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SERUM LACTOFERRIN LEVELS IN ABDOMINAL OBESE POSTMENOPAUSAL WOMEN WITH NORMAL BONE STATUS AND OSTEOPENIA*

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ABSTRACT

Background. Recently, it has been shown that lactoferrin intake might impact bone health. However, less is known about the association between blood lactoferrin levels and bone status. Therefore, this study aimed to compare serum lactoferrin concentrations between abdominally obese postmenopausal women with normal bone health and osteopenia.

Material and methods. A total of 238 women aged 50–75 years with a body mass index (BMI) ≥ 30 kg/m^2 and waist circumference ≥ 88 cm were included in the study. Their bone mineral density (BMD) and content (BMC) for the total body, lumbar spine and femoral neck were measured using the dual-energy X-ray absorptiometry method, and the T-score and Z-score were calculated. Biochemical markers of glucose and insulin homeostasis, lipid metabolism and inflammatory parameters, lactoferrin levels, and body composition were determined.

Results. Women with a T-score of the lumbar spine > –1 were significantly younger (p = 0.0005) and had a higher body weight (p = 0.0208) and BMI (p = 0.0312) than participants with a T-score ≤ –1. In the group of subjects with a T-score > –1, BMC at the lumbar spine was significantly higher in women with lactoferrin levels below than above the median (p = 0.0455), whereas in the group of those with a T-score ≤ –1, women with lactoferrin levels lower than the median had significantly lower BMC (p = 0.0298), BMD (p = 0.0428), T-score (p = 0.0216) and Z-score (p = 0.0452) at the femoral neck and BMC (p = 0.0358), BMD (p = 0.0244) and T-score (p = 0.0139) at the total body.

Conclusion. These results suggest that serum lactoferrin levels might be associated with bone health, but the effect may differ in subjects with normal bone status and osteopenia.

Keywords: lactoferrin, obesity, densitometry, bone density, women

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INTRODUCTION

Menopause is often a turning point in a woman's life and is associated with an increase in fat mass, insulin resistance, dyslipidaemia and endothelial dysfunction, as well as a higher risk of osteopenia and osteoporosis (World Health Organization, 2022). The prevalence of these diseases is expected to rise in the future due to the increasingly ageing global population (Clynes et al., 2020). Decreased ovarian oestrogen production and relative androgen excess around menopause onset are one of the most studied factors linking menopause and bone loss (Roa-Díaz et al., 2021). It seems that excessive body weight and metabolic abnormalities, often observed in postmenopausal women, also have an unfavourable effect on bone health (Rinonapoli et al., 2021), with obese subjects more at risk of reduced bone mineral density (BMD) due to the replacement of osteoblasts in bone by adipocytes (Gkastaris et al., 2020).

Lactoferrin is an iron-binding glycoprotein produced by epithelial cells and found in neutrophil granules. It is also present in most exocrine secretions, including human and bovine milk (Shini et al., 2022), and has multidirectional actions. This iron-binding glycoprotein possesses bacteriostatic properties, anti-inflammatory activity, regulates cellular growth and differentiation and protects against cancer development (Arredondo-Beltrán et al., 2023; Xu et al., 2022; Yami et al., 2023). Recently, it has been shown that lactoferrin can also promote bone health. In vivo, local injection of lactoferrin in adult mice stimulated the proliferation and differentiation of primary osteoblasts (Cornish et al., 2004), inhibiting osteoclast formation (Cornish et al., 2006). Moreover, a significant increase in the dynamic histomorphometric indices of bone formation and bone area was found (Cornish et al., 2004). Animal studies have also shown that lactoferrin intake significantly improved BMD, simultaneously affecting bone resorption and formation markers (Fan et al., 2018; Xu et al., 2020). Moreover, Bharadwaj et al. (2009) showed that RNAse-enriched-lactoferrin supplements significantly reduced bone resorption and increased osteoblastic bone in 38 healthy postmenopausal women aged 45–60 years. Unfortunately, less is known about the association between blood lactoferrin levels and bone health.

Therefore, this study compared serum lactoferrin levels between abdominally obese postmenopausal women with normal bone status and osteopenia. Moreover, as metabolic abnormalities are often present in postmenopausal obese women (Pu et al., 2017), we also compared selected cardio-metabolic markers between women with normal bone health and osteopenia, as well as contrasting densitometric parameters between metabolically healthy (MHO) and unhealthy (MUHO) obese postmenopausal women.

MATERIAL AND METHODS

Ethics

The present study is a retrospective analysis of pre-intervention data from the CLA (Łochocka et al., 2014), Endofit (Jamka et al., 2020) and Ced-Med (Bajerska et al., 2018) projects. The projects were conducted according to the guidelines of the Declaration of Helsinki (Sawicka-Gutaj et al., 2022) and obtained ethical approval from the Ethical Committee of Poznań University of Medical Sciences (protocol code: 606/12, 453/13, 358/14, 603/14, 398/15, 219/16, 984/17 and 1155/18). Subjects willing to participate in the projects received information about the study and were aware that their participation was voluntary, meaning that they could withdraw from the study at any time without providing reasons. Written informed consent was obtained from all participants before enrolment.

