Role of a Novel Insulin Resistance Index based on Combined Levels of C-peptide and Insulin in Assessment of the Heterogeneity of Diabetes Mellitus

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Role of a novel insulin resistance index based on combined levels of C-peptide and insulin in assessment of the heterogeneity of diabetes mellitus

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ABSTRACT

Background

The C-peptide-to-insulin ratio (C/I) is associated with hepatic insulin clearance and insulin resistance. We established a novel C-peptide-to-insulin ratio index (CPIRI) and explored its application in the heterogeneity of non-autoimmune diabetes.

Methods

A total of 865 adults diagnosed with new-onset diabetes mellitus (DM) within 1 year and 54 healthy controls (HC) were recruited. Our CPIRI model was established with fasting C/I as the independent variable and homeostasis model assessment of insulin resistance (HOMA-IR) as the dependent variable. Patients with negative-autoantibodies against pancreatic islets were subclassified according to CPIRI, age of onset, hemoglobin A1c (HbA1c), body mass index (BMI) and homeostatic model assessment of β-cell function (HOMA-β) via cluster analysis.

Results

Fasting C/I and HOMA-IR in both diabetic and healthy subjects were hyperbolically correlated, and log(C/I) and log(HOMA-IR) were linearly and negatively correlated; the correlation coefficient was −0.83 (P = 0.000) and −0.76 (P = 0.000), respectively. Finally, we obtained the equations $CPIRI(DM) = 670/(C/I)^{2.24} + 0.25$ and $CPIRI(HC) = 670/(C/I)^{2.24} - 1$ (F = 1904.39, P = 0.000). Pancreatic islet autoantibody-negative patients were further subclassified into three subtypes; subtype 1 (13.8%) had severe insulin resistance, subtype 2 (42.1%) suffered elderly-onset DM

with slight symptoms, and subtype 3 (44.1%) suffered severe dysfunction of pancreatic islets. Each subtype exhibited different clinical characteristics and complications.

Conclusions

CPIRI can be used to effectively evaluate insulin resistance. Adult-onset non-autoimmune diabetes can be subclassified into 3 subtypes according to CPIRI along with other symptom-based characteristics of diabetic progression.

Key Words: insulin resistance, CPIRI, HOMA-IR, diabetes heterogeneity

BACKGROUND

Diabetes mellitus (DM) is increasingly being recognized as much more heterogeneous a condition which encompasses heterogeneity in phenotype, islet β cell function, and insulin resistance [1]. The present classification of DM does not fully reflect the pathogenesis of this disease, and results in the failure of patients to receive individualized therapy [2]. Effective classification can help detail the clinical characteristics of different subtypes of DM, identify a high risk of complications, and enable individualized treatment regimens.

Several methods, including direct, indirect and surrogate indicators, are employed to estimate insulin resistance. The vast majority of indicators of insulin resistance assess the relationship between glucose and insulin. Among them, the homeostatic model assessment for insulin resistance (HOMA-IR) remains the most widely used in epidemiological studies. First proposed by Matthews *et al.* in 1985 with a standard

value of 1.0, HOMA-IR is based on analyses of feedback regulation between the liver and pancreatic-islet tissue in homeostasis [3, 4], in turn reflecting the balance between insulin secretion and hepatic glycogen output.

As C-peptide is co-secreted in equimolar amounts with insulin, and it is negligibly absorbed by hepatic tissue and is cleared at a relatively constant rate in peripheral tissues; it possesses a longer half-life than that of insulin [5, 6]. As such, the C-peptide-to-insulin ratio (C/I) reflects hepatic insulin clearance [7] and hepatic insulin clearance is closely related to insulin resistance in particular [8, 9].

Here, we incorporated fasting C/I into a novel C-peptide-to-insulin ratio index (CPIRI) based on HOMA-IR to determine the level of insulin resistance and propose a new classification system of DM with application of data-driven cluster analysis containing CPIRI with other key clinicopathological indicators of DM to provide a foundation for appropriate medical treatment of DM patients.

METHODS

Study population

A total of 865 adult patients diagnosed with new-onset DM in The First Affiliated Hospital with Nanjing Medical University were recruited to our study. All study participants were diagnosed with DM according to World Health Organization (WHO) 1999 criteria within 1 year of recruitment. Exclusion criteria were a history of pancreatic exocrine disease, gestational DM, secondary DM, severe systemic disease, severe heart failure, critical liver/kidney dysfunction, hematologic disease or mental

illness. In addition, we enrolled 54 healthy controls (HC).

Measurement of general indicators

General information on patients (including complete medical history) was obtained using questionnaires developed for this research (Supplementary File 1). Anthropometric details were collected by experienced researchers. After overnight fasting, blood samples were collected and metabolic profiles detected *via* colorimetry with an automatic biochemical analyzer (AU5800; Beckman, Coulter, Fullerton, CA, USA). Levels of insulin and C-peptide were detected using an automatic electrochemical luminescence immunoassay (Roche, Basel, Switzerland). The concentration of hemoglobin A1c (HbA_{1c}) was measured using a Variant II system (Bio-Rad Laboratories, Hercules, CA, USA). Ultrasound imaging of the cervical, abdominal and lower-extremity arteries was done using an ultrasound machine (EPIC7 L12-3; Philips, Amsterdam, the Netherlands).

Dynamic nutrient load test

All HC and DM patients requiring a diagnosis underwent an oral glucose tolerance test (OGTT) with 75g of glucose. Patients who had already been diagnosed with DM within the last year underwent a mixed meal tolerance test (MMTT) with a piece of bread made of 100 g of wheat flour. In short, after an overnight fast, venous blood was sampled before and after a 75g glucose or 100g bread load was ingested. Blood samples were collected at 0, 60 and 120 min to determine glucose, insulin and C-peptide concentretions.

