RESEARCH

Association of metabolic syndrome components with circulating levels of cytokine clusters in young women

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Abstract

Background: The purpose of this study was to investigate the relationship between circulating zinc α 2-glycoprotein (ZAG), irisin, betatrophin and adiponectin concentrations and metabolic syndrome (MetS) components and to analyze the effects of blood glucose and insulin on these cytokine concentrations *in vivo*.
Methods: A total of 196 young women, including 78 healthy women and 118 women with MetS components, were recruited for this cross-sectional study. An oral glucose tolerance test and euglycemic-hyperinsulinemic clamp (EHC) were performed in healthy subjects and women with MetS components. An ELISA kit was used to measure serum ZAG, irisin, betatrophin, and adiponectin levels, and their relationship with the MetS components was analyzed.
Results: In women with MetS components, circulating irisin and betatrophin levels were significantly higher than those in the healthy women ((207 (150–248) vs 178 (147–228); $P < 0.05$) for irisin; (0.51 (0.38–0.63) vs 0.38 (0.23–0.52); $P < 0.001$) for betatrophin), but circulating ZAG and adiponectin levels were significantly lower (39.8 (26.4–50.4) vs (46.7 (40.6–63.0); $P < 0.001$) for ZAG; (36.5 (22.0–47.6) vs 41.2 (35.7–54.7); $P < 0.01$) for adiponectin). FBG, WC, and triglyceride were significantly correlated with the circulating levels of these four cytokines ($P < 0.001$ or <0.05). All four cytokines were associated with MetS and its components. In response to increasing insulin levels, circulating ZAG concentrations were markedly increased in both healthy subjects and women with MetS components during the EHC. However, serum irisin, betatrophin, and adiponectin levels in both healthy subjects and women with MetS components were significantly reduced compared with baseline.
Conclusion: Serum ZAG, irisin, betatrophin and adiponectin were associated with MetS and might be biomarkers for screening MetS components.

Key Words
- cytokine
- insulin resistance
- metabolic syndrome
- women

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Introduction

Insulin resistance (IR) is the pathophysiological basis of metabolic diseases, such as type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS) and nonalcoholic fatty liver disease (NAFLD) (1, 2, 3, 4). Increased IR in T2DM patients, obesity and MetS individuals is a high-risk factor for cardiovascular disease (CVD) (5, 6). MetS is a group of metabolic abnormalities related to IR, T2DM and CVD (7, 8). In recent decades, the incidence of MetS has tended to be found in younger women, especially those who are obese or have polycystic ovary syndrome (PCOS) (9). In addition, there is a physiological decrease of insulin sensitivity in healthy adolescents, reaching the lowest point in mid-to-late adolescence, and returning to normal levels after adolescence (10, 11, 12). We speculate that the changes of some cytokines related to insulin sensitivity in youth may help to predict the occurrence of metabolic diseases, such as MetS and T2DM. Thus, we believe that it is of great clinical significance to explore the relationship between the main components of MetS, such as impaired glucose tolerance, IR, hyperlipidemia, hypertension and obesity, and some circulating cytokines in young women.

In recent decades, it has been reported that some cytokines play a substantial role in metabolism and IR (13, 14). In previous studies, several cytokines, such as zinc α 2-glycoprotein (ZAG), betatrophin, adiponectin and irisin, have been reported to play a role in IR and metabolic disorders (15, 16, 17). Like the classical adipokine adiponectin, ZAG is considered a type of adipokine and mainly expressed in s.c. and abdominal adipose tissue. Circulating ZAG levels are decreased in patients with T2DM (15). Betatrophin is mainly expressed in liver and adipose tissue and is considered as a hepato-adipokine (16). Irisin is a myokine and expressed in muscle as the type I membrane precursor protein FNDC5, which is proteolytically cleaved, secreted into the circulatory system, and found to improve muscle IR (17). These cytokines are also believed to be involved in metabolic diseases, such as T2DM, PCOS, and MetS (13, 18, 19). However, a single cytokine cannot reflect the whole pathological process but only one aspect of disease development. According to previous studies, both the specificity and sensitivity for prediction and diagnosis of metabolic diseases using a single cytokine are relatively low. Therefore, their clinical application has some limitations. To date, it has not been reported using multiple cytokines to investigate their relationship with metabolic disease.

