TL, 1988; Reverter JL, 2005; Reverter JL, 2010; Ribot C, 1990; Sajjinanont T, 2005; Schneider DL, 1995; Schneider P, 1991; Stěpán JJ, 1992; Toivonen J, 1998) produced some conflicting results with regard to LT_4 impact on BMD and bone metabolism.

In pre-menopausal women, most studies found no detrimental LT_4 effects on BMD at various peripheral and central skeletal sites neither in thyroid cancer patients (Eftekhari M, 2008; Florkowski CM, 1993; Franklyn JA, 1992;

Frusciante V, 1998; Gianinni S, 1994; Görres G, 1996; Görres G, 1998; Gonzales DC, 1991; Heijckmann AC, 2005; Reverter JL, 2005; Schneider P, 1991; Sajjinanont T, 2005; Stěpán JJ, 1992; Toivonen J, 1998), in goitre patients (Baldini M, 2002; De Rosa G, 1995; Nuzzo V, 1998), nor in mixed cohorts of cancer and goitre patients (Marcocci C, 1994; Ribot C, 1990) confirming baseline BMD measurement outcomes of this study.

Only three studies (Diamond T, 1991; Greenspan SL, 1991; Paul TL, 1988) yielded some opposite findings at the axial skeleton. Greenspan et al. found a decrease of trabecular BMD at the lumbar spine in a cohort including int. al. pre-menopausal thyroid cancer and goitre patients, while reduced BMD at the femoral neck was assessed by Diamond et al. in a sample of pre-menopausal thyroid cancer patients and by Paul et al. in a mixed cohort of pre-menopausal thyroid cancer and goitre patients, respectively.

In measurements of various biochemical markers of calcium and bone metabolism as well as of SHBG, results were mostly in accordance with baseline findings of this study, irrespective of underlying diagnosis and treatment characteristics (Baldini M, 2002; De Rosa G, 1995; Franklyn JA, 1992; Frusciante V, 1998; Giannini S, 1994; Gonzales DC, 1991; Greenspan SL, 1991; Marcocci C, 1994; Nuzzo V, 1998; Paul TL, 1988; Reverter JL, 2005; Schneider P, 1991; Stěpán JJ, 1992).

Three studies reported significantly higher blood levels of some bone turnover markers when compared to the respective control population. Diamond et al. assessed higher mean serum concentration of OC in pre-menopausal thyroid cancer patients on long-term LT_4 suppressive treatment than in healthy matched control subjects. In a similar patient cohort, Heijckmann et al. measured higher mean serum ICTP levels in comparison to age-matched control-sera. Those two findings were reproduced by the study of Toivonen et al.

Also in men, there was mostly no evidence of appendicular or axial BMD impairment linked to LT_4 administration neither in thyroid cancer patients

(Eftekhari M, 2008; Florkowski CM, 1993; Franklyn JA, 1992; Gonzales DC, 1991; Görres G, 1996; Görres G, 1998; Heijckmann AC, 2005; Reverter JL, 2010; Stěpán JJ, 1992; Toivonen J, 1998), in a cohort of cancer and goitre patients (Marcocci C, 1997), nor in a large investigation of patients taking LT_4 medication for various thyroid disorders (Schneider DL, 1995).

Biochemically, mainly balanced calcium homoeostasis and bone metabolism were determined in male cancer patients also not significantly different from respective controls (Franklyn JA, 1992; Gonzales DC, 1991; Reverter JL, 2010; Stěpán JJ, 1992). However, also in men, Heijckmann et al. assessed higher mean serum ICTP level as compared to age-matched control sera, while Marcocci et al. measured significantly elevated serum concentrations, but within respective reference ranges of Ca and OC in a mixed patient cohort compared to healthy controls.

Yet one study (Schneider P, 1991) showed lower total and trabecular BMD at the ultra-distal radius as well as lower mean serum P and higher mean serum AP levels in male thyroid cancer patients compared to healthy controls, which might be attributed not only to LT_4 suppressive treatment, but to calcitonin deficiency. In accordance to Schneider et al., another study (Mirzaei S, 1999) accounted thyroidectomy rather than LT_4 medication for lower BMD at the lumbar spine and total hip in male goitre patients.

