Associations Between Liver Function, Bone Turnover Biomarkers and Adipokines in Older Patients With Hip Fracture

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Abstract

Background: To examine the associations of serum liver markers with parameters of mineral and bone metabolism and their relationship with leptin, adiponectin and resistin in patients with hip fracture (HF).

Methods: In 294 older patients (mean age 82.2 ± 7.9 years, 72.1% women) with osteoporotic HF, we measured serum levels of alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), bilirubin, albumin, adiponectin, leptin, resistin, 25(OH) vitamin D (25(OH)D), intact parathyroid hormone (PTH), calcium, phosphate, magnesium, osteocalcin (OC), bone-specific alkaline phosphatase (BAP), urinary concentrations of deoxypyridinoline (DPD/Cr) and cross-linked N-telopeptide of type 1 collagen (NTx/Cr) (both corrected for urinary creatinine concentrations), as well as routine blood parameters.

Results: In the total cohort, in fully adjusted multivariate linear regression analyses, lower OC was an independent predictor of higher GGT, ALT and bilirubin, whereas higher BAP was positively associated with GGT and ALP; NTx/Cr, hemoglobin (both inversely), adiponectin, coronary artery disease (CAD) and alcohol overuse (all three positively) were also independently associated with GGT activity. However, in malnourished women, OC was not an independent predictor of GGT or ALT and NTx/Cr predicted ALP activity. OC was independently predicted by GGT, ALT (both negatively), ALP, leptin and age (all three positively), BAP by GGT and ALP, OC/BAP ratio by GGT (inversely) and leptin (positively) and both elevated NTx/Cr and DPD/Cr by higher ALP and lower leptin levels. The GGT > 20 U/L indicated increased prevalence of low OC levels (two-fold) and low OC/BAP ratio (2.6-fold) with a positive predictive value above 75%.

Conclusions: In older HF patients, bidirectional links exist between liver function (within normal range in the vast majority) and parameters of bone metabolism. Adiponectin is an independent predictor of GGT, whereas leptin is a determinant of OC and bone resorption; these relationships are modulated by nutritional status. GGT > 20 U/L may be used as a marker of impaired bone metabolism.

Keywords: Liver function; Adipokines; Bone turnover; Osteocalcin

Introduction

The importance of liver-bone interactions under both normal and disease conditions is supported by a growing number of evidence. 1) Liver plays a fundamental role in metabolism of vitamins D and K, parathyroid hormone (PTH) and minerals, essential regulators of bone homeostasis [1-5]. 2) Osteopenia/osteoporosis (hepatic osteodystrophy) is present in 20-50% of patients with chronic liver disease [6-10]. 3) Osteocalcin (OC), an osteoblast-derived hormone, is recognized as a critical determinant of energy and glucose homeostasis [11-16]. 4) Dysregulation and dysfunction of adipokines, adipose tissue-derived hormones, in particular, adiponectin, leptin and resistin, which have receptors expressed in both hepatocytes and bone cells and control a vast diversity of physiological functions, are involved in initiation and progression of many diseases including liver and bone (osteoporosis) disorders [17-23].

However, despite the intense research carried out in recent years, the relationship between liver and bone and the underlying mechanisms, including the role of adipokines, are still poorly characterized. To date, neither clinical, nor the animal models provide a uniform explanation of the liver-bone link(s). The majority of research on adipokine-liver-bone interactions has focused primarily on obesity-related metabolic syndrome, diabetes mellitus (DM) and non-alcoholic fatty liver disease (NAFLD). There has been limited evaluation (with considerable controversy in the reports) of liver function, bone metabolism and adipokines in patients with hip fracture (HF), the most devastating consequence of osteoporosis. There are no data concerning the association of serum hepatic biomarkers with indices of mineral and bone metabolism and the mechanisms involved, nor are there any data showing whether liver

function indicators predict clinically important markers of bone turnover in HF patients. Thus, the aims of this study were to evaluate 1) the cross-sectional associations of serum liver markers with parameters of mineral and bone metabolism, and 2) the relationship between serum levels of three adipokines (leptin, adiponectin and resistin) and both liver and mineral-bone indicators in older patients with HF.

Methods

Study cohort

This prospective observational study included 294 consecutive older ($\geq$ 60 years of age) patients (212 women and 82 men) with low-trauma osteoporotic HF admitted to the Canberra Hospital who did not have the following exclusion criteria: age < 60 years, high trauma, femur shaft fracture, pathological HF due to primary or metastatic bone cancer, multiple myeloma, Paget disease, or primary hyperparathyroidism. Data on sociodemographic, clinical and laboratory characteristics were collected as previously reported [24].

The study was conducted in compliance with the Declaration of Helsinki (as revised in 2008).

Informed consent was obtained from all patients or their carers. The study has approval of the local Health Human Research Ethical Committee.

Laboratory analyses

In each patient venous blood and second morning urine samples were collected between 07:00 and 11:00 after at least 12-h overnight fast, usually within 24 h after arrival at the emergency department. After centrifugation of blood, one serum sample as well as the urine sample was frozen and stored at -70 °C for later analyses of bone turnover markers and adipokines.

For all patients, the following parameters were measured: complete blood count, liver function tests, urea, creatinine and electrolytes, fasting blood glucose (and HbA_{1C} in diabetic patients), thyroid function tests (TSH, T_4), 25(OH) vitamin D (25(OH)D), intact PTH, total calcium, phosphate, magnesium, markers of bone turnover, and three adipokines (adiponectin, leptin and resistin). All routine hematological and biochemical assessments were performed by standardized methods on autoanalyzers at the day of collection. Glomerular filtration rate (eGFR) was calculated using a standardized serum creatinine-based formula normalized to a body surface area of 1.73 m² [25]. Chronic kidney disease (CKD) was defined as eGFR < 60 mL/min/1.73 m² (CKD stage $\geq$ 3).

Assessment of liver parameters

The following biochemical indicators of liver function were measured as the blood samples were collected: alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), al-

kaline phosphatase (ALP), bilirubin and albumin. These markers were evaluated by using commercially available standard enzymatic reagents and diagnostic kits by spectrophotometry on the biochemical autoanalyzer Abbott Architect CI16200 (Abbott Laboratories, IL, USA). ALT, GGT and ALP were measured with enzymatic methods, total bilirubin was analyzed using diazonium salt, albumin was measured using bromcresol green, and total protein was tested by a Biuret method. The mean inter-assay and intra-assay coefficients of variations (CVs) for these tests were within 1.1-6.6%. For liver enzymes two times the upper normal limit (UNL), cut-off levels were used to define abnormal tests.

