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Elevated serum lipocalin 2 levels are associated with indexes of both glucose and bone metabolism in type 2 diabetes mellitus

Podwyższone stężenia lipokaliny-2 w surowicy krwi są związane ze wskaźnikami metabolizmu glukozy i metabolizmu kostnego w przebiegu cukrzycy typu 2

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Abstract

Introduction: The role of lipocalin 2 (LCN2) in type 2 diabetes mellitus (T2DM) needs to be fully elucidated. Moreover, bone has been demonstrated to modulate glucose metabolism via LCN2. We thus performed this study to investigate the association of LCN2 with indexes of glucose metabolism in T2DM. The associations of LCN2 with bone metabolism were examined concurrently.

Material and methods: A total of 288 Chinese Han subjects entered in this study, including 146 patients with T2DM and 142 subjects with normal glucose tolerance. Insulin resistance was assessed by HOMA-IR, and β-cell function was assessed by HOMA-β. For patients with T2DM, bone turnover markers, type 1 N-terminal procollagen, and collagen type 1 cross-linked C-telopeptide were assayed, and bone mineral density (BMD) of the lumbar spine and femoral neck were measured.

Results: Serum LCN2 levels in T2DM were higher than those in subjects with normal glucose tolerance ($p = 0.005$). Moreover, LCN2 was positively associated with fasting serum insulin ($r = 0.262$, $p = 0.001$), HOMA-IR ($r = 0.185$, $p = 0.026$), and HOMA-β ($r = 0.306$, $p < 0.001$), respectively, and negatively associated with fasting plasma glucose ($r = –0.218$, $p = 0.006$). Additionally, femoral neck BMD ($\beta = –0.176$, $p = 0.033$), type 1 N-terminal procollagen ($\beta = 0.181$, $p = 0.026$), and collagen type 1 cross-linked C-telopeptide ($\beta = –0.168$, $p = 0.037$) were independent predictors for LCN2 in T2DM.

Conclusions: LCN2 was associated with indexes of glucose metabolism. Furthermore, BMD and bone turnover markers were independent predictors for LCN2 in T2DM. We speculate that LCN2 might play a role in the cross-talk between bone homeostasis and glucose homeostasis. **(Endokrynol Pol 2018; 69 (3): 276–282)**

Key words: *lipocalin 2, type 2 diabetes mellitus, bone mineral density*

Streszczenie

Wstęp: Wyjaśnienie roli lipokaliny-2 (*lipocalin* 2; LCN2) w przebiegu cukrzycy typu 2 jest niezbędne, w szczególności, że zostało dowiedzione, iż kość moduluje metabolizm glukozy za pośrednictwem LCN2. Niniejsze badanie przeprowadzono, aby zbadać, w jaki sposób LCN2 jest powiązana ze wskaźnikami metabolizmu glukozy w przebiegu cukrzycy typu 2. Jednocześnie zbadano powiązania LCN2 z metabolizmem kostnym.

Materiał i metody: W badaniu wzięło udział 288 Chińczyków Han, w tym 146 pacjentów z cukrzycą typu 2 i 142 pacjentów z prawidłową tolerancją glukozy. Insulinooporność oceniano za pomocą wskaźnika HOMA-IR, natomiast funkcję komórek beta trzustki za pomocą HOMA-β. W przypadku pacjentów z cukrzycą typu 2 oznaczano również markery obrotu kostnego, N-końcowy propeptyd prokolagenu typu I, C-końcowy usieciowany telopeptyd łańcucha alfa kolagenu typu I, gęstość mineralną kości (*bone mineral density*; BMD) odcinka lędźwiowego kręgosłupa i szyjki kości udowej.

Wyniki: Stężenia LCN2 w surowicy krwi w przebiegu cukrzycy typu 2 były wyższe niż u osób z prawidłową tolerancją glukozy ($p = 0{,}005$). Ponadto, LCN2 była dodatnio skorelowana ze stężeniem insuliny w surowicy krwi na czczo ($r = 0{,}262$, $p = 0{,}001$), wskaźnikiem HOMA-IR ($r = 0{,}185$, $p = 0{,}026$) i HOMA-β ($r = 0{,}306$, $p < 0{,}001$), odpowiednio, oraz ujemnie skorelowana z osoczowym stężeniem glukozy na czczo ($r = –0{,}218$, $p = 0{,}006$). Dodatkowo, BMD szyjki kości udowej ($\beta = –0{,}176$, $p = 0{,}033$), N-końcowy propeptyd prokolagenu typu I ($\beta = 0{,}181$, $p = 0{,}026$) oraz C-końcowy usieciowany telopeptyd łańcucha alfa kolagenu typu I ($\beta = –0{,}168$, $p = 0{,}037$) były niezależnymi czynnikami predykcyjnymi dla LCN2 w przebiegu cukrzycy typu 2.