Inclusion and exclusion criteria

This study included postmenopausal females aged 50–75 years old with a body mass index (BMI) ≥ 30 kg/m^2 and a waist circumference ≥ 88 cm. Subjects who had used any dietary supplements within the three months before enrolment were excluded from the analysis, as were subjects with a cancer diagnosis in the last five years, acute or chronic kidney and liver diseases, systemic inflammatory diseases, and any other serious chronic diseases with poor health status.

Anthropometric measurements

The following anthropometric parameters were measured in the morning with the participants wearing light clothing and standing barefoot: body height and weight and waist circumference. Body weight and height were assessed using electronic scales with

a stadiometer and were performed with an accuracy of 0.1 kg and 0.01 m, respectively (Norton, 2018). Waist circumference was measured on bare skin at the level of the iliac crest with the subjects at minimal respiration using a measurement tape with an accuracy of 0.1 cm (World Health Organization, 2008). Based on anthropometric parameters, BMI was calculated. The World Health Organization criteria were used to classify subjects' nutritional status. A BMI ≥ 30 kg/m^2 was considered obese (World Health Organization, 2020) and abdominal obesity was diagnosed when the waist circumference exceeded 88 cm (National Cholesterol Education Program (NCEP) Expert Panel on Detection et al., 2002).

Densitometric parameters

Densitometric parameters were assessed at the Department of Pediatric Gastroenterology and Metabolic Diseases at Poznan University of Medical Sciences. Body composition assessment, including measurement of total fat and visceral adipose tissue (VAT), was performed in accordance with the dual-energy X-ray absorptiometry (DXA) method using the Hologic Discovery DXA system (Bedford, MA, USA). BMD and bone mineral content (BMC) at the total body, lumbar spine (L1-L4) and femoral neck were also measured, and the T-score and Z-score were calculated. The T-score is the number of standard deviations of the BMD measurement above or below that of young, healthy adults of the same sex. The Z-score represents the number of standard deviations of the BMD measurement above or below that of adults of the same age and sex. All measurements were based on the International Society for Clinical Densitometry guidelines (Hangartner et al., 2013). During the assessment, participants wore light clothing and removed all metal objects, and the women were instructed not to exercise for 24 h before measurement. Calibration was performed every day. The World Health Organization Study Group criteria were used to diagnose bone health status based on DXA measurements and the T-score for the lumbar spine, as the measurement in this location was available for the total study population. A T-score > –1 indicated normal bone health, osteopenia was diagnosed for a T-score ≤ –1 and > –2.5, while a value ≤ –2.5 suggested osteoporosis (World Health Organization, 1994).

Blood pressure

Blood pressure was measured according to the European Society of Hypertension guidelines (Mancia et al., 2023) based on the oscillometric method using an electronic sphygmomanometer. Blood pressure was assessed after five minutes of rest and was expressed as systolic (SBP) and diastolic pressures (DPB). The average of three measurements was used for statistical analysis.

Biochemical parameters

A laboratory diagnostician took blood samples after 12 h of fasting, centrifuged and stored them at –80 C until analysis. The following biochemical parameters were measured: lactoferrin, glucose, insulin, lipid profile and high-sensitivity C-reactive protein (hsCRP). Standard clinical chemical assays were used to measure fasting glucose, insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and hsCRP levels. Serum lactoferrin concentrations were measured using the BIOXYTECH Lactof EIA reagent set (Oxis Research, Oxis International, Beverly Hills, CA, USA). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated (Matthews et al., 1985). Adult Treatment Panel III criteria were used to diagnose insulin resistance (Gayoso-Diz et al., 2013). Participants were classified into MHO and MUHO groups according to the criteria set forth by the National Cholesterol Education Program Adult Treatment Panel III (2002). MHO individuals exhibited fewer than three of the following conditions, while MUHO individuals displayed a minimum of three of the following abnormalities:

- waist circumference: ≥ 88 cm
- SBP: ≥ 130 mmHg and/or DBP: ≥ 85 mmHg, or current use of antihypertensive medications
- TG levels: ≥ 150 mg/dl
- fasting glucose levels: ≥ 100 mg/dl
- HDL-C levels: < 50 mg/dl.

Statistical analysis

Statistica 13.0 software (TIBCO Software Inc., Palo Alto, CA, USA) was used for statistical analyses with a two-sided $p < 0.05$ being regarded as statistically significant. The variables were tested for normality using the Shapiro-Wilk test. The characteristics of the

study population were expressed as medians and the interquartile ranges (IQR) based on non-parametric data distribution. Comparisons between two unpaired groups were conducted using the Mann–Whitney U test.

