Received: 1 August 2021 | Revised: 1 November 2021 | Accepted: 26 November 2021

DOI: 10.1111/1753-0407.13244

Journal of Diabetes

WILEY

ORIGINAL ARTICLE

New clusters of serum electrolytes aid in stratification of diabetes and metabolic risk

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Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 81941017, 81930021, 81970728, 91857205, 82088102; Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support Round 2, Grant/Award Numbers: 20152508, 20161307; Shanghai Shenkang Hospital Development Center, Grant/Award Numbers: SHDC12019101, SHDC2020CR1001A, SHDC2020CR3069B

Abstract

Background: Serum electrolytes were found to associate with type 2 diabetes. Our study aimed to stratify nondiabetes by clusters based on multiple serum electrolytes and evaluate their associations with risk of developing diabetes and longitudinal changes in glucose and lipid metabolic traits.

Methods: We performed a data-driven cluster analysis in 4937 nondiabetes individuals aged ≥40 years at baseline from a cohort follow-up for an average of 4.4 years. Cluster analysis was based on seven commonly measured serum electrolytes (iron, chlorine, magnesium, sodium, potassium, calcium, and phosphorus) by using the k-means method.

Results: A total of 4937 nondiabetes individuals were classified into three distinct clusters, with 1635 (33.1%) assigned to Cluster A, 1490 (30.2%) to Cluster B, and 1812 (36.7%) to Cluster C. Individuals in Cluster A had higher serum chlorine, were older, and more were women. Individuals in Cluster B had higher serum iron and body mass index (BMI). Individuals in Cluster C had higher serum phosphorus, were younger, and had lower BMI. Cluster B had 1.41-fold higher risk of developing diabetes and Cluster C's risk was 1.33-fold higher compared with Cluster A. Over an average follow-up of 4.4 years, Cluster A showed a moderate and stable BMI, Cluster B showed an accelerated deterioration in glucose metabolism, and Cluster C showed the most sharply increased serum low-density lipoprotein cholesterol level.

Conclusions: Clusters based on seven common serum electrolytes differed in diabetes risk and progression of glucose and lipid metabolic traits. Serum electrolytes clusters could provide a powerful tool to differentiate individuals into different risk stratification for developing type 2 diabetes.

Yanan Hou and Jiali Xiang contributed equally to the work.

KEYWORDS
cluster analysis, metabolic risk, risk stratification, serum electrolytes, type 2 diabetes

Highlights

- Three identified clusters based on seven commonly measured serum electrolytes had different metabolic profiles, associated with distinct risk of developing diabetes and progression of glucose and lipid metabolic traits.
- Individuals in Cluster A were older, more were women, and had "normal" to low risk for diabetes. Individuals in Cluster B were at a high risk and with significant deterioration in glucose metabolism. Individuals in Cluster C were younger, at a moderate risk and with more deleterious lipids metabolism.
- This provides a convenient method of risk stratification for nondiabetes.

1 | INTRODUCTION

Diabetes has become a big epidemic with 463 million adults affected globally in 2019 and the figure is projected to reach 700 million by 2045.[1] Of note, China now is facing the world's largest diabetes epidemic in that more than 12.8% of adults are estimated to have diabetes and more than 35.2% to have prediabetes in 2018.[2] Diabetes, together with its related cardiometabolic risk profiles, imposed a huge burden on cardiovascular diseases, mortality, and health care expenditure.[3,4] Early identification and stratification for risk of developing diabetes was crucial for better management and prevention of diabetes and its related cardiometabolic disorders.

Diabetes is a disease with high heterogeneity in clinical presentation, disease progression, and etiopathogenesis, which makes it difficult to implement individualized treatment, achieve adequate glucose control, and predict prognosis in affected individuals. These issues might be attributed to the fact that diabetes diagnosis is oversimplified via the evaluation of plasma glucose solely.[5] Adopting cluster analyses to refine the classification of diabetes beyond the simple use of plasma glucose is a powerful tool to detect diabetes subphenotypes with specific patterns of complications.[5] Using demographic and clinical variables, several recent studies successfully provided a refined classification of diabetes, which aids in characterizing and exploiting diabetes heterogeneity, identifying risk of complications at diagnosis, and optimizing precision medicine according to subphenotypes of diabetes.[6-11] Indeed, metabolic abnormalities characterizing prediabetes already exist 6 years before diabetes onset.[12] The macrovascular and microvascular complications of diabetes also need preventive care before diabetes onset. However, studies on risk evaluation and stratification of developing diabetes in the nondiabetes East Asian population are sparse.[13]

A very recent study adopted fat composition, insulin secretion, insulin sensitivity, and genetic risk to classify individuals at elevated risk for diabetes. They identified six distinctive subphenotypes with different metabolic risk. The study indicated adopting multivariables to recapitulate subphenotypes for individuals at elevated risk for diabetes would aid in disentangling metabolic heterogeneity, assessing diabetes and complication risks, and benefiting precision medicine.[13]

Serum electrolytes, including iron, chlorine, magnesium, sodium, potassium, calcium, and phosphorus, are important cofactors for multiple enzymes and play a pivotal role in many key biological and physiological processes, including glucose metabolism.[14-16] The excess or deficiency of these electrolytes was found to be linked to deleterious metabolism status.[14] Many studies have reported single serum electrolyte level, such as magnesium, calcium, and ferritin, was associated with risk of type 2 diabetes and related metabolic abnormalities.[17-20] The Rotterdam study with a median follow-up of 6.7 years[17] reported that each 0.1 mmol/L decrease in serum magnesium was associated with 1.12-fold greater prediabetes risk and 1.18-fold greater diabetes risk. In another two prospective cohorts,[18,20] researchers reported that a high level of baseline serum calcium was associated with an increased risk of developing type 2 diabetes. It indicated that serum electrolytes were a feasible clinical index used for predicting diabetes and prediabetes. However, few studies have systematically and comprehensively assessed the effects of serum electrolytes distribution pattern rather than specific concentration of serum electrolytes on the risk of incident diabetes.

Our study aimed to, first, create clusters in individuals who were nondiabetic at baseline based on seven systematically measured and clinical-conveniently available serum electrolytes by using cluster analysis;

and second, evaluate the associations of these clusters with risk of developing diabetes and longitudinal change patterns of metabolic traits in an average 4.4 years follow-up Chinese adults. The current study will provide a refined classification of nondiabetes into different stratifications of developing type 2 diabetes and progression of metabolic traits.