Jódar et al. (Jódar E, 2001) considered small detrimental LT_4 effects on BMD at the lumbar spine and femoral neck in male thyroid cancer patients comparable to BMD losses at the same skeletal sites in newly diagnosed hyperthyroid patients affected by Graves' disease on carbimazol therapy. Male patients receiving LT_4 medication, though, showed significantly lower mean serum AP and OC levels than men diagnosed with Graves' disease.

5. Summary (English)

The objective of this prospective observational controlled study was to evaluate potential effects and dose-response relationship of LT_4 administration on BMD, parameters of bone and muscle strength, and biochemical variables of calcium homoeostasis and bone turnover.

Ninety-seven men and pre-menopausal women after near total thyroidectomy and ^{131}I remnant ablation for well-differentiated thyroid carcinoma or after strumectomy for non-toxic goitre were stratified by degree of TSH suppression and by gender in three subgroups: 28 men and 46 women on LT_4 suppressive treatment and 23 women on LT_4 replacement therapy. Patients were matched for age, gender and BMI to 89 healthy controls with a negative history of thyroid disease. Patients and controls were followed and studied for a mean time of 1.1±0.2 years. Peripheral volumetric total and trabecular BMD as well as bone strength (pQCT) were determined at the ultra-distal radius. Central areal BMD (DXA) was measured at the lumbar spine, left and right femoral neck as well as left and right total hip. Maximum grip strength (dynamometer) of the non-dominant forearm and serum markers of calcium and bone metabolism were assessed.

Sufficient statistical power was ensured by reasonable sample size and measuring precision of methods applied. Beside the fact that this was one of the very few studies recruiting men, only pre-menopausal women were enclosed to control for confounders, int. al. oestrogen deficiency and age.

BMD at the axial skeleton and muscle strength were not impaired by LT_4 medication irrespective of gender, underlying diagnosis or treatment regimen. By contrast, a general trend of inversely affected total and trabecular BMD and of decreased bone strength was detected at the ultra-distal radius.

Only in women on LT_4 suppressive treatment, loss of total BMD at the ultra-distal radius reached a level of high significance. In women on LT_4 replacement therapy, a significant decline of maximum grip strength appeared in comparison

with female controls, while appendicular total and trabecular BMD as well as bone strength remained unchanged and did not differ from respective controls. In men on LT_4 suppressive treatment, greater reduction of bone strength as compared to female thyroid cancer patients was marginally significant.

Calcium balance was stable and serum concentrations of bone metabolism markers levelled off or rather decreased contradicting (high turnover) bone loss.

The study did not reveal any dose-related differential influence of LT_4 administration either on primary or secondary study endpoints in female patients.

The impairment of total BMD at the ultra-distal radius was attributable to cortical bone loss, which was not compensated by increased trabecular BMD. The lack of detrimental LT_4 impact on central skeletal sites might be partially due to a weight-bearing effect.

In women receiving LT_4 suppressive medication, sustained muscle strength might have preserved bone strength at the ultra-distal radius, although decrease of total BMD was highly significant and the most marked harmful LT_4 effect. Also in women on LT_4 replacement therapy, exposed to an even higher mean cumulative LT_4 dose, physical stresses and strains still seemed large enough to maintain existing bone strength and BMD at the appendicular measuring site.

A gender-related difference of bone strength in response to LT_4 suppressive treatment might not be excluded, as male thyroid cancer patients showed greater decline of bone strength despite unaffected peripheral BMD and muscle strength.

In contrast to cross-sectional investigations evaluating only prevalence of defined study endpoints, the prospective longitudinal design examined the association between LT_4 exposure and annual change rates of the assessed parameters. Possible explanations for inconsistent results of other longitudinal

studies might be due to differences regarding patient characteristics. These included age, gender or menopausal state, concomitant diseases, (prior) metabolic thyroid state, underlying diagnosis, previous and current treatment regimens. Also methodological differences such as data sourcing and presentation, sample size, time period of follow-up, lack or poor matching of a healthy control population, inhomogeneous composition of patient cohorts, techniques and skeletal sites of BMD measurement as well as laboratory procedure of biochemical assessment contributed to inconsistencies.

