CLINICAL STUDY

Prediabetes is not all about obesity: association between plasma leptin and prediabetes in lean rural Chinese adults

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Abstract

Objective: This study investigated the associations of plasma leptin levels with insulin resistance (IR) and prediabetes in relatively lean, rural Chinese men and women.

Design and methods: This study included 574 subjects aged 21–45 years from a community-based twin cohort. Plasma leptin concentrations were measured by sandwich immunoassays using flowmetric xMAP technology. Prediabetes was defined based on fasting plasma glucose and 75-g oral glucose tolerance test. Multivariate linear and logistic regression analyses were used to investigate gender-specific associations of leptin with IR measures and prediabetes, adjusting for intra-twin correlation, measures of adiposity, and other pertinent covariates.

Results: The body mass index is 22.3 ± 2.7 kg/m² in men and 22.5 ± 2.7 kg/m² in women. Leptin levels were positively associated with IR. Individuals with higher tertiles of leptin also had increased risk of prediabetes with odds ratios (OR) of 2.6 (95% confidence interval (CI): 1.4–5.1) and 4.3 (95% CI: 2.1–8.7) in men; OR of 1.1 (95% CI: 0.6–2.1) and 3.1 (95% CI 1.5–6.2) in women for second and third tertile respectively. These associations were attenuated after further adjusting for adiposity measurements only in men. The leptin–prediabetes associations disappeared after adjusting for the homeostatic model assessment of IR in both genders.

Conclusion: In this sample of relatively lean rural Chinese adults, plasma leptin levels were associated with IR and prediabetes in a dose–response fashion, which were not totally explained by adiposity. Our data emphasize that prediabetes is not all about obesity, and leptin may be an additional biomarker for screening individuals at high risk for prediabetes in this population.

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Introduction

Prediabetes, characterized by impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or a combined IFG/IGT (1), reflects glucose dysregulation intermediate between normoglycemia and type 2 diabetes mellitus. Prediabetes, often asymptomatic and under-detected, is likely fueling the epidemics of type 2 diabetes and its consequences worldwide (2). Cowie *et al.* (3) reported that the crude prevalence of prediabetes was almost 30% in the US adult population. While obesity is a well-recognized risk factor for prediabetes and type 2 diabetes in heavy western populations, there are paradoxical findings in relatively lean Asian populations. For example, 33% of US Asian Indian had prediabetes (4). Our own data showed that prevalence of prediabetes is 26% in middle-aged, rural, relatively lean (mean body mass index (BMI) 21.6 kg/m² in men and 22.1 kg/m²

in women) Chinese population. The high prevalence of prediabetes in relatively lean populations raised a possibility that prediabetes is not all about obesity. A better understanding of the role of other important determinants of prediabetes may provide new insight into the pathogenic mechanisms of prediabetes and type 2 diabetes, and offer a window of opportunity for early detection and prevention.

Leptin, an adipokine encoded by the obese (ob) gene (5), is primarily produced by white adipose cells. It has a variety of important central and peripheral actions, related to the regulation of food intake, energy balance, and metabolism (6, 7). Leptin levels in humans increase with increasing body fat, and leptin resistance has been postulated to present in obese individuals. Independent of the relationship to body fat, leptin levels are also positively correlated with fasting insulin levels in humans (8). While the association between leptin and

insulin has not been fully elucidated, several studies indicated that leptin inhibits insulin synthesis and secretion, while insulin stimulates leptin secretion. This bidirectional hormonal feedback loop between insulin and leptin has been named as the adipoinsular axis (9). To date, few data are available on the role of leptin in prediabetes, especially, in relatively lean Chinese population.

This study was to examine whether leptin is associated with insulin resistance (IR) and prediabetes and whether these associations can be explained by measures of adiposity in relatively lean, rural Chinese adults.

Subjects and methods

Study population and procedures

This report utilized data from an ongoing NIH funded study of metabolic syndrome in a large community-based Chinese twin cohort in Anqing, Anhui province in China during 2005–2006. All the study subjects completed a questionnaire interview, standardized physical examination, dual-energy X-ray absorptiometry (DEXA) scan, and oral glucose tolerance test (OGTT) after at least 10 h of overnight fasting. We utilized a nested case–control design, which included all prediabetic cases and normal glucose tolerance (NGT) controls matched by age and gender. A total of 574 subjects (290 males and 284 females) aged 21–45 years were included in this analysis. The study protocol was approved by the Institutional Review Boards of Children's Memorial Hospital and the Biomedical Institute, Anhui Medical University in Hefei, People's Republic of China. All participants gave written consent.