2 | METHODS

2.1 | Study population

The prospective study is a community-based survey on type 2 diabetes and related cardiometabolic diseases conducted in Jiading district, Shanghai, China.[21,22] In phase I (March–August 2010), 10 375 of 10 569 registered permanent residents aged 40 years or older underwent the baseline examination. Individuals with cardiovascular diseases ($n = 306$) or type 2 diabetes ($n = 1755$) or missing data on serum electrolytes, type 2 diabetes, or cardiovascular diseases ($n = 329$) at baseline were excluded. In phase II (August 2014-May 2015), 7965 nondiabetes individuals were enrolled to complete a follow-up survey. Of these, 153 individuals died during follow-up period, and 2788 individuals failed to attend the onsite follow-up visit. Seventy eight individuals were missing data on diabetes status at follow-up visit, and four individuals with extreme outliers (>2 SD from the mean of serum phosphorus level) were excluded. Finally, 4937 nondiabetes individuals were included in the current analysis. The detailed selection process of study participants is shown in Figure 1.

The study protocol was approved by the Committee on Human Research at Ruijin Hospital affiliated with Shanghai Jiao Tong University School of Medicine. All participants provided written informed consent.

2.2 | Biochemical measurements

Blood samples were collected at phase I and II after ≥10 h overnight fasting. Each individual underwent a simplified 75 g oral glucose tolerance test (OGTT). Plasma glucose (0 and 2 h) and serum insulin (0 and 2 h) was determined using glucose oxidize method (Modular P800; Roche, Basel, Switzerland) and chemiluminescence method (Modular E170; Roche, Basel, Switzerland), respectively. Serum electrolytes (iron, chlorine, magnesium, sodium, potassium, calcium, and phosphorus) were determined using chemiluminescence method (Modular E170; Roche, Basel, Switzerland). Glycated hemoglobin A1c (HbA1c) and serum creatinine were determined using high-performance liquid chromatography (VARIANT II Hemoglobin Testing System, Bio-Rad Laboratories) and picric acid method (clinical chemistry diagnostic system C16 000, Abbott Laboratories, Otawara-shi, Japan), respectively. Serum apolipoprotein A (APOA), apolipoprotein B (APOB), low-density

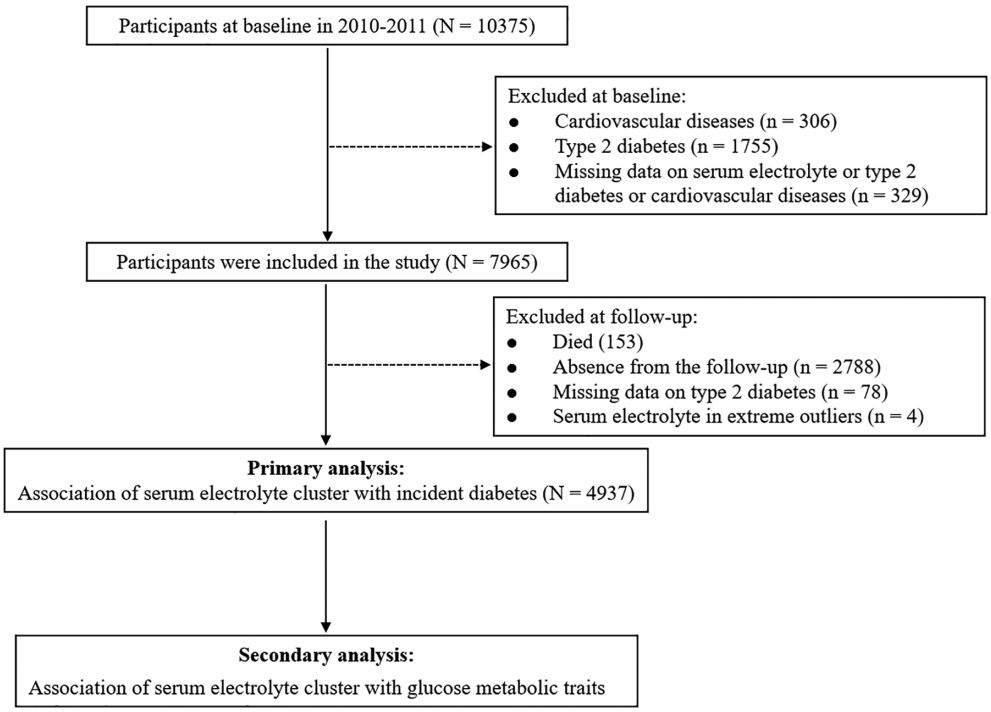

FIGURE 1 Flow chart of the present study

lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), total cholesterol (TC), aspartate aminotransferase (AST), γ- glutamyl transpeptidase (GGT), and alanine aminotransferase (ALT) were determined using chemiluminescence method (Modular E170; Roche, Basel, Switzerland).

2.3 | Ascertainment of incident diabetes

Incident diabetes cases were defined as (a) fasting plasma glucose ≥7.0 mmol/L; (b) 2 h-OGTT plasma glucose ≥11.1 mmol/L; (c) individuals were receiving antidiabetic medications; and (d) self-reported diagnosed diabetes. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula fasting plasma glucose (mmol/L) × serum fasting insulin (mIU/L) /22.5. The homeostasis model assessment of function of β cells (HOMA-β) was calculated using the formula: 20 × fasting serum insulin (mIU/L)/[fasting plasma glucose (mmol/L)-3.5].

2.4 | Covariates

Information about demographics, lifestyles, diseases history, and medication use were collected through a standard questionnaire. Individuals who consumed alcohol or smoked cigarettes regularly in the past 6 months were regarded as current drinking or smoking. Physical activity was evaluated using International Physical Activity Questionnaire[23] and divided into three levels on the basis of metabolic equivalent (MET): vigorous (≥3000 MET-min/week), moderate (600–2999 MET-min/week), and mild (≤599 MET-min/week).[24,25]

Body weight, height, diastolic blood pressure (DBP), systolic blood pressure (SBP), fat-free mass, and fat mass were measured using a standard protocol. Three seated SBP and DBP at nondominant arm were measured consecutively with 1 min intervals after 10 min of rest (OMRON Model HEM-752 FUZZY, Omron Company, Dalian, China). These three measurements were averaged for analysis. Fat-free mass and fat mass were evaluated on a body composition analyzer (Tanita TBF-300, Japan) by bioelectrical impedance analysis.