RESULTS

Study population

The characteristics of the study participants are shown in Table 1. A total of 238 postmenopausal abdominally obese women were included in the study, with a median age of 59 (55–63) years and a BMI of 34.76 (31.80–37.85) kg/m^2.

Comparison of study participants based on bone health status

The study population was divided into two groups based on their lumbar spine (L1-L4) T-score value: group one consisted of subjects with a T-score ≤ –1 (n = 61) and group two included women with a T-score > –1 (n = 177). The prevalence of osteopenia based on the T-score of the lumbar spine (L1-L4) was 25.6%, with no osteoporosis detected in any of the subjects examined. Women with a T-score > –1 were significantly younger (60 (54–62) *vs.* 62 (57–65) years, p = 0.0005) and had a higher body weight (90.00 (82.78–100.40) *vs.* 85.48 (78.80–96.70) kg, p = 0.0208) and BMI (35.18 (32.05–38.18) *vs.* 34.10 (31.27–36.52) kg/m^2, p = 0.0312) than participants with a T-score ≤ –1. A comparison between groups is presented in Table 2.

Comparison of study participants based on lactoferrin levels

Study participants with lactoferrin concentrations below the median were compared independently with those with lactoferrin levels above the median in groups of women with a T-score for the lumbar spine (L1-L4) ≤ –1 and > –1. In the group of women with a T-score > –1, subjects with lactoferrin levels lower than the median had a significantly lower BMI (34.30 (31.79–37.40) *vs.* 36.77 (32.62–40.15) kg/m^2, p = 0.0120) and hsCRP levels (2.10 (1.30–4.30) *vs.* 3.90 (2.05–6.30) mg/l, p = 0.0016) than participants with lactoferrin levels higher than the median. Moreover, significant differences in BMC at the lumbar spine (L1-L4) were found between women with lactoferrin concentrations

Table 1. Characteristics of the study population (n = 238)

	Median (IQR)
Age, years	59 (55–63)
Height, m	1.60 (1.56–1.64)
Weight, kg	88.60 (82.04–100.20)
BMI, kg/m^2	34.76 (31.80–37.85)
Waist circumference, cm	108.0 (102.5–113.5)
Fat mass, g*	37 698.5 (31 815.0–43 732.0)
Fat mass, %*	43.0 (38.3–47.3)
VAT mass, g	1 076.0 (908.0–1 276.0)
Femoral neck BMC, g**	4.24 (3.88–4.74)
Femoral neck BMD, g/cm^{2}**	0.86 (0.79–0.95)
Femoral neck T-score**	0.10 (–0.60–0.90)
Femoral neck Z-score**	1.40 (0.60–2.00)
L1-L4 BMC, g	57.53 (53.02–65.59)
L1-L4 BMD, g/cm^2	1.01 (0.93–1.09)
L1-L4 T T-score	–0.26 (–1.00–0.40)
L1-L4 Z-score	1.11 (0.46–1.77)
Total BMC, g	2 305.50 (2 114.00–2 517.00)
Total BMD, g/cm^2	1.14 (1.06–1.21)
Total T-score	0.50 (–0.30–1.20)
Total Z-score	0.90 (0.30–1.50)
SBP, mmHg	140 (130–149)
DBP, mmHg	85 (79–90)
Glucose, mg/dl	97 (90–107)
Insulin, µU/ml	12.05 (8.40–16.80)
HOMA-IR	2.89 (2.02–4.28)
TC, mg/dl	223 (192–248)
HDL-C, mg/dl	55 (47–62)
TG, mg/dl	134 (99–176)
LDL-C, mg/dl	137 (109–163)
hsCRP, mg/l***	3.00 (1.05–5.60)
Lactoferrin, µg/ml	1.64 (1.03–2.40)

*n = 226; **n = 121; ***n = 236

BMI – body mass index; BMC – bone mineral content; BMD – bone mineral density; DBP – diastolic blood pressure; HDL-C – high-density lipoprotein cholesterol; HOMA-IR – homeostatic model assessment – insulin resistance; hsCRP – high sensitive C-reactive protein; IQR – interquartile range; LDL-C – low-density lipoprotein cholesterol; L1-L4 – lumbar spine; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides; VAT – visceral adipose tissue.

