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Serum C-X-C motif chemokine ligand 14 levels are associated with serum C-peptide and fatty liver index in type 2 diabetes mellitus patients

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Keywords
C-X-C motif chemokine ligand 14, Hepatic steatosis, Insulin resistance

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ABSTRACT

Aims/Introduction: Recent studies have suggested C-X-C motif chemokine ligand 14 (CXCL14), secreted from adipose tissue, to play an important role in the pathogenesis of metabolic syndrome. However, the clinical significance of CXCL14 in humans has not been elucidated. This study aimed to assess correlations between serum CXCL14 levels and clinical parameters in patients with type 2 diabetes mellitus.

Materials and Methods: In total, 176 individuals with type 2 diabetes mellitus were recruited. Serum CXCL14 concentrations were determined by enzyme-linked immunosorbent assay. We examined the associations of serum CXCL14 levels with laboratory values, abdominal computed tomography image information, surrogate markers used for evaluating the pathological states of diabetes, obesity and atherosclerosis.

Results: Serum CXCL14 levels correlated positively with body mass index, waist circumference, subcutaneous and visceral fat areas, and serum alanine transaminase, uric acid, total cholesterol, low-density lipoprotein cholesterol, triglycerides and C-peptide (CPR) levels. In contrast, CXCL14 levels correlated inversely with age, pulse wave velocity and serum adiponectin levels. Multiple linear regression analysis showed serum levels of CPR ($\beta = 0.227$, $P = 0.038$) and the fatty liver index ($\beta = 0.205$, $P = 0.049$) to be the only parameters showing independent statistically significant associations with serum CXCL14 levels.

Conclusions: Serum CXCL14 levels were independently associated with serum CPR and fatty liver index in patients with type 2 diabetes mellitus. In these patients, a high serum CPR concentration might reflect insulin resistance rather than β-cell function, because CXCL14 showed simple correlations with obesity-related parameters. Collectively, these data suggested that serum CXCL14 levels in type 2 diabetes patients might be useful predictors of elevated serum CPR and hepatic steatosis.

INTRODUCTION

Dysregulation of adipose tissue homeostasis is a principle cause of obesity-related metabolic disorders, including insulin resistance, dyslipidemia and hepatic steatosis[1]. Accumulating evidence highlights the importance of the secretory factors from adipocytes, called adipokines, interacting with other organs and regulating metabolic homeostasis[2]. In recent decades, numerous studies have shown that brown adipose tissue (BAT) exerts beneficial effects, protecting against both obesity and obesity-related health conditions, not only in rodents, but also in humans[3]. Recently, several circulating adipokines, which influence organs and tissues other than adipose at a distance, secreted from BAT have been identified, including fibroblast growth factor-21, neuregulin-4 and C-X-C motif chemokine ligand 14 (CXCL14)[4].

CXCL14 is a relatively novel chemokine of the C-X-C family. CXCL14 is widely expressed in normal tissues, such as the kidney, liver, brain and skeletal muscle, as well as both white

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adipose tissue (WAT) and BAT[5–7]. Although neither the cellular receptor nor the signaling pathway of CXCL14 has yet been definitively identified, this chemokine reportedly exerts chemotactic activity on various inflammatory mononuclear cells, including monocytes, neutrophils, dendritic cells and natural killer cells[8]. Active investigations in the fields of immunobiology and oncology have shown the roles of this chemokine as a modulator of both inflammatory responses and cancer growth[9]. CXCL14-deficient mice are viable and reproductive with inapparent immune defects[10], but they seemed to be protected from obesity-induced insulin resistance[6,11]. These animal studies showed that CXCL14 promotes visceral obesity and inflammation of adipose tissue, leading to elevation of hepatic gluconeogenesis and the development of insulin resistance. In contrast, Takahashi *et al.*[12] reported CXCL14 to enhance insulin-dependent glucose uptake in adipocytes, suggesting amelioration of adipose insulin sensitivity. Intriguingly, a recent study showed BAT-derived CXCL14 to promote the recruitment of M2-type macrophages, inducing browning of WAT through type 2 immune cell activation[7]. This finding showed that CXCL14 plays a pivotal role in a BAT-derived signaling mechanism, and has beneficial effects on adiposity and glucose metabolism.

As shown by previous reports, the possible functions of CXCL14 in metabolic contexts have yet to be determined. Additionally, circulating CXCL14 levels have not been examined in detail in humans, especially those with metabolic disorders. To elucidate the pathological role of CXCL14 in metabolic homeostasis, we carried out an association analysis of serum CXCL14 levels with clinical parameters, including laboratory values, abdominal computed tomography (CT) image information, and surrogate markers used to evaluate pathological states of glucose metabolism, obesity and atherosclerosis, in patients with type 2 diabetes mellitus.

