Higher Serum RCAN2 Levels Are Associated with Metabolically Unhealthy Individuals, Either with Obesity or Not

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Abstract

Background: Regulator of calcineurin 2 (RCAN2) has been documented to cause weight gain and related pathology and metabolism abnormalities in animal researches. The aim of the study was to compare serum RCAN2 concentrations in metabolically healthy (MH) and metabolically unhealthy (MUH) individuals.

Methods: 872 subjects (581 MH and 291 MUH) were recruited to the study. Basic anthropometric, metabolic parameters and concentration of RCAN2 were measured.

Results: Serum RCAN2 levels differ between MH and MUH subjects (9.22 ± 3.21 vs. 11.09 ± 5.01 ng/mL, $p < 0.001$). Circulating RCAN2 level was an independent predictor of MUH compared with MH group (9.3% higher rate of MUH incidence per 1 ng/mL increment of RCAN2) after adjust other potentially confounding variables. Higher serum RCAN2 levels were significantly correlated with SBP, FBG, WHR. Receiver-operating characteristic (ROC) curve analysis suggested that serum RCAN2 [area under the curve (AUC) = 0.615, 95% CI 0.575-0.655, $p < 0.001$] might be used as a candidate biomarker for MUH. Men had considerably greater RCAN2 levels than female in the MH group, but this difference was not significant in the MUH group.

Conclusion: Higher circulating RCAN2 levels are associated with MUH individuals regardless of the BMI status.

Introduction

Obesity, measured by body mass index (BMI), is one of the most common epidemics today with resultant hazardous health implications, and development of obesity has risen to undesired levels worldwide [1, 2]. As a major independent risk factor for multiple diseases, obesity also risks to anxiety and depression prevalence [3, 4].

However, among individuals with obesity, a significant percentage of that, do not show metabolic disorders; though people with normal body weight develop metabolic disorders [5, 6]. The condition seems connected with ectopic fat accumulation in normally tissues (heart, liver, skeletal muscle, etc) [7]. The global burden of disease (GBD) group has estimated that cardiovascular disease (CVD) was the primary cause of death and disability-adjusted life-years correlated to elevated BMI globally [8]. Due to the individual metabolic heterogeneity, BMI cannot anticipate increased risk of CVD and all-cause mortality veraciously [5, 7, 9]. Metabolically unhealthy obesity (MUHO) and metabolically unhealthy overweight (MUHOW) are vital and emerging phenotypes for increased risk of CVD [9–11]. Previous research found that whatever the BMI status, CVD and total mortality risk were significantly higher in metabolically unhealthy (MUH) compared to metabolically healthy (MH) individuals; People with metabolically healthy obesity (MHO) not at increased or decreased risk for all-cause and CVD mortality [9, 11, 12].

The Regulator of Calcineurin (RCAN, formerly known as MCIPs, DSCR1) encoded in Down syndrome critical region (DSCR) region located on human chromosome 21, has been reported involving in calcineurin-NFAT signaling pathway [13–16]. RCAN2 (also termed ZAKI-4, DSCR1L1, MCIP2 or Calcipressin-2) was highly expressed in heart, brain, liver and skeletal muscle in both adult human and mice, as a thyroid hormone (T3)-responsive gene primarily identified [17–19]. RCAN2 has been reported to associate with pathological conditions including age- and diet-induced obesity, hepatic steatosis and formation of cancers in animal studies [20–23]. Importantly, C Casas, et al. found that RCAN proteins are involved in the development of cardiac hypertrophy [24]. On balance, RCAN2 may affects cardiovascular risk increase, clear RCAN2 levels vary in respect to MH and MUH is of great necessity in view of MUH is a novel phenotype for obesity and CVD.

Therefore, the aim of the present study was to compare serum RCAN2 levels with metabolically healthy (MH) and metabolically unhealthy (MUH) participants, also evaluating the association of the potential correlation between RCAN2 concentrations and individual components of the metabolic parameters.

Materials And Methods

Study Population and Design

A total of 1682 participants aged 18 to 70 years old were enlisted in the study during 1 June 2021 to 1 Sep 2021 from physical examination center of the affiliated hospital of Southwest Medical University. Anthropometric and metabolic information were collected by trained staff. Exclusion criteria of this study as follow: prior history of CVD, stroke, hyperthyroidism or hypothyroidism, cancer, use of any hypolipidemic, antihypertension and weight-affecting drugs, pregnant or lactating women, as well as individuals with BMI < 18.5 kg/m². Finally, a total of 872 participants (558 men and 314 women) were included in the analysis.