Table 2. Comparison of subjects with a T-score > –1 and ≤ –1 for L1-L4. Data presented as median and IQR

	T–score > –1 (n = 177)	T–score ≤ –1 (n = 61)	p
Age, years	60 (54–62)	62 (57–65)	0.0005
Height, m	1.60 (1.57–1.64)	1.60 (1.56–1.64)	0.3608
Weight, kg	90.00 (82.78–100.40)	85.48 (78.80–96.70)	0.0208
BMI, kg/m^2	35.18 (32.05–38.18)	34.10 (31.27–36.52)	0.0312
Waist circumference, cm	108.0 (103.0–114.0)	105.0 (102.0–110.0)	0.0845
Fat mass, g*	37 639.0 (31 979.0–44 849.0)	38 617.0 (30 362.0–42 825.0)	0.4229
Fat mass, %*	42.9 (38.1–47.3)	43.3 (38.7–47.5)	0.5510
VAT mass, g	1 070.0 (923.0–1 280.0)	1081.0 (893.0–1250.0)	0.7875
Femoral neck BMC, g**	4.31 (3.94–4.80)	3.94 (3.28–4.19)	0.0008
Femoral neck BMD, g/cm^{2**}	0.89 (0.81–0.99)	0.81 (0.75–0.84)	0.0002
Femoral neck T-score**	0.30 (–0.35–1.25)	–0.50 (–1.10—0.10)	0.0001
Femoral neck Z-score**	1.50 (0.80–2.30)	0.70 (0.20–1.30)	0.0003
L1–L4 BMC, g	60.69 (55.14–66.46)	51.07 (48.09–54.74)	<0.0001
L1–L4 BMD, g/cm^2	1.06 (0.99–1.13)	0.90 (0.86–0.93)	<0.0001
L1–L4 Z–score	1.40 (1.00–1.90)	0.09 (–0.30–0.28)	<0.0001
Total BMC, g	2 385.57 (2 206.00–2 601.00)	2 057.00 (1 909.28–2 175.13)	<0.0001
Total BMD, g/cm^2	1.17 (1.11–1.23)	1.03 (1.00–1.07)	<0.0001
Total T-score	0.90 (0.20–1.50)	–0.80 (–1.30—0.50)	<0.0001
Total Z-score	1.20 (0.70–1.70)	0.00 (–0.30–0.28)	<0.0001
SBP, mmHg	140 (130–149)	140 (130–150)	0.3349
DBP, mmHg	84 (78–90)	85 (80–95)	0.2066
Glucose, mg/dl	97 (90–107)	97 (92–107)	0.4751
Insulin, µU/ml	11.90 (8.60–17.00)	12.80 (8.00–14.90)	0.8741
HOMA-IR	2.78 (2.06–4.34)	3.06 (1.89–3.95)	0.9261
TC, mg/dl	223 (193–252)	225 (190–241)	0.8970
HDL-C, mg/dl	55 (47–62)	57 (48–64)	0.5551
TG, mg/dl	134 (97–177)	134 (103–165)	0.9210
LDL-C, mg/dl	138 (109–163)	137 (107–159)	0.6506
hsCRP, mg/l**	3.10 (1.60–6.00)	2.80 (1.30–4.60)	0.3701
Lactoferrin, µg/ml	1.64 (1.07–2.36)	1.79 (0.90–2.67)	0.9338

*T-score ≤ –1: n = 55, T-score > –1: n = 171.
**T-score ≤ –1: n = 29, T-score > –1: n = 92.
BMI – body mass index; BMC – bone mineral content; BMD – bone mineral density; DBP – diastolic blood pressure; HDL-C – high-density lipoprotein cholesterol; HOMA-IR – homeostatic model assessment - insulin resistance; hsCRP – high sensitive C-reactive protein; IQR – interquartile range; LDL-C – low-density lipoprotein cholesterol; L1-L4 – lumbar spine; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides; VAT – visceral adipose tissue.

below and above the median (61.19 (55.95–67.61) *vs.* 59.36 (54.23–65.78) g, $p = 0.0455$). In the group of subjects with a T-score ≤ –1 for the lumbar spine (L1-L4), women with lactoferrin levels lower than the median had a significantly lower fat mass than subjects with lactoferrin levels higher than the median (33632.5 (28920.5–40804.5) *vs.* 39308.2 (34314.0–43880.0) g, $p = 0.0318$). This group was also characterised by a lower BMC at the femoral neck and total body (femoral neck: 3.55 (3.23–3.83) vs. 4.11 (3.44–4.37) g, $p = 0.0298$, total body: 2 013.18 (1 912.00–2 089.00) vs. 2 136.00 (1 876.00–2 324.06) g, $p = 0.0358$), BMD at the femoral neck and total body (femoral neck: 0.75 (0.69–0.79) vs. 0.82 (0.77–0.87) g/cm^2, $p = 0.0428$, total body: 1.02 (0.99–1.05) vs. 1.06 (1.01–1.12) g/cm^2, $p = 0.0244$), T-score at the femoral neck and total body

Table 3. Comparison of subjects with lactoferrin levels lower and higher than median according to T-score values for L1-L4. Data presented as median and IQR