In conclusion, there was only little evidence of adverse LT_4 effects. For the most part, LT_4 administration irrespective of degree of TSH suppression was not associated with low or accelerated loss of BMD at the peripheral and central skeleton and loss of bone and muscle strength, a finding also confirmed biochemically. The ultra-distal radius as a non-weight bearing skeletal site might be at risk for BMD reduction. According to the results, pre-menopausal women on LT_4 suppressive therapy might be at risk of bone loss.

The more complex approach of this study also took into account biomechanical qualities of bone material as well as structural and geometrical characteristics of bone architecture implying a causal muscle-bone interrelationship.

For future prospective longitudinal studies, the preferred clinically relevant outcome should rather be incidence of bone fracture to reveal a real causal relationship between LT_4 exposure and bone.

6. Summary (German)

Diese prospektive, kontrollierte Beobachtungsstudie untersuchte den Langzeiteinfluß einer medikamentösen Behandlung mit LT_4, auch unter dem Aspekt einer Dosis-Wirkungsbeziehung, auf periphere und axiale Knochendichte, periphere Knochenfestigkeit und Muskelkraft (d.h. primäre Studienendziele) sowie auf Laborparameter des Kalziums- und Knochenstoffwechsels (d.h. sekundäre Studienendziele) bei Schilddrüsenkarzinom- und Strumapatienten/-innen.

Das Patientenkollektiv umfasste 97 Männer und prämenopausale Frauen unter LT_4-Dauermedikation wegen Thyroidektomie und ^{131}I Ablationstherapie bei differenziertem Schilddrüsenkarzinom oder einer Strumektomie bei euthyreoter Struma. Die Patienten wurden nach Geschlecht sowie Grad der TSH-Suppression, der abhängig von der vorliegenden Schilddrüsengrunderkrankung war, in drei Gruppen eingeteilt: 28 männliche und 46 prämenopausale Karzinompatienten/-innen unter TSH-suppressiver Therapie sowie 23 prämenopausale Strumapatientinnen unter physiologischer Schilddrüsenhormonsubstitution. 89 gesunde Freiwillige mit negativer Schilddrüsenanamnese dienten als Kontrollen, die bezüglich Alter, Geschlecht und BMI angepaßt waren. Patienten und Kontrollen wurden bei Studieneintritt und nach einem Zeitraum von 1.1±0.2 Jahren wiederholt untersucht. Bestimmt wurden die periphere volumetrische totale und trabekuläre Knochendichte sowie die periphere Knochenfestigkeit am ultra-distalen Radius (pQCT), die axiale planare Knochendichte an der Lendenwirbelsäule, am linken und rechten Schenkelhals sowie an der linken und rechten Hüfte (DXA), die maximale Griffstärke des nicht-dominanten Unterarms (Dynamometer) sowie Laborparameter des Kalziums- und Knochenstoffwechsels.

Als eine der wenigen, rekrutierte diese Studie männliche Probanden und schloss zudem nur prämenopausale Frauen ein, um wichtige Störfaktoren wie Östrogenmangel und Alter im Hinblick auf den Parameter Knochendichte zu kontrollieren. Die hohe Fallzahl der einzelnen Patientengruppen sowie die hohe

Messgenauigkeit der angewandten Techniken bzw. Geräte sicherten eine ausreichende statistische Aussagekraft.