2.5 | Cluster analysis

Baseline fasting serum iron, chlorine, magnesium, sodium, potassium, calcium, and phosphorus were selected as model variables for cluster analysis. Cluster analysis was done on values centered to an SD of 1 and a mean value of 0. In the TwoStep clustering, we first calculated the optimal clustering value using the elbow method (R version 4.0.3) and then dose K-means clustering (k = 3) using the k-means runs function (runs = 100).

2.6 | Statistical analysis

Baseline characteristics were described according to the three clusters. Continuous variables were presented as mean ± SD or medians (interquartile range), whereas categorical variables were presented as numbers (percentage). The multiple comparisons of baseline characteristics among clusters were conducted by Student-Newman-Keuls (SNK) test.

Logistic regression analysis was performed to determine the risk of developing diabetes in relation to clusters. Risk estimates were described as odds ratios (OR) and 95% confidence intervals (CIs). Model 1, adjusted for sex, age (years), body mass index (BMI) (kg/m^2), family history of diabetes (yes or no); Model 2, further adjusted for current smoking (yes or no), current drinking (yes or no), physical activity (mild, moderate, or vigorous), education level (≥9 years of education or not); Model 3, further adjusted for systolic blood pressure (mmHg), diastolic blood pressure (mmHg), fasting plasma glucose (mmol/L), 2 h-OGTT plasma glucose (mmol/L), serum creatinine (umol/L), TC (mmol/L), TG (mmol/L), HDL-C (mmol/L), LCL-C (mmol/L), and use of diuresis (yes or no) based on model 2. To assess the added value of serum electrolytes clusters, we included these clusters in the models of predicting diabetes. We calculated the difference (C statistic) without or with clusters, integrated discrimination improvement (IDI), and net reclassification improvement (NRI).

All statistical analyses were conducted using SAS (version 9.4, SAS Institute, Cary, NC). A two-tailed p value < 0.05 was considered as statistical significance.

3 | RESULTS

3.1 | Baseline characteristics

A total of 4937 nondiabetes individuals were included in the current analysis, with an average age of 57.3 ± 8.6 years, 1721 (35%) men, and 3216 (65%) women. We used the k-means clustering method to classify individuals into three different clusters based on the seven serum electrolytes, with the minimum sum of the squared errors. The baseline characteristics according to the three clusters were

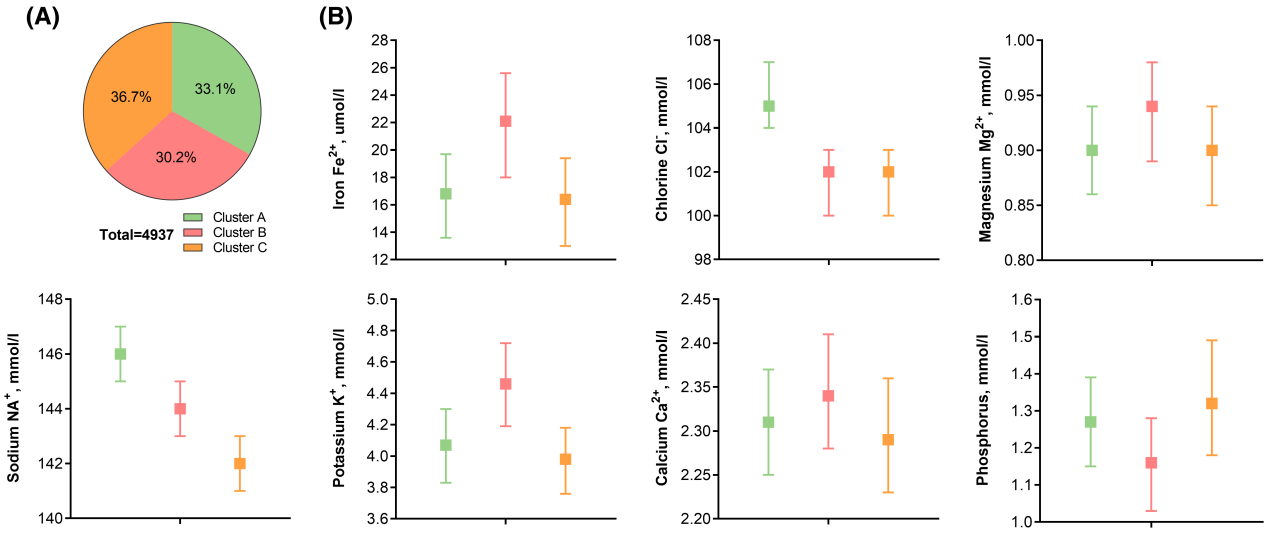

FIGURE 2 Clusters distribution and the characteristics of serum electrolytes in each cluster. (A) Proportions of the three clusters in total study participants ($n = 4937$) according to k-means clustering methods. (B) Characteristics of serum electrolytes at baseline in each cluster. Data were shown as means and SDs. Green = Cluster A, red = Cluster B, and yellow = Cluster C