Wnioski: Lipokalina-2 była powiązana ze wskaźnikami metabolizmu glukozy. Ponadto, BMD oraz markery obrotu kostnego były niezależnymi czynnikami predykcyjnymi dla LCN2 w przebiegu cukrzycy typu 2. Można sądzić, że LCN2 może odgrywać rolę w procesie wzajemnego wpływu homeostazy kości i homeostazy glukozy. **(Endokrynol Pol 2018; 69 (3): 276–282)**

Słowa kluczowe: *lipokalina-2, cukrzyca typu 2, gęstość mineralna kości*

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Introduction

Lipocalin 2 (LCN2), a protein also known as neutrophil gelatinase-associated lipocalin, 24p3, or siderocalin, belongs to the lipocalin superfamily [1]. LCN2 is abundantly expressed in various tissues such as immune cells, adipose tissue, lung, liver, kidney, and uterus, and is implicated in diseases associated with inflammation and obesity including inflammatory bowel disease, cardiovascular disease, acute kidney injury, acute pancreatitis, cancer, multiple sclerosis, and type 2 diabetes mellitus (T2DM) [2–7].

Notably, LCN2 is also involved in bone metabolism. A study by Costa et al. indicated that osteoblasts in mice expressed LCN2. Moreover, their study suggested that LCN2 exerted both a negative effect on bone formation and a positive effect on bone resorption [8]. Another study supported the role of LCN2 in inducing bone loss [9]. Most recently, Mosialou et al. found that osteoblasts in mice expressed LCN2 at higher levels than white adipose tissue and other tissues. LCN2 derived from bone improved glucose tolerance and insulin sensitivity [10]. Their findings revealed the endocrine function of bone, which modulates glucose metabolism via LCN2. However, the relationship between bone and LCN2 in humans remains little known.

In humans, clinical reports have described the relationship between LCN2 and insulin resistance, β-cell function, and risk for T2DM in humans in recent decades. Wang et al. reported that serum LCN2 levels were significantly elevated in obese subjects and positively correlated with the index of insulin resistance, homeostasis model assessment for insulin resistance (HOMA-IR), and fasting glucose [11]. A study of a large-scale Chinese population indicated that serum LCN2 levels were higher in subjects with newly diagnosed T2DM than in subjects with normal glucose tolerance (NGT) and positively associated with fasting insulin and HOMA-IR in the whole population [12]. Moreover, elevated serum LCN2 level was shown to be associated with increased risk for T2DM. A recent study also indicated that LCN2 levels were elevated in obese women and could be used as an early predictor of T2DM [13]. Nevertheless, no significant association of LCN2 with HOMA-IR was indicated in non-diabetic obese women [14]. In young healthy men, LCN2 was not shown to be associated with insulin resistance [15]. A study using euglycaemic hyperinsulinaemic clamp method in healthy middle-aged men showed no correlation between LCN2 and insulin sensitivity [16]. For patients with T2DM, Xiao et al. found that LCN2 had positive correlation with fasting insulin, but no correlation with fasting glucose or insulin resistance [17].

In addition to insulin resistance, impairment of β-cell function is one of the most important aetiological factors for the development and progression of T2DM. Remarkably, LCN2 was shown to be a biomarker for assessing severity of acute pancreatitis, which is one of the causes of impairment of β-cell function [5]. Concerning insulin secretion, a weak negative correlation between LCN2 and β-cell function index, homeostatic model assessment for insulin secretion (HOMA-β), was displayed [18]. Nevertheless, few published studies have reported on the association of LCN2 with β-cell function in patients with T2DM.