Markers related to mineral and bone metabolism

These included serum concentrations of 25(OH)D, intact PTH, total calcium, phosphate, magnesium, and markers of bone turnover - OC, and bone-specific alkaline phosphatase (BAP) as markers of bone formation and urinary concentrations of deoxypyridinoline (DPD/Cr), and cross-linked N-telopeptide of type 1 collagen (NTx/Cr) as markers of bone resorption (both corrected for urinary creatinine concentrations in the same sample). Serum calcium concentrations were corrected for serum albumin. Serum 25(OH)D was measured by radioimmunoassay kit (Dia Sorin, Stillwater, MN, USA) and intact PTH by two-site chemiluminescent enzyme-linked immunoassay on DPC Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA, USA). Serum OC was determined by electrochemiluminescent immunoassay (Elecsys 1010, Roche Diagnostics, Ltd Corp., IN, USA), serum BAP by Metra BAP ELISA (Quidel Corp., San Diego, CA, USA), urinary NTx by enzyme-linked immunosorbent assay (ELISA) (Wampole Labs, Princeton, NJ, USA), and urinary DPD by two-site chemiluminescent enzyme-labeled immunoassay (DPC Immulite 2000, Diagnostic Products, Los Angeles, CA, USA). All samples were analyzed with commercially available kits of the same lot number according to the manufacturer's protocol and blind to any clinical information. In these methods both the intra- and inter-assay CVs ranged from 2.1% to 12.7%. Insufficiency of vitamin D was defined as 25(OH)D < 50 nmol/L and secondary hyperparathyroidism (SHPT) was defined as elevated serum PTH (> 6.8 pmol/L, the upper limit of the laboratory reference range). For levels of bone turnover markers, we used the standard laboratory reference ranges and data provided by the manufacturer.

Measurements of adipokines

Serum levels of leptin were determined by ELISA method (Diagnostic System Laboratories, Webster, TX, USA), and total adiponectin and resistin by human ELISA kits (B-Bridge International, Mountain View, CA, USA). All assays were performed according to the manufactures' instructions with kits of the same lot number. Intra- and inter-assay CVs were less than 7% for these three tests. Malnutrition was defined as serum leptin concentration < 4 ng/mL in males and < 6.5 ng/mL in

Table 1. Demographic, Clinical and Laboratory Characteristics of the Studied Patients With Hip Fracture (n = 294)

Clinical characteristic		Laboratory characteristic	
Age, years (mean ± SD)	82.2 ± 7.9	ALT, U/L	23.0 ± 42.6
Females, %	72.1	GGT, U/L	54.1 ± 95.6
Cervical/trochanteric fracture, n	152/142	ALP, U/L	105.4 ± 80.1
Hypertension, %	54.0	Albumin, g/L	37.1 ± 6.3
CAD, %	21.2	Bilirubin, μmol/L	12.4 ± 7.4
Atrial fibrillation, %	13.2	OC, ng/mL	17.2 ± 15.2
History of stroke or TIA, %	21.7	BAP, U/L	26.4 ± 14.2
Type 2 DM, %	18.8	NTx/Cr, nmol/μmol	159.4 ± 169.0
Dementia, %	27.8	DPD/Cr, nmol/μmol	12.6 ± 7.2
Parkinson's disease, %	4.4	25(OH)D, nmol/L	37.3 ± 18.0
COPD, %	11.8	PTH, pmol/L	6.9 ± 5.6
CKD stage ≥ 3, %	42.6	Adiponectin, ng/mL	17.5 ± 7.4
Malnutrition*, %	33.8	Leptin, ng/mL	18.5 ± 23.2
Alcohol (≥ 3 times a week), %	9.5	Resistin, ng/mL	18.7 ± 10.5
Ex-smokers, %	10.0	Hb, g/L	124.9 ± 17.1
Current smokers, %	5.4	TSH, mU/L	1.55 ± 2.17
Vitamin D insufficiency**, %	79.7	T4, pmol/L	15.9 ± 3.54
SHPT, %	38.2	eGFR, mL/min/1.73 m^2	65.1 ± 23.7

CAD: coronary artery disease; TIA: transient ischemic attack; DM: diabetes mellitus; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; ALP: alkaline phosphatase; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone; TSH: thyroid-stimulating hormone; T4: thyroxin; eGFR: estimated glomerular filtration rate; SHPT: secondary hyperparathyroidism (PTH > 6.8 pmol/L); NTx/Cr: cross-linked N-telopeptide of type 1 collagen corrected for urinary creatinine concentration; DPD/Cr: deoxypyridinoline corrected for urinary creatinine concentration. *Malnutrition was defined as serum leptin concentration < 4 ng/mL in males and < 6.5 ng/mL in females. **Vitamin D insufficiency was defined as 25(OH)D < 50 nmol/L.

females [26].

Statistical analyses

Data are presented as mean values ± standard deviations (SDs) for continuous variables or percentages for categorical variables. Comparisons between groups were performed using analysis of variance and Student's t-test (for continuous normally distributed variables) and χ^2 test (for categorical variables). The relations between variables were also analyzed by Pearson's correlation coefficient with log-transformed data (to achieve normal distribution); Bonferroni and Sidak adjustments for multiplicity have been performed. Univariate and multivariate linear regression analyses were performed to determine the associations between liver markers and different parameters related to mineral and bone metabolism; all potential confounding variables (demographic, biochemical and clinical) with statistical significance ≤ 0.10 on univariate analyses were included in multivariate analysis. The significance of multicollinearity phenomena in regression analyses was evaluated by the variance inflation factor. Two-sided P < 0.05 values were considered statistically significant. All statistical calculations were carried out using the Stata software version 10 (StataCorp, College Station, TX, USA).

Results

Clinical characteristics of the study patients

The clinical features and laboratory characteristics in the patients are summarized in Table 1. The prevalence of abnormal liver enzyme activities defined as two times over their respective ULNs was low or moderate: for ALT (> 80 U/L) in five (1.7%) patients, for GGT (> 128 U/L) in 23 (7.8%), and for ALP (> 120 U/L) in 26 (8.8%) subjects. Elevated bilirubin (> ULN, 20 μmol/L) demonstrated 29 (9.9%) patients and low albumin levels (< 33 g/L) were found in 64 (21.8%) subjects. There were no gender differences in mean values of liver markers, OC, BAP, calcium (corrected for albumin), resistin, TSH and hemoglobin. Women, compared to men, had significantly lower mean serum 25(OH)D (35.3 ± 17.6 vs. 42.4 ± 18.2 nmol/L, P = 0.003) and eGFR (62.7 ± 22.2 vs. 71.2 ± 26.4 mL/min/1.73 m^2, P = 0.006) levels, but higher concentrations of PTH (7.4 ± 6.1 vs. 5.5 ± 3.5 pmol/L, P = 0.009), NTx/Cr (178.0 ± 90.5 vs. 112.3 ± 78.0 nmol/μmol, P = 0.005), DPD/Cr (13.3 ± 7.9 vs. 10.8 ± 4.6 nmol/μmol, P = 0.015), leptin (21.1 ± 24.3 vs. 11.7 ± 18.6 ng/mL, P = 0.002), adiponectin (18.3 ± 7.1 vs. 15.6 ± 7.6 ng/mL, P = 0.007) and free T4 (16.2 ± 3.50 vs. 15.2 ± 3.53 pmol/L, P = 0.021). Vitamin D insufficiency