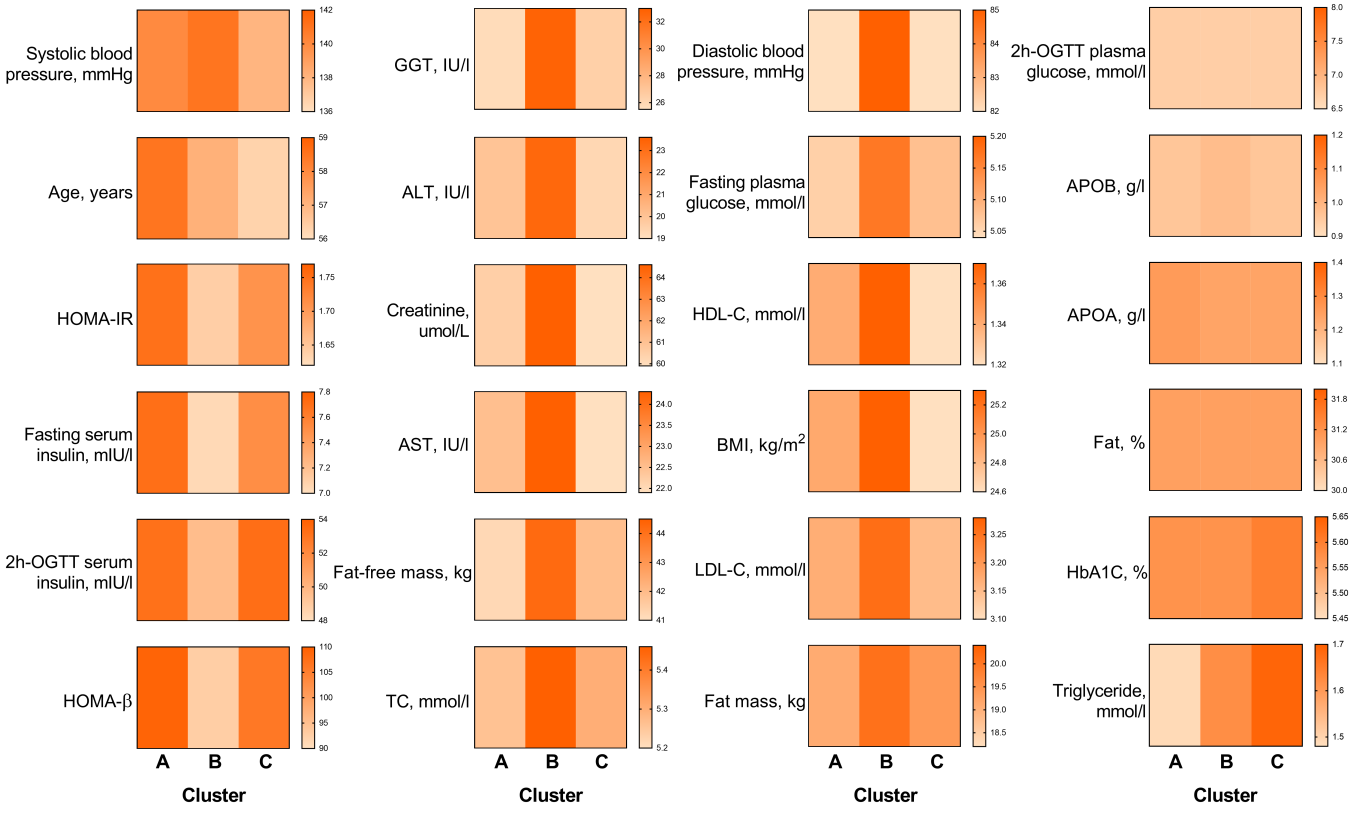

FIGURE 3 The patterns of a wide range of baseline metabolic characteristics of the participants according to the three clusters. The right bar with color gradient was presented the range of mean values of each metabolic trait. Abbreviations: APOA, apolipoprotein A; APOB, apolipoprotein B; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ- glutamyl transpeptidase; HbA1c, glycated hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cells; LDL-C, low density lipoprotein cholesterol; OGTT, oral glucose tolerance test; TC, total cholesterol

shown in Figures 2–3 and Table 1. Cluster A, including 1635 (33.1%) individuals, was characterized as elder age, more women, with higher level of serum chloride, fasting and 2 h-OGTT serum insulin and HOMA-β, and lower level of TG, Cluster B, including 1490 (30.2%) individuals, had higher level of serum iron, magnesium, potassium,

TABLE 1 Baseline characteristics of study participants according to the three clusters

	Cluster A	Cluster B	Cluster C	*p* value	SNK test
n	1635	1490	1812		
Age, years	58.5 ± 8.83	57.1 ± 8.35	56.3 ± 8.57	<0.0001	A, B, C
Women, *n* (%)	1222 (74.7)	697 (46.8)	1297 (71.6)	<0.0001	A, C, B
Body mass index, kg/m^2	24.9 ± 3.23	25.3 ± 3.00	24.6 ± 3.15	<0.0001	B, A, C
Systolic blood pressure, mmHg	140 ± 20.2	141 ± 18.6	138 ± 19.4	<0.0001	(B, A), C
Diastolic blood pressure, mmHg	82.3 ± 10.4	84.6 ± 10.0	82.0 ± 10.2	<0.0001	B, (A, C)
Current smoking, *n* (%)	220 (14.0)	454 (31.0)	266 (15.4)	<0.0001	B, (C, A)
Current drinking, *n* (%)	108 (22.8)	226 (15.4)	140 (8.05)	<0.0001	A, B, C
Physical activity, MET-h/wk	21.0 (0–38.5)	15.0 (0–35.0)	15.0 (0–33.6)	0.20	/
≥9 years of education, *n* (%)	309 (18.9)	284 (19.1)	410 (22.6)	0.009	C, (A, B)
Family history of diabetes, *n* (%)	126 (7.72)	122 (8.20)	204 (11.3)	0.0004	C, (A, B)
Use of diuresis, *n* (%)	20 (1.22)	24 (1.61)	43 (2.37)	0.03	C, (A, B)
Biochemical measurements					
Fasting plasma glucose, mmol/L	5.06 ± 0.54	5.17 ± 0.59	5.08 ± 0.56	<0.0001	B, (C, A)
2 h-OGTT plasma glucose, mmol/L	6.66 ± 1.70	6.58 ± 1.81	6.66 ± 1.69	0.32	/
Fasting serum insulin, mIU/L	6.80 (5.00–9.40)	6.28 (4.10–9.00)	6.70 (4.65–9.35)	<0.0001	A, C, B
2 h-OGTT serum insulin, mIU/L	46.2 (26.8–66.5)	39.6 (22.9–63.1)	42.5 (25.6–67.4)	<0.0001	A, C, B
HOMA-IR	1.53 (1.09–2.14)	1.43 (0.92–2.13)	1.49 (1.02–2.17)	0.02	(A, C), B
HOMA-β	91.1 (65.7–132)	77.9 (50.0–116)	88.9 (61.3–131)	<0.0001	A, C, B
Serum creatinine, umol/L	60.5 ± 13.2	64.6 ± 13.2	58.9 ± 11.9	<0.0001	B, A, C
Total cholesterol, mmol/L	5.26 ± 0.96	5.46 ± 0.95	5.30 ± 1.05	<0.0001	B, (C, A)
Triglycerides, mmol/L	1.28 (0.92–1.78)	1.35 (0.99–1.90)	1.35 (0.95–1.91)	0.0001	(C, B), A
High-density lipoprotein cholesterol, mmol/L	1.34 ± 0.31	1.37 ± 0.32	1.32 ± 0.32	<0.0001	B, (A, C)
Low-density lipoprotein cholesterol, mmol/L	3.17 ± 0.85	3.26 ± 0.81	3.15 ± 0.86	0.0005	B, (A, C)
Serum electrolytes					
Iron, umol/L	16.8 ± 4.85	22.1 ± 5.62	16.4 ± 4.99	<0.0001	B, A, C
Chlorine, mmol/L	105 ± 2.07	102 ± 2.13	102 ± 2.14	<0.0001	A, (B, C)
Magnesium, mmol/L	0.90 ± 0.07	0.94 ± 0.07	0.90 ± 0.07	<0.0001	B, (A, C)
Sodium, mmol/L	146 ± 1.41	144 ± 1.64	142 ± 1.46	<0.0001	A, B, C
Potassium, mmol/L	4.07 ± 0.35	4.46 ± 0.39	3.98 ± 0.32	<0.0001	B, A, C
Calcium, mmol/L	2.31 ± 0.10	2.34 ± 0.10	2.29 ± 0.10	<0.0001	B, A, C
Phosphorus, mmol/L	1.27 ± 0.18	1.16 ± 0.18	1.32 ± 0.19	<0.0001	C, A, B

