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ORIGINAL PAPER

Radon balneotherapy and physical activity for osteoporosis prevention: a randomized, placebo-controlled intervention study

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Abstract Low-dose radon hyperthermia balneo treatment (LDRnHBT) is applied as a traditional measure in the non-pharmacological treatment of rheumatic diseases in Europe. During the last decades, the main approach of LDRnHBT was focused on the treatment of musculoskeletal disorders, but scientific evidence for the biological background of LDRnHBT is weak. Recently, evidence emerged that LDRnHBT influences bone metabolism. We investigated, whether combined LDRnHBT and exercise treatment has an impact on bone metabolism and quality of life in a study population in an age group at risk for developing osteoporosis. This randomized, double-blind, placebo-controlled trial comprised guided hiking tours and hyperthermia treatment in either radon thermal water (LDRnHBT) or radon-free thermal water (PlaceboHBT). Markers of bone metabolism, quality of life and somatic complaints were evaluated. Statistics was performed by linear regression and a linear mixed model analysis. Significant changes over time were observed for most analytes investigated as well as an improvement in self-assessed health in both groups. No significant impact from the LDRnHBT could be observed. After 6 months, the LDRnHBT group showed a slightly stronger reduction of the osteoclast stimulating protein receptor activator of nuclear kB-ligand compared to the PlaceboHBT group, indicating a possible trend. A combined hyperthermia balneo and exercise treatment has significant immediate and long-term effects on regulators of bone metabolism as well as somatic complaints. LDRnHBT and placeboHBT yielded statistically equal outcomes.

Keywords Osteoporosis · Balneotherapy · Exercise · Radon

Introduction

Osteoporosis is a widespread systemic skeletal disease characterized by decreased bone mass leading to increased fracture risk. The WHO estimates that osteoporosis is responsible for 1700 fractures per day in the European Union (Johnell AND Hertzman 2006) and that incidence rates will rise worldwide dramatically due to a constantly ageing population. Hence, osteoporosis poses an enormous burden to the healthcare systems. The costs due to osteoporosis fragility fractures were estimated to be 799 million euro in Austria for 2010 and to increase by 28 % to 1,025 million euro in 2025 (Svedbom et al. 2013). According to recent estimates, the total of costs for treatment of osteoporosis will duplicate from 2000 to 2050, i.e., they will increase from 40 to 80 billion euro for the countries of the European Union (Dorner et al. 2009). Since fractures are

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associated with morbidity, pain, hospitalization and an increased risk of mortality, the development of strategies for the prevention and therapy of osteoporosis is a major public health concern (Bliuc et al. 2009). On the basis of these data and the fact of an "over-aged"-population, it is urgently necessary to find appropriate ways to reduce the financial burden on the healthcare system.

At the molecular level, osteoporosis is considered mainly to result from imbalanced bone remodeling, i.e., a predominance of bone reabsorption over its formation. The coupling of osteoclastic and osteoblastic bone reabsorption and formation, respectively, is under the tightly orchestrated control of various hormones, growth factors, cytokines and vitamins (Eriksen 2010). Among them, the RANKL/RANK/OPG pathway plays a crucial role in this complex maintenance of balance by regulating the differentiation and activation of osteoclasts (Trouvin and Goeb 2010; Boyce and Xing 2007). The Receptor activator of nuclear kB-ligand (RANKL) is the key regulator of osteoclast differentiation and activation. By binding to its receptor RANK, it promotes osteoclast-mediated bone reabsorption and osteolysis. In contrast, osteoprotegerin (OPG) inhibits bone reabsorption by binding to RANKL and preventing its interaction with RANK. A great variety of hormones, cytokines and growth factors have been implicated in the regulation of and interactions with the RANKL/RANK/OPG pathway, and the disturbance of the OPG/RANKL ratio is considered to be critical in the pathogenesis of numerous bone diseases including osteoporosis (Hofbauer and Schoppet 2004).

Adequate nutrition, especially sufficient intake of vitamin D and calcium (Bonjour et al. 2012; Gennari 2001), the avoidance of risk factors such as tobacco smoking and abuse of alcohol (Fini et al. 2012) and regular exercise are the most effective non-pharmacologic measures preventing osteoporosis, whereas a sedentary lifestyle and low body mass index are considered risk factors. Several in vitro studies have confirmed that mechanical loading plays an important role in bone remodeling and that the expression of OPG is enhanced and RANKL expression reduced in human osteoblasts following mechanical stress (Kusumi et al. 2005). Numerous clinical trials have confirmed the effects of exercise programs on bone metabolism and inflammatory markers in postmenopausal women, a high risk group for osteoporosis (Bergstrom et al. 2012; Roghani et al. 2012; Tartibian et al. 2011).

Chronic inflammation is another risk factor for systemic bone loss. Several pro-inflammatory cytokines such as TNFα, interleukin (IL-) 1, Il-6 and Il-17 influence bone remodeling by directly or indirectly up-regulating RANKL (De Martinis et al. 2006).

Low-dose radon hyperthermia balneotherapy/treatment (LDRnHBT) is a non-pharmacological treatment option for various inflammatory rheumatic diseases applied in more than 15 medical spas in Germany, Austria, Poland and the Czech Republic alone (Doerfelt 2014). It uses the application of low doses/activities of the radioactive noble gas ^{222}Rn and several randomized controlled clinical trials reported significant long-term improvement of pain after radon baths or radon speleotherapy in patients with *degenerative spinal disease*, *rheumatoid arthritis* and *ankylosing spondylitis* (Falkenbach et al. 2005; Franke and Franke 2013; Moder et al. 2011). However, the biological mechanisms underlying the possible effects of radon therapy and balneotherapy in general remain unclear (Verhagen et al. 2007) and controlled studies of radon therapy on relevant biomarkers are sparse. Shehata et al. showed a significant increase of the anti-inflammatory cytokine transforming growth factor-β1 (TGF-β1) after combined LDRnHBT and exercise treatment in patients with *ankylosing spondylitis*. The rise in TGF levels correlated with a significant reduction of pain in this study population (Shehata et al. 2006). As TGF-β1 stimulates the early differentiation of osteoblast cells and plays an essential role in the coupling of bone formation and bone reabsorption (Janssens et al. 2005; Zuo et al. 2012), these results suggest an influence of combined exercise and LDRnHBT on bone metabolism. Of note, from recent studies, evidence has emerged that LDRnHBT indeed may positively influence bone metabolism by modulating the OPG/RANKL system (Lange et al. 2013).