METHODS

Study participants

The study participants were 176 type 2 diabetes mellitus patients admitted to Iwate Medical University Hospital, Yahaba, Japan, during the period from January 2017 to March 2019. Enrolled patients were excluded if they had any malignancy, infectious diseases, collagen disorders or diabetic ketoacidosis. Those with renal dysfunction defined as chronic kidney disease stage G3b or G4 or G5 (estimated glomerular filtration rate with serum creatinine below 45 mL/ min/1.73 m^2) were also excluded from the present study. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or the use of antihypertensive medication. Dyslipidemia was defined as low-density lipoprotein cholesterol (LDL-C) ≥140 mg/dL and/or triglyceride ≥ 150 mg/dL and/or high-density lipoprotein cholesterol <40 mg/dL, a prior diagnosis of dyslipidemia and/or the use of antihyperlipidemic medication. The study conformed to the principles outlined in the Declaration of Helsinki, and was approved by the institutional review board of Iwate Medical University (approval number: MH2019-023). Written informed consent was obtained from all study participants.

Laboratory data analyses

Laboratory parameters were measured in blood and urine samples obtained after a 12-h overnight fast. The serum levels of C-peptide (CPR), adiponectin, malondialdehyde-modified LDL-C and plasminogen activator inhibitor-1, as well as those of urinary CPR, 8-isoprostane, 8-hydroxydeoxyguanosine and liver-type fatty acid-binding protein were measured by SRL, Inc. (Tokyo, Japan).

Human CXCL14 enzyme-linked immunosorbent assay

Serum CXCL14 concentrations were determined using a Human CXCL14 ELISA kit (ab213771; Abcam, Cambridge, MA, USA) in accordance with the manufacturer's protocol. The absorbance at 450 nm was evaluated using a Microplate Absorbance Reader (Infinite F500; TECAN Group Ltd., Männedorf, Switzerland). The CXCL14 concentration in the sample was interpolated from the standard curve.

CT imaging analysis

The total coronary artery calcification score was determined according to the method of Agatston *et al.*[13], and analyzed as previously reported[14]. Both the volume of the visceral fat area (VFA) and subcutaneous fat area (SFA) were obtained from CT scans at the level of the fourth lumbar vertebra. Plain abdominal CT defines hepatic steatosis as a liver-to-spleen density ratio <0.9[15].

Measurements of carotid artery intima-media thickness and brachial ankle pulse wave velocity

The intima-media thickness of the carotid arteries was evaluated using an ultrasonographic measuring instrument (LOGIQ 500; GE Yokogawa Medical Systems Corp., Tokyo, Japan), and the maximum intima-media thickness; that is, the thickest point observed in the scanned regions, was measured as reported previously[16]. The brachial ankle pulse wave velocity was determined using a waveform analyzer (BP-203RPE; Colin Co., Komaki, Japan).

Evaluation of hepatic steatosis

Hepatic steatosis was defined by plain abdominal CT, as described above. In addition, fatty liver index (FLI) values were used as a marker of hepatic steatosis and calculated by the formula shown below. FLI ≥60 was defined as hepatic steatosis[17,18].

$$\mathrm{FLI} = \left\{ \left(e^{0.953 \times \log e\,(\text{triglycerides}) + 0.139 \times \mathrm{BMI} + 0.718 \times \log e\,(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745} \right) / \left(1 + e^{0.953 \times \log e\,(\text{triglycerides}) + 0.139 \times \mathrm{BMI} + 0.718 \times \log e\,(\text{ggt}) + 0.053 \times \text{waist circumference} - 15.745} \right) \right\} \times 100.$$

Statistical analysis

Quantitative data are presented as the median with interquartile range for the data showing a non-normal distribution. Correlations between the variables were determined by applying a simple linear regression test. Multiple linear regression analyses were carried out to determine the factors independently showing significant correlations with the serum CXCL14 level. The level of significance was set at $P < 0.05$. All statistical analyses were carried out using SPSS version 21 (SPSS Japan Inc., Tokyo, Japan).

RESULTS

The clinical characteristics of the enrolled participants are shown in Table 1. The median age was 61.5 years, median diabetes duration was 7.0 years and 111 participants were men. The median body mass index, VFA and homeostasis model assessment of insulin resistance were 26.35, 153.38 cm^2 and 2.65, respectively, indicating moderate obesity and the presence of insulin resistance, as compared with general Japanese individuals with type 2 diabetes mellitus. The median serum CXCL14 value was 1,111 pg/mL, with the value being higher in men than in women.

The serum CXCL14 level correlated positively with age ($r = 0.308$), body mass index ($r = 0.200$), waist circumference ($r = 0.183$), VFA ($r = 0.190$), SFA ($r = 0.172$), total cholesterol ($r = 0.227$), triglycerides ($r = 0.150$), LDL-C ($r = 0.311$), alanine transaminase ($r = 0.149$), estimated glomerular filtration rate ($r = 0.149$), uric acid ($r = 0.181$), serum CPR ($r = 0.200$), urinary CPR ($r = 0.167$) and FLI ($r = 0.214$) (Table 2). The CXCL14 level showed negative correlations with adiponectin ($r = -0.164$), urinary liver-type fatty acid-binding protein ($r = -0.175$) and brachial ankle pulse wave velocity ($r = -0.215$). Although some of the variables showed statistical significance only for one sex, the tendencies remained similar in both sexes. Next, to assess whether a certain condition has an impact on serum CXCL14 level, intergroup comparisons were carried out (Table 3). The serum CXCL14 level was significantly high in female participants, and relatively high in the overweight or visceral obese participants. There was no significant difference of serum CXCL14 level in the presence of hypertension or dyslipidemia, or in participants taking a particular type of medicine. Interestingly, regarding hepatic steatosis, as evaluated by abdominal CT values or FLI, serum levels of CXCL14 were shown to be high in the participants with steatosis as compared with those without steatosis.