We hypothesized that MetS components have different effects on the circulating levels of various cytokines, including ZAG, irisin, and betatrophin. Confounding factors, including gender, age, lifestyle, and drug intervention, were controlled and the effects of these cytokines on the occurrence of MetS were investigated. The purpose of this study was to explore the relationship between circulating cytokine clusters and MetS as well as its components, such as ZAG, irisin, adiponectin, and betatrophin and to provide a basis for forecasting the occurrence of MetS in young women. Another aim of the study was to assess the impact of high insulin levels on circulating ZAG, irisin, and betatrophin concentrations by the euglycemic–hyperinsulinemic clamp (EHC). We also compared the characteristics of each MetS component in the study population.

Materials and methods

Research population

This study was performed between March 2018 and July 2019 in The Second Affiliated Hospital, Chongqing Medical University, China. A total of 196 young women (aged 20–35 years) from the community, university, and normal physical examination population (normal physical examination means a routine physical examination conducted once a year) participated in the study. Exclusion criteria for study individuals included age >35 years, known CVD, thyroid disease, neoplasms, smoking, renal impairment (serum creatinine >120 mol/L) and any other conditions not suitable for the study as evaluated by the physician.

Written informed consent was obtained from the participants before the start of any research-related activities. The clinical study was approved by the Human Ethics Committee of Chongqing Medical University. According to Chinese Diabetes Society criteria (CDS guideline 2017), MetS components were (1) central obesity (waist circumference; WC ≥85 cm); (2) triglyceride (TG) ≥1.7 mmol/L; (3) high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L); (4) blood pressure (BP) ≥130/85 mmHg; (5) a fasting blood glucose (FBG) of ≥5.6 mmol/L or a blood glucose of ≥7.8 mmol/L after glucose overload (2h-BG) (20). Individuals with MetS and with one or two MetS components were defined as the MetS-C group. The control subjects were 78 healthy women from community,

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school or routine medical checkups once a year. In the past 6 months, none of the participants took any drugs.

Anthropometric measurements and assessment

Anthropometric examines, including blood pressure, WC, hip circumference (HC), were measured by the same researcher. The BMI was calculated (BMI = body weight/height kg/m^2). Homeostasis model assessment for IR ($HOMA_{IR}$ = FBG (mmol/L) × fasting insulin (FIns, UI/L)/22.5) was used for assessing IR (21). The area under the curve for glucose (AUCg) and insulin (AUCi) during the oral glucose tolerance test (OGTT) was calculated geometrically following the trapezoidal rule by using a statistical software program (22). Body adiposity index (BAI) was calculated as (HC (cm)/height (m)$^{1.5}$ − 18) (23). Visceral adiposity index (VAI) = WC/(36.58 + (1.89 × BMI)) × (triglyceride (TG)/0.81) × (1.52/ high-density lipoprotein cholesterol (HDL-C)) (24). All measurements were made at 08:30–09:30 h after overnight fasting.

Oral glucose tolerance test (OGTT)

After 12–14 h of fasting, the OGTT test was conducted after 75 g glucose was orally administered to all subjects, as a previous report (25).

Euglycemic-hyperinsulinemic clamp (EHC)

To understand the insulin sensitivity *in vivo*, the EHC was carried out under the condition of continuous insulin infusion to achieve the normal blood glucose and high insulin level *in vivo*. A catheter was placed in the antecubital vein for insulin and glucose infusion. Blood was drawn by another catheter inserted into the dorsal vein of the opposite hand. Blood glucose was maintained at 4–6 mmol by infusion of 20% glucose solution. The glucose disposal rate (GDR) was defined as the rate of glucose infusion (GIR) during the stable-state of the EHC and was related to body weight (M-value) as the previous population (26). Blood was collected at various times. The serum was stored at −80°C until used.

Treatment of blood samples

For the determination of cytokines and biochemical parameters, blood samples were taken after 12–14 h of fasting and the indicated time during the OGTT and EHC. Blood samples were centrifuged at 3913 ***g*** for 10 min. Serum was isolated and stored at −80°C until being used for cytokine and biochemical determination.

Laboratory measurements

Blood glucose and HbA1c were measured by the glucose-oxidase method or anion exchange HPLC, respectively. Total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), and low-density lipoprotein cholesterol were determined enzymatically using an autoanalyzer (Hitachi 747; Hitachi). Insulin were measured with a commercial kit (China institute of atomic energy, China); Free fatty acids (FFAs) were measured with a commercial kit (Randox Laboratories Ltd., Antrim, UK). HbA1C were measured with a commercial kit (Bio-Rad Inc.).

