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Circulating alarin concentrations are high in patients with type 2 diabetes and increased by glucagon-like peptide-1 receptor agonist treatment

An Consort-compliant study

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Abstract

Context: Alarin has been reported to be relative to food intake and an increase in body weight. However, to date, no report has demonstrated the relationship between circulating alarin and diabetes in humans.

Objective: The objective of this study is to gain insight into the possible role of alarin in humans.

Design and methods: 164 patients with newly diagnosed type 2 diabetes mellitus (nT2DM), 112 IGT and 134 healthy subjects were recruited for this study. In an interventional study, 29 nT2DM patients were treated by a weekly GLP-1RA for 6 months. Plasma alarin concentrations were measured by ELISA.

Results: Circulating alarin concentrations were significantly higher in both IGT and nT2DM subjects than in healthy individuals (0.40 ± 0.14 and 0.54 ± 0.24 *vs* 0.37 ± 0.10 μg/L, $P < .05$ or $P < .01$), whereas in T2DM patients, circulating alarin levels were higher than in IGT subjects. Circulating alarin positively correlated with FBG, HbA1c, HOMA-IR, $AUC_{glucose}$ and TNFα ($P < .05$ or $P < .01$). Multivariate logistic regression revealed that circulating alarin levels were correlated with IGT and T2DM. GLP-1RA treatment for 6 months increased circulating alarin levels in T2DM patients (from 0.34 ± 0.10 for baseline, to 0.39 ± 0.14 for 12 weeks, and finally to 0.38 ± 0.15 μg/L for 24 weeks; *vs.* pre-treatment $P < .05$).

Conclusions: These data suggest that alarin might be involved in the pathogenesis of T2DM in humans.

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Abbreviations: $AUC_{glucose}$ = the area under the curve for glucose, BMI = body mass index, FBG = fasting blood glucose, GLP-1RA = glucagon-like peptide-1 receptor agonist, HOMA-IR = homeostasis model assessment of insulin resistance, IGT = impaired glucose tolerance, SBP = Systolic blood pressure, TNFα = tumor necrosis factor-α.

Keywords: alarin, impaired glucose tolerance, insulin resistance, type 2 diabetes

1. Introduction

Alarin is a 25 amino acid neuropeptide which is a most recent member of the galanin peptide family to be isolated from the gangliocytes of human neuroblastic tumors and is named after its N-terminal alanine and its C-terminal serine originating as a splice variant of galanin-like peptide (GALP) mRNA.[1] Since alarin expression was initially found in ganglionic cells of neuroblastic tumors, it has been reported to be localized around the blood vessels with vasoactive actions and may have a regulating role in ocular blood flow.[2,3] In accordance with its

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vascular localization, alarin inhibited neurogenic inflammation induced by substance P.[2] Subsequently, Kolk et al reported that alarin was found in the locus coeruleus and the arcuate nucleus (ARC) of the hypothalamus in rats and mice, which are central nuclei involved in energy homeostasis.[4,5] Like galanin, alarin has been found to have a much wider central distribution than does GALP.[3,6] The similarity in alarin peptide sequence identity between mice and rats is 92%, and between humans and monkeys is 96%.[1] Unlike another member of the galanin peptide family, the exclusion of exon 3 in alarin results in a loss of binding domain for galanin receptor, which is responsible for the ability of GALP to bind with galanin receptors.[2] Furthermore, alarin also promotes the secretion of luteinizing hormone (LH) in male mice.[6] In the animal study, alarin showed antidepressant-like [7] and antimicrobial [8] roles. Some studies have reported that the peripheral and intracerebroventricular (ICV) injection of alarin significantly increased food intake and body weight, and stimulated glucose uptake mediated by activation of the Akt pathway in muscle tissues of insulin resistance (IR) rats.[6,9–11] Recently, Guo et al have also found that central treatment of alarin increased body weight, adiponectin release, the 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose uptake, glucose transporter 4 (Glut4) mRNA expression, the rates of glucose infusion (GIR) during the euglycemic-hyperinsulinemic clamp (EHC), and Glut4 and vesicle-associated membrane protein 2 (VAMP2) translocation to cell surfaces, but decreased the blood glucose level and retinol-binding protein 4 (RBP4) discharge in type 2 diabetic rats.[10] Therefore, alarin has been considered as a cytokine related to body nutrition and energy balance. However, to date, no report has demonstrated the association of circulating alarin with IR or diabetes in humans.

To investigate the physiological role of circulating alarin in vivo, we conducted cross-sectional and interventional studies to evaluate whether alarin correlates with IR and diabetes. First, we examined circulating alarin levels in healthy subjects, impaired glucose tolerance (IGT) individuals and newly diagnosed patients with T2DM (nT2DM). Secondly, we evaluated the effect of PEX168 treatment, a weekly glucagon-like peptide-1 receptor agonist (GLP-1RA) on circulating alarin levels.