Multiple linear regression analyses were carried out to identify variables independently related to the serum CXCL14 level (Table 4). Multivariate analysis, with adjustment for age, sex, serum CPR, estimated glomerular filtration rate, SFA and VFA, showed the serum CPR level to be positively related to the serum CXCL14 level (model 1). Next, we carried out multivariate analysis using model 2, adding a number of dependent variables identified using model 1; that is, adiponectin, urinary albumin, free T3 and FLI $\geq$60. Intriguingly, this analysis showed serum CPR and FLI to be variables significantly related to CXCL14 levels, even after increasing the number of dependent variables, displayed as model 3.

DISCUSSION

This research report showed the significance of the serum CXCL14 level in the context of the metabolic phenotype in East-Asian individuals with type 2 diabetes mellitus. Although both the molecular mechanism of action and the functions of CXCL14 in metabolic homeostasis remain uncertain, some association of CXCL14 with fat accumulation and elevation of serum CPR was shown in the present study.

CXCL14 was first identified from breast and kidney cells[5]. It is considered to exert pleiotropic functions as an immune and inflammatory modulator on various target cells, contributing to chemotaxis, cell differentiation and angiogenesis[8]. Interestingly, it has been shown that the chemotactic activity of CXCL14 is exerted by promoting immune cells, such as monocytes, neutrophils, dendritic cells[19] and natural killer cells[20]. Consistent with these observations, the function of this chemokine in humans was investigated in patients with immune-related disorders. For instance, several human studies have presented serum CXCL14 levels to be significantly elevated in individuals with idiopathic pulmonary fibrosis[21] and acute liver injuries of various etiologies[22,23]. In contrast, a decrease in the serum CXCL14 level is linked to the stage advancement of chronic HBV infection[24] and the severity of systemic sclerosis[25]. In addition, the association between CXCL14 and oncogenesis has attracted the attention of researchers[9]. The roles of CXCL14 in cancer biochemistry are controversial, as this chemokine was shown to have either a tumor suppressive or a promotive effect, depending on the tumor type[26,27]. Taken together, these findings suggested that CXCL14 has pleiotropic functions in multifaceted pathological conditions, with differences depending on target cells.

The effects of CXCL14 on metabolism were previously examined in animal models. *CXCL14* messenger ribonucleic acid is induced in multiple tissues, such as WAT, BAT and skeletal muscle in high-fat diet-fed obese mice[6]. CXCL14-deficient mice showed no apparent phenotypic abnormalities in their immunological responses[10], but a protective phenotype from obesity-induced insulin resistance[6]. In contrast, CXCL14 is secreted from brown adipocytes in response to thermogenic stimulation and also attracts polarized M2 macrophages in WAT[7]. Recent reports showed that pioglitazone administration increased the CXCL14 level in both adipocyte messenger ribonucleic acid expression and serum concentration of women with polycystic ovary syndrome[28]. In these contexts, CXCL14 induced favorable metabolic phenotypes, such as alleviated adipose inflammation and enhanced insulin sensitivity. Furthermore, an *in vitro* study showed CXCL14 to enhance the insulin-dependent glucose uptake in adipocytes[12], supporting the insulin-sensitizing role of CXCL14. The effect of metabolic context on CXCL14 level is a subject of debate. Our