106 **Diabetes autoantibody assay**

107 A radioligand binding assay, as described previously [10, 11], was utilized to detect
108 decarboxylase antibody (GADA), zinc transporter 8 autoantibody (ZnT8A) and protein
109 phosphatase-like IA-2 antibodies (IA2A). In brief, human ^{35}S-labeled recombinant
110 antigens were produced in an *in vitro*-coupled transcription and translation system with
111 T7 (ZnT8A) or SP6 (GADA and IA2A) RNA polymerases and nuclease-treated rabbit
112 reticulocyte lysate (Promega, Fitchburg, WI, USA). Sera (5 μL) were incubated with
113 ^{35}S-antigens (≥20000 of TCA-precipitable radioactivity). After overnight incubation at
114 4°C, antibody-bound ^{35}S-antigens were separated from unbound antigens by
115 precipitation with protein A sepharose (Amersham Biosciences, Amersham, UK).
116 Immunoprecipitated radioactivity was quantified using a Wallac Microbeta Liquid
117 Scintillation Counter (Perkin Elmer Life and Analytical Sciences, Boston, MA, USA).
118 The cutoff for GADA-, ZnT8A- and IA2A-positivity was 0.042, 0.054 and 0.018,
119 respectively. The sensitivity and specificity of GADA, ZnT8A and IA2A was 60% and
120 100%, 62%, and 100%, and 70% and 100%, respectively.

121 **Cluster analysis**

122 Target DM patients who were negative for islet autoantibodies, namely adult-onset
123 non-autoimmune DM, were further assessed *via* two-step cluster analysis incorporating
124 CPIRI with body mass index (BMI), age at onset of DM, HbA_{1c} level, and the
125 homeostatic model assessment for β cell function (HOMA-β) as cluster variables.
126 Figure 1 shows the workflow of our study.

Statistical analysis

Statistical analysis was undertaken using SPSS 23.0 (IBM, Armonk, NY, USA). Quantitative data are expressed as the mean ± SD; categorical variables are expressed as frequencies or percentages. Mean values between two groups were compared using Levene's test of homogeneity of variances. The Student's *t*-test was employed in the condition of homogeneity whereas the t' test was adopted if the variances were not homogeneous. If Levene's test revealed the homogeneity of variance, one-way analysis was undertaken for the comparison of mean values among multiple groups, followed by pairwise comparison using the least-significant-difference method. If Levene's test revealed the heterogeneity of variance, the Welch test was adopted and the Dunnett T3 test was used for pairwise comparison. The comparison of classification variables between groups was evaluated using the Pearson χ^2 test. General linear regression was applied to formulate the CPIRI model. $P < 0.05$ was considered significant.

RESULTS

The average age of newly diagnosed DM patients was 48 years (Table 1). Male patients accounted for a larger proportion of this population compared with HC (57.8% *vs* 50%, P = 0.000). Diabetic patients suffered more serious metabolic disorders in the setting of increased levels of total cholesterol, triglycerides, low-density lipoprotein, apolipoprotein A, BMI, waist circumference, and aminotransferase, as well as in the setting of decreased levels of high-density lipoprotein and albumin. All aforementioned differences were statistically significant ($P < 0.05$).

Both in DM cases and HC, fasting C/I and HOMA-IR were hyperbolically dependent (Figure 2), whereas log(C/I) was in linear negative correlation with log(HOMA-IR); the correlation coefficient was −0.83 (P = 0.000) in DM cases and −0.76 (P = 0.000) in HC, respectively.

On the basis of the linearly negative relationship between log(C/I) and log(HOMA-IR), we aimed to establish an index to assess insulin resistance while considering log(C/I) as the independent variable, and log(HOMA-IR) as the dependent variable. After excluding patients with incomplete data and those found to have extreme (1%) outlying data, we constructed a univariable, general linear model in SPSS and obtained the following equation after analysis of 740 DM patients: log(CPIRI) = 6.51 − 2.24 × log(C/I) (F = 1904.39, P = 0.000). After logarithm conversion, the original CPIRI = $671.8/(C/I)^{2.24}$ model was obtained. However, when the original values for CPIRI and HOMA-IR in DM patients were paired for analyses using the Student's *t*-test, the difference in values among the two indices was 0.239 and significant (t = 4.17, P = 0.000). Therefore, correction of the constant and coefficient simplification in the original equation yielded a corrected equation: CPIRI(DM) = $670/(C/I)^{2.24}$ + 0.25. Results for the paired Student's *t*-test for CPIRI and HOMA-IR revealed no significant difference (t = 0.20, P = 0.84). Furthermore, these two indicators exhibited good correlation among data analyses of DM patients (Pearson correlation coefficient = 0.77, P = 0.000).

HC data was also analyzed by the CPIRI(DM) model to verify application of this

formula in a normal glucose tolerance population. However, the paired *t*-test revealed a significant difference between values of CPIRI(DM) and HOMA-IR in HC (t = −11.23, P = 0.000). Thus, CPIRI(DM) was not be suitable for application in healthy population. Consequently, the model applicable for a healthy population was constructed as CPIRI(HC)= $670/(C/I)^{2.24} - 1$. The paired *t*-test revealed no significant difference between values of CPIRI(HC) and HOMA-IR in HC (t = −1.22, P = 0.229), and a strong relationship was demonstrated among the two models (Pearson correlation coefficient = 0.80, P = 0.000).

Finally, values of CPIRI and HOMA-IR were compared in HC and DM cases, respectively. Both indices were significantly higher in DM patients as compared with HC (2.50±1.80 *vs*. 1.50±1.44; 2.50±2.40 *vs*. 1.42±0.88, P < 0.05; Figure 3). In general, the novel CPIRI model could be used to distinguish the severity of insulin resistance among HC and DM patients with accuracy identical to that observed with HOMA-IR. In an identical population, no significant difference among the two indicators was noted whereas the correlation between them was high. A CPIRI model for evaluating DM cases and HC was established.