2. Material and methods

2.1. Cross-sectional study

This study was performed from January 2017 to January 2018. A total of 410 subjects (164 nT2DM, 112 IGT and 134 healthy controls) were consecutively recruited to the study. According to the World Health Organization 1998 diagnostic criteria, IGT and T2DM were diagnosed.[12] Individuals with IGT (IGT group) or T2DM (nT2DM group) were newly diagnosed and had not been treated with any medicines including insulin and oral hypoglycemic agents, as well as diet control or physical exercise. Excluded criteria included type 1 diabetic mellitus (T1DM) and patients with macrovascular or microvascular complications, hypertension, liver cirrhosis, hepatic and renal failure, congestive heart failure, or any other diseases. One hundred thirty-four age-matched normal individuals (body mass index [BMI] ranged from 16 to 30 kg/m^2) without any major diseases were recruited from an unselected population of routine medical check-up, or the community or schools through advertisement as the controls (NGT group). An oral glucose tolerance test (OGTT) was performed in these subjects for confirming their nondiabetic statuses, and these subjects had no family history of T2DM and hypertension. None of the control individuals was taking medications related to glucose and lipid metabolism. Written voluntary consents were obtained from all subjects before their participation. The study was approved by the Human Research Ethics Committee of Chongqing Medical University and all experiments were performed in accordance with relevant guidelines and regulations.

2.2. Oral glucose tolerance test (OGTT)

An OGTT test was performed in all of the studied subjects. After an overnight fast of 10–14 h, included individuals underwent a standard 75 g, 2-h OGTT between 0800 and 0830 h. Blood samples were drawn at indicated times (0, 30, 60, and 120 min) for the measurements of glucose, insulin, alarin, and other parameters.

2.3. GLP-1RA treatment

This study was performed from January 2016 to January 2017. Twenty-nine patients with T2DM, including 15 females and 14 males, attended the interventional study of GLP-1RA treatment. Inclusion criteria included age 18–78 years; BMI of 20–40 kg/m^2; HbA1c levels between 7.5% and 11.0%; no history of unconscious hypoglycemia and fasting blood glucose (FBG) $<$13.9 mmol/L. These patients were given sc PEX168, a novel long-acting GLP-1RA, (200 μg/week) injection once a week for 24 weeks. To prevent acute complication, patients with three consecutive values of fasting blood glucose (FBG) $\geq$13.9 mmol/L or $\leq$3.9 mmol/L were withdrawn from this study. All patients were provided written informed consent before GLP-RA treatment. Blood samples for measuring alarin and other parameters were collected at 0800 h (pre-treatment) and on day 2 of the last treatment.

Study Design: Randomization was performed using a computer-generated list of random numbers. Four hundred six participants were assigned in a 1:1:1 ratio into group A (100 μg/week of PEX168), group B (200 μg/week of PEX168) or placebo groups in a random and double-blinded manner. Finally, 29 from 118 participants of group B were randomly selected to participate in the study of the relationship between circulating alarin and diabetes in humans. Study clinical physicians enrolled participants. Participants were consecutively assigned by a medical technologist, who was unaware of enrolment status, to treatment codes that corresponded to labels on otherwise identical concealed containers. Participants, investigators, and outcome assessors were blinded to the treatment for the duration of the study. Treatment assignments were not revealed prior to data collection and analysis. Study Outcomes: The primary outcome measure was the difference in plasma levels of glucose. Secondary outcome measures included anthropometric measurements, fasting plasma levels of insulin, lipids, and blood pressure.

2.4. Anthropometric and biochemical evaluation

Anthropometric and body composition were measured by a trained dietician in all subjects after an overnight. BMI was calculated as weight divided by height squared. Waist circumference and hip circumference were measured by the same observer and the waist-to-hip ratio (WHR) was calculated. The percentage of body fat (FAT %) was measured by bioelectrical impedance (BIA-101; RJL Systems, Shenzhen, China). The homeostasis model assessment of insulin resistance (HOMA-IR) and the area under the curve for glucose ($AUC_{glucose}$) and insulin ($AUC_{Insulin}$)