In light of these findings, we performed the current randomized, double-blind, placebo-controlled study to test whether a combined exercise and LDRnHBT have detectable effects on key markers and endocrine regulators of bone metabolism as well as on self-reported somatic complaints and quality of life as assessed by questionnaires in a healthy study population at the risk age for developing osteoporosis.

Methods

Study design and setting

We performed a longitudinal, randomized, double-blind, placebo-controlled intervention trial to investigate the effects of combined exercise and LDRnHBT on relevant bone formation and reabsorption markers. The study members participated in a combined balneotherapy and exercise program. The intervention group bathed in radon water, whereas the control group bathed in degassed thermal water. The primary outcome variables were OPG for bone formation, RANKL for bone reabsorption and their ratio (OPG/RANKL) as a measure for the balance of bone metabolism. Secondary outcomes were five other

regulators and markers of bone metabolism, i.e., osteocalcin (OC), osteopontin (OPN), leptin, parathyroid hormone (PTH) and adrenocorticotropic hormone (ACTH). In addition, somatic complaints and self-reported health were assessed using validated questionnaires, the Zerssen's list and the Qualeffo-41 (von Zerssen 1973; Lips et al. 1997, 1999; Richard 1989). The study protocol was approved by the Ethics Committee of Salzburg (E1216/8-2010, ISRCTN registration ISRCTN09441803) and conducted in the health resort area of the Gastein valley in Salzburg, Austria.

Participants

Eligible participants were working married couples aged between 50 and 65. All women were *postmenopausal*. Participants were primarily recruited all over Austria through advertisement in newspapers and communication via a web page. Written informed consent was obtained from all study members. Out of 72 potential participants 64 were enrolled after screening based on inclusion and exclusion criteria. Inclusion criteria were as follows: age, the physical ability to meet the demands of the exercise program (i.e., at least 300 m of altitude difference per day) and sufficient knowledge of German to complete the questionnaires. Exclusion criteria were as follows: acute or chronic disturbances of the immune system, hyperthyreosis, cardiac arrhythmia, renal insufficiency, severe cardiovascular diseases, acute infections or fever, iritis or an acute attack of polyarthritis. In addition, participants should not have used hormone replacement therapy or any other therapy affecting the bone metabolism during the last 12 months before enrollment.

Randomization and blinding

Study subjects were allocated by random into a "radon group" and "placebo group". Randomization was computed in blocks of four with an equal treatment allocation ratio. Radon or placebo thermal water bath filling was blinded with a personalized digital ID card that provided tub filling with either radon thermal water or radon degassed thermal water (placebo) in an automatic process. In this double-blind setting, neither the participants nor the bath attendant had knowledge of the composition of the water.

Intervention

Intervention was structured into a first time of on-site treatment lasting for 1 week (Treat-1; day 0 to day 6), followed by a 6 weeks lasting off-site non-treatment interval, followed by a second on-site "brush-up" time of treatment lasting 3 days (Treat-2; day 60 to day 63), and finally, a non-treatment follow-up period till day 240. The Treat-1 program was composed of (1) five 3- to 4-h guided GPS-monitored mountain hiking tours with a minimum of 300 m altitude difference per day, and (2) five radon or placebo thermal water baths performed at the spas Felsentherme (Bad Gastein, elevation 1,002 m a.s.l.) or Alpentherme (Bad Hofgastein, elevation 859 m a.s.l.) in either radon-containing or degassed thermal water, respectively. Each bathing session lasted 20 min. Radioactivity concentrations were between 412 and 900 Bq/liter, and the water temperature was 36-39 °C. The Treat-2 program consisted of two hiking tours plus two radon or placebo baths, respectively. No specific life style recommendations were given for the non-treatment periods.

Data collection

Forearm venous blood was collected from all participants at baseline (day 0, time point 1; T1), end of first treatment week (day 6, time point 2; T2), start and end of the brush-up (day 60 and 63, time point 3; T3 and time point 4; T4) and 6 months after the last treatment (follow-up, day 240, time point 5; T5). At each time point, 12 ml of blood from each individual were collected in tubes (BD Vacutainer® system, Becton–Dickinson AG, Vienna, Austria) according to manufacturer's guidelines and the plasma and serum aliquots immediately frozen and stored at −80 °C until analyzed.

Questionnaires assessing somatic complaints (Beschwerdenliste, von Zerssen 1976. Weinheim: Beltz Test GmbH) and quality of life (Quality of life questionnaire of the European foundation for osteoporosis; Qualeffo-41) were handed out for completion on T1, T2, T3, T4, T5 and days T1, T3, T5, respectively. Zerssen's list consists of 24 items and asks for actually perceived general and somatic complaints. Qualeffo-41 was developed by the International Osteoporosis Foundation for patients with vertebral fractures and consists of 48 questions in the domains pain, activities of daily living, jobs around the house, mobility, leisure and social activities, general health perception and mental function.

Measurements of radon concentration

^{222}Rn concentrations were measured directly from the bathing water using a Triathler Multilabel LSC single sample counter (Hidex, Finland) according to the manufacturer's instructions.

Measurements of bone marker concentrations in plasma

Measurements of individual bone marker levels were assessed with LUMINEX xMAP® technology (Millipore Corporation, 290 concord Road, Billerica, Ma 01821, USA). The following detection kits were used for the

analysis: Human Bone Panel 1A for the detection of human OPG, OC, leptin and PTH, human RANKL Single Plex for the detection of RANKL. Briefly, frozen plasma samples were thawed and centrifuged for 10 min. Twenty-five microliters of undiluted supernatant sample was used per well, with all further steps performed according to the manufacturer's protocol in 96-well plates.

Statistical analysis

Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA, version 20.0) and statistical software R GNU (General Public License, version 2.12.1). Statistical significance was set at the level of $\alpha < 0.05$ for all tests. All variables are expressed as mean ± standard deviation (SD) unless otherwise indicated. The percent changes over time of plasma concentrations and questionnaire T- and Sten-values were calculated for the PG and RG, respectively, at each time point t_i [(value t_i − value t_1)/mean t_1 *100], with mean t_1 = geometric mean of OPG/RANKL ratios at time point 1 and mean t_1 = arithmetic mean at time point 1 for all other variables.

For the linear regression analysis and linear mixed model, all values were log10-transformed. To detect short-term or long-term effects of the radon treatment and to search for influencing variables on the primary criteria OPG and RANKL and OPG/RANKL ratio and for Zerssen's list of somatic complaints, linear regression analysis was performed for T2, T3, T4, and T5. The treatment factor (radon or placebo) and the baseline values (T1) of the respective variables (OPG, RANKL, OC, leptin, OPN, ACTH and PTH) were included as predictors and further possible confounder variables were identified by the Akaike information criterion by backwards selection. The treatment factor was always kept in the model. For the five other variables (OC, OPN, leptin, PTH and ACTH), long-term effects were calculated at T3, T4 and T5. A random intercept linear mixed model with treatment and time or treatment, time and the interaction of treatment and time as fixed factors and the patient ID as a random effect was generated to account for individual differences and to analyze time and treatment effects.