Clinical and Anthropometric Measurements

Following an overnight fast, all individuals were subjected to anthropometric measurements in light clothing and without shoes. BMI was computed as weight (kg) divided by height (m^2). Waist-to-hip ratio (WHR) was calculated as Waist circumference (WC) / hip circumference (HC). Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were monitored using an automatic electronic blood pressure monitor (HBP-9020, Omron Corporation, Kyoto, Japan) after resting for at least 5 minutes.

Biochemical Measurements

Registered staff nurses used conventional venepuncture to obtain blood samples from the antecubital vein. After a 12-hour fast, blood samples were obtained from the subjects and stored at 80°C until the day of analysis.

The following parameters were included in biochemical measurements and detected by automated biochemical analyzer (ADVIA2400, SIEMENS, Germany): fasting blood glucose (FBG), HDL-C, LDL-C, TC,

TG, total protein (TP), albumin (ALB), globulin (GLO), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), urea nitrogen (Urea), uric acid (UA), creatinine (Crea), and homocysteine (HCY). An automated blood cell counter was used to count peripheral WBC) and NEU (Myriad BC-6800, Shenzhen, China). Serum RCAN2 concentrations were assessed with well-established commercial ELISA kit (Human RCAN2 ELISA Kit, Abebio, Wuhan, China).

Criteria of BMI Categories and Metabolic Profiles and Their Classification

Body weight status was determined using BMI categories based on Chinese criterion [25]: normal weight (18.5≤ BMI ≤ 23.9 kg/m²), overweight (BMI 24.0 ≤ BMI ≤ 27.9 kg/m²), and obese (BMI ≥ 28 kg/m²). Participants were classified into MH and MUH, according to the novel standard defined by Anika Zembic et al. [12], MH subjects possessed all of three following physiological status: systolic blood pressure (SBP)≤130 mmHg and no use of antihypertension medication; waist-to-hip ratio (WHR)≤0.95 (women) and ≤1.03 (men); and no prevalent hyperglycemia.

For further analysis, subjects have been divided into 6 groups combined BMI and metabolic characteristics: metabolically healthy normal weight (MHNW, n=277), metabolically unhealthy normal weight (MUNW, n=71), metabolically healthy overweight (MHOW, n=255), metabolically unhealthy overweight (MUOW, n=142), metabolically healthy obesity (MHO, n=49), and metabolically unhealthy obesity (MUO, n=78).

Statistical Analyses

SPSS 25.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA) software were used for all statistical analysis and visuals. $P < 0.05$ with two-tailed was regarded statistically significant. Continuous variables are expressed as mean SD, while categorical variables are expressed as *n*. (%). Continuous variables comparisons between unpaired groups were conducted using the Mann–Whitney *U* test and one-way analysis of variance (ANOVA) or Kruskal-Wallis test, categorical variables were accessed by Chi-square test. Spearman or Pearson coefficients correlations calculated to evaluate the relationships between serum RCAN2 concentrations and other parameters separately for groups. The area under the receiver operating characteristic (ROC) curve (AUC) was used to determine the diagnostic value of serum RCAN2 for MUH.

Results

Demographic Parameters and Serum RCAN2 Levels

The current study included 872 participants (558 males and 314 women) with an average age of 39.6 years. Table 1 presents their baseline characteristics, 291 (33.4 percent) of the participants were diagnosed with MUH. Individuals with MUH were more likely to be older, men were more represented, and have upper levels of weight, BMI, WC, HC, WHR, SBP, DBP, heart rate, WBC, NEU, ALT, AST, AST/ALT, DBIL,

GGT, ALP, UA, TC, TG, HDL-C, LDL-C, FBG, eGFR and RCAN2 (all $p < 0.05$) than those with MH. In the MH group, men had significantly higher RCAN2 levels than women, but this difference was not significant in the MUH group (Fig. 1A).

Table 1
Baseline characteristics of participants according to MH/MUH.