Table 1 | Clinical characteristics of participants

	All	Men	Women
n	176	111	65
Age (years)	61.5 (46.0–69.8)	65.0 (52.5–75.0)	58.0 (43.0–67.0)
Diabetes duration (years)	7.0 (1.0–15.0)	13.0 (3.0–20.0)	5.0 (1.0–11.5)
BMI (kg/m^2)	26.4 (23.0–31.0)	26.2 (23.5–30.4)	26.7 (22.5–30.6)
Waist circumference (cm)	95.0 (88.0–105.1)	96.5 (88.3–106.0)	95.0 (87.0–104.0)
VFA (cm^2)	153.8 (118.7–203.6)	148.7 (116.3–190. 5)	156.9 (119.2–227.1)
SFA (cm^2)	196.9 (131.0–277.0)	236.3 (169.0–326.2)	172.2 (120.7–249.9)
Total cholesterol (mg/dL)	188 (162–217)	184 (160–208)	195 (167–219)
Triglyceride (mg/dL)	119 (89–188)	107 (79–166)	124 (95–215)
HDL cholesterol (mg/dL)	45 (39–56)	48 (41–59)	43 (37–55)
LDL cholesterol (mg/dL)	109 (90–134)	102 (79–123)	113 (91–140)
ALT (U/L)	29 (18–52)	24 (16–37)	31 (20–64)
AST (U/L)	22 (17–36)	21 (17–33)	23 (17–43)
γGT (U/L)	38 (22–65)	28 (17–58)	43 (30–76)
Cr (mg/dL)	0.72 (0.61–0.87)	0.60 (0.51–0.70)	0.78 (0.70–0.90)
eGFR (mL/min/1.73 m^2)	72.3 (61.22–85.80)	72.1 (59.45–87.75)	73.2 (61.80–85.50)
UA (mg/dL)	5.8 (4.5–6.4)	4.7 (4.0–5.9)	5.9 (5.3–6.9)
hsCRP (mg/dL)	0.11 (0.04–0.27)	0.09 (0.04–0.24)	0.12 (0.04–0.34)
TSH (μlU/mL)	1.55 (1.09–2.37)	1.69 (1.07–2.98)	1.55 (1.14–2.08)
FT3 (pg/mL)	2.77 (2.52–3.14)	2.66 (2.41–2.87)	2.89 (2.61–3.21)
FT4 (ng/mL)	1.36 (1.21–1.50)	1.34 (1.18–1.40)	1.39 (1.22–1.54)
CXCL14 (pg/mL)	1,111 (544–1,587)	1,062 (524–1,394)	1,219 (616–1,783)
PAI-1 (ng/mL)	27.0 (14.0–39.0)	23.5 (14.0–44.8)	25.0 (16.0–37.0)
MDA-LDL (U/dL)	108.0 (81.0–145.5)	98.0 (78.5–137.5)	110.0 (83.0–152.0)
Adiponectin (μg/mL)	2.31 (1.35–4.25)	3.35 (1.93–5.04)	1.89 (1.11–3.28)
HbA1c (%)	9.6 (8.5–11.5)	9.6 (8.2–11.5)	9.6 (8.6–11.5)
HOMA-IR	2.7 (1.6–5.2)	2.6 (1.8–5.2)	2.7 (1.4–5.0)
HOMA-β	29.6 (13.7–61.3)	33.8 (14.5–61.0)	27.7 (12.8–62.4)
FIRI (μU/mL)	6.4 (4.1–11.9)	7.0 (4.5–13.3)	6.2 (3.8–11.5)
Serum C-peptide (ng/mL)	1.51 (0.98–2.07)	1.34 (0.88–1.93)	1.55 (1.00–2.15)
Urinary C-peptide (μg/day)	50.0 (24.40–89.25)	44.7 (18.55–67.85)	54.2 (29.50–101.00)
Urinary albumin (mg/day)	12.2 (5.2–43.6)	6.0 (4.7–14.2)	21.6 (6.8–79.2)
Urinary L-FABP (μg/g Cr)	1.8 (1.2–3.0)	2.1 (1.5–3.5)	1.7 (1.1–2.7)
Urinary 8-isoprostane (pg/mg Cr)	230.0 (140.0–387.5)	210.0 (115.0–315.0)	260.0 (150.0–420.0)
Urinary 8-OHdG (pg/mg Cr)	10.8 (5.6–16.4)	9.5 (4.7–14.9)	10.7 (6.5–16.7)
Liver : spleen ratio	1.1 (1.0–1.3)	1.2 (1.0–1.4)	1.1 (0.9–1.2)
FLI	55.3 (30.9–85.9)	48.7 (28.7–77.2)	62.4 (32.1–87.2)
CACS (AU)	27.3 (0.0–276.6)	31.0 (0.0–195.3)	21.5 (0.0–244.8)
baPWV (cm/s)	1,506 (1,327–1,834)	1,531 (1,316–1,904)	1,489 (1,326–1,825)
ABI	1.1 (1.1–1.2)	1.1 (1.1–1.2)	1.1 (1.1–1.2)
IMT (mm)	1.60 (1.15–2.15)	1.60 (1.15–2.14)	1.63 (1.15–2.20)
Sulphonylureas, *n* (%)	39 (22.2)	15 (23.1)	24 (21.6)
Glinides, *n* (%)	9 (5.1)	3 (4.6)	6 (5.4)
α-Glucosidase inhibitors, *n* (%)	26 (14.8)	13 (20.0)	13 (11.7)
Thiazolidinediones, *n* (%)	9 (5.1)	0 (0.0)	9 (8.1)
Biguanides, *n* (%)	43 (24.4)	16 (14.4)	27 (41.5)
DPP-4 inhibitors, *n* (%)	93 (52.8)	37 (56.9)	56 (50.5)
SGLT-2 inhibitors, *n* (%)	24 (13.6)	9 (13.8)	15 (13.5)
GLP-1 receptor agonists, *n* (%)	13 (7.4)	5 (7.7)	8 (7.2)
Insulin, *n* (%)	74 (42.1)	33 (50.8)	41 (36.9)

Table 1 (*Continued*)

	All	Men	Women
Statins, *n* (%)	70 (39.8)	29 (44.6)	41 (36.9)
Angiotensin receptor antagonists, *n* (%)	64 (36.4)	18 (27.7)	46 (41.4)