Table 2. Correlation of Serum Liver Markers With Indices of Bone-Mineral Metabolism, Leptin, Adiponectin, and Resistin Levels, Thyroid Markers and Hemoglobin

	GGT	ALT	ALP	Albumin	Bilirubin
OC	-0.182**	-0.158**	0.104	-0.034	-0.147*
BAP	0.307***	0.050	0.462***	0.014	0.005
OC/BAP	-0.350***	-0.178**	-0.183**	-0.035	-0.137*
DPD/Cr	0.018	-0.071	0.191**	-0.037	-0.021
NTx/Cr	-0.134*	-0.041	0.150*	-0.069	-0.035
25(OH)D	0.014	-0.022	-0.165**	0.012	-0.021
PTH	0.053	0.058	0.111	0.036	0.139*
Cac	-0.002	0.086	0.133*	-0.111	-0.039
PO4	-0.016	-0.049	0.092	0.058	-0.075
Mg	-0.087	-0.068	0.017	0.105	-0.065
Leptin	-0.082	-0.025	-0.045	0.108	-0.029
Adiponectin	0.146*	0.087	0.059	0.010	0.128*
Resistin	0.088	0.001	0.054	0.124*	0.153*
L/A	-0.116	-0.070	-0.044	0.109	-0.055
L/R	-0.109	-0.038	-0.054	0.059	-0.077
A/R	0.022	0.053	-0.011	-0.090	-0.034
TSH	0.132*	0.057	-0.037	-0.014	0.043
T4	-0.186**	0.121*	-0.144*	0.027	0.007
Hb	-0.146*	0.071	-0.129*	0.281***	0.047

Pearson's correlation coefficients for log-transformed variables after adjustment for age and sex shown. GGT: gamma-glutamyltransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; eGFR: estimated glomerular filtration rate; OC: osteocalcin; BAP: bone specific alkaline phosphatase; DPD/Cr: deoxypyridinoline corrected for urinary creatinine concentration; NTx/Cr: cross-linked N-telopeptide of type 1 collagen corrected for urinary creatinine concentration; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone; TSH: thyroid-stimulating hormone; T4: thyroxin; Cac: calcium corrected for albumin; PO4: phosphate; Mg: magnesium. *P < 0.05; **P < 0.001; ***P < 0.001.

(25(OH)D < 50 nmol/L) was found in 84.6% of females and 67.5% of males (P < 0.008) and SHPT (PTH > 6.8 pmol/L) in 43.2% and 25.3%, respectively (P = 0.016).

Associations between liver markers and parameters of mineral-bone metabolism, adiponectin, leptin and resistin, and clinical characteristics

In Pearson's analyses (all biochemical variables log-transformed), after adjusting for age and sex, both serum GGT and ALT activities correlated negatively with serum OC levels (Table 2). GGT was associated positively with BAP, adiponectin and TSH and negatively with NTx/Cr, T4 and hemoglobin concentrations, while ALT correlated positively with T4. Serum ALP activity, as could be expected, demonstrated a strong correlation with BAP, and a significant positive association with both urinary resorption markers (DPD/Cr and NTx/Cr) and a negative correlation with 25(OH)D, T4 and hemoglobin. Albumin correlated with resistin and hemoglobin. Bilirubin was inversely associated with OC and positively with PTH, adiponectin and resistin. We calculated also the OC/BAP ratio as an index reflecting osteoblast differentiation/maturation [27]. Results showed significant inverse correlations between

the OC/BAP ratio and all liver markers (GGT, ALT, ALP and bilirubin) except albumin.

Similar partial correlation analyses (after adjusting for age and sex) also demonstrated that serum GGT activity correlated with presence of CAD (β = 0.178, P = 0.003) and alcohol overuse defined as ≥ 3 times a week (β = 0.211, P < 0,001), ALT correlated negatively with Parkinson's disease (PD, β = -0.173, P = 0.004), ALP with dementia (β = 0.118, P = 0.047), albumin was inversely associated with DM (β = -0.126, P = 0.032) and positively with hemoglobin (β = 0.281, P < 0.001), and bilirubin correlated with CAD (β = 0.142, P = 0.018) and atrial fibrillation (AF, β = 0.242, P < 0.001). There was a statistically significant inverse correlation between adiponectin and leptin (β = -0.196, P = 0.002), but there was no association between adiponectin and resistin (β = 0.043, P = 0.493), or between leptin and resistin (β = 009, P = 0.885).

When comparing patients with GGT < 30 U/L (median level) and GGT > 30 U/L, the latter group had, as would be expected, higher mean ALT (31.9 vs. 17.2 U/L, P = 0.004), BAP (29.6 vs. 24.2 U/L, P = 0.002), ALP (129.5 vs. 89.3 U/L, P < 0.001) and bilirubin (13.7 vs. 11.5 µmol/L, P = 0.011) levels, but also higher adiponectin (18.8 vs. 16.6 ng/mL, P = 0.015) and resistin concentrations (20.3 vs. 17.5 ng/mL, P = 0.038) and lower NTx/Cr urinary excretion (133.5 vs. 176.6 nmol/

Table 3. Multiple Linear Regression Analyses for Liver Markers in Older Patients With Hip Fracture

	Log GGT		Log ALT		Log ALP		Log albumin		Log bilirubin	
	A	B	A	B	A	B	A	B	A	B
	β (P value)	β (P value)	β (P value)	β (P value)	β (P value)	β (P value)	β (P value)	β (P value)	β (P value)	β (P value)
Age			-0.012 (0.017)	-0.012 (0.017)						
Sex (m)	0.264 (0.027)								0.180 (0.026)	-0.109 (0.025)
Log OC	-0.219 (0.011)	-0.176 (0.029)	-0.145 (0.016)	-0.155 (0.006)						
Log BAP	0.595 (<0.001)	0.570 (<0.001)			0.490 (<0.001)	0.496 (<0.001)				
Log NTx/Cr	-0.185 (0.002)	-0.172 (0.005)								
Log 25(OH)D					-0.134 (0.015)	-0.131 (0.017)				
Log PTH	-0.897 (0.013)						0.393 (<0.001)	0.390 (<0.001)	0.103 (0.038)	
Log Hb										
Log adiponectin	0.228 (0.037)	0.215 (0.050)								0.130 (0.054)
Log resistin							0.043 (0.026)	0.439 (0.023)	0.119 (0.038)	
Alcohol +		0.883 (<0.001)								
CAD		0.309 (0.023)								
AF				-0.516 (0.007)						0.405 (<0.001)
PD										
R²	0.2500	0.2701	0.0497	0.0784	0.2380	0.2334	0.1104	0.1177	0.0678	0.1328