Variables	Total (n = 872)	MH (n = 581)	MUH (n = 291)	p value
Anthropometric parameters				
Male	558 (64.0)	354 (60.9)	204 (70.1)	**0.008**
Age (year)	39.56 ± 10.67	37.18 ± 9.60	44.37 ± 11.05	**< 0.001**
Weight (kg)	68.00 ± 12.50	65.93 ± 10.18	72.29 ± 15.35	**< 0.001**
Height (cm)	164.83 ± 7.93	164.84 ± 7.53	164.88 ± 8.65	0.777
BMI (kg/m^2)	24.97 ± 3.53	24.22 ± 2.92	26.48 ± 4.10	**< 0.001**
WC (cm)	83.96 ± 9.32	81.76 ± 7.93	88.33 ± 10.27	**< 0.001**
HC (cm)	97.11 ± 6.65	96.14 ± 5.73	99.04 ± 7.84	**< 0.001**
WHR	0.86 ± 0.06	0.85 ± 0.05	0.89 ± 0.06	**< 0.001**
SBP (mmHg)	121.92 ± 14.40	114.93 ± 9.23	136.01 ± 12.51	**< 0.001**
DBP (mmHg)	73.36 ± 10.30	69.75 ± 7.96	80.63 ± 10.63	**< 0.001**
Heart Rate	83.87 ± 11.23	82.75 ± 11.05	86.10 ± 11.24	**< 0.001**
Metabolic parameters				
WBC (10^9/L)	6.36 ± 1.58	6.19 ± 1.49	6.71 ± 1.73	**< 0.001**
NEU (10^9/L)	3.70 ± 1.21	3.59 ± 1.13	3.95 ± 1.35	**< 0.001**
ALT (U/L)	29.21 ± 24.03	26.94 ± 21.47	33.82 ± 27.85	**< 0.001**
AST (U/L)	23.92 ± 10.30	22.99 ± 9.39	25.78 ± 11.68	**< 0.001**
AST/ALT	1.00 ± 0.39	1.02 ± 0.35	0.95 ± 0.45	**< 0.001**
TP (g/L)	72.37 ± 3.35	72.27 ± 3.38	72.59 ± 3.25	0.137
ALB (g/L)	46.55 ± 2.30	46.58 ± 2.27	46.51 ± 2.35	0.638
GLO (g/L)	25.82 ± 2.69	25.69 ± 2.65	26.08 ± 2.73	0.056
A/G	1.82 ± 0.22	1.83 ± 0.22	1.80 ± 0.22	0.133
TBIL (μmol/L)	15.33 ± 6.36	15.36 ± 6.14	15.27 ± 6.77	0.389
DBIL (μmol/L)	4.32 ± 1.82	4.39 ± 1.78	4.17 ± 1.87	**0.013**

Continuous variables were expressed as mean ± SD. Categorial variables were expressed as n (%). P values were derived from Mann–Whitney U test for continuous variables, and chi-square test for the categorical variables between MH and MUH. Bold font indicated $p < 0.05$.

Variables	Total (n = 872)	MH (n = 581)	MUH (n = 291)	p value
IBIL (μmol/L)	11.02 ± 4.71	10.97 ± 4.52	11.12 ± 5.07	0.893
GGT (U/L)	34.05 ± 36.4	28.77 ± 27.69	44.84 ± 47.75	**< 0.001**
ALP (U/L)	72.72 ± 19.89	69.77 ± 18.53	78.89 ± 21.27	**< 0.001**
Urea (mmol/L)	5.00 ± 1.11	4.94 ± 1.12	5.10 ± 1.05	0.062
UA (μmol/L)	346.76 ± 84.82	340.14 ± 82.90	360.45 ± 86.63	**0.002**
Crea (μmol/L)	66.52 ± 12.38	66.38 ± 12.61	66.93 ± 11.88	0.487
TC (mmol/L)	4.82 ± 0.88	4.71 ± 0.82	5.05 ± 0.94	**< 0.001**
TG (mmol/L)	1.72 ± 1.56	1.44 ± 0.92	2.30 ± 2.29	**< 0.001**
HDL-C (mmol/L)	1.31 ± 0.33	1.34 ± 0.34	1.25 ± 0.31	**< 0.001**
LDL-C (mmol/L)	3.18 ± 0.88	3.11 ± 0.84	3.34 ± 0.95	**< 0.001**
FBG (mmol/L)	5.25 ± 1.26	4.93 ± 0.38	5.93 ± 1.97	**< 0.001**
HCY (μmol/L)	12.15 ± 7.40	12.02 ± 7.26	12.42 ± 7.65	0.278
eGFR (ml/min)	123.39 ± 21.39	124.24 ± 21.4	121.48 ± 21.21	**0.041**
RCAN2 (ng/mL)	9.84 ± 4.01	9.22 ± 3.21	11.09 ± 5.01	**<0.001**

Continuous variables were expressed as mean ± SD. Categorial variables were expressed as n (%). P values were derived from Mann–Whitney U test for continuous variables, and chi-square test for the categorical variables between MH and MUH. Bold font indicated $p < 0.05$.