Physical activity

Physical activity was assessed using the short version of the international physical activity questionnaire (IPAQ-Short) (<http://www.ipaq.ki.se>). Participants were asked information about three types of physical activity (vigorous, moderate, and walking) across various physical activity domains (i.e. leisure time, occupational or school, domestic, and transport), using the 'last 7 days' as the reference period. Using both the total volume of activity and the number of activity days/sessions per week, the IPAQ generates a categorical indicator (low, moderate, and high) of regular physical activity.

Anthropometric parameters

Height and weight were measured using standard protocols, without shoes and outerwear. Height was measured to the nearest 0.1 cm on a portable stadiometer and weight to the nearest 0.1 kg. Waist circumference (WC) was measured at the horizontal circumference midway between the lowest rib margin and the iliac crest. BMI was calculated as weight (kg)/height squared (m^2). Blood pressure (BP) was

measured by trained nurses according to a standard procedure. A standard whole-body scan was performed by DEXA (GE-Lunar Prodigy, Madison, WI, USA) to measure total body fat and trunk fat using a standard software calculation (10). Percentage body fat (PBF) and percentage trunk fat (PTF) were calculated as (total body fat in kg/weight in kg) $\times$ 100 and (trunk fat in kg/total body fat in kg) $\times$ 100 respectively.

Laboratory methods

Plasma was separated from blood cells in the field within 30 min after the blood was drawn and kept refrigerated. Plasma glucose was measured within 2 h by a glucose oxidase method (Hitachi 7020 Automatic Analyzer; Hitachi, Tokyo, Japan). Standard quality control procedures were performed each day with standard samples that came with the reagents (the coefficient of variation, CV < 8%). Triglycerides and cholesterol were measured by enzymatic methods (Boehringer Mannheim, Mannheim, Germany), and high-density lipoprotein cholesterol (HDL-c) was measured by the same enzymatic method after precipitation with dextran sulfate/magnesium chloride. Serum insulin was measured by ECL method on an Elecsys 2010 system (Roche, Basel, Switzerland). Duplicate analyses were also conducted daily using samples collected from study participants (CV < 10%, mean = 3%). Plasma leptin concentrations were determined using a sandwich immunoassays based flowmetric xMAP technology on Luminex 200 machines (luminex multi-analyte profiling system, Luminex, Corp., Austin, TX, USA). Immunoassay kit is commercially available from Millipore Corporation (Bedford, MA, USA). Each sample was duplicated, and intra-assay CV was < 5.1%.

Definition of IR and prediabetes

All the study subjects completed a standard 75-g glucose OGTT after at least 10 h of overnight fasting. Blood samples were obtained at 0 and 120 min after glucose administration, for glucose and insulin measurements. IR was estimated using fasting insulin, 2 h post-load insulin (2 h PI), and the homeostatic model assessment of IR (HOMA-IR). HOMA-IR was calculated using the following formulae: $\text{HOMA-IR} = \text{fasting insulin concentration } (\mu\text{U/ml}) \times \text{fasting glucose concentration } (\text{mmol/l}) / 22.5$ (11).

Prediabetes was defined as either IFG (i.e. fasting plasma glucose was between 5.6 and 6.9 mmol/l), and/or IGT (i.e. 2 h post-load glucose was between 7.8 and 11.0 mmol/l).

Exclusion criteria for this analysis included any of the followings: previous diagnosis of diabetes by a physician, plasma glucose concentration ≥ 7.0 mmol/l after a 10 h fast, or plasma glucose concentration ≥ 11.1 mmol/l 2 h after a 75-g OGTT, or taking any anti-diabetic medication.