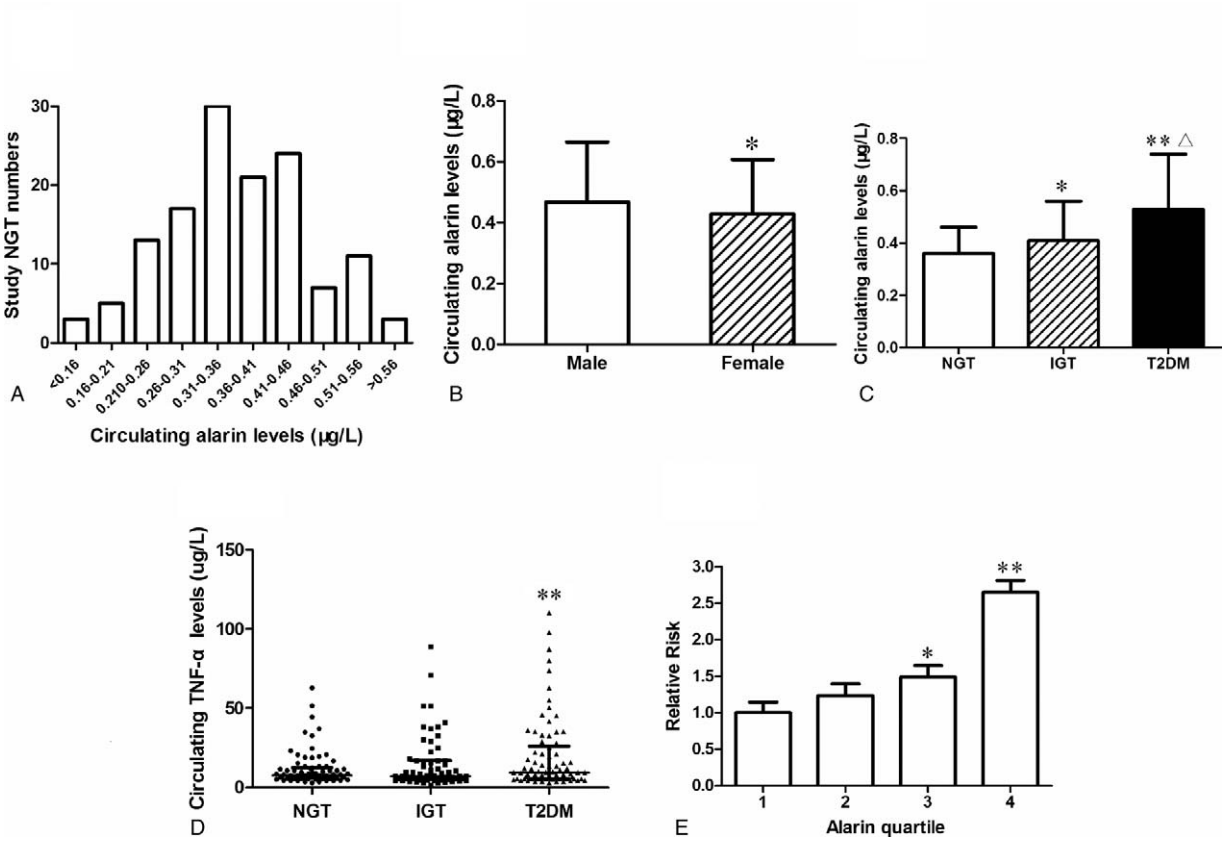

Figure 1. Distribution and concentration of circulating alarin in all study populations. (A) Distribution of circulating alarin concentrations in healthy individuals. (B) Circulating alarin levels according to sex. (C) Circulating alarin levels according to NGT, IGT, and nT2DM. (D) Circulating TNFα levels according to NGT, IGT, and nT2DM. (E) Prevalence of elevated T2DM in different quartiles of alarin: quartile 1 (Q1), <0.32 μg/L; quartile 2 (Q2), 0.32–0.39 μg/L; quartile 3 (Q3), 0.39–0.51 μg/L; quartile 4 (Q4), >0.51 μg/L. Values were given as means ± SD, $^{*}P < .05$, $^{**}P < .01$ compared with male or NGT or quartile 1; $^{\Delta}P < .05$, $\blacktriangle P < .01$ compared with IGT.

were calculated as reported previously.[13] Blood glucose, HbA1c, free fatty acids (FFAs), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) were determined as reported previously.[14]

2.5. Measurements of cytokines

Circulating alarin concentrations were determined with a commercial enzyme-linked immunosorbent assay (ELISA) Kit following the manufacturer's protocol (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA). The kit has a sensitivity of 0.08 ng/mL and a linear range of 0.08–0.78 ng/mL. Intra-assay and inter-assay variations were <10% and <15%, respectively. The ELISA kit had been validated by the dealer, showing high sensitivity and excellent specificity for detection of human alarin, but no significant cross-reactivity with other members of the galanin family. Plasma tumor necrosis factor-α (TNF-α) concentration was examined by a commercially available ELISA Kit (4A Biotech Co. Ltd, Beijing, China). The linear range of the ELISA assay was 4.3–1000 ng/L. The intra- and inter-assay coefficients (CV) were <10% and <12%, respectively.

2.6. Statistical analysis

Statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL, USA). Normal distribution of the data was tested using Kolmogorox–Smirnov test. The variables of non-normal distribution were skewed and logarithmically transformed to obtain a normal distribution. Continuous variables were presented as mean ± SD. Comparisons between groups were performed using ANOVA, an unpaired *t*-test or a paired *t*-test. Simple and multiple linear regression analyses were used to assess the association between circulating alarin levels and the other biomarkers. The trends of alarin associated with T2DM and IGT were analyzed using the Cochran–Armitage trend test and the row mean score difference. Receiver operating characteristics (ROC) curves of circulating alarin levels were delineated to investigate the optimal cut-off point for the prediction of T2DM. Sample size was calculated using the following equations: $N = [Z_{\alpha/2}\ \sigma/\varepsilon\mu]^2$ (σ, standard; μ, mean; $Z_{\alpha/2} = 1.96$, $\alpha = 0.05$, $\varepsilon = 7\%$). In all statistical tests, *P* values of <.05 were considered significant.

3. Results

3.1. Circulating alarin levels in IGT, T2DM and healthy subjects

In the present study, we measured fasting alarin levels in 134 healthy subjects (from 30 to 84 years) after overnight fasting. The distribution of plasma alarin was shown in Fig. 1a. Circulating alarin concentrations were ranged from 0.18 to 0.55 μg/L for most healthy individuals (95%).