Data are presented as means ± SD (SD), or medians (interquartile ranges) for skewed variables, or number (percentage) for categorical variables. Multiple comparisons were performed by Student-Newman-Keuls (SNK) test. Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cells; OGTT, oral glucose tolerance test; SNK test, Student-Newman-Keuls test. A, B, and C represent cluster A, cluster B, and cluster C, respectively. Letters within brackets indicate means in different clusters are not significantly different.

and calcium, BMI, DBP, fasting plasma glucose, TC, HDL-C, LDL-C, and liver enzymes (ALT, AST, and GGT), fat mass, and fat-free mass, but lower level of fasting and 2 h-OGTT serum insulin, HOMA-IR, and HOMA-β. Cluster C, including 1812 (36.7%) individuals, had higher level of serum phosphorus and HbA1c, younger age, lower level of BMI, SBP, serum creatinine and AST, and moderate level of fasting and OGTT-2 h serum insulin, HOMA-β, fat-free mass, and fat mass.

3.2 | Associations of clusters by serum electrolytes with risk of incident diabetes

A total of 601 incident diabetes cases were identified over an average of follow-up of 4.4 years, including 163 (10.0%) in Cluster A, 217 (14.6%) in Cluster B, and 221 (12.2%) in Cluster C.

Compared with Cluster A, Clusters B and C were associated with a higher risk of incident diabetes (Table 2).

TABLE 2 Risk of incident diabetes in relation to Cluster B and C, as compared with Cluster A

	Cluster A	Cluster B		Cluster C	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value
n, cases/participants	163/1635	217/1490		221/1812	
Model 1	Ref.	1.56 (1.25–1.96)	0.0001	1.37 (1.10–1.70)	0.005
Model 2	Ref.	1.57 (1.25–1.98)	0.0001	1.36 (1.09–1.70)	0.007
Model 3	Ref.	1.41 (1.09–1.82)	0.01	1.33 (1.03–1.71)	0.03

Data are presented as odds ratio (ORs) and 95% confidence interval (CI). Model 1, adjusted for sex, age (years), body mass index (kg/m^2), family history of diabetes (yes or no); Model 2, further adjusted for current smoking (yes or no), current drinking (yes or no), physical activity (mild, moderate or vigorous), education level (≥9 years of education or not); Model 3, further adjusted for systolic blood pressure (mmHg), diastolic blood pressure (mmHg), fasting plasma glucose (mmol/L), 2 h-OGTT plasma glucose (mmol/L), serum creatinine (mmol/L), total cholesterol (mmol/L), triglycerides (mmol/L), high-density lipoprotein cholesterol (mmol/L), low-density lipoprotein cholesterol (mmol/L), and use of diuresis (yes or no) based on model 2.

