Alterations in bone homeostasis and microstructure related to depression and allostatic load

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Introduction

From early childhood to late adulthood, bones are continuously remodeling and adapting to environmental changes. Various conditions can disturb this remodeling process such as medical interventions, aging and metabolic bone diseases [1]. Remodeling processes require both bone resorption and formation, which are driven by osteoclasts and osteoblasts, respectively. Both cell types are affected by physiological (*e.g.* age, gender, physical activity and mobility states, hormones) and pathological conditions (*e.g.* prematurity, growth hormone deficiency, malnutrition, malabsorption, metastatic bone disease) [1], which can negatively impact a bone's capacity to adapt to present needs.

Bone remodeling can be measured through markers of bone formation (*e.g.* osteocalcin [OC], procollagen type 1 N-terminal propeptide [P1NP]) or bone resorption (*e.g.* beta-CrossLaps [beta-CTX]), both used as clinical markers of bone health. OC is an extracellular, non-collagenous protein secreted by osteoblasts during bone formation that has several hormone-like features such as an influence on energy homeostasis [2] . P1NP is a direct quantitative measure of osteoblast activity synthesizing collagen type 1, a component predominantly located in the bone matrix. It is also used in diagnosis and management of osteoporosis [2].In contrary to OC and P1NP, beta-CTX is released during bone resorption and is a specific marker for degradation fragments of mature type I collagen from bone, which is the main component (approximately 90%) of the protein matrix of bone. Bone turnover markers provide information about rates of bone loss and gain, and therefore reflect the metabolic activity of bones that is complementary to bone health measurements like bone mineral density (BMD) [3].

Recent work has provided compelling evidence that these bone markers can be affected by psychosocial stress [4]. Traumatic events and chronic stress are suspected to provoke strong and long-lasting effects on bone remodeling, mediated by activation of endocrine stress signaling pathways. To identify a possible link between pathogenic social stressors, stressful psychological states and health outcomes, different models can be applied [5-7].

The allostatic load model is one of the primary neurobiological stress models that proposes that homeostasis in the body is maintained by adaptive processes regulated by biological mediators (*e.g.* metabolites, hormones and cytokines) for stabilizing the internal milieu during and after stressful challenges from the environment [8, 9]. Allostasis is defined as a state of adaptive responsiveness to adversity, whereby the autonomic nervous system (ANS), the hypothalamic-pituitary-axis (HPA), the hypothalamic–pituitary–thyroid axis (HPT, metabolic system) and the immune system are involved in promoting physiological stability. However, repeated overstimulation of the allostatic mediators may result in chronic over-activation of the stress response systems, inducing a domino effect in the involved biological systems that ultimately leads to allostatic overload, toxic stress, pathology and finally epigenetic adaptions [10-12][13].

Multiple independent publications provide evidence that some specific allostatic mediators, such as cortisol, GH, or IGF-I, play a major role in bone remodeling processes [14-18]. Published data suggests a connection between bone homeostasis and glucocorticoids, whereby elevated cortisol levels may inhibit osteoblast proliferation, differentiation and apoptosis, leading to decreased bone mineral density. A reduction in bone mass may also be induced by glucocorticoids that furthermore inhibit GH and gonadal steroid production [19-21]. Moreover, glucocorticoids function as an important link between the endocrine stress response system and the immune system. Glucocorticoid receptors (GR) in particular account for the suppression of NF-κB and AP-1 [22], the transportation and functioning of leukocytes and the inhibition of pro-inflammatory cytokine production (*e.g.* TNF-α, IL-1, IL-6). Furthermore, GR resistance may be involved in the development of osteoporosis [23]. In addition, the stress-related corticotrophin-releasing hormone (CRH) induces the release of IL-6 [24-26] and other cytokines (*e.g.* IL-1β, TNF-α) that influence the differentiation of mesenchymal stem cells, suppress osteoblast function and initiate osteoclastogenesis and function [27, 28]. Lastly, while GH modulates osteoblast proliferation and differentiation [19-21], IGF-1 decreases bone mineral density (BMD) [29, 30]. Considering the impact of these singular allostatic mediators on bone health, a link between bone health and stress seems reasonable.

Regarding depressive disorders, several studies have shown that higher allostatic loads [31-33] furthermore play a major role in the pathogenesis of depressive disorders, linking depression to comorbid disease states such as osteoporosis [32, 34-37]. While an enhanced physiological state is e.g. tolerable while the coping with a stressful life event, the stressor and subjective burden should be terminated to avoid neuroendocrinological and neuroanatomic alterations that could result in allostatic overload and toxic stress. The ability to regain homeostasis after stressful events is dependent on the personal and social (exposome) resources [38], as well as epigenetic adaptions [13]. There is substantial evidence that mental diseases, such as depression, could be interpreted as disorders of the stress response that interacts with states of allostatic load. The influence of depressive disorders on the homeostasis of tissues such as bones [39-43] will certainly depend on whether is the person is experiencing a chronic depressive state or undergoing an acute depressive episode that may be triggered by a critical event.