γGT, γ-glutamyl transferase; 8-OHdG, 8-hydroxydeoxyguanosine; ABI, ankle brachial index; ALT, alanine transaminase; AST, aspartate transaminase; baPWV, brachial ankle pulse wave velocity; BMI, body mass index; CACS, coronary artery calcification score; Cr, creatinine; CXCL14, C-X-C motif chemokine ligand 14; DPP, dipeptidyl peptidase; eGFR, estimated glomerular filtration rate; FIRI, fasting immunoreactive insulin; FLI, fatty liver index; GLP, glucagon-like peptide; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; L-FABL, liver-type fatty acid-binding protein; LDL-C, low-density lipoprotein cholesterol; MDA-LDL, malondialdehyde-modified low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; SFA, subcutaneous fat area; SGLT, sodium–glucose transporter; UA, uric acid; VFA, visceral fat area.

experiments focusing on metabolic phenotypes revealed the serum level of CXCL14 to show simple correlations with indices including body mass index, waist circumference, VFA, SFA, total cholesterol, triglycerides, LDL-C, ALT, elevated CPR, hepatic steatosis and low adiponectinemia, observations collectively consistent with the characteristics of metabolic syndrome. The strange increase in serum CXCL14 in metabolic disorders potentially meant a response of biological defense, aimed at counteracting the obesity-induced metabolic stress. Alternatively, obesity possibly elicits the resistance in the functions of CXCL14, leading to its compensatory increase of serum level. This supposed mechanism would be reminiscent of the effects of insulin, leptin and fibroblast growth factor-21, which are considered to be a consequence of enhanced production in compensation for obesity-related resistance. Further detailed studies are warranted to investigate the target tissues responding to the actual systemic amount of CXCL14.

Multiple regression analyses showed a mild correlation between serum CXCL14 and fasting CPR levels in our diabetes patients. Generally, a high serum CPR concentration indicates hyperinsulinemia, one of the established diagnostic markers of insulin resistance. Several studies have shown the reliability of serum CPR as a marker of insulin resistance, in both diabetes patients and individuals without diabetes[29]. Ohkura *et al.*[30] developed a new insulin resistance index calculated from fasting glucose and CPR, resulting from a glucose clamp study and meal tolerance test in diabetes patients. Another study showed the association of the serum CPR level with muscle insulin resistance, evaluated by tracer examination, during a hyperinsulinemic euglycemic clamp study in healthy individuals[31]. As these studies showed that a high serum CPR level might reflect insulin resistance, making it a potentially useful diagnostic value and also suggesting that, as in our enrolled participants, CXCL14 is thought to be associated with insulin resistance.

The serum CXCL14 level was increased in participants with hepatic steatosis, as estimated by both imaging analyses and laboratory data. Most notably, FLI values >60 were determined by multiple regression analysis to be an independent factor related to elevated serum CXCL14. Several assessment models have been advocated for the prediction of non-alcoholic fatty liver disease or non-alcoholic steatohepatitis without invasive procedures, such as hepatic biopsy[32]. However, most predictive models evaluated hepatic fibrosis rather than fatty change. As our enrolled participants had little evidence of hepatic fibrosis, we used the FLI, which is reportedly advantageous for detecting non-alcoholic fatty liver disease, before the progression to fibrosis[17]. Although a number of parameters showed a simple correlation with serum CXCL14 level, just two variables, serum CPR and a predictive value of hepatic steatosis, were identified by multiple regression analysis.

The present study had several limitations. First, the molecular mechanism(s) underlying the actions of this chemokine, as well as major cellular sources of circulating CXCL14, have not been fully clarified. Therefore, the present results are preliminary and our interpretation remains speculative. Another limitation was the cross-sectional design of the present study, because the results possibly showed only incidental associations. Thus, the Causal relationships between the serum CXCL14 level and metabolic phenotypes cannot be confirmed, and further prospective studies are thus required. Third, some of the enrolled participants with poor glycemic control might have experienced a temporary decline in their insulin secretory capacity, resulting in the serum CPR level measurements possibly being inaccurate. Fourth, the serum CXCL14 level is collectively considered to be associated with the phenotype of "metabolic syndrome", including insulin resistance. However, the correlation with the homeostasis model assessment of insulin resistance, a classic biomarker of insulin resistance, underreached a statistical significance, indicating a requirement for further investigation of the effect of CXCL14 on insulin resistance. In addition, participants were limited to those who had type 2 diabetes mellitus, meaning these results might not be applicable to the general population. Finally, due to the small number of study participants, the investigation by sex of the effects of CXCL14 on metabolism was insufficient. As shown in Tables 2 and 3, CXCL14 concentration was high and correlated with many variables in women. In fact, previous studies reported that CXCL14 was considered to bind to estrogen receptors[33], and the phenotypes related to CXCL14 were strongly shown in female mice[11]. Unfortunately, in the present

Table 2 | Correlations of C-X-C motif chemokine ligand 14 with clinical parameters