Parameters identified as significant and independent predictors of GGT, ALT, albumin and bilirubin are shown. β: β-standardized regression coefficient; GGT: gamma-glutamyltransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; OC: osteocalcin; BAP: bone specific alkaline phosphatase; DPD/Cr: deoxypyridinoline corrected for urinary creatinine concentration; NTx/Cr: cross-linked N-telopeptide of type 1 collagen corrected for urinary creatinine concentration; 25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone; CAD: coronary artery disease; AF: atrial fibrillation; PD: Parkinson's disease; R²: adjusted R-squared. A, model 1: adjusted for age, sex, OC, BAP, NTx/Cr, DPD/Cr, 25(OH)D, PTH, leptin, adiponectin, resistin, TSH, T4 and hemoglobin. B, model 2: further adjusted for alcohol use ≥ 3 drinks/week (yes/no), presence of CAD (yes/no), hypertension (yes/no), AF (yes/no), type 2 diabetes mellitus (yes/no), PD (yes/no) and chronic kidney disease (estimated glomerular filtration rate < 60 mL/min/1.73 m², yes/no).

μmol, $P = 0.048$). Similar analyses for ALT < 20 U/L (median level) vs. > 20 U/L, bilirubin (< 20 μmol/L vs. > 20 μmol/L) and albumin (< 33 g/L vs. > 33 g/L) did not show significant associations either with any of the analyzed bone turnover markers, or with any of the adipokines (data not shown).

The patients were also stratified into two groups according to nutritional status: with (n = 99) and without malnutrition (n = 195). The first group showed significantly higher mean levels of urinary NTx/Cr (in women: 258.2 vs. 136.8 nmol/μmol, $P < 0.001$, in men: 134.5 vs. 102.6 nmol/μmol, $P < 0.05$), and serum adiponectin (19.9 vs. 17.3 ng/mL, $P = 0.020$, and 17.8 vs. 14.5 ng/mL, $P = 0.05$ in women and men, respectively). Liver markers, other mineral-bone parameters and resistin concentrations did not differ by nutritional status, in spite of the highly significant difference in mean leptin levels (3.1 vs. 30.4 ng/mL in women, 2.4 vs. 16.5 ng/mL in men, $P < 0.001$ for both groups). Malnourished men demonstrated the highest partial correlation between GGT and OC (r = - 0.497, $P = 0.010$), while the inverse correlation between GGT and OC/BAP ratio was similar and significant in all groups regardless of the nutritional status and gender (r = - 0.310 or higher, $P < 0.004$). Only in the malnourished women OC significantly correlated with adiponectin (r = 0.354, $P = 0.006$).

To further evaluate the association between OC and liver markers, adipokines and other indices of mineral-bone metabolism, we compared these parameters in HF patients, grouped according to serum OC levels. Patients with low OC levels (< 14 ng/mL, n = 156, or 53.2%), compared to the rest of the cohort (n = 138), were younger (80.7 ± 8.4 vs. 83.5 ± 7.7 years, $P = 0.003$), had higher mean serum GGT (67.3 ± 28.5 vs. 40.3 ± 21.2 U/L, $P = 0.001$) and 25(OH)D levels (40.8 ± 18.3 vs. 33.6 ± 17.1 nmol/L, $P = 0.001$), lower PTH (6.3 ± 4.3 vs. 7.7 ± 6.8 pmol/L, $P = 0.034$), calcium (2.26 ± 0.13 vs. 2.30 ± 0.11 mmol/L, $P = 0.033$), phosphate (0.88 ± 0.29 vs. 0.97 ± 0.28 mmol/L, $P = 0.007$), BAP (24.6 ± 12.6 vs. 28.4 ± 15.6 U/L, $P = 0.027$), and leptin (15.3 ± 18 vs. 22.1 ± 27.5 ng/mL, $P = 0.013$) levels, as well as lower both urinary bone resorption markers (DPD/Cr: 11.6 ± 5.7 vs. 13.7 ± 8.8 nmol/μmol, $P = 0.024$; NTx/Cr: 122.9 ± 119.6 vs. 204.2 ± 202.8 nmol/μmol, $P < 0.001$). No differences in mean values of ALT, ALP, bilirubin, magnesium, adiponectin, resistin and hemoglobin were observed.

Predictors of GGT, ALT, albumin and bilirubin (multiple regression analyses)

Multiple linear regression analyses with stepwise method were used to examine significant independent associations of liver markers with laboratory and clinical characteristics. In these models, each liver marker was set as dependent variable, while age, gender, OC, BAP, NTx/Cr, DPD/Cr, 25(OH)D, PTH, leptin, adiponectin, resistin, TSH, T4 and hemoglobin were set as independent variables (model 1). In model 2 further adjustment for alcohol use ≥ 3 drinks per week (yes/no), presence of CAD (yes/no), hypertension (yes/no), AF (yes/no), type 2 DM (yes/no), PD (yes/no) and CKD stage ≥ 3 (yes/no) was made. As presented in Table 3, reduced OC was an independent

Table 4. Multiple Linear Regression Analyses for Bone Turnover Markers in Older Patients With Hip Fracture

	Log OC	Log BAP	Log NTx/Cr	Log DPD/Cr	OC/BAP ratio
	β (P value)	β (P value)	β (P value)	β (P value)	β (P value)
Age	0.017 (0.001)		0.033 (< 0.001)		
Log GGT	-0.141 (0.012)	0.111 (0.003)	-0.315 (< 0.001)		-0.113 (0.043)
Log ALT	-0.132 (0.049)				
Log ALP	0.175 (0.038)	0.246 (< 0.001)	0.362 (0.008)	0.208 (0.008)	
Log leptin	0.084 (0.014)		-0.118 (0.027)	-0.069 (0.075)	0.013 (0.032)
R^2	0.1498	0.2043	0.2225	0.1350	0.1155

