Research Square

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Serum Apelin-12 as a Novel Marker of Metabolic Inflammatory Syndrome in elderly Chinese

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Research Article

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Abstract

***Purpose**:* Apelin-12 has been proposed as a novel adipocytokine with vital roles in metabolic disorders. In this study, we aimed to explore the relationship between serum apelin-12 and metabolic inflammatory syndrome (MIS) in the elderly.

***Methods**:* The cross-sectional study involved 224 subjects aged over 60. According to their diagnoses of the four components of MIS (type 2 diabetes, non-alcoholic fatty liver disease, atherosclerosis, and obesity/overweight), they were divided into two groups: the control group (0 or 1 component n=63) and the MIS group (≥2 components, n=161). Serum levels of apelin-12, adiponectin and C-reactive protein were measured by ELISA.

***Results**:* Serum apelin-12 was significantly higher in the MIS group than in the control group (1.83 ng/mL (1.59-2.07) vs 1.66 ng/mL (1.45-1.92), P=0.001). Partial correlation analysis showed that apelin-12 levels were positively correlated with fasting insulin (r^a=0.169), HOMA-IR (r^a=0.183), ALT (r^a=0.180), AST (r^a=0.145) and CRP (r^a=0.140, all P^a<0.05) and negatively associated with adiponectin (r^a=-0.211) and HDL-C (r^a=-0.156) after controlling age, sex and body mass index. Besides, after adjustment for age, sex, fasting blood glucose, diastolic blood pressure and total triglyceride, apelin-12 was still determined as an independent predictor for MIS.

***Conclusion**:* Serum apelin-12 can be an independent risk factor predicting metabolic inflammatory syndrome and may serve as a useful potential marker for the diagnosis of MIS in elderly Chinese.

Introduction

Metabolic inflammatory syndrome (MIS) has been proposed as a novel concept with a cluster of diseases including type 2 diabetes (T2DM), non-alcoholic fatty liver disease (NAFLD), atherosclerosis (AS), and obesity/overweight [1]. Due to its high detective rate, MIS has also been proved to be more effective for the prediction of coronary heart disease than metabolic syndrome (MetS) [1]. It has been reported that macrophages played a pivotal role in the pathogenesis of MIS, indicating that inflammation could be a central part of the initiation and the development of the syndrome [2]. However, the pathophysiology of MIS has not been well understood yet. The early diagnosis in clinical practice will help a lot in the early intervention of this syndrome, which makes it urgent to identify potential related biomarkers.

Apelin was originally described as an endogenous ligand [3] of APJ which belongs to the G protein-coupled receptor family. Recognized as a new kind of adipokines, apelin is expressed and secreted by adipocytes both in humans and mice and is directly regulated by insulin [4].

Apelin-12 is one of the pharmacologically active apelin isoforms involved in various diseases such as intracranial atherosclerotic diseases [5], diabetes [6] and acute myocardial infarction [7]. Previous studies have demonstrated that serum apelin-12 was related to metabolic disorders. In Chinese obese girls,

apelin-12 was observed higher than that in non-obese girls [8]. Clinical evidence also showed significantly higher levels of serum apelin-12 in patients with metabolic syndrome both in children [9] and adults [10].

To date, the clinical relevance of serum apelin-12 levels with MIS in the elderly remains unknown. Therefore, the purpose of the current study was to explore the association of serum apelin-12 with MIS and its components in elderly Chinese to investigate the possible role of apelin-12 in MIS.

Methods

Participants and study design

This was a cross-sectional study. A total of 224 participants aged over 60 were recruited from the physical examination population of Huashan Hospital affiliated to Fudan University from May 2019 to June 2021. Among them, 63 subjects with 0 or 1 component of MIS were classified as the control group, while 161 patients with two or more of the four components–T2DM, NAFLD, AS and obesity/overweight–were defined as the MIS group. The diagnoses of these constituents were as follows:

(1) T2DM was diagnosed by the criteria of the World Health Organization in 1999 [11];

(2) NAFLD was diagnosed according to the World Gastroenterology Organisation global guidelines in 2014 [12];

(3) AS was diagnosed based on the Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017 [13];

(4) Overweight and obesity were defined as 25 $kg/m^2 \leq BMI < 30$ kg/m^2 and $BMI \geq 30$ kg/m^2, respectively [1, 14].