Results

Study participants and baseline characteristics

Figure 1 shows the number of participants at each stage of the study. Out of 72 invited persons, 64 were included in the study according to the inclusion and exclusion criteria. Two participants did not appear for the brush-up intervention and seven participants were lost for the follow-up for personal reasons. These study subjects were excluded from the linear regression analysis and the linear mixed model calculations. Baseline characteristics do not show statistically relevant differences between the two treatment groups at the start (Table 1).

OPG and RANKL and the OPG/RANKL ratio

Figure 2 shows the longitudinal relative change of mean values in plasma OPG and RANKL concentrations with respect to the baseline levels for both treatment groups. In both groups, OPG rose from T1 to T2, i.e., during the first intervention week (Treat-1). Interestingly, there was a further strong increase in OPG from T2 to T3 and from T2 to T4 in the placebo group and radon group, respectively. The increase from T3 to T4, i.e., during the brush-up period (Treat-2) was slightly more pronounced in the radon group compared to the placebo group. After 6 months (T5, day 240), the OPG concentration decreased approximately to the baseline levels (Fig. 2a).

RANKL concentrations showed a strong, sustained and significant decline in the entire study population from Treat-1 to Treat-2 and even throughout the follow-up period. At T5 (day 240), the mean RANKL levels were lower at borderline significance in the radon group compared to the placebo group ($p = 0.0783$).

The changes in OPG and RANKL resulted in a significantly elevated OPG/RANKL ratio from time Treat-1 to Treat-2 and a subsequent decrease from T4 to T5 in both groups (Fig. 2c).

Linear regression analysis was performed to identify short-term and long-term effects of LDRnHBT on the primary outcome variables OPG, RANKL and OPG/RANKL ratio. The baseline levels of all measured bone metabolism regulating factors and markers were included in the regression analysis as predictors of OPG and RANKL concentrations and the OPG/RANKL ratio at all time points. Although the time courses (Fig. 2c) suggest differences across the two treatment groups over time, no statistically significant difference between the LDRnHBT and the placeboHBT group could be demonstrated for all time points in the linear regression analysis (Table 2). To take account of the high variations in individual OPG and RANKL concentrations within the two treatment groups, in addition a linear mixed model approach was applied to the data. No significant impact of the LDRnHBT intervention could be detected when time, intervention and their interaction where included in the model (Table 2, linear mixed model 1, LMM1). The p value of the interaction of treatment and time at T5 (day 240) with RANKL as outcome variable just misses significance ($p = 0.0783$). Likewise, the p values for the OPG/RANKL ratio at T4 ($p = 0.1431$) and T5 ($p = 0.1346$) indicate borderline significances.

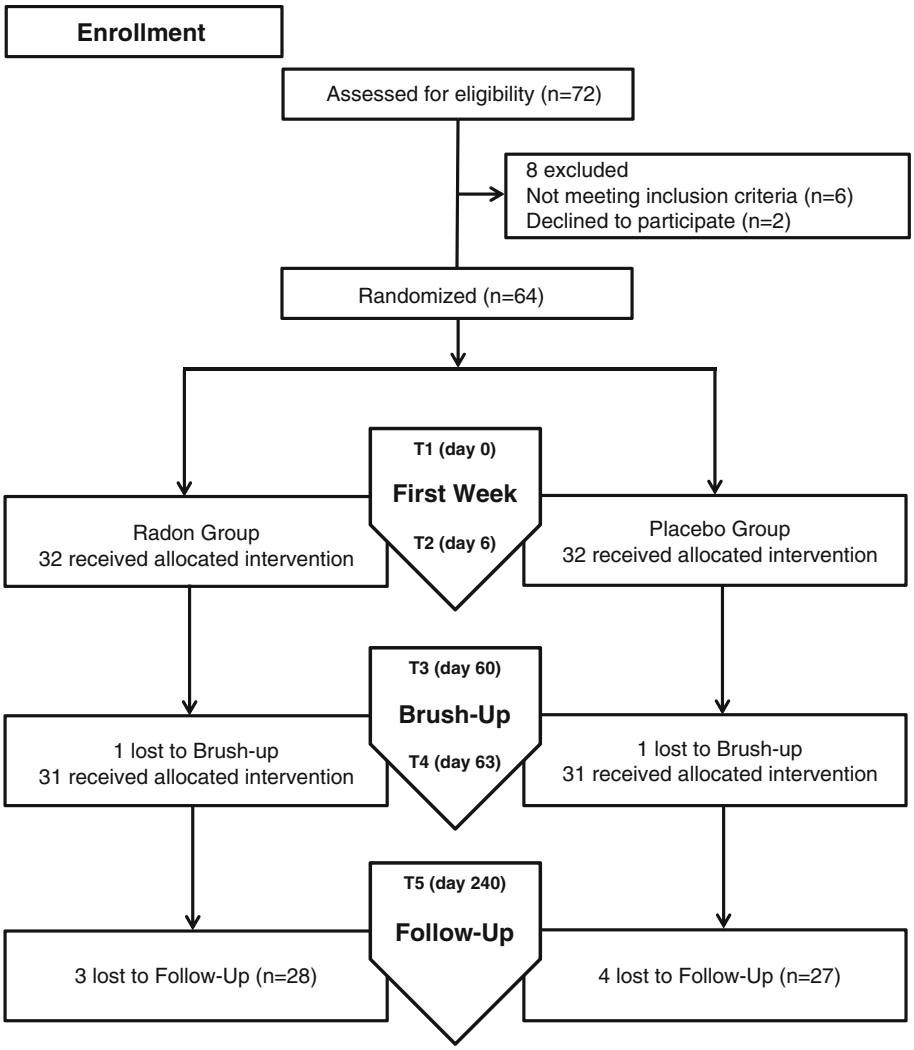

Fig. 1 Study flow chart

However, when the interaction of time and treatment are excluded from the model, a significant change over time can be demonstrated for nearly all time points in both groups (Table 2, linear mixed model 2, LMM2).

Since both men and women were included into the study population and women develop osteoporosis and osteoporotic fractures about 10 years earlier than men (Campion and Maricic 2003) we investigated whether there was a difference in the main outcome variables OPG, RANKL and the OPG/RANKL ratio between female and male participants. A linear mixed model approach did not reveal any statistically relevant influence of LDRnHBT when sex was included as a fixed factor (data not shown).

