Serum leptin level is associated with glycaemic control in newly diagnosed type 2 diabetes patients: A 1-year cohort study

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Introduction

According to guidelines for type 2 diabetes mellitus (T2DM) management issued by the Chinese Diabetes Society (CDS) and American Diabetes Association (ADA) [1], the initial regimens chosen for T2DM are mainly dependent on levels of baseline HbA_{1c}. Lifestyle interventions and metformin monotherapy are recommended as first-line therapies once HbA_{1c} levels are $\geq 7\%$, whereas combination treatment with metformin plus a second agent is recommended when HbA_{1c} is $\geq 9\%$ and insulin therapy should be considered if HbA_{1c} reaches $\geq 10\%$. If the HbA_{1c} target is not achieved after around 3 months, then second- or third-line drugs should be added. However, the initial combination therapy can increase the risk of hypoglycaemic attacks in patients who are good responders to hypoglycaemic drugs. On the other hand, the subsequent therapy will probably be of no value to patients who are poor responders to hypoglycaemic drugs. Therefore, the heterogeneity of responses to hypoglycaemic drugs among patients with diabetes should be intensively considered. Also, determining the potential characteristics that accompany responses to hypoglycaemia drugs is important to allow choosing the optimal medicine for patients in the early stages of diabetes. However, apart from baseline HbA_{1c} levels, little is known of the clinical parameters that affect glycaemic responses to hypoglycaemic drugs in routine clinical practice.

Adipocytokines (such as adiponectin, leptin and resistin), which are secreted from adipose tissue, play important roles in appetite and satiety, fat distribution, insulin secretion and sensitivity, and glucose metabolism [2]. Dysregulated secretion of these adipocytokines contribute to the pathogenesis of T2DM [3], and might serve as specific markers of responses to antidiabetic drugs. Many studies have been done on altered concentrations of adipocytokines in patients with T2DM during antidiabetic therapy [4–6]. Consequently, some adipocytokines can be considered novel biomarkers for the diagnosis and therapeutic monitoring of T2DM [6]. However, no studies are available of the associations between adipocytokines and responses to antidiabetic treatment. Accordingly, the present study followed-up patients with newly diagnosed T2DM for 1 year to assess the possible predictive effects of baseline leptin and resistin levels on glycaemic control.

Materials and Methods

Study design and participants

This was a real-world observational and longitudinal cohort study following newly diagnosed T2DM patients for 1 year. Patients who satisfied the 1999 World Health Organization (WHO) diagnostic criteria and classification standards of diabetes mellitus were screened at the diabetic outpatients clinic of Peking University People's Hospital (a

tertiary-care hospital in Beijing, China). All patients agreed to participate in the study by providing their written informed consent. Inclusion criteria were: (1) age between 20–75 years; (2) duration of diabetes < 1 year and no treatment with antidiabetic medications before screening; (3) HbA_{1c} ≥ 7.0%; and (4) body mass index (BMI) ≥ 18.5 kg/m² but ≤ 35 kg/m². Exclusion criteria included: (1) type 1 diabetes; (2) ketoacidosis; (3) renal failure; (4) hepatic failure; (5) cardiac failure; and (6) pregnancy. Patients visited the hospital every 3–6 months. A group of 55 healthy subjects who were free of diabetes according to an oral glucose tolerance test (OGTT) were recruited from the local population to serve as controls.

Therapeutic schedule

According to guidelines for the management of T2DM issued by the CDS in 2013, metformin is the first-line drug for monotherapy, although in circumstances where metformin is contraindicated or not tolerated, one of the second-line agents may be administered. Otherwise, the second-line drug is added if HbA_{1c} is still > 7% after monotherapy for 3 months, while the initial combination therapy of metformin plus a second drug is used if HbA_{1c} remains ≥ 9.0%. If patients present with significant hyperglycaemic symptoms and have overtly elevated HbA_{1c} (for example, ≥ 10%), then insulin therapy is considered initially. Once glucotoxicity has been resolved, insulin is then discontinued and the patient switched to oral

antidiabetic drugs (OADs).