The study stratified serum RCAN2 by the BMI and metabolic health status. As depicted in Fig. 1B, circulating RCAN2 levels of individuals with MUH component increased progressively with increasing BMI categories ($p < 0.001$), and **Table S1** shows their baseline characteristics.

Logistic Regression Analyses for MUH

In binary logistic regression analysis, serum RCAN2 as a continuous variable was related with an elevated OR of MUH both before and after adjustment for potential confounders (Model 1: adjusted for age and gender. Model 2: adjusted for Model 1 + Waist, Hip, WHR, WBC, NEU, ALT, AST, AST/ALT, TP, ALB, GLO, A/G, TBIL, DBIL, IBIL, GGT, ALP, Urea, UA, Crea, HCY, eGFR, Height, Heart Rate) (Table 2). 12.5%, 11.2% and 9.3% higher rate of MUH incidence per 1 ng/mL increment of RCAN2 respectively (all $p < 0.05$).

Table 2
Unconditional logistic regression analysis of MUH risk according to the serum RCAN2 concentrations (ng/mL) and lower/upper serum RCAN2 levels.

Measurement	RCAN2 concentration (ng/mL)	Lower OR (95%CI)	Upper OR (95%CI)
Range (ng/ml)	-	<9.14	≥ 9.14
RCAN2 in MUH vs. MH			
MUH/MH	291/581	112/322	179/259
Univariate	**1.125 (1.083–1.169)**	1 (reference)	**1.987 (1.491–2.648)**
p-value	**<0.001**		**<0.001**
Model 1	**1.112 (1.069–1.156)**	1 (reference)	**1.780 (1.312–2.414)**
p-value	**<0.001**		**<0.001**
Model 2	**1.093 (1.043–1.146)**	1 (reference)	**1.438 (1.011–2.047)**
p-value	**<0.001**		**0.044**

Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) from unconditional logistic regression models were applied in the analysis. Model 1: adjusted for age and gender. Model 2: adjusted for Model 1 + Waist, Hip, WHR, WBC, NEU, ALT, AST, AST/ALT, TP, ALB, GLO, A/G, TBIL, DBIL, IBIL, GGT, ALP, Urea, UA, Crea, HCY, eGFR, Height, Heart Rate. Bold font indicated $p < 0.05$.

Similar results were obtained by logistic regression after dividing participants to two groups according to the median RCAN2 level. Upper RCAN2 concentration was substantially linked with MUH (Univariate, OR = 1.987, 95% CI, 1.491–2.648, $p < 0.001$; Model 1, OR = 1.780, 95% CI, 1.312–2.414, $p < 0.001$; Model 2, OR = 1.438, 95% CI, 1.011–2.047, $p = 0.044$) compared with patients in the lower serum RCAN2 levels (Table 2).

Relationship between RCAN2 and MH-related Metabolic Parameters

According to the median RCAN2 level in all individuals, the participants were separated into two groups, as mentioned previously. Table 3 shows the clinical parameters of the two groups. In the lower and upper RCAN2 concentration groups, the prevalence of MUH was 25.8% and 40.9%, respectively ($\chi^2 = 22.240$, $p < 0.001$). SBP, FBG and WHR (stratified analysis for gender) were higher in individuals with upper RCAN2 (all $p < 0.05$), as MUH-related risk factors (Fig. 2).

Table 3
Comparison of parameters of study population by serum RCAN2 concentrations.