	T–score > –1 (n = 177)		p	T–score ≤ –1 (n = 61)		p
	lactoferrin levels lower than median (n = 89)	lactoferrin levels higher than median (n = 88)		lactoferrin levels lower than median (n = 30)	lactoferrin levels higher than median (n = 31)	
1	2	3	4	5	6	7
Age, years	58 (54–62)	59 (54–63)	0.6969	63 (60–65)	60 (56–65)	0.1521
Height, m	1.61 (1.58–1.64)	1.59 (1.55–1.64)	0.0153	1.59 (1.56–1.61)	1.61 (1.55–1.65)	0.1717
Weight, kg	88.57 (82.40–99.20)	91.16 (83.95–104.46)	0.1892	83.72 (78.20–90.00)	88.20 (78.80–101.20)	0.2017
BMI, kg/m^2	34.30 (31.79–37.40)	36.77 (32.62–40.15)	0.0120	33.24 (30.81–36.16)	35.00 (31.27–37.31)	0.5209
Waist circumference, cm	108.0 (103.0–113.0)	108.0 (102.2–115.2)	0.3204	103.7 (99.0–108.0)	109.0 (103.0–113.5)	0.0511
Fat mass, g*	38 509.0 (32 786.0–45 131.0)	36 985.5 (30 955.0–43 483.0)	0.3685	33 632.5 (28 920.5–40 804.5)	39 308.2 (34 314.0–43 880.0)	0.0318
Fat mass, %*	43.4 (38.9–47.4)	42.5 (37.4–47.1)	0.4389	41.3 (36.1–46.5)	43.7 (40.8–48.2)	0.1225
VAT mass, g	1 081.0 (937.0–1273.0)	1 057.5 (879.0–1317.5)	0.8718	1 015.0 (874.0–1 170.0)	1 117.0 (1 008.0–1 332.0)	0.1061
Femoral neck BMC, g**	4.27 (3.92–4.80)	4.34 (4.00–4.83)	0.7617	3.55 (3.23–3.83)	4.11 (3.44–4.37)	0.0298
Femoral neck BMD, g/cm^2**	0.88 (0.80–0.99)	0.89 (0.83–0.99)	0.6478	0.75 (0.69–0.79)	0.82 (0.77–0.87)	0.0428
Femoral neck T-score**	0.25 (–0.50–1.30)	0.35 (–0.15–1.20)	0.6363	–1.00 (–1.55—0.60)	–0.20 (–0.70–0.10)	0.0216
Femoral neck Z-score**	1.50 (0.70–2.40)	1.50 (0.95–2.20)	0.8469	0.20 (–0.05–0.50)	1.10 (0.50–1.40)	0.0452
L1-L4 BMC, g	61.19 (55.95–67.61)	59.36 (54.23–65.78)	0.0455	50.82 (48.32–53.64)	51.41 (47.56–55.26)	0.6809

Table 3 – cont.

1	2	3	4	5	6	7
L1-L4 BMD, g/cm^2	1.07 (1.01–1.15)	1.06 (0.99–1.11)	0.2129	0.90 (0.87–0.93)	0.91 (0.86–0.93)	0.9770
L1-L4 T T-score	0.20 (–0.40–0.90)	0.11 (–0.50–0.52)	0.2317	–1.45 (–1.60—1.15)	–1.30 (–1.60–1.10)	0.3625
L1-L4 Z-score	1.40 (1.10–2.14)	1.35 (0.93–1.80)	0.1847	0.09 (–0.19–0.28)	0.09 (–0.38–0.40)	0.6753
Total BMC, g	2 389.0 (2 236.7–2 626.7)	2 364.1 (2 185.4–2 565.2)	0.1428	2 013.18 (1 912.00–2 089.00)	2 136.00 (1 876.00–2 324.06)	0.0358
Total BMD, g/cm^2	1.17 (1.12–1.26)	1.15 (1.10–1.21)	0.2599	1.02 (0.99–1.05)	1.06 (1.01–1.12)	0.0244
Total T-score	0.80 (0.20–1.80)	0.90 (0.20–1.40)	0.6385	–1.00 (–1.30—0.60)	–0.60 (–1.10–0.20)	0.0139
Total Z-score	1.20 (0.81–1.70)	1.14 (0.60–1.60)	0.3536	–0.06 (–0.40–0.30)	0.20 (–0.30–0.50)	0.1633
SBP, mmHg	135 (126–151)	140 (130–148)	0.5347	140 (131–148)	140 (130–152)	0.8342
DBP, mmHg	84 (76–91)	85 (80–88)	0.8913	84 (80–95)	85 (78–95)	0.4700
Glucose, mg/dl	98 (92–108)	95 (88–105)	0.1074	97 (90–107)	98 (92–108)	0.5832
Insulin, µU/ml	10.90 (7.90–17.50)	13.05 (9.15–16.90)	0.1927	13.25 (8.30–14.90)	12.30 (7.40–15.60)	0.8796
HOMA-IR	2.59 (1.97–4.28)	2.89 (2.07–4.35)	0.4351	3.05 (2.07–3.95)	3.10 (1.74–4.04)	0.8343
TC, mg/dl	224 (194–254)	221 (188–247)	0.7547	227 (184–252)	223 (205–230)	0.5933
HDL-C, mg/dl	56 (47–63)	53 (46–61)	0.2271	51 (45–63)	58 (48–65)	0.1591
TG, mg/dl	135 (99–175)	132 (96–180)	0.9953	138 (107–217)	127 (102–165)	0.4068
LDL-C, mg/dl	137 (109–165)	139 (109–163)	0.8499	142 (107–165)	137 (110–159)	0.8286
hsCRP, mg/l***	2.10 (1.30–4.30)	3.90 (2.05–6.30)	0.0016	2.25 (1.40–4.60)	3.10 (1.10–4.60)	0.6339