Table 1

Main clinical features and plasma alarin levels in study population.

Group	NGT (n = 134)	IGT (n = 112)	T2DM (n = 164)
Age (yr)	53.5 ± 8.5	55.6 ± 10.1	55.4 ± 10.3
BMI (kg/m^2)	23.1 ± 2.7	24.2 ± 3.0**	24.7 ± 3.5**
WHR	0.87 ± 0.07	0.90 ± 0.06**	0.92 ± 0.05$^{**,\Delta}$
FAT (%)	28.9 ± 6.5	30.3 ± 7.2	29.8 ± 7.6
SBP (mm Hg)	118.7 ± 17.3	127.9 ± 17.1**	129.7 ± 16.3**
DBP (mm Hg)	74.6 ± 11.4	78.9 ± 9.5**	80.8 ± 10.5**
TG (mmol/L)	1.40 ± 0.70	1.93 ± 1.58**	2.07 ± 1.39**
TC (mmol/L)	4.92 ± 0.86	5.36 ± 1.02**	5.04 ± 1.04$^{\blacktriangle}$
HDL-C (mmol/L)	1.34 ± 0.29	1.34 ± 0.34	1.20 ± 0.33$^{**,\Delta}$
LDL-C (mmol/L)	2.83 ± 0.69	3.11 ± 0.91*	3.01 ± 0.91
FFA (μmol/L)	0.55 ± 0.37	0.64 ± 0.49	0.77 ± 0.57$^{**,\blacktriangle}$
FBG (mmol/L)	5.11 ± 0.50	5.82 ± 0.66**	9.42 ± 3.78$^{**,\Delta}$
2h-BG (mmol/L)	6.11 ± 1.05	9.08 ± 1.13**	19.45 ± 8.11$^{**,\Delta}$
FIns (mU/L)	9.16 ± 4.5	11.71 ± 7.66**	12.58 ± 7.39**
2h-Ins (mU/L)	47.93 ± 28.23	92.84 ± 59.69**	75.2 ± 68.71$^{**,\Delta}$
HbA1c (%)	5.55 ± 0.36	5.80 ± 0.39**	8.10 ± 2.24$^{**,\Delta}$
HOMA-IR	2.1 ± 1.07	3.03 ± 2**	4.9 ± 2.98$^{**,\Delta}$
$AUC_{insulin}$	123.72 ± 78.06	155.17 ± 88.5**	104.32 ± 75.1$^{*,\Delta}$
$AUC_{glucose}$	14.14 ± 3.21	19.46 ± 2.87**	34.38 ± 11.1$^{**,\Delta}$
TNF-α (μg/L)	10.47 ± 8.58	14.78 ± 16.92	20.23 ± 23.36**
Alarin (μg/L)	0.37 ± 0.10	0.40 ± 0.14*	0.54 ± 0.24$^{**,\Delta}$
Alarin (adjusted)[1]	0.37 ± 0.02	0.40 ± 0.02	0.54 ± 0.01

[1] Mean ± SD by general linear model with adjustment of age and gender. $AUC_{insulin}$ = the area under the curve for insulin; $AUC_{glucose}$ = the area under the curve for glucose, 2h-BG = 2 h post-glucose load blood glucose, 2h-Ins = 2 h plasma insulin after glucose overload, BMI = body mass index, DBP = diastolic blood pressure, FAT% = visceral fat%, FBG = fasting blood glucose, FFA = free fatty acid, FIns = fasting insulin, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = HOMA-insulin resistance index, IGT = impaired glucose tolerance, LDL-C = low-density lipoprotein cholesterol, NGT = normal subjects, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride, WHR = waist-to-hip ratio,TNF-α, tumor necrosis factor-α. Data are mean ± SD.

* $P < .05$.

** $P < .01$ compared with NGT.

$^{\blacktriangle}$ $P < .05$.

$^{\Delta}$ $P < .01$ compared with IGT group.

Table 2

Linear regression analysis of variables associated with plasma alarin levels in study population.

	Simple		Multiple	
	R	*P*	b	*P*
Age	0.043	.390	—	—
WHR	0.066	.180	—	—
BMI	0.070	.159	—	—
FAT (%)	0.021	.677	—	—
SBP	0.093	.061	—	—
DBP	0.036	.47	—	—
TG	0.073	.168	0.026	.044
TC	0.046	.384	—	—
HDL-C	−0.101	.056	—	—
LDL-C	0.094	.078	—	—
FFA	0.075	.195	—	—
FBG	0.278	<.001	—	—
FIns	0.019	.718	—	—
HbA1c	0.281	<.001	—	—
HOMA-IR	0.144	<.01	—	—
$AUC_{insulin}$	−0.066	.294	—	—
$AUC_{glucose}$	0.330	<.001	0.004	.013
TNF-α	0.133	<.05	—	—

In multiple linear stepwise regression analysis, values included for analysis were age, BMI, WHR, SBP, DBP, HOMA-IR, FAT%, FFA, TG, TC, HDL-C, LDL-C, FBG, FIns, HbA1c, $AUC_{insulin}$, $AUC_{glucose}$, and TNF-α.