Cytokines measurements

Serum irisin, ZAG, betatrophin and adiponectin levels were determined by using ELISA kits from the CUSABIO Life Science, Inc. (Cat#: CSB-EQ027943HU, China) and Phoenix Pharmaceuticals, Inc. (Cat#: EK-067-29,USA) for irisin, Phoenix Pharmaceuticals Inc. (Cat#: EK-051-60,USA) for betatrophin, and the Ray Biotech, Inc. (Cat#: ELH-ZAG, China) for ZAG,and CUSABIO Life Science, Inc. (Cat#. CSB-E07270h, China) for adiponectin. According to the manufacturers' protocols, the limits of detection were 3.12–200 ng/mL for irisin, 0.041–10 ng/mL for ZAG, 0–100 μg/L for betatrophin, and 1.56–100 ng/mL for adiponectin. The intra- and inter-assay coefficients of variation were <8 and <9% for irisin, <5 and 9% for ZAG, <10 and <15% for betatrophin and <8 and <10% for adiponectin, respectively, as previously described (15, 16, 17). In addition, we evaluated irisin kits from CUSABIO and Phoenix Pharmaceuticals using the same samples. The values measured using the Phoenix Pharmaceuticals' kit were slightly lower than those determined using the CUSABIO kit, the difference was not statistically significant. Therefore, we concluded that both Phoenix Pharmaceuticals and CUSABIO kits were appropriate for the analysis of clinical samples.

Statistical analysis

SPSS version 22.0 (SPSS, Armonk, NY) software was used for all statistical analyses. All data are expressed as mean ± S.D., S.E., or median (interquartile range) where appropriate. TG and HDL-C concentrations were logarithmically transformed to obtain a normal distribution. An independent sample *t*-test or nonparametric test was performed for comparisons between two groups. Cytokine concentration was used as an independent variable

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for multiple stepwise regression analysis to analyze its correlation with other variables. The occurrence of MetS and its components was predicted by binary logistic regression analysis with cytokine concentration as the independent variable. A *P*-value <0.05 (two-tailed) was considered to be significant. G*Power can be used to compute effect sizes (tail = two; effect size = 0.5; α err pro = 0.05; power = 0.95).

Results

Clinical characteristics and biochemical parameters in study participants

The demographic, anthropometric, and laboratory parameters in young females are shown in Table 1. Compared with the control group, BMI, WC, BP, TG, HOMA-$_{IR}$, FBG, 2 h post-glucose load blood glucose (2h-BG), AUCg, AUCi, FIns, 2 h serum insulin after glucose overload (2h-Ins), VAI and BAI in the MetS-C group were significantly higher, while HDL-C and M-value were lower. Importantly, in the MetS-C group, the levels of circulating irisin and betatrophin were markedly higher than those in the control group (Fig. 1C and B) but the concentrations of serum ZAG and adiponectin were substantially lower (Fig. 1A and D).

The relationship between cytokines and MetS as well as its components

Table 2 shows correlation analysis of MetS and its components with circulating cytokine levels in the study population. Results indicated that all four cytokines were highly correlated with MetS and its components. FBG, WC, and TG were significantly correlated with the circulating levels of four cytokines (Table 2). Of the four cytokines, only betatrophin was associated with BP ($P < 0.05$). As shown in Table 2, there was no relationship between any cytokine and HDL-C.

Decreasing concentrations of serum adiponectin and ZAG displayed a significant linear trend. They were independently associated with MetS, MetS component, central obesity, hypertriglyceridemia, and hyperglycemia. However, increasing serum Irisin and betatrophin levels showed a significant linear trend and were independently

Table 1 Comparison of clinical features and biochemical parameters in the study population.