We further verified whether CPIRI could distinguish different degrees of insulin resistance. DM cases were subgrouped according to BMI cutoff points of 24 kg/m^2 and 28 kg/m^2, respectively. We found that CPIRI and HOMA-IR values increased synchronously with elevated BMI, (P < 0.05; Supplementary Figure 1A). Furthermore, CPIRI and HOMA-IR values were higher in DM patients with a history of non-

190 alcoholic fatty liver disease (NAFLD) than in those without NAFLD ($P < 0.05$,
191 Supplementary Figure 1B).

192 A total of 714 DM patients underwent detection of pancreatic-islet autoantibodies
193 ultimately; 105 (14.7%) of those DM patients were found to be positive for at least one
194 type of autoantibody and, as such, suffering adult-onset autoimmune DM. The rate of
195 positivity for GADA, ZnT8A and IA2A was 10.1%, 3.6% and 5.9%, respectively. The
196 rest 609 DM patients were islet-autoantibody-negative. To classify non-autoimmune
197 diabetic patients into novel subtypes, we evaluated them utilizing CPIRI along with
198 variables including HOMA-β, age, HbA_{1c} level and BMI. After eliminating the missing
199 data, a two-step clustering methodology was applied to evaluate patients negative for
200 autoantibodies. Ultimately, we noted 3 novel subtypes of adult-onset non-autoimmune
201 DM (Figure 4A-F): subtype 1 (apparent in 79 patients (13.81%)) was characterized by
202 severe insulin resistance; subtype 2 (apparent in 241 patients (42.13%)) was defined as
203 elderly-onset DM with mild symptoms; subtype 3 (apparent in 252 patients (44.06%))
204 was defined as severe insulin-deficient DM.

205 We further compared metabolic indices, lifestyles, and associated complications
206 among the 3 aforementioned groups (Table 2). Subtype 1 patients had higher levels of
207 aminotransferase, uric acid, free triiodothyronine, as well as diastolic blood pressure,
208 as compared with those in patients in the other two subtypes ($P < 0.05$). Subtype 2
209 patients had lower levels of lymphocytes, erythrocytes, hemoglobin, platelets, and
210 glomerular filtration rate, and higher serum levels of urea nitrogen, when compared

with those of patients of other subtypes ($P < 0.05$). Apart from the changes noted in hemocytes, subtype 2 patients had an increased prevalence of peripheral vascular plaques. Subtype 3 patients encompassed a larger proportion of males, and a higher prevalence of ketosis-onset DM compared with those in patients of subtypes 1 and 2; all differences were significant ($P < 0.05$).

However, it is notable that non-autoimmune adult-onset diabetic patients can only be divided into 2 subtypes via two-step cluster analysis when HOMA-IR was employed instead of CPIRI in the cluster analysis (Supplementary Table 1), indicating CPIRI was superior to HOMA-IR in distinguishing the heterogeneity of non-autoimmune diabetes.

DISCUSSION

Insulin inhibits hepatic glucose production (HGP) *via* acute changes in metabolic pathways and long-term gene expression. Physiological studies have demonstrated that insulin inhibits HGP *via* inhibition of glycogen decomposition, suggesting that the direct effects of insulin on the liver are critical for appropriate regulation of HGP and intrahepatic glucose homeostasis. Insulin resistance develops in stages [12], in younger Zucker rats, decreased insulin sensitivity occurs first in skeletal muscle and later in fatty and hepatic tissues. The physiological mechanism of tissue-specific insulin resistance involves insufficient insulin action on target tissues. Although the liver serves a gate-keeping role in regulating the systemic insulin response to a glucose challenge, the importance of the role of insulin clearance and hepatic versus peripheral insulin resistance have received less attention[13].

Most models of insulin resistance focused on insulin and plasma glucose levels. Several studies have reported differences in metabolic kinetics among insulin and C-peptide: (i) insulin and C-peptide are secreted in equimolar quantities into the portal vein; (ii) the clearance of C-peptide from the circulation is slower than that of insulin; (iii) a large portion of insulin is cleared by hepatic metabolism, whereas the remainder enters the periphery and possesses a half-life of only 2–3 min. The liver is, therefore, the primary organ responsible for insulin clearance, whereas C-peptide is cleared mainly through the kidney. The half-life of C-peptide is about 30 min [5, 6, 14]. By virtue of the physiological relationship between C-peptide and insulin metabolism, the peripheral molar C/I ratio will reflect the changes in hepatic metabolic clearance rates of insulin, and it performs best in steady-state conditions in the consideration of the differences in the half-lives of C-peptide and insulin when their secretory responses to a stimulus [7, 15].

The relationship between impaired insulin clearance and type 2 diabetes was initially reported in 1949 [16]. Since then, several researches have identified defective insulin clearance as a critical hallmark in the pathogenesis of hyperinsulinemia [17]. The level of target-cell insulin resistance has been found to correlate with insulin clearance regardless of whether the concentration of insulin receptors is decreased or not [18]. That impaired insulin clearance causes secondary insulin resistance is supported by researches on genetically modified mouse models targeting the Ceacam1 gene globally or in a liver-specific manner [19]. Generally speaking, hepatic insulin

clearance could precede and therefore contribute to the hyperinsulinemia of obesity, suggesting a mutual interaction between insulin resistance and insulin clearance of the liver [20]. Notably, the variation of hepatic glucoregulation and insulin clearance occurred independently of changes in peripheral insulin resistance as measured by the hyperinsulinemic-euglycemic clamp [21]. Essentially, HOMA-IR reflects the dynamic balance between insulin secretion and hepatic glycogen output, which is closely related to hepatic insulin resistance. Taking this phenomenon into consideration, we believed that CPIRI may throw new light on the evaluating of insulin resistance with C/I as its independent variable and HOMA-IR as the dependent variable.