Collectively, the published observations on the relationship between LCN2 and indexes of insulin resistance and insulin secretion are discrepant in humans. The role of LCN2 in glucose metabolism needs to be fully elucidated in patients with T2DM. We carried out the study herein to investigate the associations of LCN2 with insulin resistance and insulin secretion in patients with T2DM. In addition, considering the latest finding that bone may modulate glucose metabolism via LCN2, the association between bone and serum LCN2 levels was examined concurrently. To our knowledge, this is first report on this issue in T2DM.

Material and methods

A total of 288 participants entered in this study, including 146 diabetic subjects and 142 NGT subjects matching the age, sex, and body mass index (BMI) with diabetic subjects. The ethnicity of all subjects was Han. The participants were recruited from either the Department of Endocrinology of Anhui Provincial Hospital in Hefei, China or the Sanlian community of the Shushan District and the Sanxiaokou community of the Luyang District in Hefei, China, from April 2017 to October 2017. All participants undertook 75-g oral glucose tolerance test (OGTT), and blood at 0 h and 2 h was collected in order to assay fasting and postprandial plasma glucose as well as fasting insulin levels. T2DM and NGT were diagnosed according to the criteria of the World Health Organisation (WHO) 1999. For those with T2DM, subjects fulfilling the following criteria were excluded from this study: (i) acute diabetic complications; (ii) on insulin treatment; (iii) using agents including thiazolidinediones, vitamin K,

warfarin, vitamin D, calcium supplement, bisphosphonates, vitamin A, and sex hormones; (iv) bone diseases such as bone tumours, osteoporosis, and fracture; (v) diabetic patients with albumin/creatinine ratio above 30 mg/g creatinine, liver disease, cardiovascular disease, kidney injury, lung disease, ovarian tumour, thyroid diseases, parathyroid diseases, and other endocrine diseases; (vi) infection, trauma, major operation, and other stress; (vii) type 1 diabetes mellitus, secondary diabetes mellitus, and gestational diabetes mellitus. This study was approved by the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China and was carried out in accordance with Declaration of Helsinki for experiments involving humans (approval number LLSC20160036 issued on 6 December 2016). Written, informed consent had been obtained from all participants before their inclusion in this study.

The height and weight of the total subjects were measured by trained nurses. After overnight fasting for 10–12 h, venous blood was collected from all subjects to assay total cholesterol (TC), triglyceride (TG), serum creatinine (sCr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), fasting plasma glucose (FPG), fasting serum insulin (FIns), and glycosylated haemoglobin (HbA_{1c}). For subjects who undertook OGTT, 2-h postprandial glucose (2hPG) was collected to assess postprandial plasma glucose. For subjects with T2DM, the serum bone turnover markers (BTMs), type 1 N-terminal procollagen (P1NP), and collagen type 1 cross-linked C-telopeptide (CTX) were assayed.

Serum insulin was assayed by electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannhein, Germany). HbA_{1C} was assayed by high--pressure liquid chromatography method (Bio-Rad Inc., Hercules, CA, USA). Serum LCN2 levels were measured by enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech Human Lipocalin-2 ELISA kit, RayBiotech Inc., Norcross, GA, USA). P1NP and CTX were assayed by electrochemical luminescence immunoassay on an automated Elecsys 2010 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Measurements of glucose, TC, TG, sCr, ALT, AST, and ALP were performed by a Hitachi 7600 automatic biochemical analyser (Hitachi Ltd., Tokyo, Japan).

Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) using a GE Lunar densitometer (Lunar, GE Healthcare, Madison, WI, USA) in patients with T2DM. The BMD of the lumbar spine (L1–L4) and femoral neck were measured, respectively. The coefficients of variation of measurements of the lumbar spine and femoral neck were 1.2% and 1.0%, respectively.

The BMI was calculated by dividing weight in kilograms (kg) by height in metres squared (m^2). The HOMA-β was used to assess basal insulin secretion, which was calculated as 20 × FIns (mU/L)/(FPG (mmol/L)–3.5). Insulin resistance was assessed by HOMA-IR, which was calculated as FIns (mU/L) × FPG (mmol/L)/22.5.