Although some studies have reported decreased BMD in depressed people [44, 45], most data were obtained from epidemiological studies or studies focusing on other populations and thus lack a clear operationalization of stressor forms (*e.g.* chronic vs. traumatic), detailed biomarker sets, confounder controlling (medication, smoking and alcohol use) or longitudinal design. Thus far, no distinction has been made between chronically and episodically depressed patients and their potential alteration of bone homeostasis. Furthermore, a systematic evaluation of pathways and specifically interacting mechanisms linking psychosocial stress to important bone homeostasis and osteoporosis markers (OC, P1NP, CTX, BMD) is still missing. Hence, it is unclear whether a depressive episode leads to an adaptation of the bone metabolism and, if that is the case, to what extent an interaction with traumas, life events and allostatic load gained over life span is present. Furthermore, to the best of our knowledge, there is no study in this context that applied highly sensitive mathematic models that allow to simultaneously respect the interaction of allostatic mediators and the order of their importance, while avoiding the problem of overfitting of singular testing. This indeed would be necessary to estimate the significance of singular mediators, to derive a diagnostic

tool, and subsequently to develop individual treatment strategies for bone diseases (*e.g.* osteoporosis, arthrosis, fractures) and their effect on unspecific pain syndromes.

Therefore, in the first part of this study, we aimed to evaluate cross-sectionally: a) whether people with different levels of depressive symptomatology and psychosomatic symptom severity show an altered bone metabolism and b) whether life burden in these people is associated with the pathogenesis of depression and an altered bone metabolism and lastly c) whether allostatic load is associated with depression or life burden (comorbidity check)

After gaining knowledge about alterations of the bone metabolism within an acute depression phase, we investigate in the second study part cross-sectionally and longitudinally: 1) whether a high versus low allostatic load in depressive patients is linked to disrupted bone metabolism and microstructure and 2) whether a mathematical algorithm can select singular allostatic load mediators that could be used to identify patients at higher risk of disrupted bone metabolism, for optimized medical care.

Materials and Methods

Study procedure

Study objectives presented here, were investigated at three measurement points (t_0, t_2, t_4) of an observational multicenter study (DEPREHA) which extended over four measurement points (baseline (t_0), after 5 weeks (t_1), 5 months (t_2) and 8 months (t_3) after baseline). A subsample of 54 participants took part in a further follow-up measurement at about 18 months (*M*= 15.4, *SD* = 4.5) after DEPREHA completion (t_4). At each measurement point, trained study nurses administered a comprehensive questionnaire and collected psychological data. Blood samples were drawn (morning hours, 7-9 am) from the arm and collected in plain blood collection tubes or tubes containing EDTA, citrate or sodium fluoride for subsequent analysis by laboratory partners. Participants were instructed to stay abstinent and to only drink water during the last 12 hours prior to assessment. Participants were also instructed to avoid high amounts of coffee, tea and food (e.g. bananas, cheese, almonds, nuts, vanilla, citrus fruits) on the respective previous day. Furthermore, they should refrain from intense exercise and unscheduled medication.

Participants

n=240 participants were consecutively recruited during clinic check-ins at three study sites: a psychosomatic rehabilitation clinic (multimodal antidepressant therapy), an outpatient psychiatric practice (unimodal antidepressant therapy), and a psychotherapeutic outpatient clinic (antidepressant treatment as usual: pharmacology and psychotherapy). Power calculation for the main project (evaluation of therapy context efficacy in dependence of personal risk profiles) suggested 1-β = 0.94 for a case number of *n* = 60 per therapy group [46]. Inclusion criteria were: 18 to 65 years of age, more than 21 days absent from work within the last 12 months and a depressive episode (mainly ICD-10 F32.x or F33.x). Exclusion criteria were: pregnancy, hormonotherapy (with the exception of contraceptive and thyroid hormone therapy), inability to fill in a questionnaire, intellectual disabilities (ICD-10 F70-79) or the presence of one of the following diseases: acute infection, endocrine and metabolic disorders (e.g. diabetes mellitus type II, renal or liver diseases), neurological diseases, dementia (ICD-10 F00-F03), schizophrenia (ICD-10 F20), emotional-unstable

personality disorders (ICD-10 F60.3x), disease of the immune system, substance abuse and dependency (with the exception of nicotine). All participants were fully informed in verbal and written form about the intent and content of the study. All participants gave their written informed consent. *n* = 139 participants completed all measurements.

Instruments

Psychometric measures

Besides standardized questionnaires, information regarding demographic characteristics, physical ailments, alcohol and tobacco consumption, as well as medication intake were assessed.

Depressive symptoms and severity were assessed using the Beck Depression Inventory-II (BDI-II; [47, 48]), a 21-item self-report questionnaire that addresses current affective, cognitive, motivational and physiological symptoms of depression. Internal consistency was proven to be good with Cronbach' s Alpha 0.89 [49].

Psychosomatic symptoms were assessed using the Symptom Check List-90-revised (SCL-90-R, [50, 51]) that measures the subjective impairment caused by physical, but above all psychological symptoms in the last 7 days. The SCL-90-R consists of 90 items that are to be answered on a 5-point Likert scale from 0 (not at all) to 4 (extremely), which can be assigned to a total of nine dimension of psychopathological factors (F): somatization (F1), obsessive-compulsive (F2), interpersonal sensitivity (F3), depression (F4), anxiety (F5), hostility (F6), phobic anxiety (F7), paranoid ideation (F8) and psychoticism (F9). Three supplementary global indices give information about the severity of symptoms: Global Severity Index (GSI; basic psychological distress/burden of all symptoms), the Positive Symptom total Index (PST; count of all experienced symptoms) and the Positive Symptom Distress Index, PSDI (mean intensity of distress for the reported symptoms). Cronbach´s Alpha for SCL-90-R GSI scale in the sample was 0.96 and for all subscales (F1-F9) in a range from 0.66 (psychoticism) to 0.88 (depression).