Variable	Correlation coefficient		
	All (n = 176)	Men (n = 111)	Women (n = 65)
Age (years)	−0.274**	−0.158	−0.247*
Diabetes duration (years)	−0.117	−0.061	−0.057
BMI (kg/m^2)	0.200*	0.224	0.196*
Waist circumference (cm)	0.183*	0.191	0.198*
VFA (cm^2)	0.190*	0.169	0.197*
SFA (cm^2)	0.172*	0.160	0.258*
Total cholesterol (mg/dL)	0.227*	0.185	0.204*
Triglyceride (mg/dL)	0.150*	0.068	0.159
HDL cholesterol (mg/dL)	−0.072	−0.080	−0.045
LDL cholesterol (mg/dL)	0.311**	0.344*	0.229*
ALT (U/L)	0.149*	0.148	0.112
AST (U/L)	0.117	0.211	0.063
γGT (U/L)	0.141	0.207	0.054
ALP (U/L)	−0.054	0.125	−0.175
Cr (mg/dL)	−0.011	−0.178	−0.090
eGFR (mL/min/1.73 m^2)	0.149*	0.208	0.090
UA (mg/dL)	0.181*	0.081	0.140
TSH (μIU/mL)	0.094	0.079	0.101
hsCRP (mg/dL)	0.122	0.268*	0.021
FT3 (pg/mL)	0.206*	0.134	0.191*
FT4 (ng/mL)	0.069	0.102	0.058
PAI-1 (ng/mL)	0.083	0.019	0.091
MDA-LDL (U/dL)	0.138	0.176	0.078
Adiponectin (μg/mL)	−0.164*	−0.039	−0.152
HbA1c (%)	0.029	0.039	−0.001
HOMA-IR	0.108	−0.013	0.184
HOMA-β	0.054	0.072	0.075
FIRI (μU/mL)	0.089	−0.006	0.164
Serum C-peptide (ng/mL)	0.200*	0.184	0.202*
Urinary C-peptide (μg/day)	0.167*	0.114	0.176
Urinary albumin (mg/day)	0.158*	0.169	0.077
Urinary L-FABP (μg/g Cr)	−0.175*	−0.021	−0.232*
Urinary 8-isoprostane (pg/mg Cr)	0.107	0.028	0.117
Urinary 8-OHdG (pg/mgC r)	0.158*	0.159	0.217*
CACS (AU)	−0.053	0.108	−0.159
baPWV (cm/s)	−0.215*	−0.366*	−0.120
ABI	−0.066	−0.069	−0.084
IMT (mm)	−0.057	0.088	−0.125

Spearman's rank correlation coefficient. γGT, γ-glutamyl transferase; 8-OHdG, 8-hydroxydeoxyguanosine; ABI, ankle brachial index; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; baPWV, brachial ankle pulse wave velocity; BMI, body mass index; CACS, coronary artery calcification score; Cr, creatinine; CXCL14, C-X-C motif chemokine ligand 14; DPP, dipeptidyl peptidase; eGFR, estimated glomerular filtration rate; FIRI, fasting immunoreactive insulin; FLI, fatty liver index; GLP, glucagon-like peptide; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; L-FABL, liver-type fatty acid-binding protein; LDL-C, low-density lipoprotein cholesterol; MDA-LDL, malondialdehyde-modified low-density lipoprotein; PAI-1, plasminogen activator inhibitor-1; SFA, subcutaneous fat area; SGLT, sodium–glucose transporter; UA, uric acid; VFA, visceral fat area. *P < 0.05. **P < 0.001.

study, we could not confirm the impact of sex hormones on the relationship between CXCL14 and metabolism.

In conclusion, the serum CXCL14 concentration was thought to be elevated in accordance with insulin resistance and hepatic steatosis in the participants with type 2 diabetes mellitus in the present study. CXCL14 is considered to play major roles in both the immune response and inflammation. This chemokine might thus be the link between the immune response and

Table 3 | Comparison of C-X-C motif chemokine ligand 14 values with intergroup

Sex			
Male	$n = 111$	1,062 (524–1,394)	$P = 0.034$
Female	$n = 65$	1,219 (616–1,783)	
BMI (kg/m^2)			
<25	$n = 71$	1,015 (507–1,461)	$P = 0.074$
≥25	$n = 105$	1,331 (614–1,768)	
VFA (cm^2)			
<100	$n = 31$	806 (434–1,295)	$P = 0.051$
≥100	$n = 145$	1,199 (617–1,646)	
Urinary albumin (mg/day)			
<30	$n = 110$	1,080 (467–1,448)	$P = 0.058$
≥30	$n = 48$	1,436 (604–1,937)	
Hypertension			
−	$n = 87$	1,137 (796–1,534)	$P = 0.281$
+	$n = 89$	1,077 (413–1,691)	
Dyslipidemia			
−	$n = 49$	1,199 (512–1,555)	$P = 0.847$
+	$n = 127$	1,095 (560–1,684)	
FLI			
<60	$n = 89$	1,010 (466–1,468)	$P = 0.003$
≥60	$n = 81$	1,395 (661–1,811)	
Liver : spleen ratio			
≥0.9	$n = 147$	1,072 (507–1,525)	$P = 0.017$
<0.9	$n = 28$	1,340 (894–2,175)	
Sulphonylureas			
−	$n = 137$	1,137 (614–1,617)	$P = 0.202$
+	$n = 39$	1,010 (371–1,505)	
Glinides			
−	$n = 167$	1,095 (538–1,585)	$P = 0.434$
+	$n = 9$	1,444 (534–1,691)	
α-Glucosidase inhibitors			
−	$n = 150$	1,119 (607–1,588)	$P = 0.238$
+	$n = 26$	1,074 (354–1,534)	
Biguanides			
−	$n = 133$	1,119 (614–1,589)	$P = 0.208$
+	$n = 43$	1,012 (355–1,515)	
DPP-4 inhibitors			
−	$n = 83$	1,089 (486–1,529)	$P = 0.315$
+	$n = 93$	1,144 (665–1,716)	
SGLT-2 inhibitors			
−	$n = 152$	1,094 (508–1,584)	$P = 0.268$
+	$n = 24$	1,283 (905–1,786)	
GLP-1 agonists			
−	$n = 163$	1,115 (538–1,588)	$P = 0.492$
+	$n = 13$	1,006 (567–1,593)	
Insulin			
−	$n = 102$	1,224 (598–1,762)	$P = 0.133$
+	$n = 74$	1,083 (504–1,448)	
Statins			
−	$n = 106$	1,141 (581–1,588)	$P = 0.527$
+	$n = 70$	1,083 (512–1,550)	
Angiotensin receptor antagonists			
−	$n = 112$	1,100 (662–1,520)	$P = 0.905$
+	$n = 64$	1,174 (402–1,775)	