Table 1 summarizes the anthropometric and biochemical parameters of the 410 subjects enrolled in the cross-sectional study. As expected, IGT and nT2DM subjects had higher BMI, WHR, blood pressure (BP), TG, FBG, 2-h BG, fasting inulin (FIns), 2 h plasma insulin after glucose overload (2h-Ins), HOMA-IR, $AUC_{insulin,}$ HbA1c and $AUC_{glucose}$ than the controls. In T2DM patients, FFA levels were also significantly increased, whereas HDL-C was lower compared with both IGT and controls (Table 1). We found that plasma alarin levels were significantly lower in women than in men (0.43 ± 0.19 *vs* 0.47 ± 0.19 μg/L, $P < .05$; Fig. 1b). Importantly, circulating alarin levels were higher in both IGT and nT2DM subjects than in controls ($P < .05$ or $P < .01$). When compared with IGT subjects, T2DM patients had higher circulating alarin levels ($P < .01$, Fig. 1c). These differences remained significant after adjustment for sex and age. In addition, the circulating levels of TNF-α in T2DM patients were also significantly higher than that of healthy individuals ($P < .01$; Fig. 1d). Furthermore, the relative risks for T2DM were significantly elevated along with increasing alarin quartiles (Fig. 1e).

3.2. The relationship between alarin and other parameters in the study population

Next, we investigated the association of alarin with other parameters. In all study populations, we found that there was a significant positive correlation between circulating alarin levels and FBG, HbA1c, HOMA-IR, $AUC_{glucose}$, and TNFα ($P < .05$ or $P < .01$; Table 2). These associations remained statistically significant even after adjusting for age and sex. In multiple stepwise regression analyses, we found that that plasma alarin levels were independently associated with TG and $AUC_{glucose}$ (Table 2). The multiple regression equation was: $Y_{alarin} = 0.298 + 0.004X_{AUCglucose} + 0.026X_{TG}$.

In multivariate logistic regression analysis, the results revealed that plasma alarin concentrations were significantly correlated with IGT and T2DM, even after adjustment for anthropometric parameters, age, gender, FAT%, BP (Table 3). When concentrations were analyzed by the row mean score difference and the Cochran–Armitage trend test, increasing alarin concentrations had a significant linear trend and were independently associated with IGT and nT2DM (Table 4). Finally, in all study populations, the ROC curve analyses showed that the best cut-off value for plasma alarin to predict T2DM was 0.47 μg/L (sensitivity 53%, specificity 81%, and AUC 0.721; Fig. 2a), and to predict both T2DM and IGT was 0.45 μg/L (sensitivity 59%, specificity 85.3%, and AUC 0.670, Fig. 2b).

3.3. Effect of long-acting GLP-1RA treatment on plasma alarin levels in T2DM patients

Twenty-nine patients with T2DM were treated by GLP-1RA for 24 weeks. The anthropometric and biochemical data pre- and post-treatment were shown in Table 5. As expected, after 24 weeks of GLP-1RA treatment, BMI, HbA1c, FBG, 2-hour postprandial blood glucose (2 h-BG) were significantly decreased in these patients compared with before treatment. However, plasma alarin levels after therapy for 6 months were significantly increased (from 0.34 ± 0.10 for baseline to 0.39 ± 0.14 for 12 weeks, and finally to 0.38 ± 0.15 μg/L for 24 weeks; *vs* pre-treatment $P < .05$, Fig. 3).

Table 3

Association of circulating alarin with IGT and T2DM in fully adjusted models.

Model adjust	IGT			T2DM		
	OR	95% CI	P	OR	95% CI	P
Age	15.35	1.72–136.86	<.05	1518.39	186.49–12,362.85	<.001
Age, sex	14.34	1.58–130.18	<.05	1238.49	147.36–10,408.69	<.001
Age, sex, BP	12.91	1.39–120.07	<.05	1324.93	150.61–11,655.72	<.001
Age, sex, BP, BMI	13.77	1.45–130.71	<.05	1450.51	159.42–13,197.53	<.001
Age, Sex, BP, BMI, WHR	17.68	1.76–177.48	<.05	2056.36	206.04–20,523.10	<.001
Age, Sex, BP, BMI, WHR, FAT%	17.52	1.71–179.90	<.05	1662.88	164.29–16,830.95	<.001

Results of multivariate logistic regression analysis are presented as the odds ratio of being in T2DM and IGT status increase in plasma alarin.
CI = confidence interval, OR = odds ratio.

4. Discussion

In the present study, we have demonstrated that

1. alarin levels were elevated in IGT or nT2DM subjects;
2. alarin correlated significantly with parameters of glucose metabolism (increased FBG, HbA1c, and $AUC_{glucose}$), IR (increased HOMA-IR) and TNFα;
3. circulating alarin levels were correlated with IGT and T2DM, and these associations remained statistically significant after adjusting for other variables;
4. GLP-1RA treatment in T2DM patients increased circulating alarin levels.