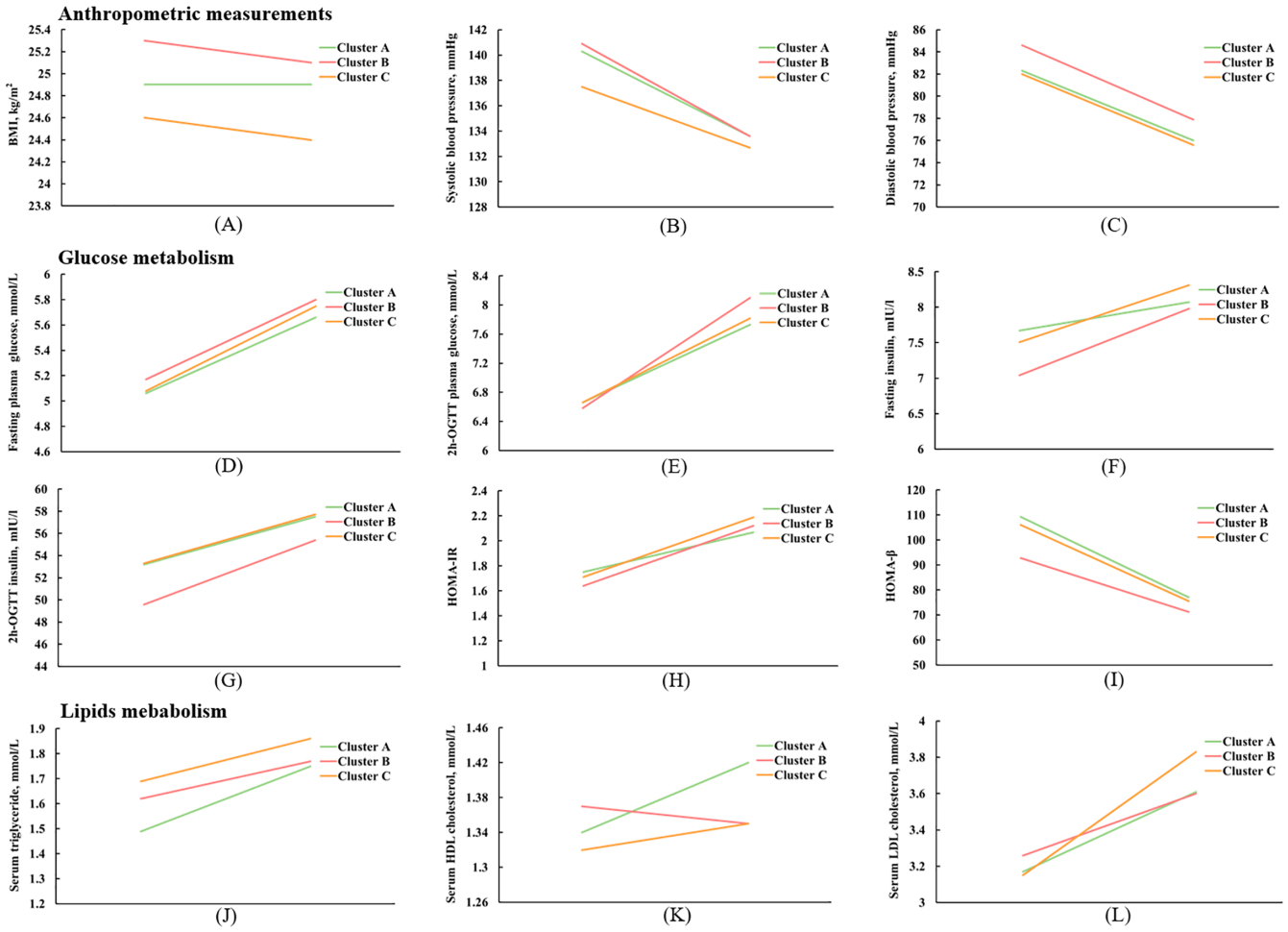

FIGURE 4 Progression of glucose and lipid metabolic traits over 4.4 years according to the three clusters. The lines show the change trends of the glucose and lipid metabolic traits from baseline to the 4.4 years of follow-up. Green = Cluster A, red = Cluster B, and yellow = Cluster C. The glucose and lipid metabolic traits included BMI (A), systolic blood pressure (B), diastolic blood pressure (C), fasting plasma glucose (D), 2 h-OGTT plasma glucose (E), fasting serum insulin (F), 2 h-OGTT serum insulin (G), HOMA-IR (H), HOMA-β (I), triglycerides (J), high-density lipoprotein cholesterol (K), and low-density lipoprotein cholesterol (L). Abbreviations: BMI, body mass index; HDL cholesterol, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β cells; LDL cholesterol, low density lipoprotein cholesterol; OGTT, oral glucose tolerance test

TABLE 3 Risk of incident diabetes in relation to selected dominant serum electrolytes in each cluster

	Each 1-SD increase			Tertile 1	Tertile 2	Tertile 3	
	1-SD	OR (95% CI)	*p* value		OR (95% CI)	OR (95% CI)	*p* for trend
Serum chlorine							
Unit, mmol/L	2.8			94–101	102–104	105–113	
n, cases/participants	603/4941			246/1507	236/2040	121/1304	
Model 1		0.75 (0.69–0.82)	<0.0001	Ref	0.69 (0.57–0.84)	0.47 (0.37–0.60)	<0.0001
Model 2		0.88 (0.80–0.97)	0.01	Ref	0.81 (0.65–1.00)	0.64 (0.50–0.83)	0.0007
Serum iron							
Unit, umol/L	5.7			3.6–15.5	15.6–20.1	32.7–54.5	
n, cases/participants	603/4941			173/1653	186/1631	244/1657	
Model 1		1.18 (1.07–1.29)	0.0006	Ref	0.99 (0.79–1.23)	1.38 (1.11–1.73)	0.003
Model 2		1.26 (1.14–1.39)	<0.0001	Ref	1.05 (0.82–1.34)	1.60 (1.25–2.05)	0.0001
Serum magnesium							
Unit, mmol/L	0.07			0.63–0.87	0.88–0.93	0.94–1.56	
n, cases/participants	603/4941			196/1523	195/1730	212/1688	
Model 1		0.96 (0.88–1.04)	0.29	Ref	0.84 (0.68–1.05)	0.93 (0.75–1.15)	0.50
Model 2		0.87 (0.80–0.96)	0.005	Ref	0.83 (0.65-1.05)	0.78 (0.62-0.99)	0.04
Serum potassium							
Unit, mmol/L	0.4			2.85–3.96	3.97–4.30	4.31–6.03	
n, cases/participants	603/4941			200/1639	200/1666	203/1636	
Model 1		0.98 (0.90–1.07)	0.67	Ref	1.02 (0.83–1.26)	1.03 (0.83–1.27)	0.82
Model 2		0.88 (0.81–0.97)	0.009	Ref	1.01 (0.80–1.28)	0.83 (0.66–1.05)	0.12
Serum sodium							
Unit, mmol/L	2.0			135–142	143–145	146–155	
n, cases/participants	603/4941			155/1163	305/2599	143/1179	
Model 1		0.93 (0.85–1.01)	0.09	Ref	0.81 (0.66–1.01)	0.80 (0.62–1.03)	0.08
Model 2		0.89 (0.81–0.98)	0.02	Ref	0.78 (0.62–0.99)	0.69 (0.53–0.91)	0.01
Serum calcium							
Unit, mmol/L	0.1			1.92–2.27	2.28–2.35	2.36–2.77	
n, cases/participants	603/4941			191/1729	186/1618	226/1594	
Model 1		1.11 (1.02–1.21)	0.01	Ref	1.03 (0.83–1.28)	1.31 (1.06–1.61)	0.01
Model 2		0.96 (0.87–1.05)	0.35	Ref	0.91 (0.72–1.16)	0.89 (0.70–1.13)	0.33

TABLE 3 (Continued)

	Each 1-SD increase			Tertile 1	Tertile 2	Tertile 3	
	1-SD	OR (95% CI)	*p* value		OR (95% CI)	OR (95% CI)	*p* for trend
Serum phosphorus							
Unit, mmol/L	0.2			0.72–1.15	1.16–1.34	1.35–3.06	
n, cases/participants	603/4941			208/1620	196/1705	199/1616	
Model 1		1.00 (0.99–1.01)	0.91	Ref	0.92 (0.74–1.14)	1.00 (0.81–1.24)	0.98
Model 2		1.00 (0.99–1.01)	0.66	Ref	0.99 (0.78–1.25)	1.03 (0.81–1.31)	0.81

Data are presented as odds ratio (ORs) and 95% confidence interval (CI). Model 1, adjusted for sex, age (years), body mass index (kg/m^2), family history of diabetes (yes or no); Model 2, further adjusted for current smoking (yes or no), current drinking (yes or no), physical activity (mild, moderate or vigorous), education level (≥9 years of education or not), systolic blood pressure (mmHg), fasting plasma glucose (mmol/L), total cholesterol (mmol/L), and triglycerides (mmol/L).