Other humoral regulators and markers of bone metabolism

In addition to OPG and RANKL, five other markers and key regulators of bone metabolism were analyzed, i.e., OC, OPN, PTH, leptin and ACTH. With the exception of ACTH, all parameters showed significant changes over time compared to the baseline values (Fig. 3a–e). No statistically measureable difference of the LDRnHBT group compared to the placeboHBT group could be detected, either by linear regression analysis or by the linear mixed model approach (Table 2).

Osteocalcin and osteopontin, both components of the non-collagenous bone matrix, exhibited significantly elevated plasma levels after Treat-1 ($p = 0.000$, T2) and Treat-2 in both groups ($p = 0.000$, T4).

Parathyroid hormone declined during Treat-1, regained the initial value during the non-intervention interval and declined again during Treat-2. Interestingly, there was a further additional decrease in both groups till T5 (day 240; $p \leq 0.001$).

A similar time course can be observed for leptin which declined significantly in the whole study population during Treat-1 ($p = 0.0176$, T2), regained the initial value during the non-intervention interval, did not change during Treat-2

but subsequently strongly declined till T5 (day 240; $p = 0.000$).

During Treat-1, ACTH remained unchanged in the placeboHBT group, but increased in the LDRnHBT group. Subsequently, ACTH levels declined in the whole study population. No significant effect of treatment or time arose on ACTH levels in linear regression or linear mixed model analysis. Complete results of the linear regression analysis including predictors of all outcome variables can be found in the supplement.

Table 1 Baseline characteristics of the study participants

	Placebo	Radon
Number	32	32
Sex		
Female	17	15
Male	15	17
Age (years)	55.94 ± 4.25	57.00 ± 5.10
Height (m)	1.72 ± 0.94	1.72 ± 0.08
Weight (kg)	77.34 ± 17.61	77.13 ±15.49
BMI	26.06 ± 4.68	26.09 ± 4.98
OPG (pg/ml)	348.64 ± 95.95	373.64 ±73.98
RANKL (pg/ml)	145.60 ± 159.42	162.18 ± 161.47
OC (pg/ml)	6,579.88 ± 2,042.64	7,521.10 ± 3,896.72
Leptin (pg/ml)	3,403.04 ± 2,700.19	3,678.94 ± 3,435.52
OPN (pg/ml)	18,310.11 ± 12,289.02	22,627.37 ± 27,598.42
PTH (pg/ml)	82.02 ± 29.14	84.98 ± 24.87
ACTH (pg/ml)	13.86 ± 15.11	10.89 ± 22.86
ZLSC (*T* values)	50.42 ± 8.09	52.26 ± 8.05

Data are presented as the mean ± SD

BMI body mass index, *OPG* osteoprotegerin, *RANKL* receptor activator of nuclear κB ligand, *OC* osteocalcin, *OPN* osteopontin, *PTH* parathyroid hormone, *ACTH* adrenocorticotropic hormone, *ZLSC* Zerssen's list of somatic complaints

Questionnaires: complaints list and quality of life

Individually experienced health and changes in quality of life were recorded by Zerssen's list of somatic complaints (von Zerssen 1976) and the Qualeffo-41 questionnaire (Lips et al. 1997). As shown in Fig. 3f, a significant decline in self-reported health complaints occurred after Treat-1 ($p \leq 0.000$, T2) and Treat-2 ($p \leq 0.000$, T4), which remained reduced until T5 (day 240; $p < 0.0004$). This decline seems more pronounced in the placebo group but the statistical analysis did not reveal a significant difference between the two treatment groups ($p = 0.1923$). Qualeffo-41 questionnaire was handed out at three time points (T1, T3 and T5) and yielded a significant reduction ($p = 0.042$) of pain (domain Qualeffo-Pain) in the whole study population 6 months after treatments (T5) (data not shown).

Individual changes of balance and bone turnover rates

Bone marker plots are a recently developed tool to visualize the balance of bone formation and bone reabsorption together with the rate of bone turnover. We applied this method to further characterize our study population. Plasma concentrations of OPG as bone formation marker and RANKL as bone reabsorption marker were used to obtain balance, rate of turnover and the 95 % confidence ellipse as described by Bieglmayer and Kudlacek (Bieglmayer and Kudlacek 2009). Figure 4a shows the bone marker plot of the whole study population at baseline (day 0, T1). The left upper quadrant of the four-field graph represents study participants with prevailing reabsorption and a high turnover rate indicating an already existing condition favoring osteopenia or osteoporosis. Further analysis of this subgroup showed a shift in balance toward bone formation after both treatment phases and a reduction of the bone turnover rate after sixth months (T5, Fig. 4b). In a next step, we selected a subpopulation of individuals who responded with a minimum rise of 20 % in the OPG/

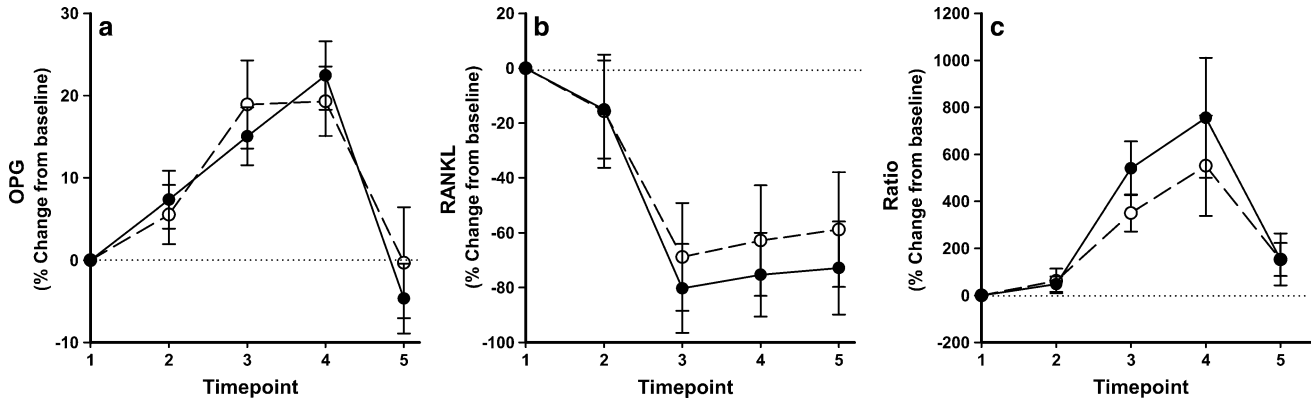

Fig. 2 Relative change of means of plasma concentrations of OPG (**a**) and RANKL (**b**) and the OPG/RANKL ratio (**c**) relative to baseline. Mean ± SEM. *Black circle* radon group, *white circle* placebo group