"Life burden" was operationalized by life events that were assessed by a modified version of the "Inventory for recording life-changing events" [52]. Participants rated 34 critical life-events regarding occurrence, frequency and year of occurrence. The accumulation of critical, life-changing events was counted for every participant over the whole life span (LE_{all}) and critical time periods prior to diseases (1 year, 5 years, 10 years). Furthermore, all participants identified their three most distressful life-events and answered additional questions concerning their strain. The scores ranged between 0 (no strain) and 4 (high strain). In the presented paper only the accumulation of life-changing events scale (LE_{all} for "life burden") was used for analysis. Cronbach´s Alpha in the sample was alpha 0.83.

Physiological measures

In the main project, 21 allostatic load mediators were assessed based on the concept of allostatic load and its influence on psychosomatic disorders [12, 53]. In detail:

Glucose metabolic biomarkers: Glycosylated hemoglobin (HbA1c) was determined with HPLC Bio-Rad Variant II (Bio-Rad Laboratories, CA, USA). Plasma glucose was measured via a hexokinase enzymatic reaction using the Roche Cobas 8000 (Roche Diagnostics Ltd., Basel, Switzerland). Fasting insulin was assessed by the electrochemiluminescence enzyme immunoassay method (ECLIA), using Roche

Cobas 8000 Modul E620 (Roche Diagnostics Ltd., Basel, Switzerland). To estimate insulin resistance, the homeostatic model assessment (HOMA) index was calculated by the formula: glucose [mg/dl] × insulin [µU/ml]/405) [54]. Body mass index was available from medical records.

Lipid metabolic biomarkers: Triglycerides, HDL cholesterol, LDL cholesterol and total cholesterol were measured using Roche/ Hitachi Cobas 701/ 702 (F. Hoffmann-La Roche, Ltd. Basel, Switzerland) via enzymatic colorimetric assays.

Sympathetic nervous system biomarkers: Epinephrine, norepinephrine and dopamine were measured in plasma and analysis was performed with enzyme-linked immunosorbent assays (RE59251 for epinephrine and dopamine and RE59261 for norepinephrine; both from IBL International GmbH, Hamburg, Germany).

H*ypothalamus-Pituitary-Adrenal Axis (HPA)*: Serum-cortisol and serum-DHEA were analyzed by immunological in-vitro-test electrochemiluminescence immune assay (ECLIA) (REF 06687733 190 for serum cortisol, REF 03000087 for serum DHEA; both Roche COBAS, MODULAR ANALYTICS E17, F. Hoffmann-La Roche, Ltd. Investor Relations Basel, Switzerland). Serum-aldosterone was measured with competitive ELISA (RE52301 from IBL, International GmbH, Hamburg, Germany).

Immune System: Soluble E-selectin, soluble ICAM-1 and TNF-α were assessed with enzyme-linked immunosorbent assays (BE59011 for sICAM-1, BE59061 for sE-selectin and BE58351 for TNF- α; all from IBL International GmbH, Hamburg, Germany). Interleukin-6 was measured with immunological in-vitro-test electrochemiluminescence immune assay (ECLIA) (REF 05109442 190 from Roche COBAS MODULAR ANALYTICS E170, Ltd. Basel, Switzerland). CRP was measured with particle-enhanced turbidimetric immunoassay (Roche cobas c 701/702 F. Hoffmann-La Roche, Ltd. Investor Relations CH-4070 Basel, Switzerland) and fibrinogen with the coagulometric method (with added heparin inhibitor, see STA Evolution from Stago).

Cardiovascular markers: Systolic and diastolic blood pressure [mmHg] and heart rate [beat per minute] were available from medical records.

Bone Markers: Measurements of osteocalcin (OC), procollagen type 1 amino-terminal propeptide (P1NP) and cross-linked telopeptides (CTX) were conducted on serum samples with electrochemiluminescence immunoassays “ECLIA” from Roche COBAS Elecsys 2010 MODULAR ANALYTICS E170 (REF 12149133 122 for Osteocalcin, REF 03141071 190 for P1NP and REF 11972308 122 for CTX, F. Hoffmann-La Roche, Ltd., Basel, Switzerland). Bone mineral density (BMD) was measured by DXA bone densitometry measurement (Lunar, Prodigy Advance, GE Healthcare, Illinois, USA) in the lumbar spine (lumbar vertebral bodies L1-L4) and both hips.

Statistical Analysis

Questionnaire data were prepared along psychometric manual rules. Biomarkers were first controlled for outliers using current literature and analysis kit recommendations, and then summarized into the multi-system allostatic load score, representing allostatic mediators of five physiological systems (SNS, HPA, glucose metabolism, lipid metabolism and inflammatory immune activity). ALI scores were calculated as the sum of single biomarkers falling within the high-risk quartile of the sample. System risk values were scored from 0 to 1 indicating 0-100% of a

participant's system biomarkers fell within the high-risk range [55]. Because of a small complete case set for ALI 21 and no differences between ALI 15 and ALI 21, hypothesis calculations were mainly based on ALI 15 score. ALI 21 is presented for completeness as far as possible. Furthermore, two sub-scores were created, namely "ALI-I" and "ALI-II". The ALI-I represents primary mediators of the stress response and physiological adaptation to the challenge (first defense line including cortisol, noradrenalin, adrenalin, DHEAS, aldosterone, dopamine, IL-6 [not available: TNF-α, IGF-1]). The ALI-II represents secondary mediators, which are involved in the prolonged (mal)adaptation to chronic stress (HbA1c, triglycerides, cholesterol, LDL, HDL, fasting glucose, insulin, CRP, fibrinogen and s-ICAM-1 [not available: WHR, exclusive of BPsys, BPdia, BMI]) [56]. TNF-α was under the detection limit of the assay (value < detectable threshold 10 ng/ml) and was excluded from further calculations. Psychometric measures were prepared along the lines of manual recommendations. Finally, the data analysis was conducted using the IBM SPSS Statistics program (IBM SPSS 24.0) and R [57, 58].