Cluster B was associated with 1.56-fold (95% CI 1.25-1.96, $p = 0.0001$) and Cluster C 1.37-fold (95% CI 1.10-1.70, $p = 0.005$) higher risk of incident diabetes, respectively, after adjustments for age, sex, BMI, and family history of diabetes (model 1). Further adjustments for the demographic and lifestyle factors, SBP, DBP, fasting, and 2 h-OGTT plasm glucose, serum creatinine, HDL-C, TG, TC, and LDL-C, and use of diuresis (model 3), the results did not appreciably change, the ORs for Cluster B and C were 1.41 (95% CI 1.09-1.82, $p = 0.01$) and 1.33 (95% CI 1.03-1.71, $p = 0.03$), respectively. However, there was no significant difference between the association of the risk of incident diabetes with Cluster B and C ($p > 0.05$).

3.3 | Progression of glucose and lipid metabolic traits over 4.4 years

The changes in major glucose and lipid metabolic traits over 4.4 years by clusters were shown in Figure 4. All the three clusters showed an obvious decreased level of SBP, DBP, and HOMA_β, the increased level of fasting and 2 h-OGTT plasma glucose, fasting, and 2 h-OGTT serum insulin, and HOMA_IR. In Cluster A, the glucose and lipid metabolic traits were characterized by a moderate and stable BMI and increased level of serum HDL-C. Cluster B showed a sharply increased level of 2 h-OGTT plasma glucose, a slightly decreased level of serum HDL-C. Cluster C was shown as in between Cluster B and A, except the most sharply increased level of serum LDL-C.

3.4 | Association of selected serum electrolyte with risk of incident diabetes

We evaluated the associations of each of the seven serum electrolytes with risk of incident diabetes (Table 3). Each 1-SD increase in serum chlorine (2.8 mmol/L) was associated with 12% lower risk of incident diabetes (OR = 0.88, 95% CI 0.80-0.97, $p = 0.01$) risk of incident diabetes. The corresponding results for serum magnesium (0.07 mmol/L) was 13% (OR = 0.87, 95% CI 0.80-0.96, $p = 0.005$), serum potassium (0.4 mmol/L) was 12% (OR = 0.88, 95% CI 0.81-0.97, $p = 0.009$), and serum solidum (2.0 mmol/L) was 11% (OR = 0.89, 95% CI 0.81-0.98, $p = 0.01$). Each 1-SD increase in serum iron (5.7 umol/L) was associated with 1.26-fold (95% CI 1.14-1.39, $p < 0.0001$) increased risk of incident diabetes. No significant association were found for calcium (0.1 mmol/L) and serum phosphorus (0.2 mmol/L) (both $p \geq 0.35$). The categorical analysis showed similar results (all $p_{\text{for trend}} \leq 0.04$, except for potassium, calcium, and phosphorus).

3.5 | Predictive effect of the serum electrolytes clusters

The C statistic (95% CI) of the predictive models of conventional risk factors (age, sex, family history of diabetes, BMI, and fasting plasma glucose) was 0.767 (0.746–0.788) for incident diabetes. The inclusion of serum electrolytes clusters to the diabetes-predictive models slightly increased the C statistic, IDI, and NRI for predicting the risk of diabetes. The C statistic, ΔC statistic, IDI, and NRI was 0.769 (0.749–0.790), 0.002 (0–0.005), 0.002 (0–0.003), and 0.13 (0.076–0.21), respectively.

4 | DISCUSSION

In this large longitudinal study of 4937 community-dwelling Chinese adults without baseline diabetes, we identified three clusters based on seven commonly measured serum electrolytes. The three clusters performed well in differentiating the risk of developing diabetes and different cardiometabolic trait progression. Cluster A was characterized as elder age, more women, with a higher level of serum chlorine, favorable β-cell function, and lower TG and showed a moderate and stable BMI and increased serum HDL-C over the follow-up periods. Cluster B had a higher baseline serum iron level and multiple metabolic disorders and showed a persistent higher BMI and unfavorable β-cell function and accelerated deterioration in glucose metabolism and progression of insulin resistance. Cluster C had a higher baseline level of serum phosphorus, younger age, and lower BMI but also an unfavorable lipids profile and the most sharply increased serum LDL-C. As compared to Cluster A, Clusters B and C were associated with a higher risk of diabetes. Cluster A could be considered as "normal" or low risk for diabetes. Cluster B could be considered as a high risk of diabetes with significant deterioration in glucose metabolism. Cluster C was comparable with A, except for the more deleterious lipids metabolism, which indicated a moderate risk of diabetes.

Individuals in Cluster A were the oldest ones but had the lowest or delayed risk of developing diabetes, which might be because of the higher level of baseline serum chlorine, higher level of baseline β-cell function, and lower level of baseline TG. Individuals in Cluster B had a higher risk of developing diabetes than those in Cluster A, which might be because of the higher level of baseline serum iron and systemic multimetabolic disorders. Individuals in Cluster B had unfavorable β-cell function, higher levels of BMI, fat mass, TC, LDL-C, and liver enzymes at baseline. Over an average follow-up of 4.4 years, individuals in Cluster B still had higher BMI and unfavorable β-cell function, together with accelerated deterioration in glucose metabolism and progression of insulin resistance. These features indicated individuals in Cluster B had systemic multimetabolic disorders. Higher level of baseline serum iron and systemic multimetabolic disorders may have jointly triggered and exacerbated the development of diabetes. Individuals in Cluster C were the younger ones, had lower level of BMI, but still had a higher risk of developing diabetes than those in Cluster A, which might be because of the higher level of baseline triglycerides, fat mass, and TC and most sharply increased level of serum LDL-C during follow-up.