Table 2 Linear regression and linear mixed model

dv	Linear regression analysis					dv	Linear mixed model			
	Time	Iv	Estimate	95 % CI	*p* value		LMM1		LMM2	
							fe	*p* value	fe	*p* value
OPG						OPG				
									Treat	0.1286 n.s.
	T2	Treat	1.0144	0.9231–1.1147	0.7632 n.s.		Treat × T2	0.7967 n.s.	T2	0.0709
	T3	Treat	1.0048	0.9024–1.1189	0.9286 n.s.		Treat × T3	0.7040 n.s.	T3	0.0000***
	T4	Treat	1.0486	0.9569–1.1490	0.3040 n.s.		Treat × T4	0.8090 n.s.	T4	0.0000***
	T5	Treat	0.9880	0.8662–1.1268	0.8539 n.s.		Treat × T5	0.4131 n.s.	T5	0.4789 n.s.
RANKL						RANKL				
									Treat	0.4452 n.s.
	T2	Treat	1.0825	0.7088–1.6532	0.7092 n.s.		Treat × T2	0.9119 n.s.	T2	0.1823 n.s.
	T3	Treat	0.9048	0.5874–1.3937	0.6447 n.s.		Treat × T3	0.1961 n.s.	T3	0.0000***
	T4	Treat	0.8812	0.5139–1.5110	0.6405 n.s.		Treat × T4	0.1398 n.s.	T4	0.0000***
	T5	Treat	0.8933	0.5796–1.3769	0.6029 n.s.		Treat × T5	0.0783	T5	0.0000***
Ratio						Ratio				
									Treat	0.2535 n.s.
	T2	Treat	0.8994	0.5731–1.4113	0.6391 n.s.		Treat × T2	0.8705 n.s.	T2	0.0939
	T3	Treat	1.0678	0.6967–1.6365	0.7596 n.s.		Treat × T3	0.2496 n.s.	T3	0.0000***
	T4	Treat	1.1970	0.6704–2.1371	0.5370 n.s.		Treat × T4	0.1431 n.s.	T4	0.0000***
	T5	Treat	1.2110	0.7514–1.9518	0.4243 n.s.		Treat × T5	0.1346 n.s.	T5	0.0000***
OC						OC				
									Treat	0.8806 n.s.
							Treat × T2	0.9672 n.s.	T2	0.0010***
	T3	Treat	0.9721	0.8096–1.1673	0.7577 n.s.		Treat × T3	0.6343 n.s.	T3	0.0000***
	T4	Treat	0.9900	0.8681–1.1290	0.8790 n.s.		Treat × T4	0.7781 n.s.	T4	0.0002***
	T5	Treat	0.9214	0.7498–1.1321	0.4286 n.s.		Treat × T5	0.2471 n.s.	T5	0.0000***
Leptin						Leptin				
									Treat	0.4185 n.s.
							Treat × T2	0.8222 n.s.	T2	0.0176*
	T3	Treat	0.8402	0.6209–1.1368	0.2537 n.s.		Treat × T3	0.3414 n.s.	T3	0.5227 n.s.
	T4	Treat	0.9720	0.7584–1.2456	0.8194 n.s.		Treat × T4	0.9503 n.s.	T4	0.3309 n.s.
	T5	Treat	0.9169	0.5721–1.4694	0.7132 n.s.		Treat × T5	0.8185 n.s.	T5	0.0000***
OPN						OPN				
									Treat	0.7102 n.s.
							Treat × T2	0.3737 n.s.	T2	0.1515 n.s.
	T3	Treat	1.1248	0.7522–1.6820	0.5605 n.s.		Treat × T3	0.4689 n.s.	T3	0.0000***
	T4	Treat	1.0406	0.6832–1.5848	0.8506 n.s.		Treat × T4	0.9136 n.s.	T4	0.0000***
	T5	Treat	1.1143	0.7285–1.7045	0.6115 n.s.		Treat × T5	0.5171 n.s.	T5	0.0000***
PTH						PTH				
									Treat	0.3615 n.s.
							Treat × T2	0.3800 n.s.	T2	0.0000***
	T3	Treat	1.0251	0.7935–1.3243	0.8473 n.s.		Treat × T3	0.9306 n.s.	T3	0.5826 n.s.
	T4	Treat	1.0917	0.9473–1.2582	0.2204 n.s.		Treat × T4	0.8179 n.s.	T4	0.0014**
	T5	Treat	1.1042	0.9073–1.3440	0.3152 n.s.		Treat × T5	0.3574 n.s.	T5	0.0000***
ACTH						ACTH				
									Treat	0.1272 n.s.
							Treat × T2	0.3865 n.s.	T2	0.1087 n.s.
	T3	Treat	0.7924	0.5345–1.1747	0.2414 n.s.		Treat × T3	0.4477 n.s.	T3	0.5767 n.s.

Table 2 continued

dv	Linear regression analysis					dv	Linear mixed model			
	Time	Iv	Estimate	95 % CI	*p* value		LMM1		LMM2	
							fe	*p* value	fe	*p* value
	T4	Treat	0.7963	0.3556–1.7830	0.5734 n.s.		Treat × T4	0.6111 n.s.	T4	0.6612 n.s.
	T5	Treat	0.8515	0.2419–2.9970	0.7983 n.s.		Treat × T5	0.1845 n.s.	T5	0.6901 n.s.
ZLSC						ZLSC				
									Treat	0.1923 n.s.
	T2	Treat	1.0136	0.9557–1.0750	0.6471 n.s.		Treat × T2	0.5933 n.s.	T2	0.0000***
	T3	Treat	1.0278	0.9649–1.0947	0.3884 n.s.		Treat × T3	0.5290 n.s.	T3	0.0005***
	T4	Treat	1.0156	0.9432–1.0937	0.6763 n.s.		Treat × T4	0.7950 n.s.	T4	0.0000***
	T5	Treat	1.0311	0.9506–1.1184	0.4534 n.s.		Treat × T5	0.6470 n.s.	T5	0.0004***

MM1 linear mixed model with time and treatment and the interaction of treatment and time (time x treatment) as fixed effects, *LMM2* linear mixed model with time and treatment as fixed effects, *dv* dependent variable, *iv* independent variable, *CI* confidence interval, *fe* fixed effect, *OPG* Osteoprotegerin, *RANKL* receptor activator of nuclear κB ligand, *OC* osteocalcin, *OPN* osteopontin. *PTH* parathyroid hormone, *ACTH* adrenocorticotropic hormone, *ZLSC* Zerssen's list of somatic complaints, *Treat* treatment (placebo or radon), *T2, T3, T4 and T5 time points* T2 = day 6, T3 = day 60, T4 = day 63, T5 = day 240, *n.s.* not significant