	Controls (*n* = 78)	**MetS-C** (*n* = 118)	***P***
Age (years)	25 (24–27)	26 (24–29)	0.052
BMI (kg/m^2)	19.9 (18.5–21.9)	23.3 (20.1–26.7)	<0.001
WC (cm)	70.0 (65.0–76.0)	78.5 (70.0–88.0)	<0.001
SBP (mmHg)	110 (104–116)	114 (105–120)	<0.01
DBP (mmHg)	73 (70–79)	78 (72–82)	<0.001
TG (mmol/L)	0.80 (0.59–1.06)	1.45 (0.82–2.24)	<0.001
TC (mmol/L)	4.14 ± 0.84	4.18 ± 1.17	0.717
LDL-C (mmol/L)	2.24 ± 0.71	2.40 ± 0.93	0.155
HDL-C (mmol/L)	1.31 (1.18–1.55)	1.12 (0.95–11.35)	<0.001
ZAG (mg/L)	46.7 (40.6–63.0)	39.8 (26.4–50.4)	<0.001
Irisin (μg/L)	178 (147–228)	207 (150–248)	<0.05
Betatrophin (μg/L)	0.38 (0.23–0.52)	0.51 (0.38–0.63)	<0.001
Adiponectin (μg/L)	41.2 (35.7–54.7)	36.5 (22.0–47.6)	<0.01
HOMA-$_{IR}$	1.50 (1.16–2.00)	2.18 (1.35–4.45)	<0.001
M-value (mg/min/kg)	9.17 (7.43–11.77)	6.04 (4.12–9.54)	<0.001
FBG (mmol/L)	4.48 (4.20–4.82)	4.77 (4.38–5.20)	<0.001
2h-BG (mmol/L)	5.41 (4.90–6.22)	6.77 (5.19–8.22)	<0.001
AUC$_g$	12.0 (11.0–13.6)	14.5 (11.8–17.5)	<0.001
FIns (mU/L)	7.55 (6.10–9.80)	9.89 (6.89–19.08)	<0.001
2h-Ins (mU/L)	47.4 (26.1–79.9)	88.8 (39.9–149.5)	<0.001
AUC$_i$	132 (76–162)	171 (109–254)	<0.001
VAI	0.96 (0.72–1.41)	2.27 (1.43–3.32)	<0.001
HbA1c	5.2 (5.0–5.3)	5.30 (5.0–5.5)	0.118
BAI	26.4 ± 2.9	29.2 ± 4.7	<0.001

Values were given as mean ± s.d. or median (interquartile range).2h-BG, 2 h post-glucose load blood glucose; 2h-Ins, 2 h plasma insulin after glucose overload; AUC, the area under the curve for glucose or insulin; BAI, body adiposity index; DBP, diastolic blood pressure; FBG, fasting blood glucose; FIns, fasting plasma insulin; HDL-C, high-density lipoprotein cholesterol; HOMA-$_{IR}$, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; M-value, the rate of glucose infusion; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; VAI, visceral adiposity index; WC, waist circumference.

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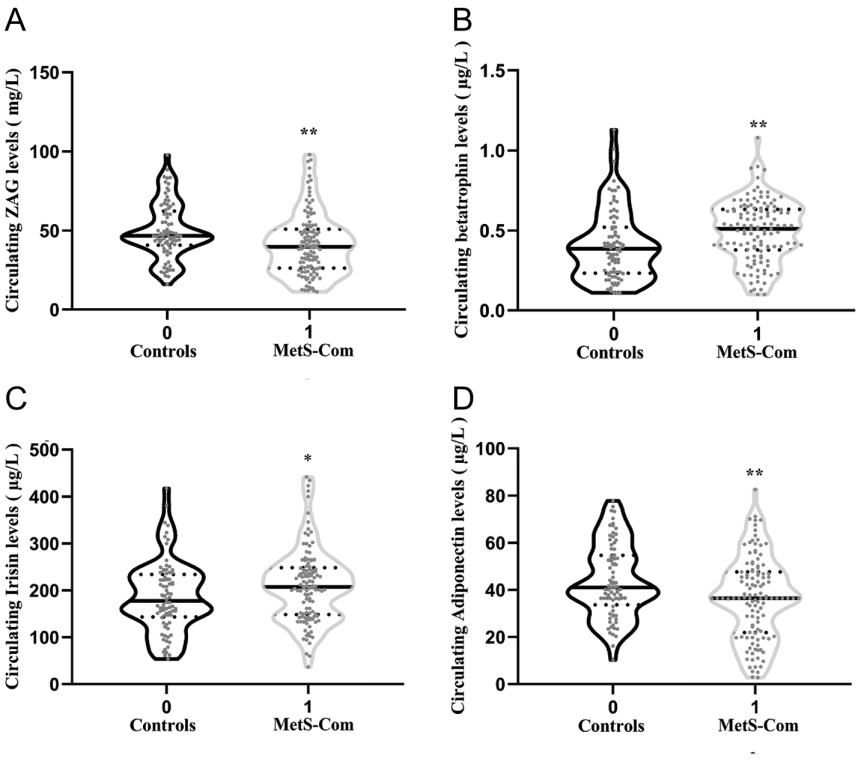