Statistical analysis

The distribution of fasting insulin, 2 h PI, HOMA-IR, and leptin was right skewed, and a logarithmic transformation was used to normalize the data for subsequent statistical analyses. In addition to analyzing leptin as a continuous variable, we also divided leptin into three tertiles, so that we could assess its functional relationship and dose–response associations with IR and prediabetes. We first examined the associations between tertiles of leptin and IR measures using gender-specific multiple linear regression models, with adjustment for age, education (primary school minor or plus), occupation (farmer or nonfarmer), active smoking and passive smoking, alcohol consumption (yes or no), and physical activity (Model A). Since the sample contains 187 twin pairs, we also accounted for intra-twin pair correlation using a generalized estimation equation. We further evaluated whether these associations could be modified by components of metabolic syndrome (Model B) and different adiposity measures (BMI, PBF, PTF, and WC for Model C–F). We also performed similar analyses among two subgroups: subjects with NGT and subjects with prediabetes. To examine explanatory contribution of leptin to IR measures, partial R^2 was calculated.

Finally, we performed gender-specific multiple logistic regression analyses to assess the associations between leptin tertiles and prediabetes, with the adjustment for the same covariates as listed above plus HOMA-IR (Model G). A two-sided P value <0.05 was regarded as statistically significant. We also used Bonferroni correction to address potentially inflated type I error (false positive) due to multiple comparisons (i.e. 4 independent tests), with corrected P value cutoff of 0.0125. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Demographic, anthropometric, and clinical characteristics

This study includes 290 males, BMI 22.3 ± 2.7 kg/m² and 284 females, BMI 22.5 ± 2.7 kg/m² and 280 prediabetes cases and 294 NGT controls. Table 1 displays the characteristics of NGT and prediabetes subjects by gender. Smoking status, education level, and occupation are compatible between prediabetes cases and NGT controls. As expected, leptin concentrations,

Table 1 Demographical and clinical characteristics of Chinese men and women aged 21–45 years.

Variables	Men		Women	
	NGT	PDM	NGT	PDM
<i>n</i>	147	143	147	137
Age (years)	36.4 $\pm$ 6.9	34.6 $\pm$ 7.3**	38.1 $\pm$ 5.4	36.3 $\pm$ 5.6†
Education (% primary school minor)	84.4	83.2	96.6	99.3
Occupation (% farmer)	76.9	67.1	70.8	70.1
Smoke status (% yes)				
Active smoking	67.4	59.4	3.4	4.4
Passive smoking	70.8	73.4	76.9	70.1
Alcohol consumption (% yes)	39.5	43.4	4.8	2.9
Physical activity				
Low	29 (19.7)	17 (11.9)	24 (16.3)	30 (21.9)
Moderate	21 (14.3)	28 (19.6)	27 (18.4)	19 (13.9)
High	70 (47.6)	73 (51.1)	76 (51.7)	65 (47.5)
Unknown	27 (18.4)	25 (17.5)	20 (13.6)	23 (16.8)
BMI (kg/m ²)	21.7 $\pm$ 2.3	22.8 $\pm$ 2.9†	22.3 $\pm$ 2.5	22.8 $\pm$ 2.8
PBF (%)	13.3 $\pm$ 6.8	16.9 $\pm$ 8.0†	28.2 $\pm$ 5.8	29.3 $\pm$ 6.2
PTF (%)	53.3 $\pm$ 6.1	55.9 $\pm$ 6.0†	50.7 $\pm$ 4.5	51.4 $\pm$ 4.9
WC (cm)	73.2 $\pm$ 7.3	78.1 $\pm$ 9.0†	72.9 $\pm$ 7.2	74.2 $\pm$ 8.6
SBP (mmHg)	108.7 $\pm$ 14.3	113.2 $\pm$ 12.9	108.4 $\pm$ 16.4	107.6 $\pm$ 12.5
DBP (mmHg)	73.4 $\pm$ 11.9	75.8 $\pm$ 10.4	69.9 $\pm$ 12.5	70.4 $\pm$ 9.3
Triglyceride (mmol/l)	1.0 $\pm$ 0.6	1.3 $\pm$ 0.8†	1.0 $\pm$ 0.7	1.0 $\pm$ 0.5
Cholesterol (mmol/l)	4.1 $\pm$ 0.7	4.4 $\pm$ 0.9	4.1 $\pm$ 0.7	4.2 $\pm$ 0.8
HDL-c (mmol/l)	1.7 $\pm$ 0.5	1.8 $\pm$ 0.6	1.6 $\pm$ 0.5	1.7 $\pm$ 0.5
FPG (mmol/l)	5.1 $\pm$ 0.3	5.8 $\pm$ 0.3†	5.1 $\pm$ 0.3	5.8 $\pm$ 0.4†
2 h PG (mmol/l)	4.8 $\pm$ 1.3	5.7 $\pm$ 1.7†	5.8 $\pm$ 1.1	6.8 $\pm$ 1.6†
FSI (μ U/ml)	4.8 $\pm$ 2.8	7.1 $\pm$ 5.0*	6.0 $\pm$ 2.6	8.2 $\pm$ 5.2*
2 h PI (μ U/ml)	21.6 $\pm$ 19.9	30.8 $\pm$ 33.8*	30.1 $\pm$ 16.6	45.9 $\pm$ 36.5*
HOMA-IR	1.1 $\pm$ 0.7	1.8 $\pm$ 1.3*	1.4 $\pm$ 0.6	2.1 $\pm$ 1.5*
Leptin (ng/ml)	1.9 $\pm$ 1.9	3.4 $\pm$ 3.4*	6.8 $\pm$ 4.0	9.5 $\pm$ 6.8*