Besides descriptive statistics, paired sample t-tests ($t_0 - t_0$, t_0 - t_2) and multiple regression models ($t_0 - t_0$ and for DXA t_0 - t_4) were applied for the pretest and hypotheses 1. Least Absolute Shrinkage and Selection Operator Model (LASSO) [59] were applied in hypothesis 2 for the selection of the most important allostatic mediators (of 19 potential single mediators, *n*=91 observations, $t_0 - t_0$). Because first descriptive results and paired sample t-tests indicated no significant changes either in bone biomarkers (OC, CTX, P1NP nor in allostatic load indices ALI-15, ALI-I, ALI-II from therapy start (t_0) to follow-up measurement 5 months later (t_2)), the calculation of hypothesis 1 was performed cross-sectionally with the advantage of a higher complete case set. The same procedure was done for LASSO models (only complete allostatic mediator calculation), although here significant differences between t_0 - t_2 were detected in 6 mediators (DHEA-S, fasting glucose, HbA1c, sICAM-1, adrenalin, sE-selectin see result section). All statistic models were controlled for age, gender and study sites. DXA measurement analysis were also controlled for weight.

Results

Descriptive

n = 208 patients completed the initial examination. Of those, *n* = 101 patients were from the psychosomatic rehabilitation clinic (age: *M* = 48.3, *SD* = 8.9, *f* = 86.1%, *BDI*: *M* = 25.7, *SD* = 10.3, antidepressants = 66.3%), *n* = 62 from the outpatient psychiatric practice (age: *M* = 51.6, *SD* = 9.1, *f* = 72.6%, *BDI*: *M* = 20.5, *SD* = 8.8, antidepressants = 83.9%), and *n* = 45 from the psychotherapeutic outpatient clinic (age: *M* = 36.0, *SD* = 11.5, *f* = 55.5%, *BDI*: *M* = 23.7, *SD* = 10.7, antidepressants = 42.2%). 65% of the patients suffered from severe to moderate depression and presented severe psychosomatic symptoms (GSI: 41.8%; PSDI: 42.3%). 29.3% of the sample showed higher allostatic load (values above mediansplit). Descriptive statistics are presented in table 1, 2 and 3.

Mean values of all biomarkers were within the normal range. Significant differences between allostatic load mediators from therapy start (t_0) to follow up measurement (t_2) were only seen in single biomarkers: in detail DHEAS ($t(70)$=-2.115, P=.038), fasting glucose ($t(63)$=-4.266, P<.001), HbA1c ($t(65)$=-3.367, P=.001), sICAM-1 ($t(70)$=-3.022, P=.004) decreased and adrenalin ($t(58)$=2.689, P=.009) and sE-selectin ($t(24)$=5.689, P<.001) increased over time (t_0- t_2).

A subsample of *n* = 18 patients (age: *M* = 53.8, *SD* = 6.37, *f* = 77.8%, mean body-mass index 25.6 kg/m2 (*SD* = 4.7 kg/m2)) was available for DXA bone densitometry measurements. Bone mineral density (BMD) in the trochanter region of the proximal femur was *M* = 0.942 g/cm2 (*SD* = 0.19 g/cm2). In two patients, asymmetry (defined as a *t*-value difference > 0.2) was detected. For one female, the measurement was performed unilaterally (left) because of an implanted total hip replacement (THR) in the right femur. Mean *t*-value was 0.56 (*SD* = 1.35). 16 subjects had normal scores regarding BMD. Minimal t-value was -1.3; maximum t-value was 3.3. Mean z-value was 94.6% (*SD* = 15.4%). Osteoporosis (*t*-value <-2.5) was not detected, osteopenia (*t*-value between <-1.0 and -2.5) was seen in two females.

Mean BMD in the lumbar spine (mean of lumbar vertebral bodies L1-L4) was 1.144 g/cm2 (*SD* = 0.22 g/cm2). Mean *t*-value was -0.38 (SD = 1.75). Thirteen subjects had normal scores regarding mean BMD in the lumbar spine. Minimal *t*-value was -4.1; maximum *t*-value was 2.1. Mean z-value was 100.2% (*SD* = 14.0%). Osteoporosis (defined as a *t*-value <-2.5) was detected in three females, osteopenia (*t*-value between <-1.0 and -2.5) was seen in two females. In case of discordance of the *t*-value between the trochanter region of the proximal femora and the lumbar spine, the diagnosis of the spine was assumed as overall diagnosis, because of its larger bone volume.

Table 1. Descriptive clinical characteristics (*M, SD*) at baseline (t_0, therapy start)

Clinical characteristic	***M***	***SD***	***N***	***%***	***NA***	***n.ob***
Gender (female)			157.00	75.48	0	208
Study site						
• psychosomatic rehabilitation clinic			101.00	48.56	0	101
• psychotherapeutic outpatient clinic			45.00	21.63	0	45
• outpatient psychiatric practice			62.00	29.81	0	62
Regular cigarette use (yes)			47.00	22.71	1	207
Regular alcohol use (yes)			36.00	17.56	3	205
Antidepressiva (yes)			138.00	93.24	60	148
BDI-II[a] severity					15	193
none			11.00	5.67		
minimal			23.00	11.92		
mild			33.00	17.10		
moderate			66.00	34.20		
severe			60.00	31.09		
Age (years)	46.63	11.13			0	208
Weight (kg)	78.88	17.97			47	161
Height (cm)	169.31	8.33			48	160
Body Mass Index (weight/height^2)	27.46	5.80			48	160
BDI-II sum score	23.61	10.14			15	193
LE all[b]	13.91	9.96			43	165
SCL-90[c] PSDI[c]	2.05	0.53			33	175
SCL-90 PST[d]	42.49	19.85			16	192
SCL-90 GSI[e]	1.11	0.55			34	174