Mann–Whitney *U*-test.

Table 4 | Determinants of C-X-C motif chemokine ligand 14 in multivariate regression

Factors	Model 1		Model 2		Model 3	
	β	*P*-value	β	*P*-value	β	*P*-value
Age	−0.170	0.073	−0.055	0.616	-0.021	0.852
Sex	0.067	0.389	0.122	0.159	0.130	0.139
C-peptide	0.250	0.004	0.227	0.038	0.221	0.044
eGFR	0.076	0.379	0.149	0.126	0.128	0.194
SFA	−0.058	0.468	−0.095	0.335	−0.043	0.635
VFA	0.002	0.984	−0.085	0.332	−0.111	0.264
Adiponectin			−1.184	0.238	−0.135	0.174
Urinary albumin			−0.681	0.497	−0.080	0.340
FT3			−0.996	0.321	−0.073	0.461
FLI ≥60			0.205	0.049	0.234	0.031
8-OHdG					0.047	0.577
LDL-C					0.139	0.166
TG					−0.109	0.243

Model 1: dependent variables: age, sex, C-peptide, estimated glomerular filtration rate (eGFR), subcutaneous fat area (SFA) and visceral fat area (VFA). Model 2: dependent variables: age, sex, C-peptide, eGFR, SFA, VFA, adiponectin, urinary albumin, free triiodothyronine (FT3), fatty liver index ≥60. Model 3: dependent variables: age, sex, C-peptide, eGFR, SFA, VFA, adiponectin, urinary albumin, FT3, FLI ≥60, 8-hydroxydeoxyguanosine (8-OHdG), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). β is the standard coefficient, the multiple coefficient of determination (R^2), model 1: $R^2 = 0.144$, model 2: $R^2 = 0.179$ and model 3: $R^2 = 0.128$.

metabolic disorders, and thereby possibly serve as a novel biomarker and/or a novel therapeutic target for obesity-related insulin resistance and hepatic steatosis.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Crewe C, An YA, Scherer PE. The ominous triad of adipose tissue dysfunction: inflammation, fibrosis, and impaired angiogenesis. *J Clin Investig* 2017; 127: 74–82.
2. Katagiri H, Yamada T, Oka Y. Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. *Circ Res* 2007; 101: 27–39.
3. Shinoda K, Luijten IH, Hasegawa Y, *et al*. Genetic and functional characterization of clonally derived adult human brown adipocytes. *Nat Med* 2015; 21: 389–394.
4. Villarroya F, Gavalda-Navarro A, Peyrou M, *et al*. The lives and times of brown adipokines. *Trends Endocrinol Metab* 2017; 28: 855–867.