Figure 1
Circulating levels of cytokines in healthy women and women with MetS components. (A) circulating ZAG levels. (B) circulating betatrophin levels. (C) circulating irisin levels. (D) circulating adiponectin levels. MetS-Com, with MetS components. Data are expressed as median (interquartile range). *P < 0.05, **P < 0.01.

related to MetS, MetS component, WC, TG, and FBG (Table 2).

Table 3 shows the multiple stepwise regression analysis of four cytokines as independent variables for predicting MetS and its components. Among the two decreased cytokines, adiponectin remained independently and negatively related to WC and TG, while serum ZAG was negatively associated with WC, SBP, TG, and blood glucose, after adjusting for age. Of the two increased cytokines, irisin was independently and positively associated with WC, TG, HDL-C, and 2h-BG, whereas betatrophin was positively associated with WC and SBP (Table 3).

Prediction of MetS and its components from circulating cytokines

As shown in Table 4, binary logistic regression analyses were performed for serum adiponectin, ZAG, irisin, and betatrophin levels as independent variables to predict MetS and its components. After adjustment for age, adiponectin was negatively associated with central obesity (WC ≥85 cm) and hyperglycemia (FBG ≥5.6 mmol/L or 2h-BG ≥7.8 mmol/L), and the OR-values were 0.957 and 0.96, respectively. ZAG was negatively associated with MetS, obesity (WC ≥85 cm), hypertriglyceridemia (TG ≥1.7 mmol/L), and hyperglycemia, and the OR-values were

Table 2 Correlation analysis of MetS and its components with circulating cytokine levels in the study population.

Variables	Adiponectin		ZAG		Irisin		Betatrophin	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P
MetS (12.1%)[a]	19.76	<0.001	33.69	<0.001	9.584	<0.01	17.09	<0.001
MetS-C (59.3%)	7.949	<0.01	13.47	<0.001	12.882	<0.001	11.02	<0.05
WC ≥85 cm (20.6%)	23.98	<0.001	27.14	<0.001	17.056	<0.001	28.28	<0.001
TG ≥1.7 mmol/L (24.6%)	8.001	<0.01	18.47	<0.001	5.830	<0.05	10.60	<0.001
HDL-C <1.04 mmol/L (25.6%)	1.612	0.204	0.960	0.327	2.299	0.129	2.868	0.090
BP ≥130/ 85 mmHg (13.1%)	1.214	0.271	2.182	0.140	0.140	0.708	4.539	<0.05
FBG ≥5.6 or 2h-BG ≥7.8 mmol/L (21.6%)	29.44	<0.001	44.30	<0.001	19.052	<0.001	17.30	<0.001

[a]% of the study population; MetS-C, Individuals with at least one MetS component.

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Table 3 Multiple line regression analysis (stepwise) predicting MetS components with various cytokines.

Variable	R^2	Adiponectin		ZAG		Irisin		betatrophin	
		β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
WC	0.397	−0.174 (−0.256, −0.091)	<0.001	−0.141 (−0.214, −0.067)	<0.001	0.031 (0.014, 0.047)	<0.001	11.599 (5.079, 18.120)	<0.001
SBP	0.067	–	–	−0.091 (−0.165, −0.018)	<0.05	–	–	6.942 (0.124, 13.760)	<0.05
DBP	0.059	–	–	–	–	–	–	–	–
FBG	0.059	–	–	−0.012 (−0.019, −0.005)	< 0.001	–	–	–	–
TG[a]	0.163	−0.003 (−0.005, 0.000)	<0.05	−0.002 (−0.004, 0.000)	<0.05	0.001 (0.000, 0.001)	<0.001	–	–
HDL-C[a]	0.021	–	–	–	–	0.000 (0.000, 0.001)	<0.05	–	–
2h-BG	0.29	–	–	−0.060 (−0.075, −0.044)	<0.001	0.006 (0.002, 0.010)	<0.01	–	–

[a]Variables were logarithmically transformed to obtain a normal distribution.