[a] Beck Depression Inventory, [b] life events total score (LE all), Inventory of critical life events (ILE), [c] Symptom Check List 90 – revised (SCL-90-R) Positive Symptom Distress Index (PSDI), [d] SCL-90-R Positive Symptom total (PST), [e] SCL-90-R Global severity index (GSI)

Table 2. Descriptive statistics (*M, SD*) of biomarkers at baseline (t_0, therapy start)

at baseline (t_0, therapy start)						
Biomarker characteristics	***min***	***M***	***max***	***SD***	***NA***	***n***
AL-indices						
ALI 21[a]	0.00	3.64	11.00	2.64	46	162
ALI 15[a]	0.00	3.42	10.00	2.41	107	101
ALI-I[b]	0.00	1.50	5.00	1.19	74	134
ALI-II[c]	0.00	2.83	10.00	2.51	56	152
Metabolic markers						
HbA1c (IFCC) [mmol/mol]	26.00	33.90	43.00	3.48	56	152
Insulin [µU/ml]	1.94	8.98	27.92	5.36	51	157
HDL [mg/dL]	12.40	51.71	90.00	16.18	47	161
LDL [mg/dL]	30.00	113.86	213.00	37.49	48	160
Cholesterol [mg/dL]	63.00	193.77	322.00	48.18	50	158
Triglyceride [mg/dL]	14.88	110.38	276.00	52.77	53	155
Glucose (NaF) [mg/dL]	57.00	85.56	124.00	11.28	59	149
HOMA Index	0.40	2.04	8.00	1.50	59	149
BMI [weight/height^2]	18.07	27.44	47.75	5.78	48	160
SNS und HPA markers						
Adrenalin [pg/mL]	8.00	168.38	490.60	117.44	64	144
Aldosterone [pg/ml]	56.10	169.45	324.20	56.69	67	141
Cortisol [nmol/l]	28.50	390.73	807.50	144.45	51	157
DHEAS [µmol/l]	0.25	3.89	9.45	2.10	50	158
Dopamine [pg/mL]	4.00	32.62	259.50	47.47	72	136
Noradrenalin [pg/mL]	20.00	390.770	875.00	184.66	64	144
Immune markers						
CRP [mg/L]	0.06	0.19	0.79	0.20	52	156
Fibrinogen [mg/dL]	146.00	309.08	501.00	69.95	54	154
IL-6 [pg/ml]	1.50	2.11	7.80	1.22	47	161
TNF-α [pg/ml]	--	--	--	--	--	--
sE-Selectin [ng/ml]	13.80	52.22	102.50	17.30	101	107
Cardiovascular markers						
Blood pressure systolic [mmHg]	99.00	127.50	180.00	17.65	164	44
Blood pressure diastolic [mmHg]	60.00	85.07	111.00	10.34	164	44
Heart rate [beat per minute]	56.00	78.42	104.00	11.95	165	43
Bone marker						
CTX [ng/ml]	0.06	0.32	0.62	0.12	67	141
Osteocalcin [ng/ml]	6.70	16.65	31.30	5.31	65	143
P1NP [µg/ l]	14.90	47.75	94.80	17.02	65	143

[a] allostatic load index with 15 indicators (cortisol, adrenalin, noradrenalin, DHEAS, IL-6, CRP, fibrinogen, sE-selectin, sICAM-1, Hba1c, fasting glucose, HOMA, triglycerides, HDL, LDL) and for ALI 21 further: aldosterone, dopamine, BMI, RR, HRV

[b] ALI primary indicators (cortisol, noradrenalin, adrenalin, DHEAS, aldosteron, dopamin, IL-6, [not available: TNF-α, IGF-1]) [56]

[c] ALI secondary indicators (HbA1c, triglycerides, cholesterol, LDL, HDL, fasting glucose, insulin, CRP, fibrinogen, sICAM-1 [not available: exclusive of BPsys, BPdia, BMI, WHR]) [56]

Table 3. Descriptive statistics (*M, SD*) of biomarkers at follow up (t_2: t_0 + 5 month, therapy end) and BMD measures at follow up (t_4: t_0 + 18 month)