Statistical analyses

Data were examined for normal distribution by Kolmogorov-Smirnov test, and the skewed distributed data were log transformed to normality before further analysis. Data were expressed as mean ± standard deviation (SD). The differences in variables between two groups were assessed by unpaired two-tailed t-test except the ratio of male to female, which was analysed by X^2 test. Pearson correlation analysis was used to examine the correlation between LCN2 and various indexes of glucose and bone metabolism. Multiple stepwise regression analysis was performed to assess multivariate correlations. All analyses were performed using the SPSS Statistical Package version 20.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was considered as $p < 0.05$.

Results

The anthropometrical and biochemical parameters from both the T2DM group and the NGT group are shown in Table I. No significant differences in BMI, the ratio of male to female, age, sCr, AST, and ALP were observed between the T2DM group and the NGT group (all $p > 0.05$), whereas TG, TC, and ALT were significantly different in the two groups (all $p < 0.05$). As expected, the T2DM group had higher FPG, 2hPG, FIns, HbA_{1c}, and HOMA-IR and lower HOMA-β than the NGT group (all $p < 0.05$). Moreover, serum LCN2 levels were higher in the T2DM group compared to the NGT group (166.54 ± 45.31 vs. 122.53 ± 26.15, $p = 0.005$).

Next, all T2DM subjects were stratified into two groups according to serum LCN2 levels (Table II). The unpaired t-test showed that diabetic patients with low LCN2 levels were older and had lower sCr, FIns, HOMA-IR, HOMA-β, and P1NP compared to diabetic patients with high LCN2 levels (all $P < 0.05$). FPG, lumbar BMD, femoral neck BMD, and CTX were higher in the low-LCN2 group than in the high-LCN2 group (all $P < 0.05$). No significant differences were observed between the two groups in regard to the ratio of male to female, BMI, ALT, AST, ALP, TG, TC, and HbA_{1c}, respectively (all $P > 0.05$).

Subsequently, the correlations between the serum LCN2 levels and various indexes of glucose and bone metabolism were assessed. As shown in Table III, the

Table I. *Comparison of clinical parameters between NGT group and T2DM group*

Tabela I. *Porównanie parametrów klinicznych osób z grupy z prawidłową tolerancją glukozy i osób z cukrzycą typu 2*

Group	NGT (n = 142)	T2DM (n = 146)	p
Male/Female (n)	72/70	74/72	0.831#
Age (years)	56.82 ± 11.57	57.74 ± 11.06	0.104
BMI [kg/m^2]	24.67 ± 3.13	24.55 ± 3.31	0.820
sCr [μmol/L]	63.18 ± 15.07	62.07 ± 16.35	0.379
ALT [U/L]	20.50 ± 10.75	24.29 ± 15.43*	0.017
AST [U/L]	22.01 ± 6.32	22.88 ± 9.69	0.332
ALP [U/L]	71.00 ± 29.64	72.48 ± 23.92	0.633
TG [mmol/L]	1.52 ± 0.94	2.11 ± 1.55*	< 0.001
TC [mmol/L]	4.83 ± 0.75	4.46 ± 1.11*	< 0.001
FPG [mmol/L]	5.16 ± 0.57	8.73 ± 2.36*	< 0.001
2hPG [mmol/L]	7.11 ± 0.66	14.85 ± 4.78*	< 0.001
Log10 FIns [mIU/L]	0.83 ± 0.13	0.89 ± 0.25	0.045
HbA_{1c} (%)	5.35 ± 0.39	9.21 ± 2.15*	< 0.001
Log10 HOMA-IR	0.20 ± 0.15	0.46 ± 0.21*	< 0.001
Log10 HOMA-β	1.95 ± 0.26	1.53 ± 0.32*	< 0.001
LCN2 [ng/mL]	122.53 ± 26.15	166.54 ± 45.31*	0.005

Normally distributed data are presented as mean ± SD. Skewed distributed data were log transformed to normality. 2hPG — 2-h postprandial glucose; ALT — alanine aminotransferase; AST — aspartate aminotransferase, ALP — alkaline phosphatase; BMI — body mass index; Fins — fasting serum insulin; FPG — fasting plasma glucose; HbA_{1c} — glycosylated haemoglobin; HOMA-β — homeostatic model assessment for β-cell function; HOMA-IR — homeostasis model assessment for insulin resistance; LCN2 — lipocalin 2; NGT — normal glucose tolerance; sCr — serum creatinine; T2DM — type 2 diabetes mellitus; TC — total cholesterol; TG — triglycerides. #Chi-squared test. *P < 0.05 vs. NGT group