Parameters identified as significant and independent predictors of each bone turnover marker are shown. Adjusted for age, sex, GGT, ALT, ALP, albumin, bilirubin, 25(OH)D, PTH, leptin, adiponectin and resistin (all variables were log-transformed). β: β-standardized regression coefficient; GGT: gamma-glutamyltransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; OC: osteocalcin; BAP: bone specific alkaline phosphatase; NTx/Cr: cross-linked N-telopeptide of type 1 collagen corrected for urinary creatinine concentration; DPD/Cr: deoxypyridinoline corrected for urinary creatinine concentration.

predictor of higher GGT, ALT and bilirubin, whereas higher BAP was associated with GGT and ALP. These analyses also showed that NTx/Cr, hemoglobin (both inversely), adiponectin, alcohol overuse and CAD (all three positively) were independent predictors of GGT activity. Higher ALP was predicted by elevated BAP and lower 25(OH)D levels. The independent predictors of serum albumin were hemoglobin and resistin. In the fully adjusted model, higher bilirubin levels were predicted by lower OC, higher adiponectin and presence of AF, although male gender, PTH and resistin were independent predictors in model 1 (included only laboratory parameters), suggesting that these variables are incorporated in clinical factors used in the model 2. The full panel of determinants predicted 27.0%, 7.8%, 23.3%, 11.8% and 13.2% of variance in serum GGT, ALT, ALP, albumin and bilirubin levels, respectively. The liver markers' variance was only partially explained by the panel of determinants indicating the contribution of other regulating factors.

To further explore these observations, we constructed similar multiple linear regression analyses to predict liver markers in patients with and without malnutrition. These revealed that only in malnourished women, OC was not an independent predictor of GGT or ALT, and NTx/Cr predicted ALP activity (β = 0.108, P = 0.042), while all other abovementioned correlations in the entire cohort were present regardless of the nutritional status.

Predictors of bone turnover markers (multiple regression analyses)

Results from linear multiple regression with each bone turnover marker as a dependent variable and age, sex, GGT, ALT, ALP, albumin, bilirubin, 25(OH)D, PTH, leptin, adiponectin and resistin (all biochemical variables were log-transformed) as independent variables are shown in Table 4. Five factors were significantly and independently associated with OC: age, ALP, leptin (all three positively), GGT and ALT (both negatively). BAP was independently predicted by GGT and ALP. Both elevated NTx/Cr and DPD/Cr were predicted by higher

ALP activity and lower leptin concentrations; age also demonstrated a strong relationship with NTx/Cr. Finally, the independent determinants of OC/BAP ratio were GGT (inverse) and leptin (positive). Overall, the panel of determinants independently contributed to 15% of OC variance, to 20.4% of BAP, to 22.3% of NTx/Cr, to 13.5% of DPD/Cr, and to 11.6% of OC/BAP ratio variance.

Multivariate regression modeling, using abovementioned laboratory and clinical variables and eGFR confirmed also a significant inverse bidirectional link between leptin and adiponectin (β = -0.331, P = 0.036 for adiponectin as an independent correlate of leptin, and β = -0.065, P = 0.036 for leptin as a predictor of adiponectin).

Clinical implications

From the practical point of view, we tried to evaluate the diagnostic value of hepatic markers as indicators of the mineral-bone metabolism status. We focused on GGT activity which seems to be more relevant to bone metabolism and performed logistic regression analyses with GGT as a dependent variable (GGT ≤ 20 U/L (cut-off level of the first tertile) vs. > 20 U/L). Among patients with the GGT > 20 U/L, there was a 2.3-fold increase of subjects with low circulating OC levels (< 14 ng/mL, lower limit of normal range), about a 4.8-fold increase of subjects with elevated of BAP activity (> 43 U/L, upper limit of normal range) and, consequently, a 2.6-fold increase of subjects with low OC/BAP ratio (< 0.6, median level). These associations remained significant after adjustments for age, sex, serum adiponectin, leptin and resistin levels, alcohol overuse, presence of CAD or DM (Table 5). Diagnostic value of GGT > 20 U/L was as follows: for presence of low OC (< 14 ng/mL) sensitivity 59.9%, specificity 60.2%, positive predictive value (PPV) 75.7%, negative predictive value (NPV) 42.1%, positive likelihood ratio (LR) 1.51; for high BAP (> 43 U/L) 9.5%, 97.8%, 90.0%, 34.6%, 4.41, respectively; for low OC/BAP ratio (< 0.6) 57.9%, 65.6%, 77.5%, 43.3%, 1.68, respectively. Although the diagnostic value was relatively low, this still would have translated in identifying about 76% of patients

Table 5. Serum Gamma-Glutamyltransferase Activity (GGT > 20 U/L) as a Predictor of Abnormal Bone Formation Markers in Older Patients With Hip Fracture

Adjustments	OC < 14 ng/mL			BAP > 43 U/L			OC/BAP ratio < 0.6		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Unadjusted	2.26	1.32 - 3.87	0.003	4.76	1.03 - 30.33	0.044	2.62	1.52 - 4.54	0.001
+Age, sex	2.06	1.23 - 3.46	0.006	4.42	0.99 - 19.68	0.051	2.68	1.58 - 4.54	< 0.001
+Alcohol use*	2.07	1.22 - 3.50	0.007	4.88	1.10 - 21.75	0.038	2.81	1.64 - 4.82	< 0.001
+CAD	2.10	1.23 - 3.57	0.006	5.05	1.38 - 22.66	0.035	2.79	1.65 - 4.79	< 0.001
+DM	2.07	1.22 - 3.53	0.007	4.81	1.06 - 21.73	0.041	2.70	1.57 - 4.66	< 0.001
+Adipokines**	2.20	1.22 - 3.94	0.008	7.55	0.99 - 60.32	0.053	2.29	1.28 - 4.12	0.005

GGT: gamma-glutamyltransferase (> 20 U/L, above the level in the first tertile); OC: osteocalcin; BAP: bone specific alkaline phosphatase; CAD: coronary artery disease; DM: diabetes mellitus. *Three or more times a week. +Adjustments for all covariates in the above model. **Adipokines: adiponectin, leptin, resistin.

with abnormal bone formation status (PPV = 75.7% for low OC and PPV = 77.5% for low OC/BAP ratio) indicating that GGT > 20 U/L in the elderly can be used as a simple first step in detecting bone disease.

Interestingly, GGT > 20 U/L was also associated with a two-fold increase of presence of CAD (OR: 1.94, 95% CI: 1.01 - 3.65, P = 0.050) (sensitivity 25.0%; specificity 85.6%, PPV 76.7%, NPV 37.6%, LR 1.73).