Die langfristige Gabe von LT_4 verringerte weder die Knochendichte am axialen Skelett noch die periphere Muskelkraft. Am ultra-distalen Radius zeigte sich eine Tendenz zu verminderter Knochenfestigkeit sowie zu verringerter totaler Knochendichte bei erhöhter trabekulärer Knochendichte. Nur bei Frauen unter TSH-suppressiver Therapie erreichte der Verlust an peripherer totaler Knochendichte ein hohes Signifikanzniveau. Frauen in Substitutionsbehandlung wiesen im Vergleich zu den weiblichen Kontrollen einen signifikanten Verlust an peripherer Muskelkraft auf bei unveränderter peripherer totaler und trabekulärer Knochendichte sowie unveränderter peripherer Knochenfestigkeit. Männer unter TSH-suppressiver Therapie zeigten tendenziell einen größeren Verlust an peripherer Knochenfestigkeit als Frauen. Die biochemischen Resultate, die einen normalen Kalziumhaushalt sowie konstante bzw. abfallende Serumkonzentrationen an Knochenstoffwechselmarkern umfassten, untermauerten die ausgebliebenen bis geringfügigen morphologischen Veränderungen am Knochen. Weder bei primären noch sekundären Studienendzielen lies sich eine Dosis-Wirkungsbeziehung nachweisen.

Die verminderte periphere totale Knochendichte ist auf einen Verlust an kortikaler Knochendichte zurückzuführen, der nicht vollständig durch die erhöhte trabekuläre Knochendichte ausgeglichen wurde. Am axialen Skelett könnte die körpergewichtstragende Funktion und damit die Knochenneubildung stimulierende Wirkung einem Verlust an Knochendichte entgegengewirkt haben. Die unveränderte periphere Muskelkraft bei Frauen unter TSH-suppressiver Therapie hat möglicherweise zu einer unveränderten peripheren Knochenfestigkeit beigetragen trotz eines hoch signifikanten Verlustes an peripherer Knochendichte. Auch bei Frauen in Substitutionsbehandlung, die einer höheren durchschnittlichen kumulativen Dosis ausgesetzt waren, war die periphere Muskelkraft wahrscheinlich groß genug, um die periphere Knochendichte und -festigkeit ausreichend kompensatorisch aufrechtzuerhalten. Ein geschlechtsspezifischer Unterschied hinsichtlich der peripheren Knochenfestigkeit konnte bei Patienten unter TSH-suppressiver

Therapie nicht ausgeschlossen werden. Männer zeigten einen größeren Verlust, obwohl periphere Knochendichte und Muskelkraft unverändert blieben.

Querschnittsstudien erfassen lediglich die Prävalenz der definierten Studienendziele. Dagegen untersuchte diese prospektive, longitudinal angelegte Studie die Assoziation zwischen einer langfristigen LT_4-Therapie und der jährlichen Veränderungsrate der erhobenen Parameter. Mögliche Erklärungen für widersprüchliche Ergebnisse anderer prospektiver Studien sind in Unterschieden hinsichtlich Ein- und Ausschlußkriterien des Patientenkollektivs sowie methodischen Aspekten und praktischen Vorgehensweisen zu suchen.

Eine langfristige medikamentöse Behandlung mit LT_4 wirkte sich nicht bzw. nur sehr geringfügig nachteilig auf die (Funktions-)Einheit von Knochen und Muskel aus. Die Gabe von LT_4 war weder assoziiert mit niedrigen Ausgangswerten peripherer und axialer Knochendichte, peripherer Knochenfestigkeit und Muskelkraft noch mit einem (beschleunigten) Verlust an peripherer und axialer Knochendichte, peripherer Knochenfestigkeit und Muskelkraft. Dieses Resultat spiegelte sich auch biochemisch wider. Der ultra-distale Radius könnte einen hinsichtlich Knochendichteverlustes gefährdeten Skelettabschnitt darstellen. Für die Patientengruppe der Frauen unter TSH-suppressiver Therapie könnte sich daraus ein erhöhtes Risiko für periphere Frakturen ergeben.

Diese Studie berücksichtigte auch bisher außer Acht gelassene biomechanische, strukturelle und geometrische Eigenschaften von Knochensubstanz und -architektur, denen eine eng miteinander verknüpfte, sich gegenseitig stark beeinflussende Funktionseinheit von Knochen und Muskel zugrunde liegt.

In zukünftigen prospektiven Studien sollte die Inzidenz von Knochenfrakturen bzw. das Frakturrisiko als klinisch relevantes, primäres Studienendziel definiert werden, um einen kausalen Zusammenhang zwischen einem möglichen schädlichen Langzeiteinfluss auf den Knochen und einer Dauermedikation mit LT_4 zu prüfen.

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