The exclusion criteria were any of the following: (1) type 1 diabetes, or diabetes induced by drugs or other endocrine disorders. (2) active infection or fever within two weeks before admission; (3) self-reported severe alcohol abuse; (4) autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, anaphylactoid purpura; (5) neuropathy or psychiatric diseases, such as multisystem atrophy, epilepsy, depression; (6) severe organ diseases; (7) tumors; (8) patients with incomplete clinical information or laboratory results.

Written informed consent was obtained from all the subjects. The research was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Boards of Huashan Hospital, Fudan University.

Questionnaire investigation, anthropometric and blood pressure measurements

Questionnaires were used to obtain detailed medical histories of all the subjects and the results were recorded by trained physicians. Participants who smoked at least 1 cigarette every day for more than 6 months were defined as smokers. Drinking was defined as an alcohol dose of more than 25 g per day for over 3 times every week. The weight of each individual was measured with an electronic scale with precision to 0.1 kilogram. The height was measured barefoot without heavy clothing and was recorded with an accuracy of 1 centimeter. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Resting blood pressure including systolic blood pressure (SBP) and diastolic blood pressure (DBP) was calculated as the average of three measurements measured by an electronic sphygmomanometer in a sitting position after 15-min rest.

Biochemical measurements and laboratory procedures

Venous blood samples were collected in the morning after overnight fasting, and immediately sent to the laboratory for detection. Fasting blood glucose (FBG), total cholesterol (TC), serum triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), blood urea nitrogen (BUN), serum creatinine (Scr), alanine aminotransaminase (ALT), aspartate transaminase (AST) and uric acid (UA) were detected using the Hitachi Automatic 7600 – 210 analyzer (Hitachi, Ltd., Tokyo, Japan). Serum insulin, C-reactive protein (CRP), and adiponectin were measured by ELISA kit (DiLab, Shanghai, China). Glycated hemoglobin A1c (HbA1c) levels were determined using the Variant device (Bio-Rad Laboratories, Hercules, CA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin (FINS) (milliunits per liter) × FBG (millimoles per liter)/22.5 [15]. The evaluation of carotid arteries, vertebral arteries, lower extremity arteries and liver were performed by ultrasound.

Measurement of apelin-12

The serum samples were stored at -80°C until analyzed. Serum apelin-12 concentrations were measured in duplicate using a commercial enzyme-linked immunosorbent assay kit (CSB-E08639h, CUSABIO, Wuhan, China) according to the manufacturer's instructions. The corresponding intra- and inter-assay coefficients of variation for apelin-12 the ELISA were 5.9% and 8.4%, respectively.

Statistical analysis

Kolmogorov-Smirnov test was used to determine the distribution of continuous variables. Normally distributed data are presented as the mean ± standard deviations, while non-parametric data were described as median with the interquartile range (IQR). Categorical variables were presented as percentages (%). Comparisons between the control and the MIS groups were carried out by unpaired Student's t-test and Mann-Whitney U test for variables with normal or skewed distribution, respectively. Chi-squared test and continuity correction test were used for categorical variables. Kruskal-Wallis test was used to compare the difference in apelin-12 among the four groups. Firth's bias-reduced logistic regression analysis was adopted to clarify the relationship between serum apelin-12 and MIS. Correlations between apelin-12 and various clinical parameters were analyzed using Spearman's rank correlation and partial correlation analysis. Statistical analyses were performed by SPSS version 26.0

(IBM Corp., Armonk, NY), Prism 9 (GraphPad, San Diego, CA) and R version 4.1.2 (Foundation for Statistical Computing). All tests were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Characteristics of participants