*T-score ≤ –1: lactoferrin levels higher than median: n = 31, lactoferrin levels lower than median: n = 24, T-score > –1: lactoferrin levels higher than median: n = 84, lactoferrin levels lower than median: n = 97.
**T-score ≤ –1: lactoferrin levels higher than median: n = 21, lactoferrin levels lower than median: n = 8, T-score > –1: lactoferrin levels higher than median: n = 40, lactoferrin levels lower than median: n = 52.
***Lactoferrin levels lower than median: n = 87.
BMI – body mass index; BMC – bone mineral content; BMD – bone mineral density; DBP – diastolic blood pressure; HDL-C – high-density lipoprotein cholesterol; HOMA-IR – homeostatic model assessment - insulin resistance; hsCRP – high sensitive C-reactive protein; IQR – interquartile range; LDL-C – low-density lipoprotein cholesterol; L1-L4 – lumbar spine; SBP – systolic blood pressure; TC – total cholesterol; TG – triglycerides; VAT – visceral adipose tissue.

Table 4. Comparison of densitometric parameters between metabolically healthy (MHO) and unhealthy (MUHO) obese postmenopausal women. Data presented as median and IQR

	MHO (n = 84)	MUHO (n = 154)	p
Femoral neck BMC, g*	4.13 (3.89–4.60)	4.24 (3.87–4.79)	0.4579
Femoral neck BMD, g/cm²*	0.85 (0.76–0.92)	0.87 (0.79–0.96)	0.2422
Femoral neck T-score*	0.00 (–0.80–0.70)	0.10 (–0.60–1.00)	0.2630
Femoral neck Z-score*	1.20 (0.50–2.00)	1.40 (0.70–2.10)	0.1591
L1-L4 BMC, g	58.72 (51.31–66.15)	57.23 (53.64–64.19)	0.7347
L1-L4 BMD, g/cm²	1.03 (0.93–1.10)	1.01 (0.93–1.09)	0.5453
L1-L4 T-score	–0.18 (–0.83–0.58)	–0.33 (–1.00– 0.40)	0.3790
L1-L4 Z-score	1.20 (0.55–1.80)	1.10 (0.40–1.70)	0.4323
Total BMC, g	2 316.50 (2 103.56–2 584.57)	2 279.71 (2 119.00–2 467.00)	0.4690
Total BMD, g/cm²	1.14 (1.06–1.22)	1.13 (1.06–1.19)	0.2598
Total T-score	0.50 (–0.30–1.40)	0.40 (–0.40–1.10)	0.2513
Total Z-score	1.05 (0.33–1.55)	0.90 (0.30–1.40)	0.4151

*MHO; n = 46, MUHO: n = 75.
BMC – bone mineral content; BMD – bone mineral density; IQR – interquartile range; L1-L4 – lumbar spine.

(femoral neck: –1.00 (–1.55–(–0.60)) vs. –0.20 (–0.70–(–0.10)), p = 0.0216, total body: –1.00 (–1.30—0.60) vs. –0.60 (–1.10–0.20), p = 0.0139) and Z-score at the femoral neck (0.20 (–0.05–(–0.50)) vs. 1.10 (0.50–1.40), p = 0.0452). There were no differences between the groups for the other densitometric parameters (Table 3).

Comparison of densitometric variables between metabolically healthy and unhealthy obese subjects

Table 4 presents a comparison of densitometric parameters between MHO and MUHO postmenopausal women. No differences between the groups were observed for all the densitometric markers analysed.

DISCUSSION

Our findings showed that in subjects with a normal bone status, BMC at the lumbar spine was significantly higher in women with lactoferrin levels below the median than above the median, whereas those with osteopenia and lactoferrin levels lower than the median had a significantly lower BMC, BMD, T-score and Z-score at the femoral neck and BMC, BMD and T-score at the total body.