Data are presented as percent and mean $\pm$ s.d.; * $P < 0.05$ for t -test based on log-transformed variables; ** $P < 0.05$ for t -test compared with NGT groups; † $P < 0.01$ for t -test compared with NGT groups; NGT, normal glucose tolerance; PDM, prediabetes (including impaired fasting glucose and impaired glucose tolerance); BMI, body mass index; PBF, percentage body fat; PTF, percentage trunk fat; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; 2 h PG, 2 h post-load glucose; FSI, fasting serum insulin; 2 h PI, 2 h post-load insulin; HOMA-IR, homeostasis modeling accessible of insulin resistance.

plasma glucose, insulin measurements, and leptin levels were significantly higher in prediabetes subjects compared with NGT subjects in men and women ($P < 0.05$). Also as expected, adiposity measurements and triglycerides were significantly higher in prediabetes subjects compared with NGT subjects, most notably in men. There were no differences in BP, and plasma HDL-c concentrations between NGT and prediabetes groups. Moreover, the leptin levels were much higher in women than men in both NGT and prediabetes subjects.

Association of plasma leptin with IR

In both genders, we observed a dose-response relation between the tertiles of leptin and fasting serum insulin (FSI), 2 h PI, and HOMA-IR among all the subjects as well as among the subgroups: subjects with NGT and subjects with prediabetes (Fig. 1). Table 2 showed that higher tertiles of leptin were positively associated with

FSI, 2 h PI, and HOMA-IR in both genders. For example, compared with the lowest tertile of leptin, the second and third tertiles of leptin were associated with elevated HOMA-IR with β and s.e.m. estimates of 0.32 ± 0.08 and 0.71 ± 0.09 in men, when adjusting for ages, education, occupation, active smoking and passive smoking, alcohol consumption, and physical activity (Model A). The corresponding estimates for women were 0.15 ± 0.07 and 0.50 ± 0.08 . These associations did not change after adjusting BP and lipid profile (Model B), while they became attenuated but remained significant after adjusting for adiposity measurements (Model C–F). The explanatory contribution of leptin to HOMA-IR was 21% in both genders, which was attenuated to 12 and 13% in men and women respectively after adjusting for BP and lipid profile. It was further attenuated to 2–10% after adjustment for adiposity measures. Similar patterns were observed for FSI and 2 h PI levels.

Association of leptin with prediabetes

As shown in Table 3, compared with individuals with the lowest tertiles of leptin, those with higher leptin tertiles had increased risk of prediabetes (OR = 2.6, 95% CI: 1.4–5.1 and OR = 4.3, 95% CI: 2.1–8.7 for second and third tertiles in men respectively; OR = 1.1, 95% CI: 0.6–2.1 and OR = 3.1, 95% CI: 1.5–6.2 for second and third tertiles in women respectively) after adjusting age, education, occupation, smoking and alcohol consumption, and physical activity (Model A). The associations remained after adjusting for BP and lipid profile (Model B). With further adjustment for adiposity measures in the model: BMI (Model C), PBF (Model D), PTF (Model E), and WC (Model F), the associations of leptin with prediabetes were attenuated in men. Adjustment for adiposity measures had no impact on leptin–prediabetes associations in women. Finally, the leptin–prediabetes associations disappeared after adjusting for HOMA-IR (Model G) in both genders.