Variables	Lower RCAN2 (*n* = 434)	Upper RCAN2 (*n* = 438)	*p* value
MUH	112 (25.8)	179 (40.9)	**< 0.001**
Anthropometric parameters			
Male	259 (59.7)	299 (68.3)	**0.008**
Age (year)	38.62 ± 10.59	40.78 ± 10.74	**0.001**
BW (kg)	66.38 ± 12.17	69.69 ± 12.64	**< 0.001**
Height (cm)	164.45 ± 7.76	165.35 ± 8.08	0.089
BMI (kg/m^2)	24.46 ± 3.38	25.40 ± 3.60	**< 0.001**
WC (cm)	82.99 ± 9.01	84.97 ± 9.59	0.976
HC (cm)	96.94 ± 6.52	97.22 ± 6.90	**< 0.001**
WHR	0.86 ± 0.06	0.87 ± 0.06	**< 0.001**
SBP (mmHg)	119.68 ± 13.74	123.73 ± 14.70	**< 0.001**
DBP (mmHg)	71.75 ± 10.05	74.66 ± 10.32	**< 0.001**
Heart Rate	83.47 ± 11.64	84.32 ± 10.93	0.122
Metabolic parameters			
WBC (10^9/L)	6.31 ± 1.64	6.41 ± 1.55	0.219
NEU (10^9/L)	3.71 ± 1.23	3.70 ± 1.22	0.987
ALT (U/L)	26.93 ± 20.88	31.66 ± 27.04	**< 0.001**
AST (U/L)	22.79 ± 8.66	25.22 ± 11.81	**< 0.001**
AST/ALT	1.03 ± 0.42	0.97 ± 0.36	**0.025**
TP (g/L)	72.00 ± 3.13	72.66 ± 3.53	**0.007**
ALB (g/L)	46.40 ± 2.25	46.67 ± 2.36	**0.046**
GLO (g/L)	25.61 ± 2.55	26.00 ± 2.84	0.115
A/G	1.83 ± 0.22	1.82 ± 0.23	0.437

Continuous variables were expressed as mean ± SD. Categorial variables were expressed as *n* (%). All subjects were divided into two groups according to the median RCAN2 concentrations in total participants. *P* values were derived from Mann–Whitney *U* test for continuous variables, and chi-square test for the categorical variables between lower and upper RCAN2 concentrations. Bold font indicated $p < 0.05$.

Variables	Lower RCAN2 (*n* = 434)	Upper RCAN2 (*n* = 438)	*p* value
TBIL (μmol/L)	15.23 ± 6.12	15.37 ± 6.51	0.967
DBIL (μmol/L)	4.43 ± 1.80	4.18 ± 1.80	**0.011**
IBIL (μmol/L)	10.80 ± 4.48	11.19 ± 4.86	0.275
GGT (U/L)	28.79 ± 28.88	39.37 ± 42.09	**< 0.001**
ALP (U/L)	71.68 ± 20.05	74.20 ± 19.63	**< 0.001**
Urea (mmol/L)	4.95 ± 1.14	5.05 ± 1.04	0.192
UA (μmol/L)	337.16 ± 82.00	357.06 ± 87.05	**0.001**
Crea (μmol/L)	66.22 ± 12.39	67.26 ± 12.30	0.236
TC (mmol/L)	4.63 ± 0.83	5.02 ± 0.88	**< 0.001**
TG (mmol/L)	1.38 ± 0.80	2.03 ± 1.82	**< 0.001**
HDL-C (mmol/L)	1.33 ± 0.32	1.29 ± 0.34	**0.012**
LDL-C (mmol/L)	3.08 ± 0.82	3.29 ± 0.93	**< 0.001**
FBG (mmol/L)	5.17 ± 1.16	5.38 ± 1.41	**< 0.001**
HCY (μmol/L)	12.10 ± 7.07	12.14 ± 7.77	0.913
eGFR (ml/min)	123.48 ± 21.64	122.40 ± 20.86	0.511
RCAN2 (ng/mL)	7.03 ± 1.32	12.61 ± 3.66	**<0.001**

Continuous variables were expressed as mean ± SD. Categorial variables were expressed as *n* (%). All subjects were divided into two groups according to the median RCAN2 concentrations in total participants. *P* values were derived from Mann–Whitney *U* test for continuous variables, and chi-square test for the categorical variables between lower and upper RCAN2 concentrations. Bold font indicated $p < 0.05$.

Following that, the study performed a Pearson correlation analysis, SBP, FBG and WHR were positively correlated with serum RCAN2 ($r = 0.200, 0.097, 0.195$, respectively, $p < 0.01$) (**Table S2**).