Table II. *Comparison of clinical parameters of T2DM patients stratified by serum LCN2 levels below/equal or above median*

Tabela II. *Porównanie parametrów klinicznych osób z cukrzycą typu 2, podzielonych pod względem stężenia lipokaliny-2 w surowicy krwi poniżej/równo lub powyżej średniej*

Group	LCN2 ≤ median (n = 73)	LCN2 > median (n = 73)	p
Male/Female (n)	37/36	35/38	0.833#
Age (years)	62.17 ± 10.22	57.95 ± 10.39*	0.010
BMI [kg/m^2]	24.08 ± 3.09	24.62 ± 3.03	0.892
sCr (μmol/L]	57.52 ± 14.71	65.84 ± 12.75*	0.002
ALT [U/L]	26.23 ± 16.54	22.34 ± 14.04	0.090
AST [U/L]	23.12 ± 10.44	22.65 ± 8.92	0.747
ALP [U/L]	75.18 ± 20.45	74.52 ± 16.56	0.890
TG [mmol/L]	1.91 ± 1.60	2.22 ± 1.29	0.124
TC [mmol/L]	4.45 ± 1.05	4.47 ± 1.10	0.905
FPG [mmol/L]	9.10 ± 2.44	8.29 ± 2.20*	0.037
Log10 FIns [mIU/L]	0.81 ± 0.25	0.98 ± 0.23*	< 0.001
HbA_{1c} (%)	9.11 ± 2.19	9.16 ± 2.13	0.808
Log10 HOMA-IR	0.40 ± 0.21	0.53 ± 0.22*	0.003
Log10 HOMA-β	1.41 ± 0.35	1.65 ± 0.38*	< 0.001
P1NP [ng/mL]	33.58 ± 12.66	39.08 ± 16.75*	0.031
CTX [ng/mL]	459.12 ± 167.90	401.27 ± 182.06*	0.044
Femoral neck BMD [g/cm^2]	0.95 ± 0.13	0.87 ± 0.17*	0.004
Lumbar BMD [g/cm^2]	1.17 ± 0.19	1.07 ± 0.19*	0.003

BMD — bone mineral density; CTX — collagen type 1 cross-linked C-telopeptide; P1NP — type 1 N-terminal procollagen
#Chi-squared test, *p < 0.05 vs. low LCN2 group

serum LCN2 levels positively correlated to FIns (r = 0.270, p = 0.001), HOMA-IR (r = 0.185, p = 0.026), and HOMA-β (r = 0.316, p < 0.001), respectively, and negatively correlated to FPG (r = –0.228, p = 0.006). Negative correlation between serum LCN2 levels and lumbar BMD (r = –0.223, p = 0.007) as well as femoral neck BMD (r = –0.237, p = 0.004) was shown. Moreover, serum LCN2 levels positively correlated with P1NP (r = 0.213, p = 0.010) and negatively correlated with CTX (r = –0.194, p = 0.019). For the other indexes, no significant correlations were observed (all p > 0.05).

To further determine whether LCN2 and other parameters were independently associated with indexes of glucose metabolism, multivariate stepwise regression analysis was performed. Results showed that LCN2 was positively associated with FIns (β = 0.262, p = 0.001), HOMA-IR (β = 0.185, p = 0.026), and HOMA-β (β = 0.306, p < 0.001) , respectively, and negatively associated with FPG (β = –0.218, p = 0.006) independently of age, sex, BMI, and duration of T2DM,

Table III. *Correlation analysis between LCN2 and various indexes of glucose and bone metabolism in T2DM*

Tabela III. *Analiza korelacji między lipokaliną-2 i różnymi wskaźnikami metabolizmu glukozy i metabolizmu kostnego w cukrzycy typu 2*

	r	p
FPG	–0.228	0.006
Log10 FIns	0.270	0.001
HbA1c	–0.041	0.621
Log10 HOMA-IR	0.185	0.026
Log10 HOMA-β	0.316	< 0.001
ALP	–0.013	0.875
P1NP	0.213	0.010
CTX	–0.194	0.019
Femoral neck BMD	–0.237	0.004
Lumbar BMD	–0.223	0.007