*** < 0.001, ** < 0.01, * < 0.05, < 0.1

RANKL ratio after Treat-1. This subpopulation was denoted as "responders". Twelve participants in the radon group and eleven participants in the placebo group fulfilled this condition, with more LDRnHBT group members in the upper left quadrant. Figure 4c reveals a considerable short- and long-term improvement in both balance and turnover rate in this responder subgroup. This improvement is revealed by a contraction and a change of slope of the main axes of the 95 % confidence ellipse. The changes over time in the bone marker plots of the total study population (not shown) prompted us to investigate, how different individuals with different starting conditions responded to the treatment. Figure 4d gives an example of three individuals with unfavorable starting conditions, who showed a desirable response over time (Individual 1 and Individual 2) or no response at all (Individual 3). In comparison to those three, Individual 4 with fairly good balance and low turnover rate at the beginning did not exhibit noticeable changes over time.

Discussion

The aim of the present study was to assess the influence of combined exercise and low activity radon hyperthermia therapy/treatment (LDRnHBT) on important regulators of bone remodeling and on quality of life parameters in a randomized, double-blind controlled design. We observed significant changes over time in the concentrations of almost all analyzed bone markers and humoral factors as well as on quality of life parameters.

Osteoprotegerin increased not only during the two periods of intervention (Treat-1 and Treat-2) but also during the intermediate non-intervention period. This was paralleled by a decrease in RANKL. Of note, while OPG returned to basal levels after Treat-2, RANKL remained suppressed up to the 240 days and this was more pronounced in the radon group compared to the placebo group ($p = 0.078$). These findings could indicate a prolonged systemic up-regulation of OPG and a sustained down-regulation of RANKL by the applied treatment regimen which tends to be eventually favored by LDRnHBT. The concerted action of these factors results in a shift toward a more balanced bone metabolism as indicated by a lasting increase of the OPG/RANKL ratio favoring a mitigation of bone reabsorption and an enhancement of bone formation.

In addition, the five other parameters of bone metabolism and turnover analyzed in this study revealed similar immediate and long-term changes.

Increasing plasma levels of osteocalcin and osteopontin suggest heightened osteoblast activity and bone formation on the one hand and augmented bone remodeling most likely due to mechanical stress on the other. The osteoblast produced hormone osteocalcin is mainly considered as marker of osteoblast activity and bone formation. The significant rise over time in our study population in agreement with recently published results on the effects of exercise on osteocalcin levels (Coiro et al. 2012). Interestingly, osteocalcin is also involved in glucose metabolism by stimulating insulin production, insulin sensitivity and energy expenditure as well as adiponectin production (Confavreux 2011; Veldhuis-Vlug et al. 2013) and hence may be tightly related to the changes of leptin observed in this study (discussed below).

The changes of osteocalcin are paralleled by those of osteopontin. Osteopontin is required for stress induced

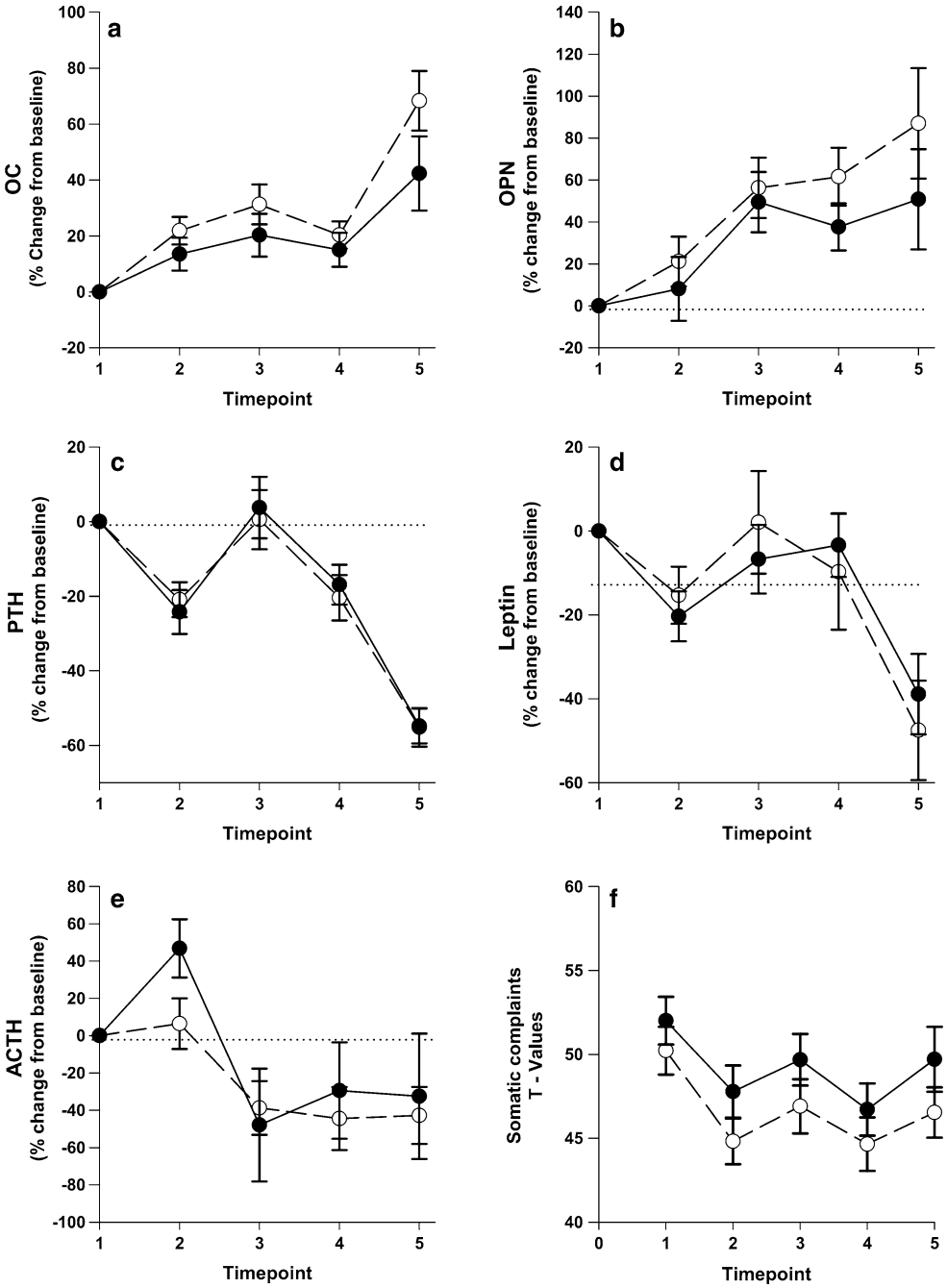

Fig. 3 Changes of plasma concentrations relative to baseline (in %) of osteocalcin (OC, **a**), osteopontin (OPN, **b**), parathyroid hormone (PTH, **c**), leptin (**d**) and adrenocorticotropic hormone (ACTH, **e**); time course of *T* values in Zerssen's list of somatic complaints (**f**), Mean ± SEM. *Black circle* radon group, *white circle* placebo group