Biomarker for Predicting MUH Using ROC Analysis

The study assessed the diagnostic value of RCAN2 levels on MUH. The specificity and sensibility of the serum RCAN2 for predicting MUH was explored by ROC analysis, 64% and 55%, respectively. The AUC of circulating RCAN2 concentrations for MUH was 0.615 (95% CI 0.575–0.655, $p < 0.001$) (**Figure S1**), compared with MH.

Discussion

This is the first time, to our knowledge, that the connection between serum RACN2 concentrations and MUH has been investigated. The main conclusion of the present study is that serum RCAN2 is highly associated with MUH regardless of BMI status, even when other conventional risk variables are taken into account. RCAN2 may play a role in blood pressure, glucose and lipid metabolism, according to our findings. Furthermore, in the upper serum RCAN2 group, the prevalence of MUH was more visible. RCAN2 abnormal increase could be a workable indicator of MUH or perhaps a risk factor.

In the present study, RCAN2 levels are higher in men than in women of MH participants, but this difference was not significant in MUH. Ding LC et al. found that RCAN2 increased food intake, whereas 17β-estradiol (E2) in female mice promoted energy expenditure [26]. We speculate that 17β-estradiol may be a negative regulator of RCAN2, but there is a limit to this effect.

Similar to our findings, exogenous calcineurin inhibitors (CNIs) such as cyclosporine and tacrolimus have multiple adverse effects, including hypertension, new-onset diabetes and hyperlipidemia [27], and may be related to inhibition of calcineurin in non-immune tissues [28]. Studies have found that CNIs may affect the production and release of vasodilators such as endothelin and nitric oxide (NO), leading to vasoconstriction and thus hypertension [29, 30]. In addition, CNIs may affect glucose homeostasis through multiple mechanisms, mainly by affecting pancreatic β-cell function, which may lead to reduced insulin secretion [28].

Various illnesses, including as heart hypertrophy and diabetes, have been attributed to elevated expression levels of RCAN homologs [31]. Previous experimental animal research has discovered the potential pathophysiological function of RCAN2. Sun et al found that the mRNA levels of RCAN2-3 (splicing variant of RCAN2) were significantly upregulated in hypothalamus of mice fed with high fat, and increase in food intake, weight gain, impaired glucose tolerance, insulin resistance was related to the RCAN2 [23]. Little is currently known with respect to the putative function of RCAN2 in the CVD. RCAN2 may also affect endothelial cell function and angiogenesis, previous research reveals that RCAN2 is highly expressed in numerous kinds of pathologic angiogenesis in vivo [32]. several angiogenic factors such as VEGF-A upregulated expressions of RCAN2 mRNA and proteins [32, 33]. Yang et al. found that RCAN2 was concentrated in striated muscle and was implicated in cardiac hypertrophy, and responded to thyroid hormone [34]. Date of the study shows there were no significant variations in serum FT3 concentrations between the lower and upper RCAN2 groups (**Figure S2**). Serum RCAN2 were not connected with FT3 in both Pearson correlation and binary logistic regression analyses (data not shown). Participants in this study had no thyroid dysfunction and were relatively healthy, so we speculate that abnormal levels of FT3 may participate in the levels of RCAN2 and pathogenesis of hyperthyroid cardiopathy. Whether exogenous FT3 affects RCAN2 remains to be proven.

Simply, MUH have more cardiometabolic abnormalities than those defined as MH, MH may shift to MUH in the long term [11, 35], and diabetes risk may be increased in these people of all BMI groups [36]. Apart from that, MUH might raise the risk of metabolic dysfunction-associated fatty liver disease (MAFLD, formerly known as NAFLD), gallstones and critical coronavirus disease 2019 (COVID-19) [37–39].

Admittedly, people with obesity more likely to classified as MUH [35], MUH can present in persons of any BMI, thus individuals with all BMI categories should maintain normal metabolism.

Previous researches corroborate our conclusions, findings of the study imply that RCAN2 anomalies can be used to detect metabolic issues in the body. Individuals with elevated serum RCAN2 should be considered to have an "increased risk" medical condition, behavioral intervention and treatment should be prompted as soon as possible to eschew the farther metabolic disorder and higher risk of CVD.

There were several drawbacks to this study as well. RCAN2 concentrations were detected in blood, extrapolating data to intracellular levels and tissue distribution should be done with caution. Criteria for defining metabolically unhealthy conditions are not uniform worldwide, therefore, using different definition criteria may lead to different study results. Additionally, despite careful controlling and adjustments for major available confounders, there may still be residual covariates. All participants were Chinese and came from a single medical center, as a result, the applicability of the current results may be limited. More experimental investigations are required to prove the causal connection between RCAN2 and blood pressure and glucolipid metabolism.