Detection of C-peptide involves use of two monoclonal antibodies which recognize human C-peptide specifically and exhibit cross-reactivity with the C-chain or the cleavage product of proinsulin. The concentration of proinsulin and the cleavage product is 100-fold lower than that of C-peptide. Consequently, cross-reactivity does not significantly influence C-peptide detection [5, 22]. Studies have reported that lispro, aspart or glargine synthetic insulin in the absence of insulin and in the setting of insulin concentrations of 30, 100, 300 and 1000 mIU/L yielded results inferior to the lower limit at all concentrations (<0.20 μU/mL or <1.39 pmol/L) [23]. Patients who received intensive insulin therapy were treated with lispro or aspart through continuous subcutaneous insulin infusion, and the insulin pump was closed before the tolerance test. Thus, the CPIRI model was scarcely affected by use of exogenous insulin in our study.

Importantly, CPIRI findings may be higher than actual levels when applied to patients with insulin autoantibody-positivity or those undergoing therapy using exogenous insulin derived from animals or humans. This, in turn, may account for the fact that the constant of CPIRI(DM) was higher than that of CPIRI(HC).

Evidence suggests that target tissues possess a type of “metabolic memory” [24], indicating that appropriate, early therapy is crucial for prevention of chronic complications. Reports of heterogeneous clustering analysis among various ethnic groups in terms of DM are limited. After comparison of cluster results among European and Chinese populations [2], we were surprised to find that the consensus on subgrouping adult-onset non-autoimmune diagnosed DM resulted in classification into three subtypes: severe insulin-resistant, severe β cell-dysfunctional and age-related DM. Another obesity-related subtype was demonstrated in the European DM population in which BMI ranges were not positively correlated with insulin resistance. In Chinese patients, however, BMI ranges were positively correlated with insulin resistance. Whether this difference is due to racial variation, the number of patients studied, or the diversity of cluster variables requires further investment.

An increased circulating level of gamma-glutamyl transpeptidase is an important risk factor for the development of T2DM, and increased transaminase levels are closely related to insulin resistance [25]. Patients of subtype 1 had higher aminotransferase levels as compared with those of other subtypes, indicating that hepatocyte injury in subtype-1 patients was more serious than that in other groups. Subtype-1 patients also

had the highest triglyceride:high-density lipoprotein ratio (TG/HDL) and uric-acid level. The TG/HDL ratio was first proposed by McLaughlin and colleagues to evaluate insulin resistance [26]. Hyperuricemia interferes with glucose homeostasis and insulin sensitivity by activating the inflammatory response, which results in oxidative stress, endothelial dysfunction and inhibition of insulin pathways, resulting in the chronic complications of DM. Patients of subtype 2 exhibited a higher prevalence of peripheral vascular plaques, increased blood urea nitrogen levels and decreased glomerular filtration rate. Arambewela et al. reported that age is an important risk factor for macro- and microvascular complications [27]. Therefore, the necessity to screen potential vascular complications should be reinforced especially in elderly-onset DM patients, even if their plasma glucose level is well controlled, suggesting that glucose-lowering therapy is not the optimum method for preventing this complication.

Our novel clustering of patients suffering adult-onset DM is superior to the traditional classification of DM because it provides information about underlying disease mechanisms and defines patients at high risk of DM complications even at the diagnosis, thereby guiding more appropriate therapy. It is worth mentioning that CPIRI was superior to HOMA-IR in distinguishing the heterogeneity of adult-onset non-autoimmune diabetes.

Conclusions

Our analysis suggests that CPIRI can evaluate insulin resistance effectively and provide a better discrimination of heterogeneity of diabetes than HOMA-IR. Likewise,

the combined information from a few variables central to DM development precedes the assessment of glucose as the sole variable. Our study provides a significant step towards application of "precision" medicine in DM treatment.

Abbreviations

C/I: C-peptide-to-insulin ratio; CPIRI: C-peptide-to-insulin ratio index; DM: diabetes mellitus; HOMA-IR: homeostatic model assessment of insulin resistance; HbA1c: hemoglobin A1c; BMI: body mass index; HOMA-β: homeostatic model assessment of β-cell function; HC: healthy controls; HGP: hepatic glucose production; HbA1c: glycated hemoglobin; OGTT: oral glucose tolerance test; MMTT: mixed meal tolerance test; GADA: decarboxylase antibody; ZnT8A: zinc transporter 8 autoantibody; IA2A: phosphatase-like IA-2 antibodies; NAFLD: non-alcoholic fatty liver disease; TG/HDL: triglyceride to high-density lipoprotein ratio; WHO: World Health Organization.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' Contributions

HD and TY convinced and carried out the experiments. YS collected the data. MS and MZ analyzed data. HC and YG carried out experiments. QF generated figures. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of the First Affiliated Hospital with Nanjing Medical University (2015-SR-071) in Nanjing, China. All participants provided written informed consent.

Consent for publication

Not applicable.

References

1. Sladek R. The many faces of diabetes: addressing heterogeneity of a complex disease. Lancet Diabetes Endocrinol. 2018; 6(5): 348-9.

2. Ahlqvist E, Storm P, Käräjämäki A, Martinell M, Dorkhan M, Carlsson A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol. 2018; 6(5): 361-9.

3. Matthews D R, Hosker J P, Rudenski A S, Naylor B A, Treacher D F, Turner R C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7): 412-9.

4. Wallace T M, Levy J C, Matthews D R. Use and abuse of HOMA modeling. Diabetes Care. 2004; 27(6): 1487-95.

5. Wahren J, Kallas A, Sima A A. The clinical potential of C-peptide replacement in type 1 diabetes. Diabetes. 2012; 61(4): 761-72.

6. Faber O K, Kehlet H, Madsbad S, Binder C. Kinetics of human C-peptide in man. Diabetes. 1978; 27 Suppl 1: 207-9.

7. Henry R R, Brechtel G, Griver K. Secretion and hepatic extraction of insulin after weight loss in obese noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1988; 66(5): 979-86.

8. Jimenez J, Zuniga-Guajardo S, Zinman B, Angel A. Effects of weight loss in massive obesity on insulin and C-peptide dynamics: sequential changes in insulin production, clearance, and sensitivity. J Clin Endocrinol Metab. 1987; 64(4): 661-8.