0.897, 0.965, 0.972, and 0.922, respectively. Serum irisin was positively associated with obesity and hyperglycemia, and the OR-values were 1.007 and 1.011, respectively. Serum betatrophin levels were positively associated with MetS and obesity, and OR-values were 17.76 and 20.76, respectively (Table 4).

Association of four cytokines with hyperinsulinemia

To investigate the impact of hyperinsulinemia on the circulating levels of four cytokines *in vivo*, we performed an EHC study in both healthy women and women with MetS components.

In healthy women, the serum insulin level increased from 8.0 ± 3.1 to 103 ± 22 μm/L. In women with MetS components, the insulin level increased from 19.0 ± 4.4 to 96.2 ± 21.6 μm/L during the EHC. Blood glucose was maintained at 4.0–6.0 mmol/L with 20% glucose infusion. During the stable-state of the clamp, women with MetS components had a lower M-value than healthy controls (6.04 (4.12–9.54) vs 9.17 (7.43–11.77) mg/kg per min; $P < 0.01$; Table 1) indicating IR in women with MetS components. In response to increased insulin levels, serum ZAG concentrations were markedly increased in women with or without MetS components during the EHC ($P < 0.05$ or $P < 0.01$ vs 0 min; Fig. 2A). However, on the contrary, serum irisin, betatrophin, and adiponectin levels in both healthy and MetS-C women dropped significantly compared with baseline (Fig. 2B, C and D). Our data indicate that the circulating levels of four cytokines were affected by hyperinsulinemia.

Discussion

Study of novel cytokines linking metabolic disorder to related co-morbidities has become an important topic. However, the increasing number of new cytokines has led to an increased need to identify the function of these cytokines, their molecular targets, and potential clinical relevance as biomarkers for obesity and metabolic diseases. In this study, we revealed the different effects of MetS and its components on the circulating levels of four cytokines, including irisin, ZAG, betatrophin, and adiponectin, in young women. Different severity or conditions of MetS may lead to different metabolic disorders and circulating cytokine levels, and oral drugs may also affect the measurement results. Therefore, to avoid undetectable confusion and deviation, in this study, we enrolled a cohort of homogenous women with one or more MetS components who did not receive medication or other interventions. This study differs from previous studies in the following ways: (1) four cytokines were studied at the same time, and their relationship with each component of MetS was compared; (2) to eliminate the interference of age with IR, we selected young women for the study; and (3) to exclude the interference of gender and sex hormones, women were used as study subjects.

Study results revealed that in women with MetS components, the levels of serum irisin and betatrophin were significantly higher, while the levels of serum ZAG and adiponectin were significantly lower than those in healthy women. These results indicated that these four cytokines have a two-way change in response to metabolic disorders. Similar to some previous studies, serum betatrophin and irisin levels were high in women with PCOS or T2DM patients (16, 17, 27, 28). However, in other studies, serum betatrophin and irisin levels were found to be low in metabolic-related diseases (18, 29). These differences might be related to age, gender, included population, measurement methods, and different metabolic characteristics in these diseases.

The mechanism of abnormal increase in serum betatrophin and irisin levels in women with MetS-C is unclear at present, and we speculate that increased betatrophin and irisin may be an adaptive response that compensates for IR and metabolic disorders associated

Table 4 Binary logistic regression analyses predicting MetS and its components with cytokines as independent variables.