A total of 224 participants were recruited in our study. The basic demographic and clinical characteristics of two groups are presented in Table 1. The median ages of the control and the MIS group were 73 and 75, respectively (Table 1). Compared with controls, the MIS group exhibited higher BMI, SBP and DBP. Concerning metabolic parameters, significantly higher levels of FBG, fasting insulin, HOMA-IR, HbA1c, TG, BUN, Scr, UA, ALT and CRP were found in the MIS group (all $P < 0.05$, Table 1). However, TC, LDL-C, HDL-C and adiponectin were lower in the MIS group compared to the control group (all $P < 0.05$, Table 1). In general, the values of FBG, HbA1c and blood lipids (TC, TG, LDL-C, HDL-C) in both groups were mostly within the normal range, indicating that glycolipid metabolism indexes were basically well-controlled in most subjects. Besides, no statistical differences were observed in sex, smoking status, alcohol consumption, and the levels of AST between the two groups (all $P > 0.05$, Table 1).

Table 1
Characteristics of study population

Characteristic	Total	Control	MIS	*P* value
Subjects	224	63	161	-
Age (years)	74.00 (69.00–82.00)	73.00 (70.00–80.00)	75.00 (69.00–82.00)	0.381
Sex (male/female)	215/9	59/4	156/5	0.464
Smoking, n (%)	10 (4.46)	1 (1.59)	9 (5.59)	0.345
Drinking, n (%)	18 (8.04)	3 (4.76)	15 (9.32)	0.260
BMI (kg/m^2)	25.80 (23.10-27.25)	22.20 (20.60–23.10)	26.60 (25.50–27.70)	< 0.001
SBP (mmHg)	145.13 ± 17.85	133.95 ± 14.69	149.85 ± 16.97	< 0.001
DBP (mmHg)	77.14 ± 10.37	73.37 ± 6.31	78.73 ± 11.31	< 0.001
FBG (mmol/L)	5.70 (5.23–6.70)	5.30 (5.10–5.50)	6.10 (5.40–7.40)	< 0.001
Fasting insulin (mIU/L)	8.66 (7.59–10.06)	6.43 (5.50–7.52)	9.48 (8.46–10.45)	< 0.001
HOMA-IR	2.28 (1.81–2.83)	1.46 (1.30–1.80)	2.57 (2.21–3.12)	< 0.001
HbA1c (%)	6.10 (5.70–6.70)	5.80 (5.60–5.90)	6.40 (5.95–6.90)	< 0.001
TC (mmol/L)	4.61 ± 0.94	4.85 ± 0.86	4.51 ± 0.95	0.014
TG (mmol/L)	1.43 (1.10–1.86)	1.22 (0.95–1.60)	1.52 (1.13–1.92)	0.001
LDL-C (mmol/L)	2.86 (2.10–3.46)	3.12 (2.45–3.60)	2.75 (1.96–3.38)	0.027
HDL-C (mmol/L)	1.15 (0.98–1.31)	1.15 (0.98–1.31)	1.08 (0.95–1.27)	< 0.001
BUN (mmol/L)	5.80 (5.00-6.68)	5.40 (4.80–6.20)	6.10 (5.10–6.80)	0.003
Scr (μmol/L)	77.00 (68.00-87.75)	72.00 (65.00–80.00)	80.00 (70.50–92.50)	< 0.001
UA (μmol/L)	389.39 ± 84.29	338.14 ± 70.49	409.45 ± 80	< 0.001
ALT (IU/L)	20.00 (15.00-28.75)	18.00 (14.00–23.00)	21.00 (15.00–32.00)	0.002

Characteristic	Total	Control	MIS	*P* value
AST (IU/L)	20.00 (17.00–25.00)	20.00 (17.00–25.00)	21.00 (17.00–25.00)	0.990
CRP (mg/L)	2.96 (2.55–3.44)	2.42 (2.05–2.61)	3.18 (2.89–3.60)	< 0.001
Adiponectin (μg/mL)	7.15 (5.73–8.11)	8.84 (7.80–9.62)	6.37 (5.43–7.48)	< 0.001
Apelin-12(ng/mL)	1.78 (1.56–2.05)	1.66 (1.45–1.92)	1.83 (1.59–2.07)	0.001