Notably, our results demonstrated that circulating alarin was higher in diabetic patients than prediabetic (IGT) subjects, suggesting a progressive increase of circulating alarin concentrations from prediabetes to diabetic state. Another notable is the independent association between T2DM, IGT, and plasma alarin in our logistic regression analysis. Importantly, in the present study, some confounding factors in the study cohort have been limited as much as possible. For example, our patients were newly diagnosed and had not been treated with hypoglycemic agents or diet control. The effects of disease duration, diet, and hypoglycemic agents were excluded. Therefore, our data revealed a real relationship between circulating alarin and T2DM, and alterations in plasma alarin levels over time from prediabetes (IGT) to diabetic state. However, the mechanisms underlying increased alarin levels in IGT and nT2DM individuals remain elusive. Animal studies have revealed the beneficial effects of alarin on ameliorating IR, insulin levels and blood glucose *in vivo*.[10,11] Recently, it has been reported that circulating alarin is relative to the regulation of energy balance[15] and blood glucose levels.[11] We speculate that the elevation of circulating alarin in subjects with IGT and T2DM might be a compensatory up-regulation *in vivo* for counteracting the metabolic stress produced by obesity, hyperglycemia, or hyperlipemia. In addition, it is also possible that adiposity or metabolic disorder may cause the resistance of alarin actions, like insulin or leptin resistance, leading to the increase of alarin secretion and release.

Table 4

Row mean scores differ and Cochran-Armitage trend analysis of the impact of circulating alarin level on IGT and T2DM.

Model adjusted	IGT		T2DM	
	χ^2	P	χ^2	P
Row mean scores test	5.4421	.0197	43.3814	<.001
Cochran–Armitage trend test	2.3373	.0097	–6.5967	<.001

The circulating alarin levels of all subjects were cut-off, and adjusted for age, BMI, WHR, SBP, DBP and lipid profile.

In the present study, our results show a higher level of circulating alarin in males. This distinct sexual dimorphism suggests that this protein may possess gender-specific activity and/or be regulated by sex hormones. As in animal-based studies, it was reported that alarin stimulates the secretion of luteinizing hormones and gonadotropin-releasing hormones in murine models.[6] Further detailed studies are needed to conclusively address these topics.

In addition, consistent with animal studies,[9–11] in our study populations, multiple linear regression analysis revealed TG, $AUC_{glucose}$ as independent contributors to plasma alarin concentrations. These findings suggest that alarin might act as a biomarker of adiposity and be related metabolic diseases, including T2DM and hypertension. Our results also showed that circulating alarin was positively associated with the parameters of IR (HOMA-IR and $AUC_{glucose}$) and glucose metabolism. Therefore, we speculate that increasing alarin levels in IGT and T2DM might be related to a defensive response, which may represent an ability for adaptation to IR or increased blood glucose concentrations.

The roles of systemic low-grade inflammation in IR have been initially reported by the finding that TNFα promoted IR and that its neutralization improved insulin sensitivity in diabetic animals.[16] Since then, a large number of studies have further revealed the relationship between chronic inflammation and IR.[17–19] However, a large-scale prospective study found that circulating IL-6 was an independent predictor of T2DM, while the expression of TNF-α or IL-1β was not a predictor of T2DM.[20] In the present study, we found that the circulating levels of TNFα were increased in newly diagnosed T2DM patients and associated with IR, further supporting that TNFα, as a marker of inflammation, is associated with IR. Importantly, we also found that there is a significant positive correlation between the circulating levels of alarin and plasma TNFα in our study population. In addition, these two proteins were positively correlated with HOMA-IR. Therefore, we speculate that, like TNFα, alarin may also be a cytokine that promotes IR. However, the roles of alarin in IR still need further extensive investigations.

GLP-1 has been shown to ameliorate IR and to promote or inhibit some cytokines release, such as adiponectin and visfatin.[21–23] However, the effect of GLP-1 RA on alarin release has not been reported. Therefore, we designed an interventional study in T2DM patients to address this question. After 6 months of GLP-1RA treatment, BMI and BG were significantly reduced, suggesting an improvement in glucose metabolism and weight