bone reabsorption and is a potential coupling factor between reabsorption and formation in the process of bone remodeling (Ishijima et al. 2007; Terai et al. 1999). The long-term rise of osteopontin levels during the study could therefore reflect the enhanced mechanical stress loaded on the skeleton by the exercise program.

Leptin is an adipocytes-derived hormone acting as key regulator of body weight. A significant correlation between plasma leptin levels and body mass index is known (Shah and Braverman 2012) and also exists in the given study population (data not shown). Therefore, it is feasible to assume that the observed changes of leptin (Fig. 3d) might arise from changes of body fat mass caused by the exercise program. Moreover, leptin is also formed by osteoblasts and has been recognized as an important regulator of bone metabolism (Cirmanova et al. 2008; Isaia et al. 2005; Jurimae et al. 2008; Zaidi et al. 2012). Leptin's central effect on bone metabolism via the sympathetic nervous system seems to be catabolic. This is consistent with several clinical trials showing that the use of β-blockers is associated with higher bone mineral density (Pasco et al. 2004). On the other hand, in vitro and in vivo experiments in

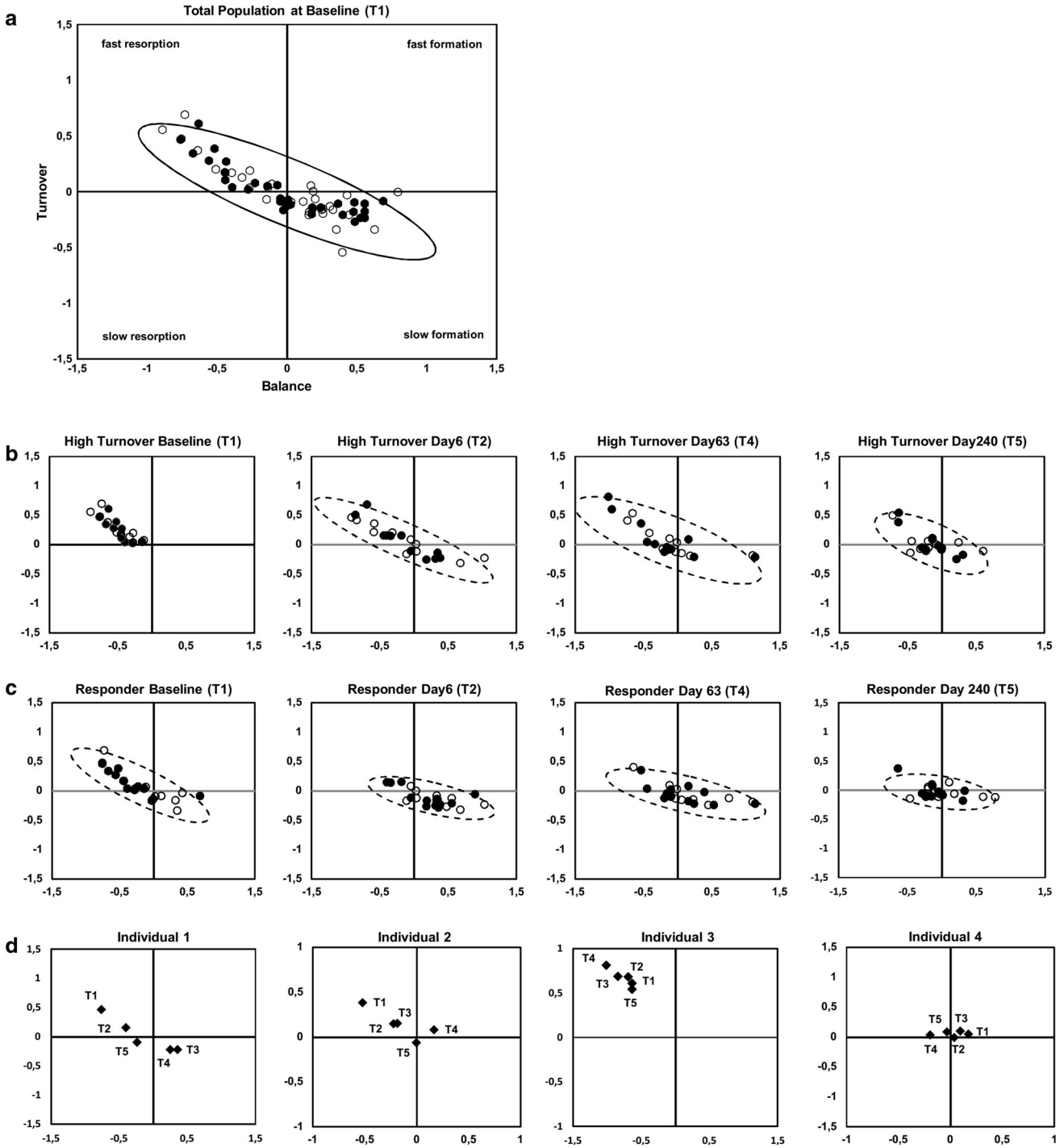

Fig. 4 **a** Bone marker plots of the total study population at baseline (T1); **b** high turnover subgroup at T1, T2, T4 and T5; **c** responder subgroup at T1, T2, T4 and T5; **d** four different study participants (Individuals 1, 2, 3 and 4) at all time points. *Black circle* radon group, *white circle* placebo group

rodents have shown a direct stimulating influence of leptin on osteoblasts and a reduction of bone fragility (Cornish et al. 2002). These results are supported by the finding that high serum levels of leptin are associated with low risk of non-traumatic fracture in men and women older than 50 years (Schett et al. 2004). Interestingly, the strongest decrease of leptin occurred after Treat-2, again indicating a sustained effect of the applied treatment regimen. Similar to leptin, PTH concentrations showed a significant decline during the periods of intervention (Treat-1 and Treat-2) as well as a sustained decrease of up to 6 months after Treat-2. Hence, both leptin- and PTH levels coincide with the