The participants' initial regimens were evaluated and adjusted every 3–6 months by an endocrinologist, based on HbA_{1c} levels, tolerability and hypoglycaemic symptoms.

Physical examination and biochemical analyses

Medical histories were collected and recorded at each visit. Body weight, height, waist circumference and blood pressure were measured using standard methods. BMI was calculated as weight (kg) divided by the square of height (m²). All blood samples were obtained in a fasting state in the morning. HbA_{1c} was measured by the high-performance liquid chromatography (HPLC) method (Primus Diagnostics, Kansas City, MO, USA). Serum total cholesterol (TC), triglycerides (TG), low- and high-density lipoprotein cholesterol (LDL-C and HDL-C, respectively), uric acid (UA) and creatinine (CRE) were determined, using enzymatic methods and an automated biochemical analyzer (7170A; Hitachi, Tokyo, Japan). Leptin and resistin were measured using enzyme-linked immunosorbent assay (ELISA) kits (EMD Millipore, St. Charles, MO, USA). Insulin resistance was assessed by homoeostasis model assessment (HOMA-IR) index score, calculated as: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5.

Statistical analyses

Continuous variables were expressed as means $\pm$ SD. Values that followed non-normal distributions were log-transformed (such as Ln leptin, Ln resistin and Ln TG), and expressed as medians and interquartile ranges (IQR). Differences in continuous parameters among groups were analyzed by one-way analysis of variance (ANOVA). A chi-square test was performed to analyze categorical variables, and Pearson's correlation analysis was performed to determine the relationship between two variables that were normally distributed. Independent factors associated with the magnitude of HbA_{1c} reduction were determined by stepwise linear-regression analysis, while reductions were calculated as the HbA_{1c} reduction in 1 year divided by baseline HbA_{1c}. All statistical analyses were performed using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

Results

Participants' baseline clinical characteristics

A total of 83 newly diagnosed T2DM patients and 55 gender-matched non-diabetic control subjects were included in the baseline analysis. In the T2DM group, 50 (60.2%) were men and 33 (39.8%) were women, with a mean age of 50 ± 11 years and a mean BMI score of 26.5 ± 3.7 kg/m². The mean HbA_{1c} at baseline was $9.4\% \pm 1.9\%$, and median levels of leptin and

resistin were 2.71 ng/mL and 5.83 ng/mL, respectively. Compared with the diabetes-free controls, T2DM patients were older, and had higher levels of HbA_{1c}, fasting blood glucose (FBG), UA, insulin, HOMA-IR, systolic blood pressure (SBP), BMI scores and lipids. However, concentrations of both leptin and resistin did not differ between the controls and T2DM patients. Also, levels of leptin were significantly higher in women than in males in both healthy controls and T2DM patients (data not shown).

Follow-up data

Treatments and biochemical parameters

Of the 83 newly diagnosed T2DM patients, 65 (78.3%) returned for the 1-year visit (Fig. 1): 11 (13.3%) patients had initially received lifestyle interventions only; 42 (50.6%) patients were given one OAD; 26 (31.3%) patients were given two or more OADs; and four (4.8%) needed regimens including insulin. After 6 months to 1 year of a stable therapeutic regimen, 7.7% of patients were still being treated with lifestyle interventions only, while most patients (56.9%) were taking one OAD and 35.4% of patients needed two or more OADs. No patient continued to need insulin injections.