5. Hromas R, Broxmeyer HE, Kim C, *et al*. Cloning of BRAK, a novel divergent CXC chemokine preferentially expressed in normal versus malignant cells. *Biochem Biophys Res Commun* 1999; 255: 703–706.
6. Nara N, Nakayama Y, Okamoto S, *et al*. Disruption of CXC motif chemokine ligand-14 in mice ameliorates obesity-induced insulin resistance. *J Biol Chem* 2007; 282: 30794–30803.
7. Cereijo R, Gavalda-Navarro A, Cairo M, *et al*. CXCL14, a brown adipokine that mediates brown-fat-to-macrophage communication in thermogenic adaptation. *Cell Metab* 2018; 28: 750–763.e6.
8. Lu J, Chatterjee M, Schmid H, *et al*. CXCL14 as an emerging immune and inflammatory modulator. *J Inflamm* 2016; 13: 1.
9. Hara T, Tanegashima K. Pleiotropic functions of the CXC-type chemokine CXCL14 in mammals. *J Biochem* 2012; 151: 469–476.
10. Meuter S, Schaerli P, Roos RS, *et al*. Murine CXCL14 is dispensable for dendritic cell function and localization within peripheral tissues. *Mol Cell Biol* 2007; 27: 983–992.
11. Tanegashima K, Okamoto S, Nakayama Y, *et al*. CXCL14 deficiency in mice attenuates obesity and inhibits feeding behavior in a novel environment. *PLoS One* 2010; 5: e10321.
12. Takahashi M, Takahashi Y, Takahashi K, *et al*. CXCL14 enhances insulin-dependent glucose uptake in adipocytes and is related to high-fat diet-induced obesity. *Biochem Biophys Res Commun* 2007; 364: 1037–1042.
13. Agatston AS, Janowitz WR, Hildner FJ, *et al*. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990; 15: 827–832.
14. Hangai M, Takebe N, Honma H, *et al*. Association of advanced glycation end products with coronary artery calcification in Japanese subjects with type 2 diabetes as assessed by skin autofluorescence. *J Atheroscler Thromb* 2016; 23: 1178–1187.
15. Murai T, Takebe N, Nagasawa K, *et al*. Association of epicardial adipose tissue with serum level of cystatin C in type 2 diabetes. *PLoS One* 2017; 12: e0184723.
16. Todate Y, Uwano I, Yashiro S, *et al*. High prevalence of cerebral small vessel disease on 7T magnetic resonance imaging in familial hypercholesterolemia. *J Atheroscler Thromb* 2019; 26: 1045–1053.
17. Zelber-Sagi S, Webb M, Assy N, *et al*. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. *World J Gastroenterol* 2013; 19: 57–64.
18. Bedogni G, Bellentani S, Miglioli L, *et al*. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; 6: 33.
19. Cao X, Zhang W, Wan T, *et al*. Molecular cloning and characterization of a novel CXC chemokine macrophage inflammatory protein-2 gamma chemoattractant for human neutrophils and dendritic cells. *J Immunol* 2000; 165: 2588–2595.
20. Starnes T, Rasila KK, Robertson MJ, *et al*. The chemokine CXCL14 (BRAK) stimulates activated NK cell migration: implications for the downregulation of CXCL14 in malignancy. *Exp Hematol* 2006; 34: 1101–1105.
21. Jia G, Chandriani S, Abbas AR, *et al*. CXCL14 is a candidate biomarker for Hedgehog signalling in idiopathic pulmonary fibrosis. *Thorax* 2017; 72: 780–787.
22. Chalin A, Lefevre B, Devisme C, *et al*. Serum CXCL10, CXCL11, CXCL12, and CXCL14 chemokine patterns in patients with acute liver injury. *Cytokine* 2018; 111: 500–504.
23. Li J, Gao J, Yan D, *et al*. Neutralization of chemokine CXCL14 (BRAK) expression reduces CCl4 induced liver injury and steatosis in mice. *Eur J Pharmacol* 2011; 671: 120–127.
24. Lin Y, Chen BM, Yu XL, *et al*. Suppressed expression of CXCL14 in hepatocellular carcinoma tissues and its reduction in the advanced stage of chronic HBV infection. *Cancer Manag Res* 2019; 11: 10435–10443.
25. Fukui Y, Miyagawa T, Hirabayashi M, *et al*. Possible association of decreased serum CXCL14 levels with digital ulcers in patients with systemic sclerosis. *J Dermatol* 2019; 46: 584–589.
26. Hata R, Izukuri K, Kato Y, *et al*. Suppressed rate of carcinogenesis and decreases in tumour volume and lung metastasis in CXCL14/BRAK transgenic mice. *Sci Rep* 2015; 5: 9083.
27. Sjoberg E, Augsten M, Bergh J, *et al*. Expression of the chemokine CXCL14 in the tumour stroma is an independent marker of survival in breast cancer. *Br J Cancer* 2016; 114: 1117–1124.
28. García-Beltran C, Cereijo R, Quesada-López T, *et al*. Reduced circulating levels of chemokine CXCL14 in adolescent girls with polycystic ovary syndrome: normalization after insulin sensitization. *BMJ Open Diabetes Res Care* 2020; 8: e001035.
29. Perseghin G, Caumo A, Lattuada G, *et al*. Elevated fasting plasma C-peptide occurs in non-diabetic individuals with fatty liver, irrespective of insulin resistance. *Diabet Med* 2009; 26: 847–854.
30. Ohkura T, Shiochi H, Fujioka Y, *et al*. 20/(fasting C-peptide × fasting plasma glucose) is a simple and effective index of insulin resistance in patients with type 2 diabetes mellitus: a preliminary report. *Cardiovasc Diabetol* 2013; 12: 21.
31. Kaga H, Tamura Y, Takeno K, *et al*. Higher C-peptide level during glucose clamp is associated with muscle insulin resistance in nonobese Japanese men. *J Endocr Soc* 2019; 3: 1847–1857.
32. Vilar-Gomez E, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: clinical prediction rules and blood-based biomarkers. *J Hepatol* 2018; 68: 305–315.
33. Lin CY, Vega VB, Thomsen JS, *et al*. Whole-genome cartography of estrogen receptor alpha binding sites. *PLoS Genet* 2007; 3: e87.