Discussion

The major findings of this cross-sectional study are the significant and independent associations and bidirectional links between liver markers, indices of bone and mineral metabolism and adipokines. Two main points arise from our results: 1) the liver-bone interactions, and 2) the role of adipokines in these relationships.

Liver-bone interactions

Our results are consistent with many studies that have shown that elevated liver markers are not only sensitive indicators of hepatobiliary diseases and alcohol consumption, but even within reference range, as in the present study, are associated with a variety of common diseases, such as CAD, AF, DM, dementia, PD [28-33], and mortality [34-37] independently of alcohol consumption. Reported in the literature age and gender-based differences in liver [32, 34-36] and bone turnover markers [38-42], as well as in three studied adipokines [43-46] were confirmed within our study population, specifically, the decrease of transaminase activities and increase of bone turnover (OC and NTx/Cr) with ageing, and higher levels of urinary bone resorption markers and circulating adiponectin and leptin in women than in men.

In the present study, we focused on associations between hepatic markers (in the majority of our patients, they were within the normal range), parameters of mineral and bone metabolism, and the concentrations of three adipokines. We found significant bidirectional links between liver and bone-mineral

parameters, specifically between: 1) GGT and OC, NTx/Cr (both inverse) and BAP (positive), 2) ALT and OC (inverse), and 3) ALP and BAP (positive). In addition, ALP was an independent predictor of OC, NTx/Cr and DPD/Cr, and was negatively associated with 25(OH)D. OC was an independent negative correlate of bilirubin, and in the model which included only laboratory data, there was a significant association between PTH and bilirubin. Interestingly, the activity of ALT, a more specific hepatic marker than GGT, has shown similar to GGT inverse associations with OC but to a lesser degree.

Consistent with our findings, an inverse association of serum OC concentrations with ALT has been demonstrated in non-obese and obese subjects [13] and in patients with NAFLD [47, 48], independent of insulin resistance and the metabolic syndrome [47].

There are clinical and epidemiological reports, as well as in vitro and animal studies showing important physiological effects of GGT on bone metabolism. In postmenopausal women urinary GGT excretion exhibited a high correlation with DPD [49]. A large longitudinal study (16,036 Korean men aged ≥ 50 years) demonstrated that a higher serum GGT level was associated with increased development of osteoporotic fractures over a mean 3-year follow-up period [50]. Experimental data indicate that both a deficiency and an excess of GGT result in osteoporosis [49, 51-53]. GGT in vitro stimulates osteoclast formation (independently of its enzymatic activity) and expression of receptor activator of nuclear factor-kB ligand (RANKL) mRNA and protein from bone marrow stromal cells, and in transgenic mice promotes osteoporosis [51-53]. It has been proposed that osteopenia/osteoporosis in GGT-deficient (GGT-/+) mice is caused by suppression of bone formation, while excess of GGT results mainly in acceleration of bone resorption [53]. In our cohort of older HF patients, we found significant bidirectional inverse associations between GGT and both OC (an osteoblast-specific noncollagen protein, a bone formation marker) and NTx/Cr (a recognized bone resorption marker) and a positive association between GGT and BAP (a bone formation marker); GGT was an independent negative predictor of the OC/BAP ratio (an index of osteoblast differentiation) [27]. Our observations are in agreement with reports that OC and BAP are not uniformly associated.

For example, BAP correlates positively with OC in healthy women and negatively with OC in women with liver disease [54]. OC is in a positive and BAP is in a negative relationship with serum insulin growth factor-I (GF-1) [27] and the OC/BAP ratio is inversely associated with the number of vertebral fractures. In the current study, the OC/BAP ratio adjusted for age and sex was significantly and negatively associated with GGT, ALT, ALP and bilirubin indicating that dysregulated osteoblast formation and differentiation is linked to liver status. Moreover, GGT was an independent negative predictor of the OC/BAP ratio. The opposite associations of GGT with two osteoblast-derived bone formation markers (OC and BAP) could point to its specific effect on osteoblast differentiation, as OC is a protein that binds to hydroxyapatite deposited in the matrix, whereas BAP is essential to the process of mineralization of the matrix after formation of osteoid [55, 56]. The negative correlation between GGT and NTx/Cr (in adjusted multivariate linear regression analyses higher serum GGT was a significant independent predictor of lower NTx/Cr, and vice versa) may reflect the complex role of GGT in coordinating the tightly coupled bone formation and resorption. It appears that GGT acts as a factor that maintains bone homeostasis, and plays dual role in physiopathology: higher GGT levels may affect osteoblast differentiation and decreases bone resorption. However, in malnourished women both serum OC and urine NTx/Cr were not independent predictors of GGT activity, while BAP was significantly associated with GGT (as it was observed in the total cohort and in non-malnourished subjects), suggesting that the links between liver markers and specific metabolic indices of bone remodeling are modulated by nutritional status and gender, and these may contribute to an individual's propensity to osteoporosis and bone frailty.

The bidirectional link between BAP and ALP is not surprising as approximately half of circulating total ALP comes from bone [55, 56].

Our study also showed that serum hemoglobin is a significant and independent predictor of GGT (inverse association) and albumin, another determinant of bone health in the elderly [57]. These results are in agreement with previous epidemiologic studies that demonstrated that hemoglobin level is positively associated with risk of development of NAFLD independently of body mass index, type 2 DM, and other metabolic diseases [58]. Taken together, our results suggest reciprocal liver-bone cross-talk. Liver plays an important role in the regulation and dysregulation of bone remodeling and vice versa (bone-mineral metabolism parameters modulate various hepatic functions). In these interactions, GGT seems to be a major factor acting on and being influenced by bone turnover markers, as well as multiple other metabolic factors and conditions.

Adipokines and markers of hepatic, mineral and bone metabolism

Extensive studies of leptin, adiponectin, and resistin, the three best-studied adipokines, in both hepatic and bone metabolism have produced conflicting results, especially when comparing data from clinical and animal reports. In HF patients, studies on adipokines are limited [44, 59-62], and the relationship of adipokines with liver and bone turnover markers have not been evaluated systematically.

The present study, to our knowledge for the first time, documents the links between adiponectin, leptin and resistin, and liver and bone-mineral indices in patients with HF. In our multiple linear regression analyses, adiponectin, but not leptin and resistin, was an independent predictor of GGT activity, resistin (in humans, unlike in rodents, it is produced mostly in macrophages) [63] was a determinant for albumin and bilirubin (the latter in a model adjusted only for laboratory variables), whereas leptin independently predicted OC, OC/BAP ratio, NTx/Cr and DPD/Cr levels (inversely for both resorption markers).