Of note, we evaluated the associations between leptin and IR measures, leptin and prediabetes stratified by gender, and thus, the significant findings may be false due to the multiple comparisons (i.e. four independent tests). To address this concern, we used Bonferroni correction to address potentially inflated type I error (false positive). Our results showed that the majority of the associations remained statistically significant even after Bonferroni correction, using a cutoff of $P < 0.0125$.

Figure 1 The distribution of insulin resistance measures classified by leptin tertiles, grouped for all subjects (all); subjects with normal fasting glucose (NGT); subjects with prediabetes (PDM). (A) Log-transformed mean fasting serum insulin levels for each tertile of plasma leptin concentrations, FSI, fasting serum insulin. (B) Log-transformed mean 2 h post-load insulin levels for each tertile of plasma leptin concentrations; 2 h PI, oral glucose tolerant 2 h post-load insulin. (C) Log-transformed mean HOMA-IR levels for each tertile of plasma leptin concentrations, HOMA-IR, homeostasis modeling accessible of insulin resistance. * $P < 0.05$ compared with first leptin tertile; ** $P < 0.01$ compared with first leptin tertile.

Discussion

This is the first study that assessed gender-specific plasma leptin levels in relation to prediabetes in relatively lean middle-aged rural Chinese subjects. We found significant associations between higher leptin levels and increased risk of prediabetes in both men and

Table 2 Association between leptin tertiles and insulin resistance measurements in Chinese men and women aged 21–45 years.

	Males			Females		
	Second tertile β (S.E.M.)	Third tertile β (S.E.M.)	Inleptin Partial R^2	Second tertile β (S.E.M.)	Third tertile β (S.E.M.)	Inleptin Partial R^2
HOMA-IR						
Model A	0.32 (0.08) [‡]	0.71 (0.09) [‡]	0.2084	0.15 (0.07) [*]	0.50 (0.08) [‡]	0.2062
Model B	0.29 (0.08) [‡]	0.56 (0.09) [‡]	0.1181	0.09 (0.07)	0.41 (0.08) [‡]	0.1342
Model C	0.20 (0.07) [†]	0.36 (0.09) [‡]	0.0369	0.03 (0.07)	0.28 (0.09) [†]	0.0560
Model D	0.15 (0.08)	0.29 (0.11) [†]	0.0201	0.03 (0.07)	0.26 (0.08) [‡]	0.0532
Model E	0.29 (0.08) [‡]	0.57 (0.09) [‡]	0.0996	0.07 (0.07)	0.34 (0.08) [‡]	0.0887
Model F	0.17 (0.07) [*]	0.32 (0.09) [‡]	0.0305	0.03 (0.07)	0.24 (0.08) [†]	0.0392
FSI						
Model A	0.30 (0.07) [‡]	0.66 (0.08) [‡]	0.1989	0.14 (0.07) [*]	0.47 (0.07) [‡]	0.2062
Model B	0.27 (0.07) [‡]	0.52 (0.08) [‡]	0.1116	0.07 (0.06)	0.37 (0.07) [‡]	0.1269
Model C	0.19 (0.07) [†]	0.34 (0.08) [‡]	0.0342	0.02 (0.06)	0.25 (0.08) [‡]	0.0510
Model D	0.14 (0.08)	0.26 (0.10) [†]	0.0161	0.02 (0.06)	0.24 (0.08) [†]	0.0462
Model E	0.28 (0.07) [‡]	0.53 (0.08) [‡]	0.0961	0.06 (0.06)	0.31 (0.07) [‡]	0.0828
Model F	0.17 (0.07) [*]	0.30 (0.09) [‡]	0.0287	0.02 (0.06)	0.22 (0.07) [†]	0.0338
2 h PI						
Model A	0.36 (0.13) [†]	0.75 (0.14) [‡]	0.1129	0.12 (0.07)	0.48 (0.09) [‡]	0.1042
Model B	0.36 (0.14) [†]	0.68 (0.16) [‡]	0.0908	0.08 (0.08)	0.40 (0.10) [‡]	0.0664
Model C	0.30 (0.14) [*]	0.59 (0.19) [†]	0.0549	0.10 (0.08)	0.43 (0.11) [‡]	0.0595
Model D	0.10 (0.13)	0.11 (0.19)	0.0054	0.06 (0.08)	0.33 (0.11) [†]	0.0219
Model E	0.33 (0.13) [*]	0.58 (0.17) [‡]	0.0569	0.09 (0.08)	0.39 (0.10) [‡]	0.0512
Model F	0.31 (0.14) [*]	0.56 (0.19) [†]	0.0504	0.09 (0.08)	0.42 (0.10) [‡]	0.0521