Pearson correlation analysis was used. r — Pearson correlation coefficient
P < 0.05 (significant difference)

Table IV. *Multiple stepwise regression analysis for indexes of glucose metabolism in T2DM*

Tabela IV. *Analiza regresji wielokrotnej krokowej dla wskaźników metabolizmu glukozy w cukrzycy typu 2*

Dependent variables	Predictors	β (95% CI)	p
FPG	LCN2	–0.218	0.006
	HbA_{1c}	0.244	0.002
Log10 FIns	LCN2	0.262	0.001
	HbA_{1c}	–0.212	0.008
Log10 HOMA-IR	LCN2	0.185	0.026
Log10 HOMA-β	LCN2	0.306	< 0.001
	HbA_{1c}	–0.243	0.002

Skewed distributed data were log transformed to normality before analysis. Age, body mass index (BMI), duration of diabetes, sex, glycosylated haemoglobin (HbA_{1c}), lipocalin 2 (LCN2), total cholesterol (TC), triglycerides (TG), alkaline phosphatase (ALP), collagen type 1 cross-linked C-telopeptide (CTX), type 1 N-terminal procollagen (P1NP), femoral neck BMD (bone mineral density), and lumbar BMD served as independent variables

Table V. *Multiple stepwise regression analysis for the independent predictors of LCN2 in T2DM*

Tabela V. *Analiza regresji wielokrotnej krokowej dla niezależnych czynników predykcyjnych lipokaliny-2 w cukrzycy typu 2*

Predictors	β (95% CI)	p
femoral neck BMD	–0.176	0.033
P1NP	0.181	0.026
CTX	–0.168	0.037

Skewed distributed data were log transformed to normality before analysis. Alkaline phosphatase (ALP), collagen type 1 cross-linked C-telopeptide (CTX), type 1 N-terminal procollagen (P1NP), femoral neck BMD (bone mineral density), and lumbar BMD served as independent variables

TG, TC, ALP, P1NP, CTX, femoral neck BMD, and lumbar BMD (Table IV).

Because a recent study in an animal model revealed that bone-derived LCN2 is implicated in glucose metabolism, the relationship between LCN2 and bone was examined [10]. The multiple stepwise regression analysis showed that femoral neck BMD ($\beta = -0.176$, $p = 0.033$), P1NP ($\beta = 0.181$, $p = 0.026$), and CTX ($\beta = -0.168$, $p = 0.037$) were independent predictors of LCN2 in T2DM (Table V). The lumbar BMD and ALP were not significantly associated with serum LCN2 levels (both $p > 0.05$).

Discussion

In this study, serum LCN2 levels were shown to be higher in the T2DM group than in the NGT group, consistent with previous reports [12, 19]. Since BMI and sex have been shown to be related to serum LCN2 levels in human, the subjects in the NGT group who had no statistical differences in those parameters compared to T2DM group were chosen as controls in this study [14, 20]. A number of factors correlating to serum LCN2 levels have been identified in the past decade, including lung injury, kidney injury, pancreatic injury, and cardiovascular disease [3, 5, 21, 22]. Moreover, diabetic complications such as diabetic retinopathy and diabetic kidney disease have been demonstrated to be positively correlated to serum or urinary LCN2 levels [23, 24]. Additionally, serum and urinary LCN2 levels have been reported to be linked to the risk of cardiovascular disease in T2DM [25]. Therefore, subjects included in this study had none of the diseases or diabetic complications mentioned above.

The serum LCN2 levels were higher in T2DM than in NGT and negatively associated with FPG, suggesting a potential role in T2DM. This study showed that serum LCN2 levels were positively associated with FIns, as well as HOMA-β, the index assessing basal β-cell function, suggesting that LCN2 may contribute to insulin secretion in T2DM. These data are in agreement with the findings in transgenic mice [10]. In these transgenic mice lacking LCN2 in osteoblasts, insulin secretion was shown to be reduced in response to glucose or arginine challenge while islet number and size, β-cell mass, and β-cell proliferation decreased in the pancreas. It is noteworthy that a study by Zhang et al. showed that insulin stimulated LCN2 expression and secretion in a glucose-dependent manner [26]. Thus, further studies are required to elucidate the casual relationship between LCN2 and insulin secretion.