Conclusion

Altogether, present research demonstrates that serum RCAN2 is a significant candidate indicator of MUH. The addition of RACN2 to typical risk factors may improve early risk stratification for individuals with any BMI category. More in-depth experimental studies and long-term prospective cohort researches are still warranted to confirm the value of RCAN2.

Abbreviations

ALT, Alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; ALB, albumin; AUC, area under the curve; BW, body weight; Crea, creatinine; DBP, diastolic blood pressure; FBG, fasting blood glucose; GGT, gamma-glutamyl transpeptidase; GLO, globulin; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HCY, homocysteine; LDL-C, low-density lipoprotein cholesterol; MHNW, metabolically healthy normal weight; MUNW, metabolically unhealthy normal weight; MHOW, metabolically healthy overweight; MUOW metabolically unhealthy overweight ; MHO, metabolically healthy obesity ; MUO, metabolically unhealthy obesity ; NEU, neutrophil; RCAN2, Regulator of Calcineurin 2; ROC, receiver-operating characteristic; SBP, systolic blood pressure; TP, total protein; TC, total cholesterol; TG, triglycerides; Urea, urea nitrogen; UA, uric acid; WC, waist circumference; WHR, waist-to-hip ratio; WBC, White Blood Cell Count.

Declarations

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Author's Contributions

JHF and YX conceived, designed and supervised the study; XZT, QR provided research guidance; HYW, XF and QR collected the data and biological samples; HYW, XF and QR performed the measurements of serum RCAN2 concentrations; HYW and XF analyzed the data and wrote the manuscript; and QR, XZT, JHF and YX critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

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

Ethics approval and consent to participate

All participants in the study received the information of the research, and written informed consent was obtained. Protocols of the present study was conducted according to the ethical guidelines of the 1964 Declaration of Helsinki and were approved by the Southwest Medical University Hospital Human Research Ethics Committee (license number: KY2021086).

Consent for publication

All authors agreed to publish this article in Diabetology & Metabolic Syndrome.

Competing interests

The authors declare no conflicts of interest.