9. Meistas M T, Margolis S, Kowarski A A. Hyperinsulinemia of obesity is due to decreased clearance of insulin. Am J Physiol. 1983. 245(2): E155-9.

10. Petersen J S, Hejnaes K R, Moody A, Karlsen A E, Marshall M O, Høier-Madsen M, et al. Detection of GAD65 antibodies in diabetes and other autoimmune diseases using a simple radioligand assay. Diabetes. 1994; 43(3): 459-67.

11. Wenzlau J M, Juhl K, Yu L, Moua O, Sarkar S A, Gottlieb P, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci U S A. 2007; 104(43): 17040-5.

12. Clark J B, Clark C M Jr. Age-related changes in insulin receptor regulation in liver membranes from Zucker fatty rats. Endocrinology. 1982; 111(3): 964-9.

13. Kim S H, Reaven G M. Insulin clearance: an underappreciated modulator of plasma insulin concentration. J Investig Med. 2016; 64(7): 1162-5.

14. Yosten G L, Maric-Bilkan C, Luppi P, Wahren J. Physiological effects and therapeutic potential of proinsulin C-peptide. Am J Physiol Endocrinol Metab. 2014; 307(11): E955-68.

15. Polonsky K S, Rubenstein A H. C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. Diabetes. 1984; 33(5): 486-94.

16. Broh-Kahn R H, Mirsky I A. The inactivation of insulin by tissue extracts; the effect of fasting on the insulinase content of rat liver. Arch Biochem. 1949; 20(1): 10-4.

17. Pivovarova O, Bernigau W, Bobbert T, Isken F, Möhlig M, Spranger J, et al.

Hepatic insulin clearance is closely related to metabolic syndrome components. Diabetes Care. 2013; 36(11): 3779-85.

18. Flier J S, Minaker K L, Landsberg L, Young J B, Pallotta J, Rowe J W, et al. Impaired in vivo insulin clearance in patients with severe target-cell resistance to insulin. Diabetes. 1982; 31(2): 132-5.

19. Najjar S M, Perdomo G. Hepatic Insulin Clearance: Mechanism and Physiology. Physiology (Bethesda). 2019. 34(3): 198-215.

20. Hilton C W, Mizuma H, Svec F, Prasad C, et al. Relationship between plasma cyclo (His-Pro), a neuropeptide common to processed protein-rich food, and C-peptide/insulin molar ratio in obese women. Nutr Neurosci. 2001; 4(6): 469-74.

21. Lundsgaard A M, Sjøberg K A, Høeg L D, Jeppesen J, Jordy A B, Serup A K, et al. Opposite Regulation of Insulin Sensitivity by Dietary Lipid Versus Carbohydrate Excess. Diabetes. 2017; 66(10): 2583-95.

22. Zilkens T M, Eberle A M, Schmidt-Gayk H. Immunoluminometric assay (ILMA) for intact human proinsulin and its conversion intermediates. Clin Chim Acta. 1996; 247(1-2): 23-37.

23. Owen W E, Roberts W L. Cross-reactivity of three recombinant insulin analogs with five commercial insulin immunoassays. Clin Chem. 2004; 50(1): 257-9.

24. Reddy M A, Zhang E, Natarajan R., Epigenetic mechanisms in diabetic complications and metabolic memory. Diabetologia. 2015; 58(3): 443-55.

25. Simental-Mendía L E, Rodríguez-Morán M, Gómez-Díaz R, Wacher N H,

421 Rodríguez-Hernández H, Guerrero-Romero F. Insulin resistance is associated with
422 elevated transaminases and low aspartate aminotransferase/alanine aminotransferase
423 ratio in young adults with normal weight. Eur J Gastroenterol Hepatol. 2017; 29(4):
424 435-40.
425 26. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of
426 metabolic markers to identify overweight individuals who are insulin resistant. Ann
427 Intern Med. 2003; 139(10): 802-9.
428 27. Arambewela M H, Somasundaram N P, Jayasekara H B, Kumbukage M P, Jayasena
429 P M, Chandrasekara C M, et al. Prevalence of Chronic Complications, Their Risk
430 Factors, and the Cardiovascular Risk Factors among Patients with Type 2 Diabetes
431 Attending the Diabetic Clinic at a Tertiary Care Hospital in Sri Lanka. J Diabetes Res.
432 2018; doi: 10.1155/2018/4504287.
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Figure 1. The workflow of our research design. Abbreviations: GADA, decarboxylase antibody; ZnT8A, zinc transporter 8 autoantibody; IA2A, protein phosphatase-like IA-2; CPIRI, C-peptide to insulin ratio index; HOMA-β, homeostasis model assessment of beta cell; BMI, body mass index; HbA1c, hemoglobin A1C.

Figure 2. The relationship between C/I and HOMA-IR in diabetic patients and healthy controls. C/I and HOMA-IR are hyperbolically related in diabetics (A) and healthy controls (B). Log(C/I) and log(HOMA-IR) are negatively linearly correlated in diabetic patients (C) and healthy controls (D). Abbreviations: C/I, the ratio of C-peptide to insulin; HOMA-IR, homeostasis model assessment of insulin resistance.

Figure 3. Insulin resistance among DM and HC. Both CPIRI and HOMA-IR are higher in DM than HC ($P<0.05$). Abbreviations: DM, diabetes mellitus; HC, healthy controls; HOMA-IR, homeostasis model assessment of insulin resistance; CPIRI, C-peptide to insulin ratio index.

Figure 4. Heterogeneity of adult-onset non-autoimmune diabetes. Adult-onset non-autoimmune diabetes can be divided into three distinct subtypes according to results of cluster analysis: subtype 1 (n = 79, 13.81%), subtype 2 (n = 241, 42.13%), and subtype 3 (n = 252, 44.06%) (A). Distribution of age (B), HbA1c (C), BMI (D), CPIRI (E) and HOMA-β (F) for subtype1-3 reveals that subtype 1 is characterized by severe insulin resistance; subtype 2 is defined as elderly-onset DM with mild symptoms, and subtype 3 demonstrates severe insulin-deficient DM. Abbreviations: HbA1c, hemoglobin A1C; BMI, body mass index; CPIRI, C-peptide to insulin ratio index; HOMA-β, homeostasis model assessment of beta cell.