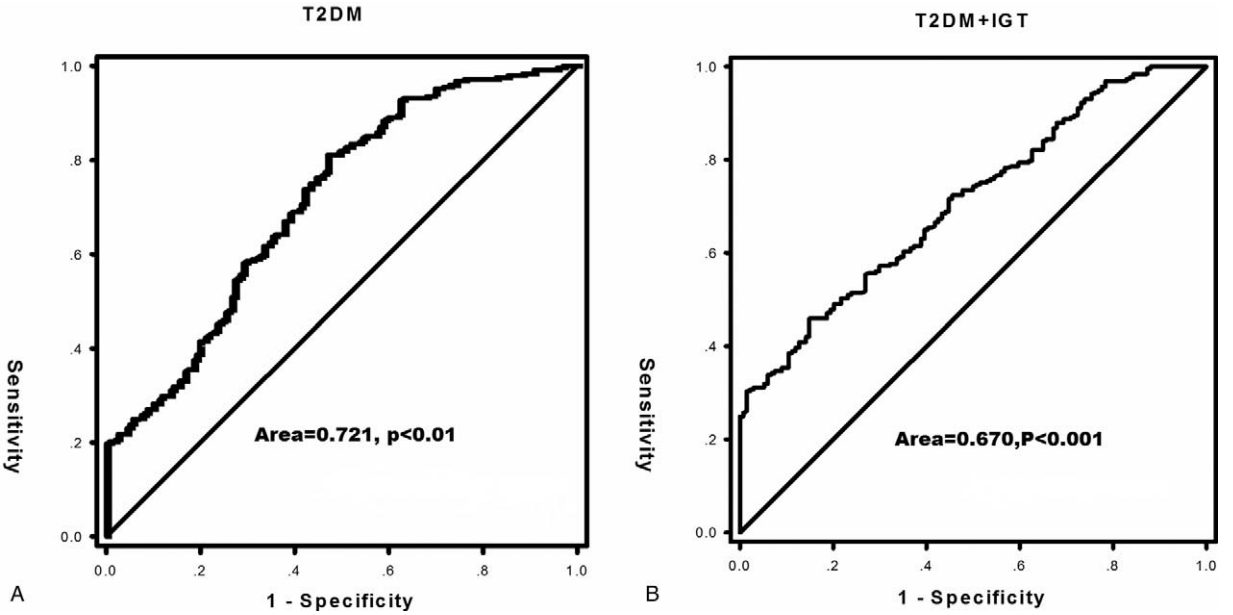

Figure 2. ROC curve analyses were performed for the prediction of T2DM (A) and IGT + T2DM (B).

loss. However, GLP-1RA treatment led to an increase in circulating alarin levels with the amelioration of glucose metabolism. Based on these findings, we speculate that long-term GLP-1RA treatment and/or the amelioration of glucose metabolism may contribute to alarin secretion and release. In addition, due to the improvement of glucose metabolism, the secretion of other cytokines, such as adiponectin and FGF-21, decreased leading to circulating alarin increased relatively. However, the precise mechanism underlying the effect of GLP-1RA on circulating alarin needs to be further studied.

Table 5

Clinical characteristics pre- and post-treatment with PEX168 in T2DM patients.

Variable	Baseline	Post-treatment 3 months	Post-treatment 6 months
BMI (kg/m^2)	25.3 ± 3.2	24.7 ± 3.1**	24.4 ± 3.4**
WHR	0.92 ± 0.06	0.92 ± 0.05	0.91 ± 0.05
SBP (mm Hg)	122.6 ± 12.6	121.2 ± 14.9	121.7 ± 13.6
DBP (mm Hg)	74.5 ± 9.8	74.0 ± 9.15	74.3 ± 12.0
HbA1c (%)	7.82 ± 1.12	7.00 ± 1.23**	6.92 ± 1.43**
HDL-C (mmol/L)	1.34 ± 0.41	—	1.38 ± 0.5
LDL-C (mmol/L)	2.57 ± 0.78	—	2.57 ± 0.73
FFA (umol/L)	0.61 ± 0.23	—	0.58 ± 0.25
TG (mmol/L)	1.93 ± 1.19	—	2.49 ± 1.95
TC (mmol/L)	4.76 ± 0.76	—	4.99 ± 0.85
FBG (mmol/L)	9.07 ± 2.03	7.63 ± 1.85**	7.70 ± 2.49**
2h-BG (mmol/L)	12.98 ± 2.52	10.73 ± 1.97**	9.99 ± 2.70**
FIns (mU/L)	11.87 ± 10.71	—	13.33 ± 11.64*
HOMA-IR	4.71 ± 4.14	—	4.68 ± 4.32

Values were given as means ± SD.

2h-BG = 2-hour postprandial glucose, BMI = body mass index, DBP = diastolic blood pressure, FBG = fasting blood glucose, FFA = free fatty acid, FIns = fasting plasma insulin, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride, WHR = waist-to-hip ratio.

* $P < .05$.

** $P < .01$ compared with baseline.

Our study has also some limitations. First, the cross-sectional design of this study leads to our inability to deduce a causal relationship between circulating alarin and IGT or T2DM. Prospective studies are needed to clarify their precise interrelationship. Second, alarin levels were not a pre-specified endpoint for recruited patients with T2DM in the experiment, and stored samples were used for alarin measurements, although the samples were relatively fresh. Finally, our results could be improperly influenced by some outliers due to the sample size. Despite these limitations, this study demonstrated clinically significant associations and differences between groups and used state-of-the-art methodology.

5. Conclusions

In summary, our data, for the first time, demonstrated that circulating alarin was significantly elevated in both IGT and

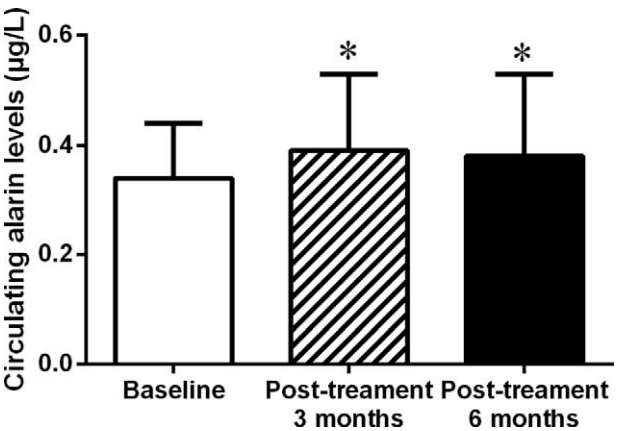

Figure 3. Circulating alarin levels in T2DM patients after both 3 and 6 months of GLP-1RA treatment. Data are means ± SD. * $P < .05$ *vs* baseline.