Difference in serum apelin-12 among participants without and with MIS

The distribution of serum apelin-12 in the study population was skewed with a median of 1.78 ng/mL (1.56–2.05) (Fig. 1). As shown in Fig. 2, median apelin-12 levels in participants without and with MIS were 1.66 ng/mL (1.45–1.92) and 1.83 ng/mL (1.59–2.07), respectively ($P = 0.001$, Table 1). Additionally, serum apelin-12 levels had an increasing trend with the increasing number of MIS components, except that the median value between patients with two and three components was of no statistical difference (Fig. 3). Notably, the median levels of apelin-12 in subjects with two (1.77 ng/mL) or four (2.05 ng/mL) components were significantly higher than that with zero/one (1.65 ng/mL) components (all $P < 0.05$, Fig. 3).

Association between serum apelin-12 and MIS

Firth's bias-reduced logistic regression model was adopted to determine the relationship between serum apelin-12 and MIS. After adjustment for potential confounding factors including age, sex, FBG, DBP and TG, four predictors–apelin-12 (OR = 10.381), BMI (OR = 1.654), HbA1c (OR = 1.512) and SBP (OR = 1.048) were determined as independent risk factors for MIS (all $P < 0.05$, Table 2).

Table 2
Firth's bias-reduced logistic regression analysis: independent predictors for MIS

	B	OR	95%CI		*P* value
			Lower	Upper	
Age	0.033	1.033	0.957	1.120	0.404
Sex	-0.540	0.583	0.031	8.426	0.724
BMI	0.503	1.654	1.399	2.020	< 0.001
FBG	0.411	1.509	0.617	2.672	0.320
HbA1c	0.414	1.512	1.041	8.162	0.029
SBP	0.047	1.048	1.012	1.090	0.007
DBP	0.012	1.012	0.947	1.081	0.720
TG	0.334	1.396	0.696	3.506	0.371
Apelin-12	2.340	10.381	1.842	69.050	0.007

Correlations between serum apelin-12 and other measured parameters

Spearman's rank analysis showed that apelin-12 had positive associations with BMI (r = 0.135), SBP (r = 0.159), FBG (r = 0.144), fasting insulin (r = 0.191), HOMA-IR (r = 0.195), ALT (r = 0.240), AST (r = 0.145) and CRP (r = 0.153), and negative associations with HDL-C (r=-0.178) and adiponectin (r=-0.221, all P < 0.05, Table 3).

Table 3
Correlations between serum apelin-12 and other measured parameters

variable	Apelin-12		Apelin-12 (age, sex, BMI-adjusted)	
	r	*P* value	r^a	P^a
Age	-0.076	0.258	-	-
BMI	0.135	0.046	-	-
SBP	0.159	0.021	0.135	0.054
DBP	0.099	0.155	0.090	0.199
FBG	0.144	0.031	0.118	0.083
Fasting insulin	0.191	0.004	0.169	0.013
HOMA-IR	0.195	0.003	0.183	0.007
HbA1c	0.112	0.095	0.055	0.417
BUN	0.012	0.857	0.026	0.701
Scr	-0.003	0.964	-0.010	0.879
UA	0.077	0.249	0.057	0.404
TC	-0.088	0.189	-0.131	0.053
TG	0.090	0.181	-0.029	0.670
LDL-C	-0.072	0.284	-0.090	0.183
HDL-C	-0.178	0.008	-0.156	0.021
ALT	0.240	< 0.001	0.180	0.008
AST	0.145	0.030	0.145	0.032
CRP	0.153	0.022	0.140	0.038
Adiponectin	-0.221	0.001	-0.211	0.002

r: Spearman's correlation coefficient.

r and *P* are the unadjusted correlation coefficients and associated p-value. r^a and P^a are values after adjustment with age, gender and BMI.

Furthermore, after adjusting for age, sex and BMI, serum apelin-12 remained positively correlated with fasting insulin (r^a=0.169), HOMA-IR (r^a=0.183), ALT (r^a=0.180), AST (r^a=0.145) and CRP (r^a=0.140, all P^a<0.05) by partial correlation analysis. Remarkably, negative correlations of apelin-12 with HDL-C (r^a=-0.156) and adiponectin (r^a=-0.211) were also observed (all P^a<0.05, Table 3). In addition, the associations between serum apelin-12 and other clinical parameters were not statistically significant.