Figures

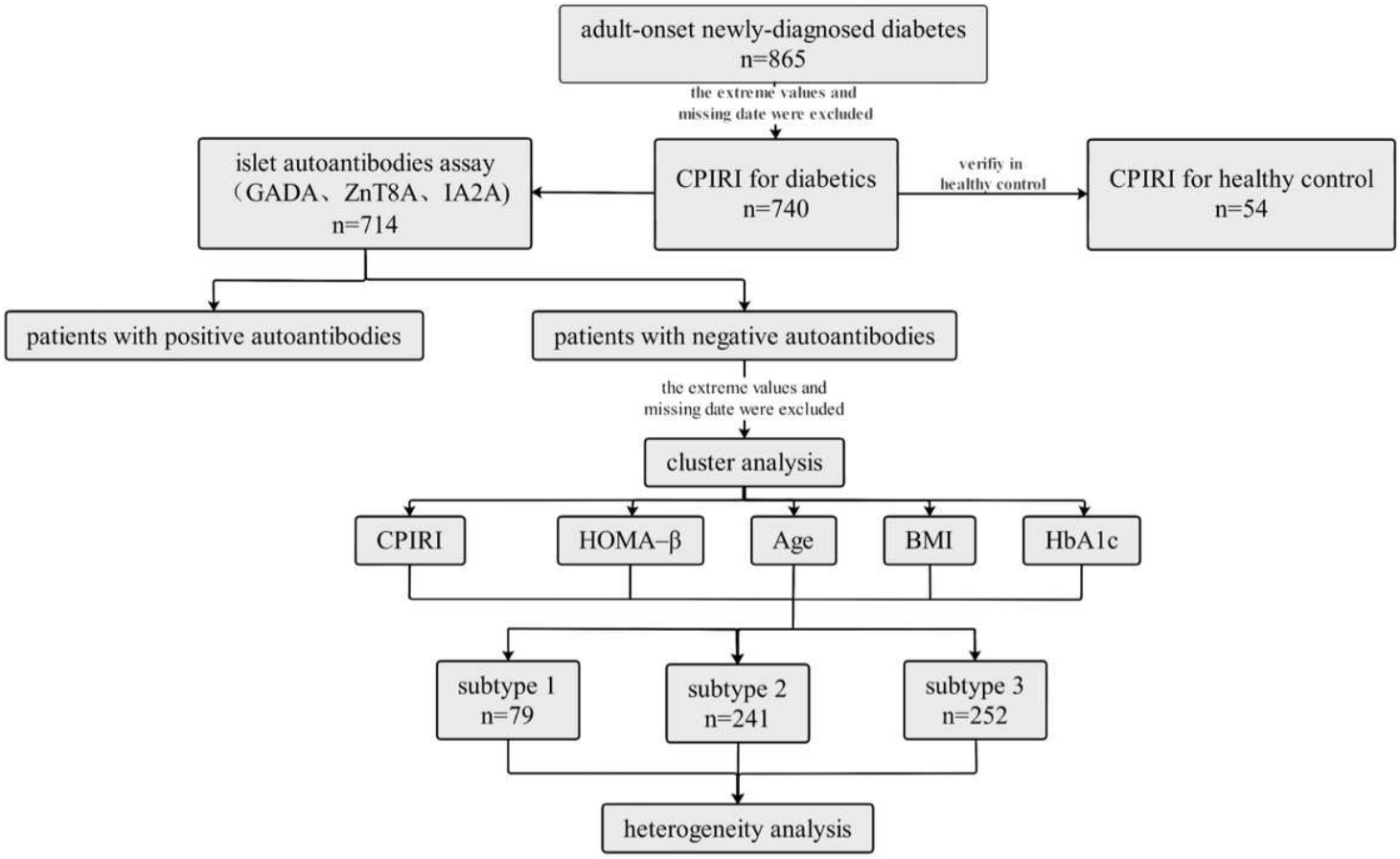

Figure 1

The workflow of our research design. Abbreviations: GADA, decarboxylase antibody; ZnT8A, zinc transporter 8 autoantibody; IA2A, protein phosphatase-like IA- 2; CPIRI, C-peptide to insulin ratio index; HOMA-β, homeostasis model assessment of beta cell; BMI, body mass index; HbA1c, hemoglobin A1C.