Herein, we report that the prevalence of osteopenia based on the T-score of the lumbar spine (L1-L4) in postmenopausal abdominally obese Polish women aged 50–75 years was 25.6%. However, no case of osteoporosis was reported in the study population, as women with any previously diagnosed chronic severe diseases were excluded. Women with normal bone health were significantly younger but had a higher body weight and BMI than participants with osteopenia, which is consistent with previous studies. It is well known that age negatively impacts BMD loss, especially in the lumbar spine (Li et al., 2023; Singh et al., 2018). Moreover, obese subjects are more protected against osteoporosis than normal-weight subjects (Hyassat et al., 2017; Khinda et al., 2022). However, in contrast to these findings, some studies have also shown an inverse relationship between BMD and VAT mass (Jain and Vokes, 2023) and

reported that obesity did not protect against fractures in postmenopausal women (Tanaka et al., 2013).

Considering women with a T-score > –1, we proved that in the subgroup with lactoferrin concentrations below the median, BMC at the lumbar spine (L1-L4) was significantly higher than in the subgroup of women with lactoferrin levels above the median. On the contrary, in the group of subjects with osteopenia, women with lactoferrin levels lower than the median had a significantly lower BMC, BMD, T-score and Z-score at the femoral neck and BMC, BMD and T-score at the total body. This is one of the first studies investigating the association between blood lactoferrin levels and densitometric parameters. Our findings may suggest that lactoferrin might play a role in preserving bone health, but the protective effect might be visible only in subjects with previously decreased bone mass. In this group, lactoferrin might protect against further deterioration of bone health and slow down the process of bone loss. Nevertheless, further studies are needed to explain and confirm these findings.

Previously, Chailurkit et al. (2011) assessed the relationship between lactoferrin with bone mass in 82 elderly women and showed positive associations between circulating lactoferrin, parathormone and C-terminal telopeptide of type I collagen levels (a marker of bone resorption), although no association with BMD at the lumbar spine or femoral neck was detected. Nevertheless, a positive effect of lactoferrin supplementation on bone status in humans (Bharadwaj et al., 2009) and animals (Blais et al., 2009; Fan et al., 2018; Hou et al., 2012; Li et al., 2018; Xu et al., 2020) has been reported. Blais et al. (2009) used a postmenopausal animal model to show that bovine lactoferrin added to a diet for 27 weeks dose-dependently improved BMD and femoral failure. Furthermore, treatment with bovine lactoferrin at concentrations ranging from 1 to 1000 μg/ml of primary cultures of murine bone cells stimulated cell growth and differentiation of osteoblastic cells and inhibited the growth of preosteoclast cells in vitro. Fan et al. (2018) also found that lactoferrin treatment of ovariectomised mice for 12 weeks improved BMD and increased the serum levels of alkaline phosphatase but decreased the serum levels of tartrate-resistant acid phosphatase. Moreover, Hou et al. (2012) showed that lactoferrin elevated the bone volume, trabecular thickness, and trabecular number and reduced trabecular separation in rats. Furthermore, higher doses of lactoferrin increased BMD, osteocalcin and bone alkaline phosphatase levels and decreased β-C-terminal telopeptide and N-telopeptide cross-links. Additionally, Li et al. (2018) supplemented piglets with recombinant human lactoferrin and observed an increase in BMD and BMC at the tibial level of 14.81% and 28.57% compared to control groups, while Xu et al. (2020) noted improved bone formation, reduced bone resorption, enhanced femoral BMD and microarchitecture and upregulation of osteocalcin, osterix, and runt-related transcription factor 2 expression of the femur in lactoferrin-supplemented Sprague-Dawley rats. While the effect of lactoferrin supplementation on bone health in animals is well known, only one study has investigated the impact of lactoferrin supplementation in humans, showing that the supplementation of RNAse-enriched-lactoferrin in a group of 38 healthy, postmenopausal women aged 45–60 years reduced bone resorption and increased bone formation markers (Bharadwaj et al., 2009).

Several mechanisms have been proposed to explain the impact of lactoferrin on bone health. Lactoferrin may affect bone health by acting on the inflammatory and oxidative systems. It is well known that postmenopausal bone loss is a consequence of oestrogen deficiency, which increases pro-inflammatory cytokines and the receptor-activator of nuclear factor κB ligand-induced osteoclastogenesis (Faienza et al., 2013). Therefore, it is speculated that the immunomodulatory effects of lactoferrin may induce decreased secretion of osteolytic cytokines (Berthon et al., 2022; Yami et al., 2023), which may contribute to an anabolic impact on the skeleton by counterbalancing the catabolic osteoclastogenesis caused by some of the mediators of the inflammatory bone turnover response (Epsley et al., 2020; Mbalaviele et al., 2017). Indeed, Yanagisawa et al. (2022) demonstrated that administration of lactoferrin decreased tumour necrosis factor α productions in an animal model of rheumatoid arthritis. Moreover, Guo et al. (2009) showed that lactoferrin orally administered to ovariectomised rats for three months suppressed the production of pro-inflammatory markers, elevated serum calcitonin levels, protected against reduced bone volume, trabecular number and thickness, and elevation of trabecular separation, as