at 5 month follow up (t_2 therapy end)						
Biomarker characteristics	**min**	**M**	**max**	**SD**	**NA**	***n***
AL-indices						
ALI 15[a]	0.00	3.50	8.00	1.84	170	38
ALI-I[b]	0.00	1.52	5.00	1.13	131	77
ALI-II[c]	0.00	2.37	8.00	2.13	129	79
Metabolic markers						
HbA1c (IFCC) [mmol/mol]	26.00	35.44	49.00	4.79	130	78
Insulin [µU/ml]	2.09	9.48	31.40	5.84	132	76
HDL [mg/dL]	19.30	53.61	95.00	16.72	127	81
LDL [mg/dL]	35.00	120.49	227.00	39.30	127	81
Cholesterol [mg/dL]	75.00	197.70	315.00	49.50	128	80
Triglyceride [mg/dL]	25.00	121.71	286.13	66.41	129	79
Glucose (NaF) [mg/dL]	69.00	92.68	141.00	14.91	130	78
HOMA Index	0.40	2.13	6.80	1.41	134	74
SNS und HPA markers						
Adrenalin [pg/mL]	27.50	137.33	352.00	73.70	131	77
Aldosterone [pg/ml]	37.70	145.88	268.80	53.99	135	73
Cortisol [nmol/l]	52.90	339.71	609.80	116.72	129	79
DHEAS [µmol/l]	0.83	3.98	9.56	2.27	128	80
Dopamine [pg/mL]	4.00	22.068	259.50	19.53	135	73
Noradrenalin [pg/mL]	20.00	410.65	927.90	180.54	136	72
Immune markers						
CRP [mg/L]	0.06	0.18	0.78	0.18	133	751
Fibrinogen [mg/dL]	186.00	298.04	186.00	71.06	127	81
IL-6 [pg/ml]	1.50	2.02	5.20	0.93	130	78
TNF-α [pg/ml]	--	--	--	--	--	--
sE-Selectin [ng/ml]	12.50	51.77	86.50	17.81	171	37
Bone marker						
CTX [ng/ml]	0.10	0.31	0.59	0.11	132	76
Osteocalcin [ng/ml]	7.60	16.62	29.50	5.07	133	75
P1NP [µg/ l]	15.00	47.08	92.20	17.64	131	77
at 18 month follow up (t_4)						
Bone density						
BMD_GMW[d]	0.62	0.92	1.09	0.15	39	16
BMD_HMW[d]	0.66	0.86	1.04	0.12	39	16
BMD_L1L4[d]	0.69	1.12	1.43	0.21	39	16
BMD_SMW[d]	0.72	1.08	1.37	0.17	40	15
BMD_TMW[d]	0.43	0.74	0.93	0.15	39	16
sICAM-1 [ng/ml]	151.90	355.58	616.30	84.65	49	159

[a] allostatic load index with 15 indicators (cortisol, adrenalin, noradrenalin, DHEAS, IL-6, CRP, fibrinogen, sE-selectin, sICAM-1, Hba1c, fasting glucose, HOMA, triglycerides, HDL, LDL) and for ALI 21 further: aldosterone, dopamine, BMI, RR, HRV

[b] ALI primary indicators (cortisol, noradrenalin, adrenalin, DHEAS, aldosteron, dopamin, IL-6, [not available: TNF-α, IGF-1]) [56]

[c] ALI secondary indicators (HbA1c, triglycerides, cholesterol, LDL, HDL, fasting glucose, insulin, CRP, fibrinogen, sICAM-1 [not available: exclusive of BPsys, BPdia, BMI, WHR]) [56]

[d] Mean value of different bone mineral density measurements

First study part / Work Package

Bone metabolism related to depressive symptomatology level and psychosomatic symptom severity

Multiple linear regression models indicate that the magnitude of the depressiveness (OC: *b* =.083 [95%CI -.008,.173], *P* =.075) and symptom severity (PSDI: P1NP: *b* = 5.304 [95%CI -.585, 11.194], *P* = .081; OC: *b* = .91 [95%CI .855,4.407], *P* = .005; CTX: *b* = .052 [95%CI .011,.093], *P* =.016); GSI: OC: *b* = 2.096 [95%CI .447,3.745], *P* = .015) influence the extend of the anabolic bone marker expression, whereby higher severity coincides with higher bone marker expression (details see table 4).

Life burden and bone metabolism

A regression model confirms that life burden is associated with depressiveness (BDI-II: *b* = .237 [95%CI .064,.411], *P* =.008). Furthermore, people with high life burden show significantly less anabolic bone marker expression in an acute depression episode as the other depressed patients in this sample (see table 4).

Table 4. Main effects (regression coefficient b) of depressiveness, life events, psychosomatic symptoms severity, and AL-indices on bone marker at baseline (t_0)

	P1NP			**OC**			**CTX**		
	coefficient	***CI***	*p*	***coefficient***	***CI***	*p*	***coefficient***	***CI***	*p*
BDI–II sum score[a]	0.121	-0.170, 0.413	0.417	**0.083**	-0.008, 0.173	0.075	0.0004	-0.002, 0.002	0.727
SCL-90 PSDI[b]	**5.304**	-0.585, 11.194	0.081	**2.631**	0.855, 4.407	0.005	**0.052**	0.011, 0.093	0.016
SCL-90 PST[c]	0.061	-0.134, 0.256	0.543	0.045	-0.015, 0.105	0.145	-0.0002	-0.002, 0.001	0.729
SCL90 GSI[d]	3.222	-2.103, 8.548	0.239	**2.096**	0.447, 3.745	0.015	0.020	-0.020, 0.059	0.333
LE all[e]	0.158	-0.153, 0.470	0.317	**0.111**	0.017, 0.206	0.021	**0.002**	0.000, 0.004	0.049
ALI 21	-0.523	-1.650, 0.604	0.365	**-0.438**	-0.779, -0.096	0.014	**-0.009**	-0.017, -0.001	0.025
ALI 15	-0.435	-1.759, 0.888	0.521	**-0.462**	-0.864, -0.059	0.027	**-0.009**	-0.018, 0.0005	0.065
ALI-II	-0.351	-1.585, 0.884	0.579	**-0.492**	-0.875, -0.108	0.014	**-0.008**	-0.017, 0.001	0.088
ALI-I	0.734	-1.824, 3.291	0.576	0.467	-0.318, 1.253	0.246	-0.005	-0.023, 0.014	0.614

Multiple interaction regression models, adjusted for age, gender, study sides. Significant Regression coefficients are bold (p <.01, p <.05, p <.10 two sided testing)

[a] Beck Depression Inventory; sum score, [b] Symptom Check List - revised SCL-90-R, Positive symptom distress index, [c] Symptom Check List - revised SCL-90-R, Positive symptom total, [d] Symptom Check List - revised SCL-90-R, Global severity index, [e] Inventory of critical life events (ILE), count of all life events

Allostatic load association with depression

There were no significant associations between allostatic load (ALI 15) and depression (BDI-II: *b* = -.186 [95%CI -.937,.565], *P* = .625) as well as between allostatic load (ALI 15) and life burden (LE all: *b* = -.611 [95%CI -1.397,.175], *P* = .127) detected.