Discussion

In the current study, we found that serum apelin-12 was significantly higher in the MIS group than that in the control group and was independently correlated with MIS. To the best of our knowledge, this is the first study to evaluate the correlation of apelin-12 with MIS in elderly Chinese, thus indicating the potential role of apelin-12 in the pathogenesis and the development of MIS.

As a ubiquitously expressed peptide, apelin was identified as a novel kind of adipokine expressed and secreted by adipocytes in both murine and human [16]. Due to the wide expression of apelin and its receptor APJ, it has been reported that apelin participated in various physiological processes, including energy metabolism such as glucose and lipid metabolism [17], endocrine response to stress, homeostasis and so on [18]. Previous clinical studies reported increased concentrations of apelin in T2DM and obesity in humans and animals [19, 20], which further confirms its essential role in metabolic disorders.

There are several isomers produced by enzymatic cleavage of proapelin that are bioactive, such as apelin-12 [21], which also has a variety of physiological and biological functions. Few studies have explored the levels of apelin-12 in patients with metabolic disorders [22]. Significantly elevated apelin-12 was found in obese children with metabolic syndrome (MetS) compared to those without MetS and was determined to be the independent risk factor for MetS [9]. Similar findings were reported by Helske et al. in middle-aged individuals [10]. This was also verified in our study that apelin-12 was increased in elderly patients with MIS in comparison with the control group. When divided by the number of four components, a general increasing trend of apelin-12 was found as the number of components increased, although no difference was found between the third group and other groups, which might be attributed to the small number of individuals.

To determine the predictive power of apelin-12 for MIS, Firth's bias-reduced logistic regression analysis has been performed. As a result, we identified apelin-12 as a risk predictor for MIS, even after the adjustment for several variates, which was also in accordance with the previous study in obese children [9]. In addition to apelin-12, BMI, HbA1c and SBP were also determined as independent factors for the presence of MIS, indicating the involvement of obesity, blood glucose and blood pressure in MIS.

Several studies described MIS as a state characterized by chronic low-grade inflammation (CLGI) [1, 14, 23, 24]. It had been widely accepted that CRP plays a huge part in the process of inflammation and the response to infection. During inflammatory conditions, the expression of CRP increases. Hence, it is considered to be a traditional marker of inflammation [25]. As one of the most abundant adipocytokines, adiponectin has been proved to be a classic adipocytokine with anti-inflammatory properties, as well as antidiabetic and anti-atherogenic activity [26, 27]. Various reports suggest the decline of plasma adiponectin concentration in obesity, diabetes and MetS and can be considered a potent predictor for MetS [28, 29]. Recently, circulating adiponectin was observed lower in elderly patients with MIS at the cross-sectional population level [24]. In our study, the MIS group exhibited higher levels of CRP and lower levels of adiponectin compared to controls, revealing the relatively inflammatory conditions in patients.

Most importantly, apelin-12 showed a significant positive correlation with CRP and a negative correlation with adiponectin in our research, which further confirmed the potential role of apelin-12 in MIS.

It is well-known that insulin resistance is a common mechanism of obesity, diabetes [30], NAFLD [31] and atherosclerosis [32], the four components of MIS. Insulin sensitivity is compromised in metabolic deterioration. The current study found that apelin-12 was positively related to fasting insulin and HOMA-IR after age-, sex- and BMI-adjustment, suggesting the underlying role of apelin-12 in insulin resistance. Besides, the expression of apelin has been reported to be directly up-regulated during adipocyte differentiation stage. This may partly explain the possible link between apelin and insulin [4]. In our study, positive associations of apelin-12 with liver function indexes (ALT and AST) and a negative correlation with HDL-C were also observed.

Both clinical and experimental studies illustrated that UA was a contributory causal factor in metabolic disorders [33]. We found an apparent increase in UA in the MIS group, whereas no significant association was found between apelin-12 and UA. Contrary to our expectations, lower levels of TC and LDL-C were found in the MIS group, which could be explained by the use of lipid-lowering drugs in these patients.