well as increasing BMD and parameters of mechanical strength. The positive effect of lactoferrin supplementation on inflammatory markers was also confirmed in a recent meta-analysis by Berthon et al. (2022), who reported that lactoferrin supplementation significantly decreased interleukine 6 levels but did not affect CRP concentrations. It has been suggested that the positive effect of lactoferrin on bone mass may also result from the transport properties of this protein. Lactoferrin may facilitate the absorption of many essential minerals and nutrients to deliver the nutrients to key locations in bones and joints (Bharadwaj et al., 2009). Lactoferrin may also interact with low-density lipoprotein receptor-related protein 1 (a protein found on the osteoblast cell membrane), increasing mitogenic activity by activating p42/44 mitogen-activated protein kinase, thereby stimulating bone growth (Grey et al., 2004). Other mechanisms include the activation of phosphoinositide 3-kinases, protein kinase B and upregulation of insulin-like growth factor 1 receptor, as well as increased activity of cyclooxygenase 2 enzyme and nuclear factor of activated T-cells cytoplasmic (Cornish and Naot, 2010; Icriverzi et al., 2020; Naot et al., 2011).

We also found an association between lactoferrin levels, anthropometric parameters and hsCRP concentrations. In the group of women with normal bone health, there were significant differences in BMI and hsCRP concentrations between subjects with lactoferrin levels lower than the median compared to participants with lactoferrin levels higher than the median. Women with lower lactoferrin levels had significantly lower BMI and hsCRP levels than participants with higher lactoferrin levels. Additionally, in the group of subjects with osteopenia, women with lactoferrin levels lower than the median had a significantly lower fat mass than subjects with lactoferrin levels higher than the median. A weak positive association between serum lactoferrin concentrations and anthropometric parameters (body weight, BMI, waist circumference, fat mass) and hsCRP levels in obese women were also reported in our previous study (Jamka et al., 2019). Similarly, Kim et al. (2015) noted a positive association between anthropometric markers and lactoferrin levels in Latino youth. The relationship between lactoferrin levels and anthropometric parameters was also previously suggested by Moreno–Navarette et al. (2013) but their obese subjects had lower lactoferrin concentrations compared to overweight individuals (Moreno-Navarrete et al., 2013). Moreover, they found a negative correlation between lactoferrin levels, BMI and waist-to-hip ratio in participants with altered glucose tolerance (Moreno-Navarrete et al., 2009). Additionally, Fernández-Real et al. (2010) reported an inverse association between lactoferrin concentrations and hsCRP levels in severely obese participants, whereas Aasbren et al. (2019) did not find a correlation between changes in CRP and lactoferrin levels after conservative and surgical weight loss interventions in individuals with morbid obesity.

Here, we also compared densitometric parameters between MHO and MUHO women. Our findings showed no differences between groups in all the bone markers analysed. These results are in line with the study by Camhi and Katzmarzyk (2014), which reported no difference in whole-body BMC and BMD between MHO and MUHO men and women. Contrary, Mirzababaei et al. (2017) showed significant differences between MHO and MUHO subjects in total but not in lumbar spine BMD.

This study has some limitations, including the relatively small number of women with a T-score < -1 and no subjects with osteoporosis in the study population. Also, we measured lactoferrin levels in serum and we did not assess neutrophil levels. During blood clotting, lactoferrin secretion from neutrophils might lead to an overestimation of serum lactoferrin concentrations when compared to plasma samples (Adeyemi and Hodgson, 1988). Nevertheless, a positive strong correlation ($r = 0.82$, $p < 0.0005$) between plasma and serum lactoferrin concentrations was previously reported (Vengen et al., 2010). Furthermore, we did not assess parathyroid hormone, vitamin D and calcium levels and markers of bone formation and resorption and among inflammatory markers, and only hsCRP levels were evaluated. In addition, women's menopausal status was recognised based on subjects' declarations and was not confirmed by the measurement of female sex hormones in the blood. Furthermore, information about hormone replacement therapy was not collected. Moreover, our results cannot be generalised to other populations, as

only centrally obese postmenopausal women were included in the study to minimise the influence of sex. Nonetheless, this study had some strengths, including high homogeneity and a well-characterised study population with clear inclusion and exclusion criteria.

In conclusion, serum lactoferrin levels might be associated with bone health, but the effect may differ in subjects with normal bone status and osteopenia. However, further studies are needed to confirm these findings.

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