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Figures

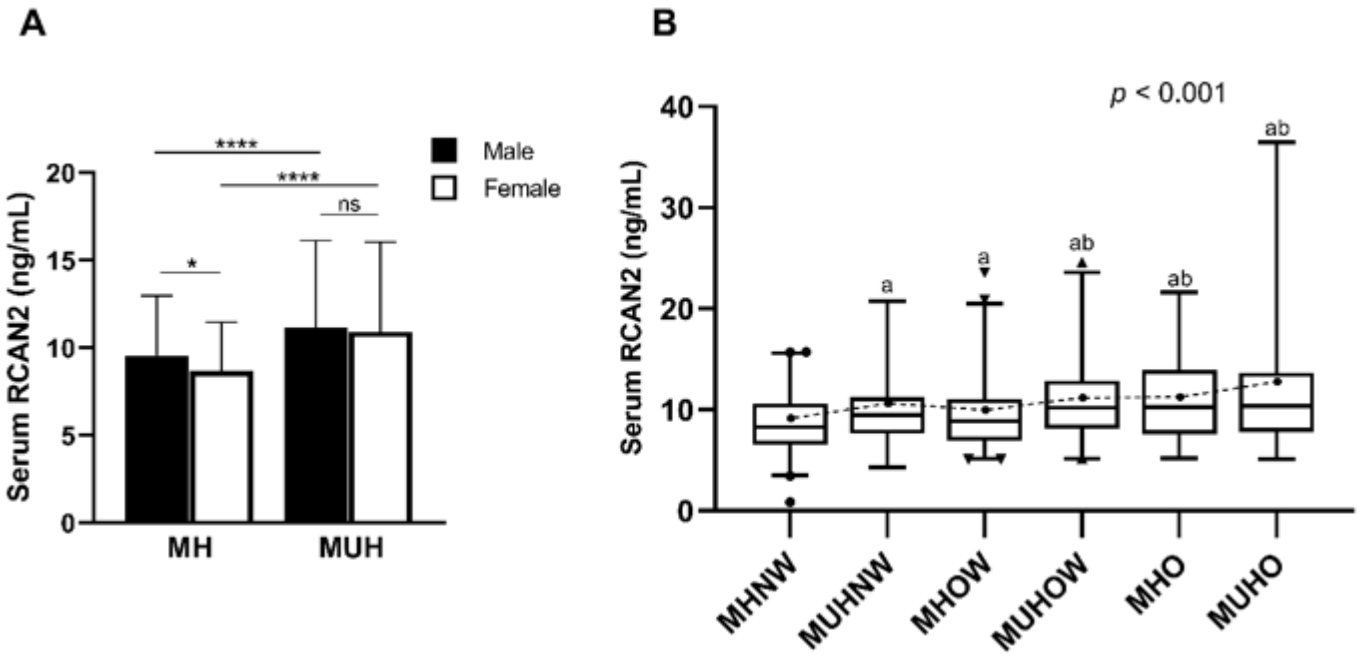

Figure 1

Serum RCAN2 concentrations of MH/MUH in various genders (A). Serum RCAN2 concentrations in subjects with MHNW, MUHNW, MHOW, MUHOW, MHO and MUHO (B). *P* values were derived from Mann–Whitney *U* test or Kruskal-Wallis test for serum RCAN2 concentrations. $^{*}p < 0.05$, $^{****}p < 0.0001$, $^{ns}p > 0.05$. $^{a}p < 0.05$ compared with MHNW group; $^{b}p < 0.05$ compared with MHOW group.

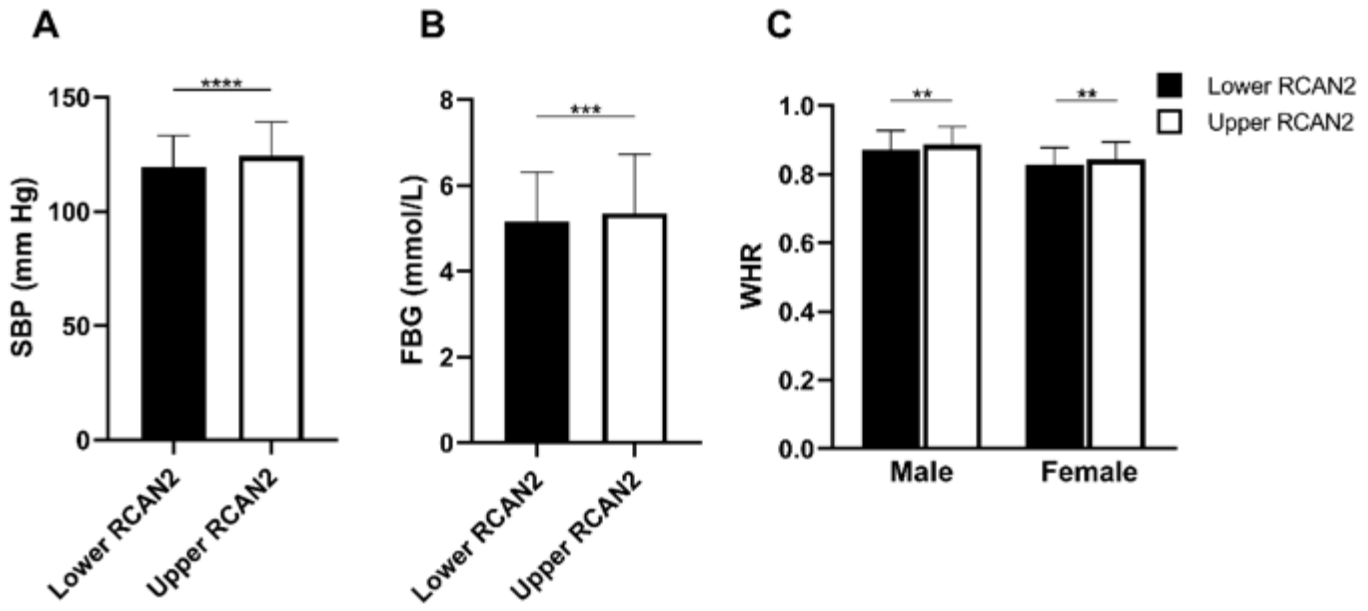

Figure 2

Levels of SBP (A), FBG (B) and WHR (C) in lower and upper RCAN2 concentrations. WHR ratio stratified analysis by gender. *P* values were derived from Mann–Whitney *U* test. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

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