* $P < 0.05$; [†] $P < 0.01$; and [‡] $P < 0.001$ compared with first leptin tertile; HOMA-IR, homeostasis modeling accessible of insulin resistance; FSI, fasting serum insulin; 2 h PI, 2 h post-load insulin. Model A, adjusted for age, education, occupation, active and passive smoking, alcohol consumption, and physical activity; Model B, Model A plus triglyceride, cholesterol, high-density lipoprotein cholesterol (HDL-c), systolic blood pressure (SBP), diastolic blood pressure (DBP). Model C, Model B plus body mass index (BMI); Model D, Model B plus percentage body fat; Model E, Model B plus percentage trunk fat; Model F, Model B plus waist circumference.

women in a dose–response fashion. The leptin–prediabetes associations were independent of BP and lipid levels. Furthermore, the leptin–prediabetes associations cannot be totally explained by comprehensive measures of adiposity, because further adjusting for adiposity measurements only attenuated these associations in men, and had little effect in women. Our findings reinforce the notion that prediabetes is not all about obesity. If confirmed in future studies, leptin may be an additional biomarker for identifying individuals at high risk of prediabetes in this lean Chinese population.

The biological mechanism by which leptin affects the risk of prediabetes is not completely clear. Notably, the leptin–prediabetes association did disappear after adjusting for HOMA-IR in both genders, suggesting that IR may be a possible biological pathway by which leptin affects prediabetes. The relationship between leptin and insulin appears to be bidirectional, that is, leptin inhibits insulin synthesis and secretion while insulin stimulates leptin secretion (9). The effect of leptin also appears to vary by species (mice versus human) and by clinical conditions. Although leptin improves glucose homeostasis and IR in humans with lipodystrophy or congenital leptin deficiency (12, 13), results in humans with ‘typical’ obesity were disappointing in this regard (14). In the majority of cases of obesity, despite both an intact leptin receptor and high circulating leptin levels, leptin fails to induce weight loss and IR improvement. This diminished response to the anorexigenic and

insulin-sensitizing effects of leptin is called ‘leptin resistance’. Moreover, attenuation of leptin sensitivity in the brain leads to excess triglyceride accumulation in adipose tissue, as well as in muscle, liver, and pancreas, resulting in impaired insulin sensitivity and secretion (15). Consequently, this could be a sign of decreased inhibition of insulin secretion, and the resulting hyperinsulinemia will further stimulate leptin production. This scenario could lead to what we have observed, that is, high leptin is associated with hyperinsulinemia and prediabetes.

Our finding is consistent with a recent study, in which serum leptin level was higher in those with prediabetes than in the controls in Saudi subjects (16). It is also consistent with previous longitudinal studies on the effects of increased leptin concentrations on diabetes in Caucasian (17) and Japanese American populations (18). In addition, Nowak *et al.* (19) found that leptin may antagonize some functions of insulin by the attenuation of insulin receptor capacity in liver.

While leptin was independently associated with IR measures and prediabetes in both genders, our data did show some gender differences. First, we found higher leptin levels in females than in males, as reported in previous studies (20). However, this difference was attenuated after adjusting for PBF (data not shown), which is consistent with a previous report that gender difference disappeared when leptin values were compared across similar body fat percentages (21).

Table 3 Association between leptin levels and the risk of prediabetes in Chinese men and women aged 21–45 years.