Classified individuals at risk for diabetes and complications would aid in detecting diabetes pathophysiological subphenotypes and predicting future metabolic trajectories.[26] Adopting indicators link to diabetes pathogenesis, accurate indicators of insulin secretion and insulin sensitivity, individuals at elevated risk for type 2 diabetes were classified into six distinct pathophysiologic subphenotypes titled (1) low risk, (2) very low risk, (3) β-cell failure, (4) low risk obese, (5) high risk insulin resistant fatty liver, and (6) high risk visceral fat nephropathy.[13] Of these, subphenotype 4 was characterized by obesity but low glycemic deterioration; subphenotype 5 was characterized by obesity, insulin resistance, high level of fatty liver, and elevated risk of type 2 diabetes, vascular disease, renal disease, and mortality; and subphenotype 6 was characterized by high amounts of visceral fat and elevated risk of microalbuminuria and chronic kidney disease. Our study used the clustering of seven common serum electrolytes to replicate the classification of nondiabetes. We found three distinct clusters and analyzed their diabetes risk and metabolic profile, including body composition, blood pressure, glucose and insulin levels, insulin resistance and insulin secretion, lipids profile, and liver and kidney function. We found Cluster B (systemic multimetabolic disorders with elevated risk for diabetes) in our study similar to subphenotype 5 in Tübingen Lifestyle Program (TUEF/TULIP) cohort,[13] but Cluster A (old age but with low risk of diabetes) and Cluster C (young age, lower level of BMI but with elevated risk for diabetes) were significantly different. These results suggested that introducing additional traits to stratify individuals susceptible to diabetes is feasible to disentangle pathophysiologic subphenotypes and implement efficient prevention strategies.

The distinct metabolic profiles and diabetes risk between clusters were validated by the association of the dominant serum electrolytes with risk of diabetes. Mechanically, excessive iron could promote β cell damage and apoptosis, impair insulin signaling, diminish insulin induced glucose transport, and result in deleterious glucose homeostasis.[27] Magnesium deficiency could inhibit β-cells proliferation and mass, reduce β-cells activity, increase insulin

resistance, and decrease insulin secretion.[16,28,29] In turn, serum insulin would decrease serum magnesium level by promoting excretion in renal, resulting in a vicious cycle.[30] Potassium deficiency could decrease insulin secretion and increase ratio of proinsulin to insulin secretion.[31] Excessive calcium could inhibit insulin exocytosis.[32,33] Low serum sodium would active sympathetic nervous system and renin-angiotensin-aldosterone system, which would reduce insulin sensitivity, promote compensatory insulin secretion, and, in turn, induce insulin resistance and diabetes.[34] These factors deleterious glucose homeostasis and exacerbate type 2 diabetes and related complications.

To the best of our knowledge, it is the first investigation reporting the stratification of risk of developing type 2 diabetes and progression in glucose and lipid metabolic traits based on multiple common serum electrolytes. Multivariable defined clusters performed well in differentiating individuals to different classification for diabetes risk. Besides, our study included comprehensive metabolic indicators to show the distinct characteristics of each cluster. Second, the relatively large sample size, well-defined community setting, and the highly homogeneous population were a great foundation for our analysis. We also considered a comparable full list of covariates in the analysis, which allowed us to control for the confounding factors between the clusters and risk of incident diabetes to the greatest extent. Several limitations should be acknowledged. First, the serum electrolytes level was measured at baseline by using one blood sampling. It forbids us to assess the association between the dynamic changes in serum electrolytes and diabetes risk. Multiple follow-up measurements of serum electrolytes are needed to support our findings. In addition, according to the 2021 American Diabetes Association criteria, in the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples. The diagnosis of diabetes in our study was based on a single measurement of fasting and 2 h-OGTT plasma glucose, which may result in an overestimate of diabetes incidence, and need to be cautious when generalizing the results to clinical practice. Second, serum electrolyte levels could change when an abnormality occurs. For example, individuals at high risk of diabetes might increase urinary electrolytes loss, which may lead to potential reverse causation. However, our study was based on a follow-up prospective design to assess the baseline serum electrolyte with risk of future diabetes. We excluded individuals with known diabetes or cardiovascular diseases at baseline, the status of the diseases that might influence serum electrolyte levels. Third, we used elbow method in the selection of the optimal number of clusters. Whether the approach was inferior to conventional predictions from multivariable modeling need further warranted,[6] but cluster analysis shows superiority when exploring the association of various elements with a certain disease, which can aggregate and classify similar characteristics to identify the group with high risk.[35] Fourth, this classification was derived primarily from middle-aged and elderly adults in Chinese, which minimizes the confounding effects by ethnic background but generalizing this conclusion to the youngers or other ethnicity population need to be cautious.

In conclusion, our study took seven common serum electrolytes simultaneously into account to stratify non-diabetes and found three distinct clusters differing in diabetes risk and progression of glucose and lipid metabolic traits. The investigation provides a powerful, convenient, and easily available method of risk stratification for non-diabetes. These analysis strategy aid in identifying more individuals susceptible to diabetes and benefit for early prevention and reducing the risk of long-term diabetes and cardiovascular disease.

ACKNOWLEDGEMENTS

The investigators are grateful to all field workers for their participation and contribution. This work was funded by the National Natural Science Foundation of China (81941017, 81930021, 81970728, 91857205, and 82088102), the Shanghai Municipal Education Commission–Gaofeng Clinical Medicine Grant Support Round 2 (20152508 and 2016307), and the Shanghai Shenkang Hospital Development Center (SHDC12019101, SHDC2020CR1001A, SHDC2020CR3069B). M. X., M. L., T.W., Y. X., J.L., Y. B., W. W., and G. N. are members of innovative research team of high-level local universities in Shanghai.

DISCLOSURE

The authors declare that they have no conflict of interest in this work.

CONTRIBUTION STATEMENT

Yanan Hou, Jiali Xiang, Yufang Bi, Zhiyun Zhao, and Min Xu conceived and designed the study. Yanan Hou, Jiali Xiang did the statistical analysis. Yanan Hou, and Min Xu drafted the manuscript. Zhiyun Zhao, Yufang Bi, and Min Xu supervised the study. Huajie Dai, Mian Li, Hong Lin, Shuangyuan Wang, Yu Xu, Jieli Lu, Yuhong Chen, Weiqing Wang, and Guang Ning contributed to acquisition, analysis, or interpretation of data. All authors revised the report and approved the final version before submission. Min Xu is guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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How to cite this article: Hou Y, Xiang J, Dai H, et al. New clusters of serum electrolytes aid in stratification of diabetes and metabolic risk. *Journal of Diabetes*. 2022;14(2):121-133. doi:10.1111/1753-0407.13244