Variables	Nagelkerke	Adiponectin			ZAG			Irisin			Betatrophin		
	R^2	β	OR (95% CI)	*P*	β	OR (95% CI)	*P*	β	OR (95% CI)	*P*	β	OR (95% CI)	*P*
MetS	0.467	−0.02	0.98 (0.945, 1.016)	0.266	−0.109	0.897 (0.848 ,0.949)	<0.001	0.005	1.005 (0.998, 1.013)	0.170	2.877	17.762 (1.033, 305.435)	<0.05
MetS-C	0.12	−0.016	0.984 (0.964, 1.004)	0.109	−0.014	0.987 (0.969, 1.004)	0.129	0.002	1.002 (0.998, 1.007)	0.248	1.199	3.317 (0.672, 16.383)	0.141
WC (≥85 cm)	0.422	−0.044	0.957 (0.928, 0.987)	<0.01	−0.036	0.965 (0.936, 0.994)	<0.05	0.007	1.007 (1.002, 1.013)	<0.01	3.033	20.756 (2.323, 185.411)	<0.01
TG (≥1.7 mmol)	0.158	−0.011	0.989 (0.966, 1.012)	0.339	−0.029	0.972 (0.949, 0.995)	<0.05	0.003	1.003 (0.998, 1.008)	0.219	1.114	3.047 (0.517, 17.961)	0.218
HDL-C (<1.04 mmol)	0.043	0.005	1.005 (0.983, 1.027)	0.666	0.002	1.002 (0.983, 1.022)	0.836	−0.004	0.996 (0.992, 1.001)	0.14	−0.863	0.422 (0.074, 2.416)	0.332
BP (≥130/ 85 mmHg)	0.027	−0.007	0.993 (0.965, 1.021)	0.602	−0.001	0.999 (0.974, 1.024)	0.923	0.00	1.00 (0.994, 1.005)	0.897	1.426	4.164 (0.502, 34.536)	0.186
FBG (≥5.6 mmol) or 2 hBG (≥7.8 mmol)	0.522	−0.04	0.96 (0.930, 0.992)	<0.05	−0.081	0.922 (0.887, 0.959)	<0.001	0.011	1.011 (1.005, 1.017)	<0.001	0.869	2.385 (0.248, 22.912)	0.451

with MetS components as previously reported (17). In addition, the positive correlation between irisin and WC, TG, HDL-C, and blood glucose may also reflect a compensatory increase in irisin levels in MetS-C women to counterbalance metabolic disorders. However, in line with previous publications related to T2DM and MetS (15, 30, 31, 32, 33), serum ZAG and adiponectin were reduced in women with MetS components in the current study. Unlike our results, a previous study found no significant difference in circulating ZAG levels between MetS patients and healthy controls (34). The reason for decreasing ZAG and adiponectin in MetS women is unclear. We speculate that during the EHC, acute hyperinsulinemia stimulates ZAG secretion and increases serum ZAG leves. However, in MetS patients, long-term hyperinsulinemia may lead to decreasing ZAG secretion or increased consumption in the liver or adipose tissues, which leads to a decrease in circulating ZAG level. However, the underlying mechanism needs further study. In addition, we believe that the alteration of the circulating levels of these cytokines might reflect their different roles in metabolic disorders. Linear regression analysis showed that FBG, WC, and TG were significantly correlated with the circulating levels of the four cytokines, which further suggests that these cytokines are closely related to glucose and lipid metabolism, as well as obesity, which are important components of MetS. However, there have been different reports on the correlation between these cytokines and MetS component. For example, Souichi *et al*. reported that serum ZAG was associated with BP, but not with body weight, blood glucose and blood lipids (35). Our results are also consistent with those of previous basic studies. For example, studies have found that ZAG regulates the expression of genes and enzymes related to lipid metabolism, such as PPARγ, SREBP-1 and UCP1 (36). In addition, the current results are similar to those reported by us and others in T2DM and PCOS population (13, 16, 17, 18), which further supports the hypothesis that these cytokines are significantly related to glucose and lipid metabolism and IR when mixed factors such as gender and age are excluded. Therefore, we believe that in young women, the greater the number of MetS components, the more obvious the cytokine disorder.

To further investigate the relationship between these four cytokines and IR, we performed EHCs, a gold standard for evaluating insulin sensitivity, to observe the effects of hyperinsulinemia on cytokines in healthy controls and individuals with MetS components. We found that in response to hyperinsulinemia, serum ZAG levels increased significantly in both healthy individuals and subjects