After the 1-year treatment, the percentage of patients with HbA_{1c} levels $\leq 7\%$ and $\leq 6.5\%$ was 75.9% and 64.2%, respectively. Also, compared with baseline, all patients had significantly lower levels of HbA_{1c}, FBG, aspartate and alanine aminotransferases (AST and ALT, respectively) and

1 TC. Mean HbA_{1c} decreased by $2.3 \pm 2.0\%$ and the magnitude of HbA_{1c}
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3 reduction was $22.8 \pm 15.4\%$. BMI, UA, LDL-C and TG did not change
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5 significantly.
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8 9 10 11 Baseline measures by HbA_{1c} reduction categories

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13 Patients were stratified into three groups based on tertiles of magnitude of
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15 HbA_{1c} reduction. Participants with the best glucose control (third tertile)
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17 tended to have higher HbA_{1c} levels and lower leptin levels at baseline.
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19 There were no differences in BMI, age, blood pressure, resistin and insulin
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21 levels, HOMA-IR, UA, CRE and lipid profile among categories of HbA_{1c}
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23 reduction. Also, the percentages of patients using any therapy did not differ
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25 between subgroups, nor were there any differences in the types of drugs
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27 used during follow-up across the three tertiles ([Table 1](#)).
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39 Correlation of baseline variables with glycaemic control

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41 Pearson's correlation analysis showed that baseline HbA_{1c} and FBG were
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43 significantly and positively correlated with the magnitude of HbA_{1c}
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45 reduction, whereas leptin was negatively correlated with HbA_{1c} reduction
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47 ($r = -0.459$, $P = 0.008$); there was also no correlation with age, BMI, blood
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49 pressure, UA, CRE, HOMA-IR or lipid profiles.
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59 *Possible predictors at baseline of glucose control*

1 The effect of baseline variables on glucose control was estimated by
2 multiple linear stepwise regression analyses using the magnitude of HbA_{1c}
3 reduction as a dependent variable, and age, gender, BMI, HOMA-IR, SBP,
4 diastolic blood pressure (DBP), TC, TG, HDL-C, LDL-C, CRE, UA, leptin
5 and resistin as independent variables. In the multivariate model, leptin was
6 independently and inversely associated with the magnitude of HbA_{1c}
7 reduction ($\beta = -0.543$, $t = -3.418$, $P = 0.002$) after adjusting for gender and
8 changes in body weight during treatment.
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25 Discussion

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27 Our present real-world longitudinal study presents new evidence that
28 serum leptin is an independent factor in the magnitude of HbA_{1c} reduction
29 in T2DM after 1 year of standard antidiabetic therapy, thereby suggesting
30 that leptin may serve as a predictor of glucose control. To the best of our
31 knowledge, this is the first clinical cohort study to explore the predictive
32 power of adipocytokines for glycaemic control.
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45 In fact, mounting evidence has suggested that leptin may be critical in
46 the management of diabetes and glycaemic control [2, 7, 8]. Previous
47 studies reported that several antidiabetic therapies had different effects on
48 serum leptin concentrations in patients with T2DM [9, 10]. However, there
49 has been little information available on the association of baseline serum
50 leptin levels and responses to antidiabetic therapies in T2DM patients.
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1 The present study has demonstrated that leptin levels were higher in
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3 poorly controlled patients compared with well-controlled patients, and the
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5 difference was statistically significant ($P = 0.016$). In addition, linear
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8 stepwise regression analysis showed that baseline leptin levels are an
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10 independent predictive factor for the magnitude of HbA_{1c} reduction. Also,
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12 the inverse association between leptin level and HbA_{1c} reduction was
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14 independent of the type of therapeutic regimen and OAD, as the regimens
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16 and drugs used during the stabilization period did not differ between poorly
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18 controlled and well-controlled patients. This suggests that, for patients
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20 with low levels of leptin and despite baseline HbA_{1c} levels $\geq 9\%$,
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22 monotherapy may still achieve a good response. In contrast, for patients
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24 with high levels of leptin at baseline, a more intensive regimen should be
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26 considered. Nevertheless, a better understanding of the mechanisms
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28 underlying the association between leptin and glucose metabolism will
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30 improve the management of T2DM.
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42 However, several limitations need to be considered when interpreting
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44 our present results. First, the inherent nature of real-world observations
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46 can lead to a mismatch in the hypoglycaemic regimens used in clinical
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48 practice and the recommendations of guidelines. Second, the small
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50 sample size of our study diminishes its statistical power. Third, the short
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52 follow-up period weakens the effects of leptin on glucose control in
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54 T2DM patients. Thus, a longer-term follow-up study with a larger sample
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size is needed to validate these preliminary results, and to better describe the effects of leptin on both glucose control and chronic complications.