Comparable to the reports in subjects with IGR and newly diagnosed T2DM, we observed a positive association of LCN2 with HOMA-IR, the index of insulin resistance, in patients with T2DM [12]. Given that the diabetic study population in this study were a combination of long disease duration and short disease duration patients, this result suggests that LCN2 might be involved in the whole pathological processes of T2DM. The association of LCN2 with insulin resistance is supported by the *in vitro* study in which knockdown of LCN2 in cultured adipocytes improved insulin action and glucose uptake in H4IIe hepatocytes [27]. Moreover, recent studies on pro-inflammatory cytokines, such as interleukin-1β, interferon-γ, and TNF-α, which are closely linked to insulin resistance, coincidently up-regulated LCN-2 expression and secretion [23, 28–30]. Nitric oxide (NO), commonly involved in inflammatory processes, also affected LCN2 protein stability [31]. Conversely, LCN2 has been demonstrated to antagonise the detrimental effects of inflammatory molecules on inflammation and metabolism in adipocytes

and macrophages [32]. Furthermore, data from patients with T2DM confirmed the positive correlation of serum LCN2 with inflammatory markers, including CRP, IL-6, and TNF-α [11, 12, 33–35]. However, although preclinical and clinical studies suggest a positive association of LCN2 with insulin resistance, some contradictory data exist. On the whole, more studies in large-scale populations are required to clarify the relationship between LCN2 and insulin resistance.

Emerging evidence has revealed the association of LCN2 with bone. Costa et al. found that transgenic mice over-expressing LCN2 in bone displayed bone micro-architectural changes and reduced bone mass, including a thinner layer of cortical bone and a decreased trabecular number [8]. LCN2 was also shown to reduce osteoblast differentiation and be involved in osteoblast-osteoclast coupling [8, 9]. The latest study demonstrates that bone is the main source of LCN2 in mice [10]. In agreement to the findings in the animal models mentioned above, this study is the first report showing that serum LCN2 levels correlate to both BMD and BTMs in patients with T2DM. Furthermore, femoral neck BMD, P1NP, and CTX were found to be independent predictors of LCN2 in T2DM.

As noted in a recent meta-analysis, patients with T2DM had a greater risk of fracture compared with that in non-diabetic subjects, although the underlying mechanisms remain unclear [36]. Growing evidence has demonstrated that hyperglycaemia has harmful effects on bone microarchitecture and bone strength in T2DM, whereas high insulin levels contribute to bone formation [37–40]. Since bone remodelling occurs continuously in the body, and requires energy consumption, good glycaemic control is beneficial to maintain bone homeostasis in T2DM. In this study, the negative association of LCN2 with FPG and positive association with insulin secretion suggest its role in anti-hyperglycaemia in T2DM. Of note, a recent study indicated that bone is the main source of circulating LCN2, and osteoblasts expressed higher levels of LCN2 compared to white adipose tissue and other tissues [10]. Therefore, we propose a feedback regulation loop in the cross-talk between bone homeostasis and glucose homeostasis via LCN2. Hyperglycaemia inhibits bone metabolism, reduces bone mass and strength, stimulates the expression and secretion of bone-derived LCN2 into circulation. Elevated serum LCN2 levels promote the secretion of insulin, thereafter decreasing blood glucose, which contributes to improved bone metabolism.

Conclusions

In this study, we found serum LCN2 levels were higher in T2DM than in NGT. Serum LCN2 levels were negatively associated with FPG and positively associated with the indexes of insulin secretion and insulin resistance in T2DM. Serum LCN2 levels correlated to both BMD and BTMs in patients with T2DM. Furthermore, femoral neck BMD and BTMs, P1NP, and CTX were independent predictors of serum LCN2 levels in T2DM. We hereby speculate that LCN2 plays a role in the cross-talk between bone and glucose homeostasis. However, because it was a cross-sectional study, we could not identify the casual relationship between LCN2 and glucose metabolism or bone metabolism. Large-scale prospective studies are required to address this issue. Another limitation is that some of the patients in this study had received several treatments. Thus, we could not completely exclude the interference of these drugs when interpreting the associations of LCN2 with indexes of glucose and bone metabolism.

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Disclosure

The authors declare no conflicts of interest.

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