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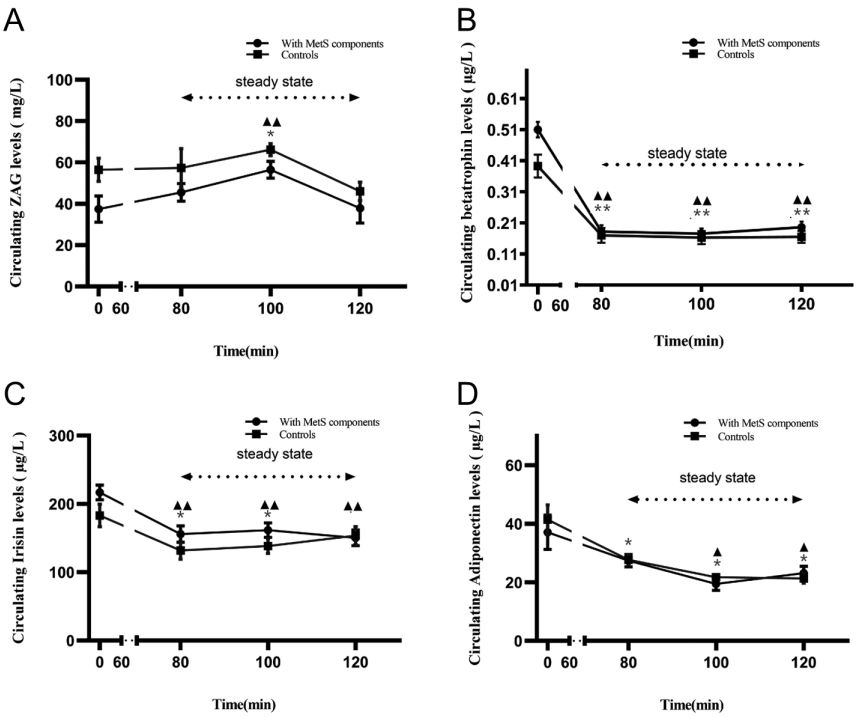

Figure 2
Circulating levels of cytokines in healthy women and women with MetS components during the EHC. (A) circulating ZAG levels. (B) circulating betatrophin levels. (C) circulating irisin levels. (D) circulating adiponectin levels. MetS-Com, with MetS components. Data are expressed as means ± S.E. *P < 0.05, **P < 0.01 vs 0 min for controls; ▲P < 0.05, ▲▲P < 0.01 vs 0 min for MetS-Com.

with MetS components, suggesting that hyperinsulinemia stimulates ZAG synthesis or release into the circulation. This result also indicated that acute hyperinsulinemia increased ZAG release regardless of the presence or absence of metabolic disorder *in vivo*. In contrast to ZAG, in response to acute hyperinsulinemia, circulating levels of the other three cytokines were significantly reduced in both healthy controls and subjects with MetS components, indicating that their release was inhibited or their consumption was increased due to acutely elevated insulin concentrations in the circulation. Our data demonstrate that insulin levels regulate these cytokines. It is important to note that we measured insulin sensitivity by the most sensitive method, an EHC, and can therefore exclude the possibility that other factors, which regulate both these cytokines and insulin sensitivity, may affect these established associations. However, the underlying mechanisms need further research.

There are several limitations of the current research: (1) the sample size is relatively small, especially in the intervention study. Therefore, further studies with a larger sample size are needed; (2) some cytokines have been found to act as bioactive regulators that ameliorate energy metabolism and IR. It is better to observe the changes in circulating cytokine levels in a long-term and dynamic way; (3) The current data do not include a follow-up measurement of these cytokines, therefore, the final relationship between these cytokines and MetS components needs further evaluation in future studies; (4) our study does not add to knowledge on the functions of these cytokines, and it is impossible to tell whether the changes of these cytokines in circulation have any pathophysiological relevance; (5) when an irisin ELISA kit is used to detect complex protein samples, such as serum, the signal will include other irisin proteins and cross-reacting proteins (37). In addition, the relationship between IR and serum factors, such as retinol binding protein-4 (RBP4) and fatty acid-binding protein 4 (FABP4), has been evaluated by EHCs (38, 39). These serum factors were not determined in the current study. However, these limitations will stimulate further study on cytokine patterns related to metabolic disease. We believe that the present study will help us to understand the relationship between these cytokine clusters and MetS, and attract the interest of other researchers.

Conclusions

We identified cytokine clusters related to MetS components and identified the impacts of MetS components on circulating cytokine levels in young women with different MetS components. We analyzed the regulating roles of blood glucose and insulin on these cytokines and explored their relationship with metabolic disorders. Our results revealed that ZAG, irisin,

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adiponectin, and betatrophin may be biomarkers for screening MetS components.

Declaration of interest

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

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Data availability

The clinical data used to support the findings of this study are included within the article.

Ethics approval

Before participating in the study, all subjects signed informed consent. The study was approved by the Human Research Ethics Committee of Chongqing Medical University and is consistent with the institutional guidelines. The study was performed in accordance with the Helsinki Declaration.

Author contribution statement

X T, W H, S T, S Y and H D researched and analyzed data. L L and G Y wrote and edited the manuscript. Y W is the guarantor of this work and, S X reviewed and edited the manuscript as such, had full access to all of 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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