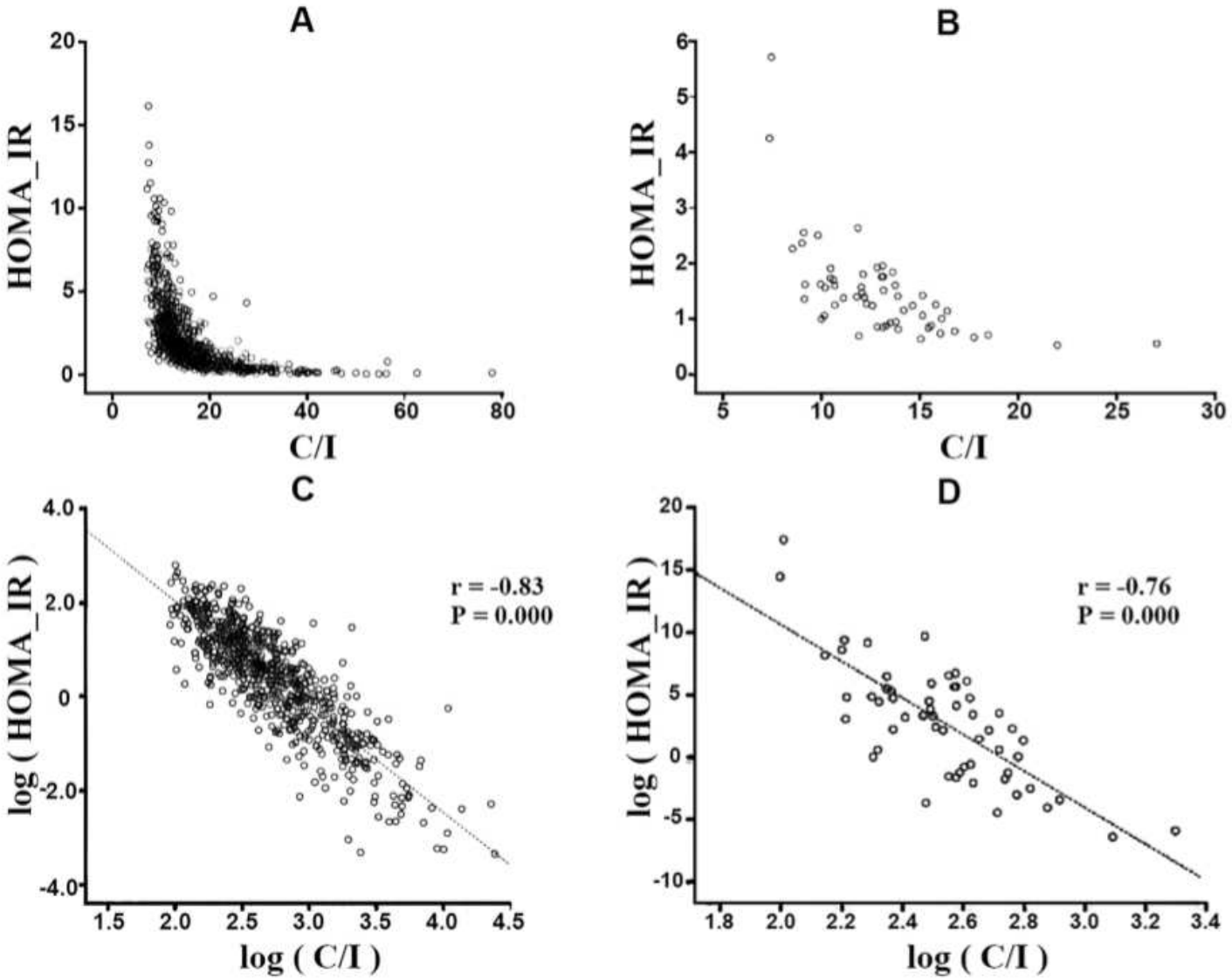

Figure 2

The relationship between C/I and HOMA-IR in diabetic patients and healthy controls. C/I and HOMA-IR are hyperbolically related in diabetics (A) and healthy controls (B). Log(C/I) and log(HOMA-IR) are negatively linearly correlated in diabetic patients (C) and healthy controls (D). Abbreviations: C/I, the ratio of C-peptide to insulin; HOMA-IR, homeostasis model assessment of insulin resistance.