In regard to adipokines-liver relationship, our observations are consistent with current evidence that the liver is a major target organ for many of its effects [18, 64]. In our study, as in previous reports, adiponectin was negatively associated with GGT [65, 66], although others found only a weak inverse association [67], or even a positive correlation [68] between these variables; some researchers demonstrated that adiponectin was negatively associated with circulating ALT activity [65-67, 69]. In a previous study, as in ours, serum levels of resistin positively correlated with bilirubin [68]. Our data are also in line with recent experiments showing that the majority of metabolic effects of leptin essential for energy homeostasis are dependent on its action in nonhepatocyte cells and/or the central nervous system [70].

Concerning the adipokine-bone links, we found that in the entire cohort leptin (but not adiponectin or resistin) was an independent predictor of OC, OC/BAP ratio and both bone resorption markers. These findings are in contrast with the widespread (based mainly on animal studies) view that leptin acting primarily through the centrally (hypothalamus and brainstem) mediated pathways suppresses bone formation [14, 71, 72], but are in accord with some, but not all [34, 44, 73-76], clinical studies that reported a positive association between serum leptin and OC levels [77, 78] and with experiments showing that peripheral leptin increases osteoblast proliferation, differentiation and activity, inhibits osteoclast activity and bone resorption, increasing bone mass [79-81]. Furthermore, a meta-analysis (59 studies) found that leptin was positively associated with bone mineral density (BMD) and high levels of leptin were predictive of lower risk of fractures [82].

There is considerable controversy on the role of adiponectin in bone metabolism. Although *in vitro* studies demonstrated that adiponectin stimulates the differentiation and mineralization of osteoblasts and the expression of OC, directly inhibits osteoclast activity and indirectly stimulates osteoclast differentiation [83-86], it is less clear whether adiponectin plays the same role in humans. We found that in malnourished women (but not in other groups) adiponectin significantly correlated with OC, suggesting that bone-adipocyte interaction in these subjects may be regulated through adiponectin and OC. Adiponectin was positively associated with total OC [87, 88] and BAP [89] in postmenopausal women and in Chinese men [90], but in other studies no correlation with any of bone formation
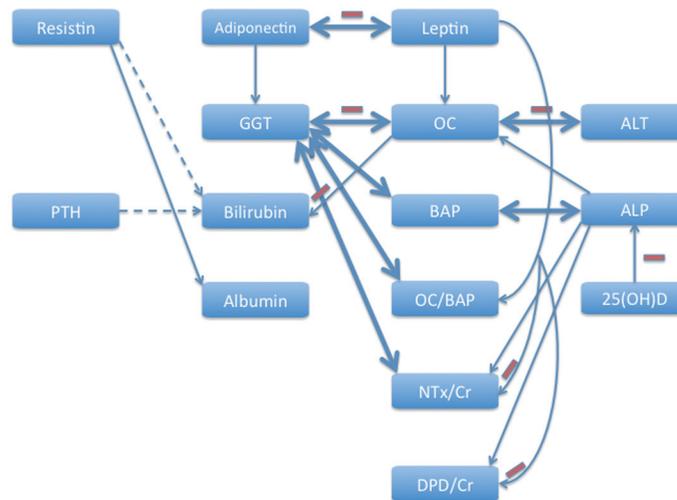
Figure 1. Links between liver markers, indices of bone metabolism and adipokines in older patients with hip fracture. GGT: gamma-glutamyltransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; OC: osteocalcin; BAP: bone specific alkaline phosphatase; NTx/Cr: cross-linked N-telopeptide of type 1 collagen corrected for urinary creatinine concentration; DPD/Cr: deoxypyridinoline corrected for urinary creatinine concentration; PTH: parathyroid hormone; Hb: hemoglobin. Bidirectional links are shown in bold arrows; -- indicates inhibitory effect. The interactions between GGT and OC, BAP, and NTx/Cr, as well as between ALT and OC, and between ALP and BAP are bidirectional: the activity of hepatic enzymes contributes to synthesis and release of molecules reflecting bone formation and bone resorption, and these, in turn, influence the circulating levels of liver enzymes. ALP activity correlates with levels of serum OC and urinary resorption markers. There is a strong reciprocal association between adiponectin and leptin. Adiponectin is positively associated with GGT activity, while leptin is positively associated with OC and OC/BAP ratio and inversely with both resorption markers; therefore, adiponectin and leptin may exert opposite effects on bone metabolism; resistin is associated with serum albumin concentration. In multiple logistic regression analyses adjusted for age, sex and confounding laboratory (but not clinical) variables both resistin and PTH were independently associated with bilirubin levels (shown in dashed lines). All other data are based on fully adjusted model. Of note, in malnourished patients circulating adiponectin and OC levels are positively associated, while adiponectin does not correlate with GGT activity (not shown).

or bone resorption markers was found [45, 91]. Several clinical studies have shown an inverse relationship between circulating adiponectin and BMD independent of gender and menopausal status, and some reviewers concluded that adiponectin may be a negative regulator of bone mass [82, 92-94], while other emphasized that the data on this association are inconsistent [95].

It is also uncertain whether resistin is related to bone turnover markers. Resistin is expressed in primary human bone marrow stem cells and in mature human osteoblasts [96], but no association between resistin and BMD was found in clinical studies [92]. In our study, circulating resistin levels in line with the latter data were not associated with bone turnover markers.

In line with many previous studies, we observed a significant inverse relationship between adiponectin and leptin, both by paired comparisons adjusted for age and sex, as well as multivariate regression modeling. This indicates that metabolic functions of leptin and adiponectin, which generally affect cellular behavior in an opposing manner, should not be considered in isolation, but rather as complementary. Our data suggest that the bone remodeling, as measured by bone formation and resorption markers, depends, at least partially, on the regulatory interaction between adiponectin and leptin which may exert opposite effects on bone metabolism through different pathways. In other words, the adiponectin-leptin link appears as an important mediator of the association between

GGT and OC.

Our analysis also identified that in malnourished women, in contrast to subjects without such condition, adiponectin is not associated with GGT, but strongly correlates with OC. It appears that in different diseases and in particular conditions adiponectin may be regulated in the opposite directions and may exert opposite activities [97, 98]. At the molecular level, variations have been found at all sites of adiponectin action including different subtypes of adiponectin receptors operating in the liver and bones [94, 99, 100]. Our observations are in agreement with a recent animal study, which concluded that adiponectin has the ability to regulate the same function in two opposite manners depending on where it acts and it opposes leptin's influence [101]. Our results show that adiponectin may exert a negative net effect on bone metabolism by increasing GGT and decreasing circulating leptin levels (inverse adiponectin-leptin relationship); on the other hand, OC and adiponectin have opposite effects on GGT. However, these relationships are not consistently maintained, but expressed differently under various pathophysiological conditions indicating the level of complexity of the homeostatic system(s). Specifically, in malnourished women increases in serum adiponectin, which is produced almost exclusively by adipocytes, are associated with higher OC levels and do not correlate with GGT.