sustained decrease of RANKL. Both leptin and PTH are linked to bone metabolism via the RANK/RANKL/OPG system. High leptin levels have been shown to correlate with high levels of proinflammatory cytokines in obese patients, which in turn led to an increased osteoclastic activity and increased bone turnover via up-regulation of RANKL (Cao 2011). PTH can exert both anabolic and catabolic actions on bone metabolism. Bone reabsorption is favored by stimulated osteoclast formation and activity via the RANK/RANKL/OPG system (Silva et al. 2011) and continuous excess circulating PTH reduces bone mass by stimulating osteoclastic bone resorption via RANKL (Huang et al. 2004). Exercise has been shown to lower serum PTH levels and low PTH levels have been associated with high bone mineral density (Vainionpaa et al. 2009). The decrease of PTH during the sojourn in Gastein in the whole study population might eventually also be evoked by an increase of serum 25-hydroxyvitamin D (25-OHD) levels which can be assumed due to intensified exposure to UV-B radiation during the hiking tours. 25-OHD suppresses secretion of PTH (Lips and van Schoor 2011), and 25-OHD deficiency is associated with secondary hyperparathyroidism, which leads to an increased fracture risk (Yamauchi et al. 2011).

A stimulating effect of radon therapy on ACTH production has been described previously (Yamaoka et al. 2004) and in vitro experiments revealed enhancing effects of ACTH on osteoblast proliferation (Isales et al. 2010). ACTH also exerts pain reducing and anti-inflammatory functions via up-regulation of cortisol. Interestingly, we could detect some minor but significant correlations between ACTH levels and self-reported somatic complaints and pain (not shown). Although ACTH levels show a clearly marked rise in the LDRnHBT group during Treat-1 (48.3 % change from baseline in the LDRnHBT group, 5.6 % change from baseline in the placeboHBT group), we could not demonstrate a substantial influence of LDRnHBT on ACTH levels in the linear regression or linear mixed model analysis.

The effective dose for the patients during LDRnHBT lies considerably below the mean annual radiation dose from natural sources. Equivalent doses to skin, kidney and lung have been estimated to be at 0.12, 0.04 and 0.13 mSv, respectively, during a typical thermal bath therapy treatment in the Gastein area (Tempfer et al. 2010). To our knowledge, no noxious effects of the low-dose radon exposure have been proven and there is no hard evidence for an increased risk of radiation prone mortality in patients receiving this treatment for therapeutic reasons (Deetjen et al. 2010; Becker 2004; Takatori et al. 2013; Nghiep and Anh 2006; Becker 2003). Nonetheless, LDRnHBT has to be applied under stringent estimation of risk–benefit ratio especially to apparently healthy persons. As the parameters analyzed displayed a high degree of inter- and intra-individual variability over time within our study population, we sought to unravel individual responses to the intervention in order to eventually identify responders from non-responders using individual bone marker plots (Bieglmayer and Kudlacek 2009). Examples are shown in Fig. 4. As clearly evident, this method unravels highly heterogenic pattern of turnover and balance subtypes within our healthy study population: Some individuals with unfavorable starting conditions showed markedly improved balance and bone turnover after the intervention whereas others did not. Thus, we are able to demonstrate that bone marker plots are useful tools for monitoring exercise LDRnHBTFuture studies shall address the question whether the assessment of distinct reaction patterns by individual bone marker plots could be useful for an early detection and exclusion of non-responders and hence protecting them from unnecessary exposure to low radiation.

The rationale for the present study were the effects of radon therapy on the OPG/RANKL and TGF-β1 pathway and on functional ability, patient's global well-being and pain in patients suffering from *Ankylosing spondylitis* (Moder et al. 2010; Shehata et al. 2006; van Tubergen et al. 2001). However, while this study unravels strong and unexpected sustained effects of the combined exercise balneo treatment in both groups no apparent differences between LDRnHBT and placeboHBT could be unmasked. Several reasons may account for this: (1) The described effects of LDRnHBT on bone metabolism apply to patients suffering from inflammatory rheumatic diseases in whom deranged bone metabolism and secondary osteoporosis is a hallmark of disease. As this study was designed to investigate changes in bone metabolism in favor of osteoporosis prevention, apparently healthy subjects were enrolled in this study. LDRnHBT might simply be without effect in such a population. (2) The current study was designed for considerably shorter periods of intervention and hence LDRnHBT, i.e., 1 week of intervention followed by a 3 days brush-up weekend compared to a typical 3 weeks lasting cure regimen. Hence, the effective time of exposure to low-dose radon might be too short. (3) We were able to reduce analytical variability by applying the Luminex xMAP® technology, which allows the simultaneous measurement of numerous components in one sample. Nevertheless, high individual variability led to large standard deviations and hence did not provide sufficient statistical power to detect a difference between the two treatments. However, due to the statistical trends observed in some of the investigated parameters, possible effects of LDRnHBT cannot be ruled out. In light of the study limitations discussed, these possibilities need to be resolved in separate studies.

Physical exercise has frequently been shown to improve bone mineral density (Kelley et al. 2013; Roghani et al.

2012; Yamazaki et al. 2004). Many of the short- and long-term effects shown in our study might be attributed to the positive effects of the hiking program. The evaluation of a non-intervention group, preferably at the same time of the year, could give additional information about the specific effects of the exercise program and about the possible seasonal variations in the outcome variables. Further research should clarify the effects of exercise and change of lifestyle effects during the intervention.

Conclusion

In conclusion, the combined exercise and thermal water bath intervention regimen applied to an apparent healthy population as included in this study revealed marked and unexpectedly sustained changes in humoral regulators and markers of bone turnover as well as self-reported somatic complaints and pain. These factors certainly indicate a delicate and complex physiological interplay of central, systemic and local regulators of bone metabolism which needs to be unraveled. However, given the changes of the primary outcome parameters OPG, RANKL and the OPG/RANKL ratio, it is feasible to assume that the overall bone metabolism is tuned toward an anabolic condition. Moreover, these effects are accompanied by significant improvements of perceived quality of life and pain. Although low-dose radon hyperthermia balneo treatment does not significantly outmatch conventional thermal water treatment in this study, borderline significant differences of RANKL and the OPG/RANKL ratio indicate a possible additive effect of radon balneo treatment on the achieved biological effects. Mountain hiking is a popular form of exercise in the alpine region and a low cost non-pharmacologic intervention. Even though men develop osteoporosis later than women, as a matter of fact age-related bone loss begins at the age of fifty in both sexes (Campion and Maricic 2003). The immense and continuously rising costs caused by osteoporosis make a preventive intervention as performed in this study worth considering and useful for both men and women.

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