In conclusion, low circulating leptin levels may be used as predictive factors for glucose control in T2DM patients.

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Figure legend

Fig. 1. Clinical characteristics and treatment regimens of our newly diagnosed type 2 diabetes patients at baseline and at follow-up.

Figure 1

Table 1

Baseline characteristics of participants according to HbA_{1c} reduction categories

Variables	Magnitude of HbA _{1c} reduction			<i>P</i>
	First tertile	Second tertile	Third tertile	
Age (years)	53 ± 9	55 ± 7	54 ± 11	0.889
FBG (mmol/L)	9.65 ± 3.40	9.33 ± 2.07	11.07 ± 4.44	0.474
BMI (kg/m ²)	26.5 ± 3.0	24.6 ± 2.8	25.4 ± 2.2	0.316
SBP (mmHg)	122 ± 9	125 ± 12	128 ± 20	0.604
DBP (mmHg)	74 ± 10	78 ± 7	78 ± 8	0.426
UA (μmol/L)	305 ± 55	293 ± 46	265 ± 60	0.275
CRE (μmol/L)	61 ± 15	68 ± 16	66 ± 15	0.620
TC (mmol/L)	5.44 ± 1.40	5.44 ± 1.50	4.64 ± 1.04	0.343
TG (mmol/L)	5.86 (4.43, 6.67)	5.09 (4.62, 5.49)	5.00 (3.84, 5.37)	0.358
HDL (mmol/L)	1.26 ± 0.36	1.19 ± 0.25	1.05 ± 0.22	0.257
LDL (mmol/L)	3.04 ± 1.31	3.61 ± 1.19	2.88 ± 1.08	0.386
Insulin (μIU/mL)	13.36 ± 5.73	8.64 ± 2.76	11.81 ± 7.14	0.140
HOMA-IR	5.33 ± 2.94	3.48 ± 1.13	6.18 ± 4.15	0.119
Leptin (ng/mL)	3.30 (1.98, 12.29)	1.37 (0.98, 3.93)	1.49 (1.03, 2.13)	0.016
Resistin (ng/mL)	3.70 (2.73, 7.44)	5.76 (2.04, 8.50)	5.49 (4.83, 8.78)	0.553
HbA _{1c} (%)	7.6 ± 1.0	8.8 ± 1.8	10.0 ± 1.5	0.007
Treatment regimens:				
Lifestyle intervention	4 (6.2%)	1 (1.5%)	2 (3.1%)	0.342
1 OAD	14 (21.5%)	14 (21.5%)	12 (18.5%)	0.981
≥ 2 OADs	4 (6.2%)	8 (12.4%)	6 (9.2%)	0.630
Drugs used:				
Metformin	18 (27.7%)	22 (33.8%)	18 (27.7%)	0.342
Secretagogues	4 (6.2%)	8 (12.4%)	6 (9.2%)	0.630
Acarbose	2 (3.1%)	2 (3.1%)	4 (6.2%)	0.688
Thiazolidinedione	0 (0.0%)	0 (0.0%)	2 (3.1%)	0.321

Data are means ± SD or medians (interquartile range);

FBG: fasting blood glucose; BMI: body mass index; SBP/DBP: systolic/diastolic blood pressure;

UA: uric acid; CRE: creatinine; TC: total cholesterol; TG: triglyceride; HDL/LDL: high-density/low-density lipoprotein cholesterol; HOMA-IR: homoeostasis model assessment of insulin resistance; OAD: oral antidiabetic drug