Second study part / Work-Package

Metabolic bone adaption abilities of depressive people with high allostatic load and bone microstructure alterations

People with high ALI show a decreased bone metabolism and an overall catabolic shift (counter regulation of the anabolic expression (ALI-15: OC: *b* = -.462 [95%CI -.864,-.059], *P* = .027; CTX: *b* = -.009 [95%CI-.018, .001], *P* = .065); ALI-II: OC: *b* = -1.107 [95%CI -1.772,-.441], *P* = .002) during a depression episode (see table 4).

Furthermore, descriptive analysis confirmed reduced bone mineral density for subjects with high ALI. Similarly, multiple regression models (controlled for age, gender weight) indicate that high allostatic load is associated with lower BMD in different areas (BMD_GMW *b* = -.028 [95%CI -0.070, 0.013], *P* = 0.220; BMD_HMW *b* = -.011 [95%CI -0.053, 0.030], *P* = .605; BMD_SMW *b* = -.038 [95%CI -0.087, 0.010], *P* = .161; BMD_TMW *b* = -.032 [95%CI -0.071, 0.007] *P* = .148; BMD_L1L4 *b* = -.029 [95%CI -0.077, 0.019], *P* = .027) (see also figure 1).

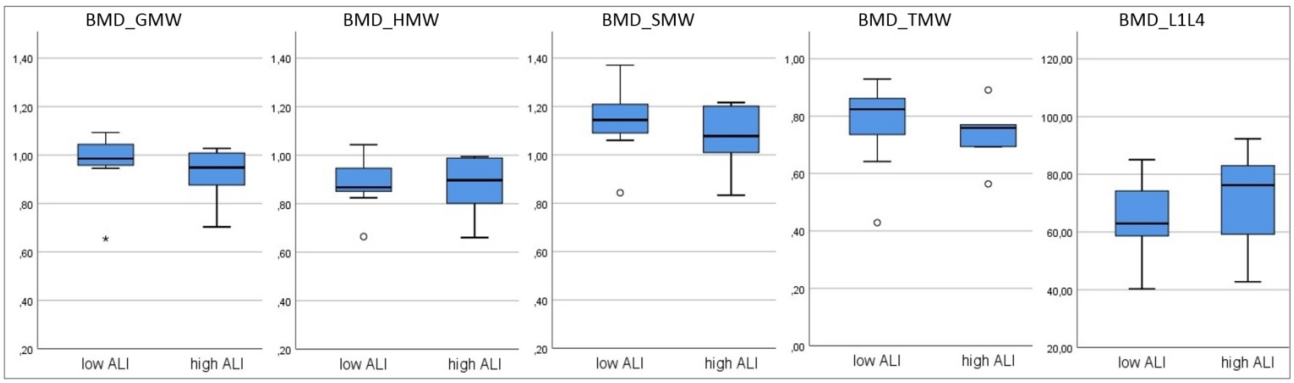

Fig. 1. Bloxplots showing bone mineral density measurements for Mean value of all bone mineral density measurements (GMW, HMW, SMW, TMW) and lumbar vertebral bodies L1-L4 (in this graph not controlled for gender, age, weight) stratified for low and high allostatic load (AL-Index)

Selection of predictors for diagnosis and personalized treatment (Hypothesis 2)

The LASSO Model with *n*=91 observations revealed that none of 19 potential single allostatic mediators or mediator sets could be selected for a prediction of (altered levels of) osteocalcin, CTX or P1NP in depressive subjects. A diagnosis failed on the base of blood biomarkers in this study and did not suggest further analysis such as root mean square errors or ROC analysis.

Discussion

Combining longitudinal design and baseline conditions with an appropriate patient selection is crucial for gaining insights into (physiological) adaptations during a depressive episode. Around 1/3 of the patients in this study suffered from severe depression and psychosomatic symptoms, around 1/3 showed a high allostatic load (not excessively, nobody reached the scale maximum) and each patient had, on average, experienced 14 life changing events. Life burden, operationalized by the mentioned life events, was positively associated with the development of depression as described in the literature [36, 60, 61]. In our cohort, we do not have a broader spectrum of comorbid patients confirmed by non-significant associations between life burden and allostatic load as well as allostatic load and depression severity. Most of the patients suffered from an acute (and mostly a first or second) depression episode (ICD-10 F32.x or F33.x).

This enables us to determine whether people during an acute depressive episode show alterations in bone metabolism and whether this depends on depression and psychosomatic symptom severity. On the one hand, we observed that higher depression and symptom severity were associated with a higher expression of anabolic bone metabolism in bone formation (OC; P1NP) and bone turnover marker (CTx). On the other hand, we observed that people suffering from higher life burden showed significantly less anabolic expression (OC and CTx) during the acute depression episode. These findings describing a metabolic adaptation in bones during a depression episode are novel, highly valuable and clinically significant.