T2DM, and higher in T2DM patients than prediabetic individuals, indicating a progressive elevation of circulating alarin from pre-diabetic state to diabetes. In addition, circulating alarin is associated with FBG, HbA1c, HOMA-IR, $AUC_{glucose}$, and TNFα. We also found that circulating alarin was impacted by GLP-1RA treatment. Therefore, our data indicate that circulating alarin is likely a cytokine related to nutrition and metabolism and is associated with the progression of IR in humans. However, future large-scale prospective studies including different ethnic groups are necessary.

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Author contributions

W.H., X.F. B.T. and X.X. contributed to data collection. W.H. analyzed data. L.L. and H.L. drafted the manuscript. G.Y and Y. L. designed the analytic strategy, reviewed and edited the manuscript. G.Y. and L.L. was the guarantor of this work and, as such, had full access to all the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

[1] Santic R, Fenninger K, Graf K, et al. Gangliocytes in neuroblastic tumors express alarin, a novel peptide derived by differential splicing of the galanin-like peptide gene. J Mol Neurosci 2006;29:145–52.
[2] Santic R, Schmidhuber SM, Lang R, et al. Alarin is a vasoactive peptide. Proc Natl Acad Sci USA 2007;104:10217–22.
[3] Schrödl F, Trost A, Strohmaier C, et al. Distribution of the regulatory peptide alarin in the eye of various species. Exp Eye Res 2013;106: 74–81.
[4] Van Der Kolk N, Madison FN, Mohr M, et al. Alarin stimulates food intake in male rats and LH secretion in castrated male rats. Neuropeptides 2010;44:333–40.
[5] Wang M, Chen Q, Li M, et al. Alarin-induced antidepressant-like effects and their relationship with hypothalamus-pituitary- adrenal axis activity and brain derived neurotrophic factor levels in mice. Peptides 2014;56:163–72.
[6] Fraley GS, Leathley E, Lundy N, et al. Effects of alarin on food intake, body weight and luteinizing hormone secretion in male mice. Neuropeptides 2012;46:99–104.
[7] Zhuang F, Zhou X, Gao X, et al. Cytokines and glucocorticoid receptors are associated with the antidepressant-like effect of alarin. Peptides 2016;76:115–29.
[8] Wada A, Wong PF, Hojo H, et al. Alarin but not its alternative-splicing form, GALP (galanin-like peptide) has antimicrobial activity. Biochem Biophys Res Commun 2013;434:223–7.
[9] Zhang Z, Wu Y, Sheng S, et al. Intracerebroventricular injection of alarin increased glucose uptake in skeletal muscle of diabetic rats. PLoS One 2015;10:e0139327.
[10] Guo L, Fang P, Yu M, et al. Central alarin ameliorated insulin resistance of adipocytes in type 2 diabetic rats. J Endocrinol 2014;223:217–25.
[11] Mikó A, Füredi N, Tenk J, et al. Acute central effects of alarin on the regulation on energy homeostasis. Neuropeptides 2017;64:117–22.
[12] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabete mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539–53.
[13] Li K, Xu X, Hu W, et al. Glypican-4 is increased in human subjects with impaired glucose tolerance and decreased in patients with newly diagnosed type 2 diabetes. Acta Diabetol 2014;51:981–90.
[14] Li K, Liao X, Wang K, et al. Myonectin predicts the development of type 2 diabetes. J Clin Endocrinol Metab 2018;103:139–47.
[15] Boughton CK, Patterson M, Bewick GA, et al. Alarin stimulates food intake and gonadotrophin release in male rats. Br J Pharmacol 2010;161:601–13.
[16] Hotamisligil GS, Budavari A, Murray D, et al. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 1994;94:1543–9.
[17] Visser M, Bouter LM, McQuillan GM, et al. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131–5.
[18] Ouchi N, Parker JL, Lugus JJ, et al. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85–97.
[19] Mraz M, Lacinova Z, Drapalova J, et al. The effect of very-low-calorie diet on mRNA expression of inflammation-related genes in subcutaneous adipose tissue and peripheral monocytes of obese patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2011;96:E606–13.
[20] Spranger J, Kroke A, Möhlig M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003;52:812–7.
[21] Li L, Yang G, Shi S, et al. The adipose triglyceride lipase, adiponectin and visfatin are downregulated by tumor necrosis factor-a (TNF-α) in vivo. Cytokine 2009;45:12–9.
[22] Li L, Miao Z, Liu R, et al. Liraglutide prevents hypo- adiponectinemia-induced insulin resistance and alterations of gene expression involved in glucose and lipid metabolism. Mol Med 2011;17:1168–78.
[23] Li L, Yang G, Li Q, et al. Exenatide prevents fat-induced insulin resistance and raises adiponectin expression and plasma levels. Diabetes Obes Metab 2008;10:921–30.