Leptin tertiles (ng/ml)	n			OR (95% CI)						
	NGT	PDM		Model A	Model B	Model C	Model D	Model E	Model F	Model G
Men										
First tertile (low)	67	29		1.0	1.0	1.0	1.0	1.0	1.0	1.0
Second tertile	45	52		2.6 (1.4–5.1) [†]	2.5 (1.3–4.9) [†]	2.3 (1.2–4.7) [*]	2.4 (1.2–4.9) [*]	2.3 (1.1–4.7) [*]	2.0 (1.0–4.0)	1.7 (0.9–3.4)
Third tertile (high)	35	62		4.3 (2.1–8.7) [†]	3.6 (1.7–7.8) [†]	3.4 (1.4–8.2) [†]	3.4 (1.3–8.9) [*]	3.1 (1.3–7.2) [†]	2.2 (0.9–5.3)	2.0 (0.9–4.7)
P trend				<0.0001	0.0009	0.0056	0.0120	0.0073	0.0652	0.0878
Women										
First tertile (low)	57	37		1.0	1.0	1.0	1.0	1.0	1.0	1.0
Second tertile	55	40		1.1 (0.6–2.1)	1.1 (0.6–2.1)	1.1 (0.6–2.3)	1.3 (0.6–2.6)	1.1 (0.6–2.2)	1.1 (0.6–2.1)	1.0 (0.5–1.9)
Third tertile (high)	35	60		3.1 (1.5–6.2) [†]	3.1 (1.5–6.3) [†]	3.2 (1.4–7.3) [†]	3.8 (1.6–9.3) [†]	2.9 (1.4–6.3) [†]	3.0 (1.3–6.8) [†]	1.9 (0.9–4.0)
P trend				0.0014	0.0022	0.0056	0.0029	0.0048	0.0102	0.0993

* $P < 0.05$; $^{\dagger}P < 0.01$; and $^{\ddagger}P < 0.001$ compared with first leptin tertile; NGT, normal glucose tolerance; PDM, prediabetes; Model A, adjusted for age, education, occupation, active and passive smoking, alcohol consumption, and physical activity; Model B, Model A plus triglyceride, cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, and diastolic blood pressure; Model C, Model B plus body mass index (BMI); Model D, Model B plus percentage body fat; Model E, Model B plus percentage trunk fat; Model F, Model B plus waist circumference; Model G, Model B plus homeostasis modeling accessible of insulin resistance (HOMA-IR).

This is consistent with the concept that women have a higher body fat content at a given BMI, and leptin levels are affected by total adiposity. Secondly, our data suggest that adiposity mediated a proportion of the association between leptin and IR measures in both genders and between leptin and prediabetes only in men. One possible explanation for the gender difference is different distributions of fat mass by gender, e.g. more abdominal visceral adipose tissue in men and more subcutaneous adipose tissue mass in women (22). An alternative explanation for the gender difference is that leptin may have differential effects in men and women.

Our study has several strengths. First, we were able to examine a wide array of adiposity measures including BMI, WC, and DEXA-based measurements of body fat, which can more accurately reflect body composition than BMI and WC. Secondly, this study included a relatively homogenous population, in which most of them were farmers, had similar lifestyle and physical activity levels, and dietary intake. The most important feature of our study is that we focused on prediabetes instead of diabetes. This minimizes several sources of confounding variables, such as drug treatment effect, unmeasured metabolic alterations or other subclinical pathology as a result of diabetes, and decreased β -cell function within diabetic individuals that might obscure secretion and effects of leptin. As such, our study was unlikely to be confounded by the above-mentioned factors. Finally, this population had relatively low plasma insulin levels, which would minimize the confounding of high insulin level on the association between leptin levels and prediabetes.

Our study also has limitations. This cross-sectional analysis only allows for the establishment of an association and not a temporal and causal relationship between leptin and prediabetes. The study sample was nested within a large twin cohort, but we limited to one twin per family for the majority of the sample. For the small number of twin pairs ($n = 187$) included in the analysis, we used general estimating equations to account for intra-pair correlation. Of note, the selected subjects were quite comparable with those excluded from this study with regards to age, gender, and adiposity measures. Furthermore, this community-based twin population is comparable to local general population as demonstrated in our previous report (23). Although this is among the largest study of this kind in Chinese population, the sample size of our study is modest. We performed a power analysis using QUANTO (<http://hydra.usc.edu/gxe>) and demonstrated that we have reasonable power ($> 85\%$) to detect an association with ORs of 2.5 and above (based on the estimates in Table 3 and frequency of exposure to higher leptin levels in normal controls: 0.3–0.4). Lastly, our findings have not been replicated in independent study samples.

In summary, this study demonstrated significant dose–response associations between plasma leptin level and IR measures and risk of prediabetes in this relatively lean,

middle-aged rural Chinese population. Leptin appeared to offer additional information beyond adiposity on the risk of prediabetes in this nonobese Chinese sample. Continued follow-up of these subjects will help to evaluate the utility of leptin as a useful predictor of prediabetes and future development of type 2 diabetes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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