Furthermore, it was of interest whether patients suffering from high allostatic load burden are at higher risk for bone diseases through altered bone metabolism in short term and altered bone mineralization in long-term. Patients with high allostatic load showed a significantly decreased bone metabolism, indicated by an overall catabolic shift of bone formation markers (P1NP, OC) and a relative stagnancy /decreased bone turnover (CTx) in the short-term. People with allostatic load didn't show a "normal" allostatic response related to the stressful depression episode, maybe due to a previous accumulation of toxic stress in tissues and cells [62]. The disrupted bone metabolism of patients with ALI was also reflected by reduced bone mineral density in the lumbar spine and both hips (DXA) after 18 months. Although these results were only descriptively evaluated due to the small group size, they are nonetheless consistent with the highlighted serum marker changes, thus confirming the relevance of the data. Knowing about the vicious circle of intercorrelations between bone disorders (*e.g.* osteoporosis, insufficient fractures, arthrosis), pain syndromes and their reinforcement of the depressive symptomatology, the results seem of high significance for the medical care of depressed patients.

In the organization of an appropriate medical care, diagnostic tools are essential. For this purpose, a mathematical selection algorithm was applied to detect an explanatory set that would provide information about risk profiles and adequate treatment preventing comorbidities for bone diseases. Using the LASSO technique, we were not able to identify a single allostatic mediator or mediator set that would be reliable enough to serve as an easy diagnostic tool for depressive patients at higher risk for metabolic bone alterations. Furthermore, and in contrast to other (rare) studies [26, 27], no moderator for an interaction between the "osteoblast suppressor", cortisol, as well as the pro-inflammatory cytokine, IL-6, and the bone markers, osteocalcin, P1NP and CTx was detected. One possible reason could be that the LASSO technique simultaneously penalized or

unpenalized all influencing factors within one model, thus reducing intercorrelations and confounders. Only very strong and unambiguous influencing factors remain. This is in line with the idea that complex neuroendocrinologic and neuroanatomic alterations of biography should be assessed by multidimensional indices and not by single mediators which can be affected by daily changes, assessment techniques or be an effect of another alteration. Several studies show that consequences of biographical and chronic stress are based on early multifactorial alterations of mitochondrial biology that influence different functional and molecular indicators (for mitochondrial allostatic load, MAL see [63-65], for catabolic neuronal factor patterns NGF, NPYR1, VIPR1 and TACR [66]).

Summary: Our findings suggest that an acute depressive episode leads to an anabolic activation of bone metabolism, with the level of activation increasing with higher severity of the depression and psychosomatic symptoms. However, this metabolic adaptation to an exceptionally high stress episode is restricted in patients with a higher life burden and completely absent in patients with allostatic overload. The latter group shows a catabolic and bone-damaging response that is reflected in reduced bone mineral density in the long term. The development of diagnostic tools seems important, yet was not possible in this cohort. Nevertheless, the results of this study may be of high importance for the understanding of the mechanism of bone development and treatment of depressive patients in clinical routines [67].

Limitations: The empirical results reported herein must be considered in the light of some limitations that should be addressed in future research. Firstly, the report focused only on the explanation of physiological associations and ignored interactions between bone markers and psychometric biographical measures. Secondly, due to the nature of an observational multicenter study that comprises participants from three different study sites and is reliant on voluntary participation of patients, potential selection bias or effect of preexisting differences between study sites cannot to be ruled out. However, as we controlled for confounding variables (*e.g*. study site, sociodemographic variable) in all analysis, the impact of these biases should be limited. Thirdly, although the baseline sample size was sufficient, missing data and subsample analysis had a potential impact on the statistical power and margin of error to identify effects. There was only a small subsample available for bone mineral density (BMD) measurements at an additional fourth follow-up. Therefore, moderator analyses must be dispensed, and the generalization of the results is strongly limited. Furthermore, LASSO models were calculated cross-sectionally, although prediction analyses are usually performed in longitudinal format. However, since there are little to no differences between the biomarkers and the follow up measurement, a cross-sectional calculation was chosen in favor of a higher N. And lastly, clinical implications would be based on a diagnostic tool; a derivation of such a tool was not possible till now. Further analyses with a combination of psychometric and biological data might help to be successful in this task.

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List of Abbreviations

Abbreviation	Description
Allostasis[1]	Active process of maintaining homeostasis
Allostatic load[1]	Cumulative change (*e.g.*, body fat; remodeling of neuronal circuitry)
Allostatic overload[1]	Wear-and-tear, pathophysiology (*e.g.,* neuronal damage, cell loss)
ALI-Total	cortisol, adrenalin, noradrenalin, DHEAS, IL-6, CRP, fibrinogen, sE-selectin, sICAM-1, Hba1c, fasting glucose, HOMA, triglycerides, HDL, LDL
ALI-primary	cortisol, noradrenalin, adrenalin, DHEAS, aldosterone, dopamine, IL-6
ALI-secondary	HbA1c, triglycerides, cholesterol, LDL, HDL, fasting glucose, insulin, CRP, fibrinogen and s-ICAM-1
ANS	Autonomic nervous system in which the stress response is driven by the sympathetic-nervous-system (SNS), sympathetic-adrenal-medullary-system (SAM) and the dopamine systems (DOPA)-
BMD	Bone mineral density
CTX	Cross Laps, bone turnover marker, resorption
CRH	Corticotropin-releasing hormone
GH	Growth Hormons
GR	Glucocorticoid receptors
HPA axis	Hypothalamus-pituitary-adrenal axis
HPT axis	Hypothalamic–pituitary–thyroid axis
Homeostasis[1]	Essential parameters of life
IGF-I	Insulin-like growth factor-I
IL-6, IL-1	Interleukins, cytokines
n	number of participants
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
Osteocalcin	Bone turnover marker, formation
P1PN	Procollagen type 1 amino-terminal propeptide, bone turnover marker, formation
p-values	Significance level: $p < 0.01$, $p < 0.05$ or $p < 0.10$
TNF-α	Tumor necrose factor α

[1] Definition from McEwen [10, 68]