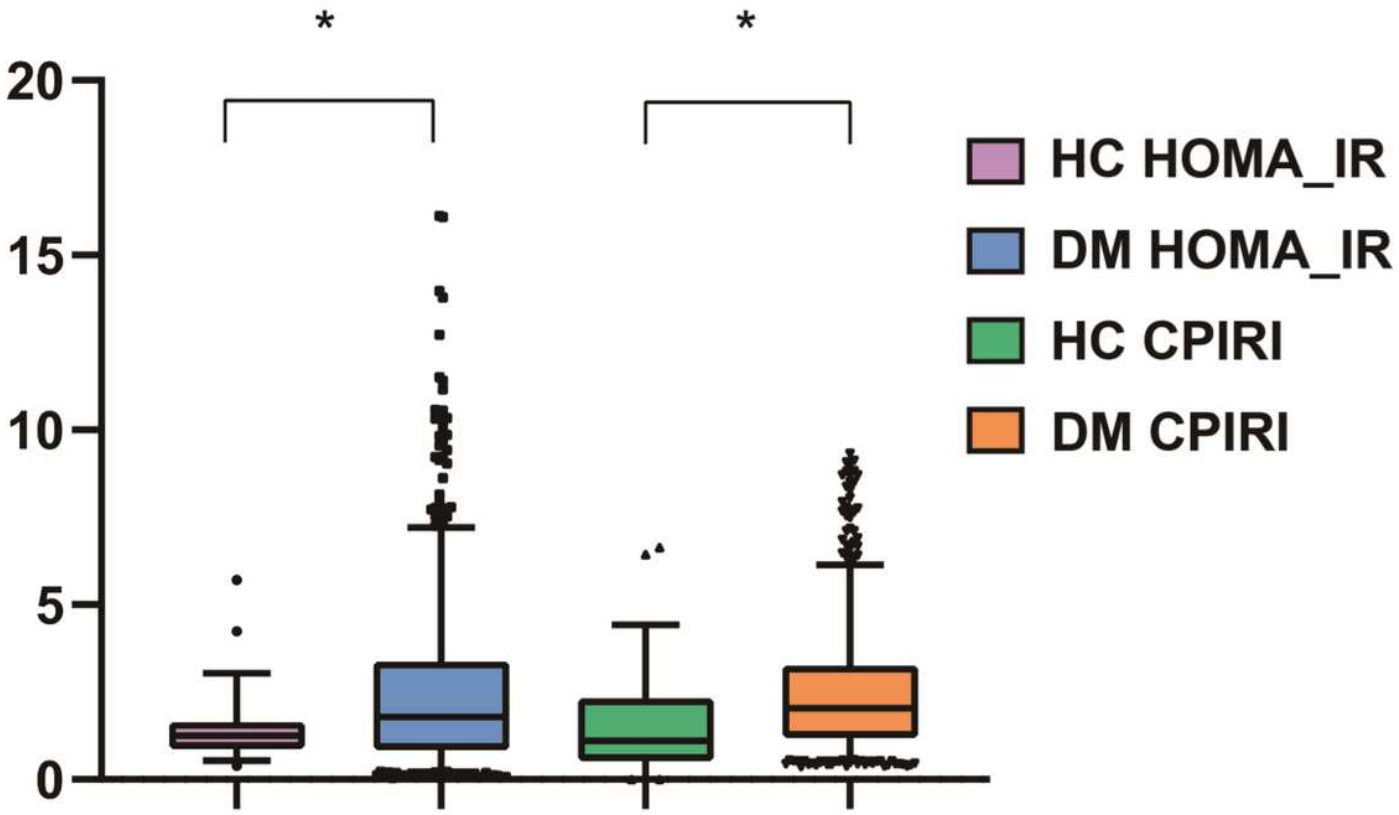

Figure 3

Insulin resistance among DM and HC. Both CPIRI and HOMA-IR are higher in DM than HC ($P<0.05$). Abbreviations: DM, diabetes mellitus; HC, healthy controls; HOMA-IR, homeostasis model assessment of insulin resistance; CPIRI, C-peptide to insulin ratio index.

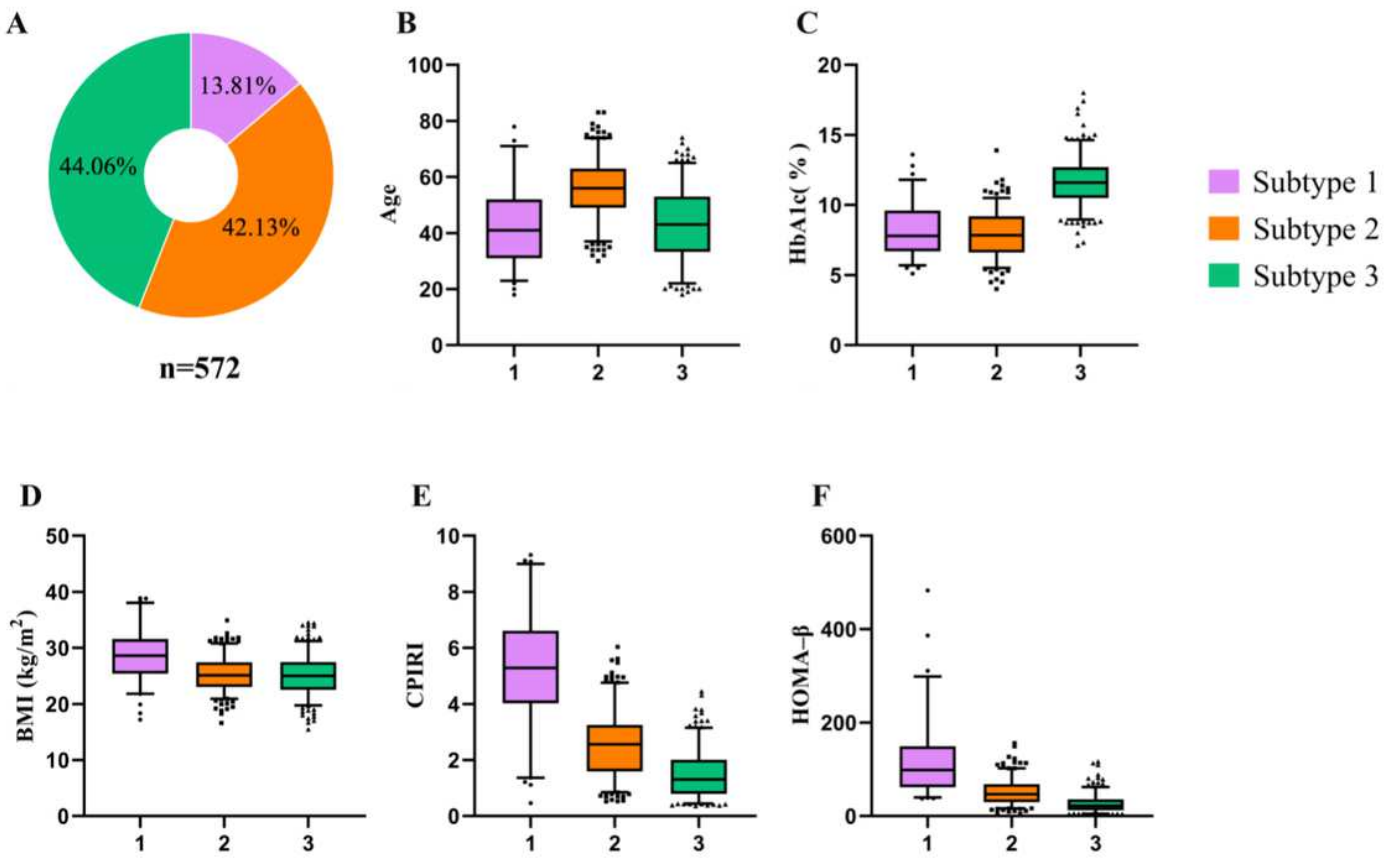

Figure 4

Heterogeneity of adult-onset non-autoimmune diabetes. Adult-onset non- autoimmune diabetes can be divided into three distinct subtypes according to results of cluster analysis: subtype 1 (n = 79, 13.81%), subtype 2 (n = 241, 42.13%), and subtype 3 (n = 252, 44.06%) (A). Distribution of age (B), HbA1c (C), BMI (D), CPIRI (E) and HOMA-β (F) for subtype1-3 reveals that subtype 1 is characterized by severe insulin resistance; subtype 2 is defined as elderly-onset DM with mild symptoms, and subtype 3 demonstrates severe insulin-deficient DM. Abbreviations: HbA1c, hemoglobin A1C; BMI, body mass index; CPIRI, C-peptide to insulin ratio index; HOMA-β, homeostasis model assessment of beta cell.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- Questionnaire.pdf
- SupplementaryFigure1.pdf
- SupplementaryTable1.pdf