Alterations in vitamin D-PTH status are factors substantially contributing to the high prevalence of osteoporosis and fractures in the elderly [1]. Although vitamin D insufficiency and elevated PTH levels were recorded in 79.7% and 38.2% of our patients, respectively, in multivariate analyses no significant correlations were detected between GGT and ALT activities, on the one hand, and 25(OH)D and PTH concentrations, on the other, suggesting that the vitamin D-PTH axis is not an independent determinant of the transaminase activities. Also, 25(OH)D and PTH were not independent predictors of bone turnover markers, indicating that vitamin D and PTH are not involved in the relationship of these liver enzymes with bone remodeling. We found positive correlations between 25(OH)D and ALP, and between PTH and bilirubin, which indicate that vitamin D insufficiency and SHPT may affect specific liver functions, as it was previously reported for the PTH-bilirubin association [102, 103].

Potential mechanisms: combining regulatory elements

Our results illustrate complex interactions between different adipokines, liver function and mineral-bone metabolism, and extend this notion by showing these links in HF patients. The findings suggest liver function as an important component involved in bone remodeling. Significant independent correlates for OC included GGT, ALT (both negative), ALP, leptin and age (all three positive), for BAP-GGT and ALP, for NTx/Cr-GGT and leptin (both negative), ALP and age (both positive), for DPD/Cr-ALP (positive) and leptin (negative), and for OC/BAP ratio-GGT (negative) and leptin (positive). Integrating multidirectional interrelationship between adipokines, hepatic and bone metabolism, factors acting on and/or being influenced by each other, it is possible to propose a model that unifies a number of functions and observations that have been considered contradictory in the past (Fig. 1). This model based on the results of multivariate regression analyses takes into account that homeostasis necessitates reciprocal signaling between hepatic function, components of mineral-bone metabolism and adipokines. It should be viewed as a part of a complex network that integrates and orchestrates the liver-bone-adipose tissue axis in health and diseases. Of major pathophysiological interest is the feedback loop between GGT and OC, in which adiponectin and leptin appear as two important counterplayers: the former acts as a positive regulator of GGT, while the latter is a positive regulator of OC (Fig. 1, upper part). Importantly, these relationships are not invariant, but depend on multiple underlying conditions, among which the nutritional status and gender may be the key contributors.

The stronger (compared to other liver indices) and bidirectional association of GGT, an indicator of oxidative stress [28, 104], with bone turnover markers suggests that the liver-bone links might reflect systemic rather than solely hepatic processes. Several lines of evidence indicate oxidative stress, a systemic process implicated in the regulation of ageing and longevity [105], as well as numerous pathological conditions [106, 107], including liver diseases [108] and osteoporosis [109, 110], as a possible unifying factor. 1) GGT is respon-

sible for the extracellular catabolism of the main antioxidant in mammalian cells - glutathione [28, 104, 111]. 2) The liver plays a key role in the systemic glutathione (GSH) homeostasis [112, 113]. 3) Adiponectin significantly contributes to oxidative damage [114]. 4) Oxidative stress produces deleterious effects in osteoblasts [115]. In addition, our study showed involvement of other factors which have also been proposed as indices of oxidant stress status, namely serum hemoglobin levels were significantly and independently associated with GGT activity (inversely) and albumin concentration (positively), while OC correlated with bilirubin level. Hemoglobin [116, 117], albumin [118-120] and bilirubin [118, 121] exhibit potent antioxidant properties. Increases in GGT activity may reflect the responses of counteracting mechanisms to protect against oxidative damage. Our findings suggest the need to explore in depth the role of liver function in bone metabolism, in particular, the participation of GGT in these processes.

Potential clinical implications

Of practical interest is the finding that in the HF patients GGT > 20 U/L (above the cut-off level of the first tertile) corresponds with a two times higher prevalence of CAD, low OC levels (< 14 ng/mL, lower limit of normal range) and 2.7 times higher prevalence of low OC/BAP ratio. Consistent with our results, previous studies have found GGT to be associated with CAD [31, 33, 122, 123]. Collectively these results indicate that GGT within the normal range is broadly linked to health conditions, including osteoblast dysfunction, and older patients with GGT > 20 U/L need an examination of their bone status and consideration of an antiosteoporotic medication with anabolic properties. Currently, osteoporosis is predominantly treated with antiresorptive medications, despite the fact that in a significant proportion of elder patients bone loss is primarily attributed to the impaired osteoblastic activity [124]. Serum GGT > 20 U/L may be a promising indirect marker useful as the first and easy step in diagnostic evaluation of impaired bone metabolism in the elderly and pointing to the need of individualized therapy; its PPV for low OC and for low OC/BAP ratio is above 75%. The clinical significance of this marker should be confirmed and validated by longitudinal studies.

Limitations

The main limitations of this study are the cross-sectional nature of the analysis and the single point-in-time assessment of the hepatic indices, mineral-bone turnover markers and adipokines. Therefore, the associations between the variables found in the study suggest a link, but a causal relationship cannot be established. Secondly, we did not measure the undercarboxylated OC (unOC), RANKL, osteoprotegerin, high molecular weight adiponectin, and sexual hormones, each of which may be involved in the liver-bone interaction. Thirdly, we could not eliminate the possible effect of medications used on the present findings, although we attempted to control most potential confounders. Finally, because the study population

consisted predominantly of Caucasians, generalization of the results should be done with caution. The strengths of our study include the use of data from an unselected well-characterized cohort of HF patients, simultaneous measurements of multiple hepatic, mineral-bone biomarkers and three adipokines. Pearson's correlation coefficients preserved statistical significance after Bonferroni and Sidak adjustments. The variance inflation factor in our multivariate regression analyses ranged between 1.07 and 1.32 indicating that the multicollinearity phenomena were not significant.

Conclusions

In older HF patients liver functions (within normal range in the vast majority) are associated with indices of bone metabolism. The complex liver-bone-adipokine interactions include bidirectional links between GGT and markers of bone formation (OC, BAP), differentiation (OC/BAP ratio) and resorption (NTx/Cr), as well as between ALT and OC. Adiponectin is an independent predictor of GGT, whereas leptin is a determinant of OC and bone resorption markers; these relationships are modulated by nutritional status and gender. GGT > 20 U/L is associated with a two times higher prevalence of low OC levels and 2.7 times higher prevalence of low OC/BAP ratio; it may be used as a marker of impaired bone metabolism.

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