There are several limitations of our study to be noted. First, this is a cross-sectional study with no follow-up data. Therefore, the causal role of apelin-12 in MIS remains unclear. Second, since the participants in our study were over 60, it is unknown whether our results can be generalized to middle-aged or younger subjects. Moreover, the number of subjects was not large enough in this study, resulting in the wide width of the confidence interval of OR of apelin-12 by Firth's bias-reduced logistic regression analysis. Subsequent studies with a larger population should be conducted to follow up on the changes in serum apelin-12 over a few years both in healthy controls and MIS patients.

Conclusions

In conclusion, we found that serum apelin-12 was an independent risk predictor for MIS and was closely correlated with CRP and adiponectin, which could be a potential diagnostic indicator for MIS. Our findings provide deeper insights into the potential role of apelin-12 in the pathogenesis of MIS.

Declarations

Ethics approval and consent to participate

All procedures performed in the study were approved by the ethical standards of the Institutional Review Board of Huashan Hospital, Fudan University and were in accordance with the 1964 Helsinki declaration. Written informed consent was obtained from each participant.

Consent for publication

Not applicable

Availability of data and materials

Datasets are available from the corresponding author Houguang Zhou on reasonable request.

Competing interests

The authors report no declarations of interest.

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Authors' contributions

XRW, JTW, JQR, YHT, JCG and HGZ conceived and designed the study; JTW, JXW, YW, YTZ, YH, JQR, YTZ, CFH, WJX and LXX collected and managed the database; XRW, JTW, JQR and XMZ analyzed and interpreted the data; YHT, JCG and HGZ supervised the project administration; XRW wrote the first draft of the paper; YHT, JCG, HGZ reviewed the article. All authors commented on the results and read the submitted manuscript.

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References

1. R. Hu, Y. Xie, B. Lu, Q. Li, F. Chen, L. Li, J. Hu, Y. Huang, Q. Li, W. Ye, R. Li, N. Liu, J. Huang, Z. Zhang, L. Zhou, M. He, W. Fan, J. Liu, J. Wen, X. Zhu, Metabolic inflammatory syndrome: a novel concept of holistic integrative medicine for management of metabolic diseases. AME Med. J. **3**, 51–51 (2018). https://doi.org/10.21037/amj.2018.03.11
2. R. Li (2017) Progress in the pathogenesis of metabolic inflammatory syndrome. Fudan Univ J Med Sci 380–384
3. K. Tatemoto, M. Hosoya, Y. Habata, R. Fujii, T. Kakegawa, M.X. Zou, Y. Kawamata, S. Fukusumi, S. Hinuma, C. Kitada, T. Kurokawa, H. Onda, M. Fujino, Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem. Biophys. Res. Commun. **251**, 471–476 (1998). https://doi.org/10.1006/bbrc.1998.9489
4. J. Boucher, B. Masri, D. Daviaud, S. Gesta, C. Guigné, A. Mazzucotelli, I. Castan-Laurell, I. Tack, B. Knibiehler, C. Carpéné, Y. Audigier, J.-S. Saulnier-Blache, P. Valet, Apelin, a Newly Identified Adipokine Up-Regulated by Insulin and Obesity. Endocrinology **146**, 1764–1771 (2005). https://doi.org/10.1210/en.2004-1427

5. W. Hu, W. Jiang, L. Ye, Y. Tian, B. Shen, K. Wang, Prospective evaluation of the diagnostic value of plasma apelin 12 levels for differentiating patients with moyamoya and intracranial atherosclerotic diseases. Sci. Rep. **7**, 5452 (2017). https://doi.org/10.1038/s41598-017-05664-8
6. W.-Y. Ma, T.-Y. Yu, J.-N. Wei, C.-S. Hung, M.-S. Lin, Y.-J. Liao, D. Pei, C.-C. Su, K.-C. Lu, P.-H. Liu, C.-H. Lin, L.-M. Chuang, H.-L. Kao, J.-W. Lin, Y.-J. Chuang, H.-Y. Li, Plasma apelin: A novel biomarker for predicting diabetes. Clin. Chim. Acta **435**, 18–23 (2014). https://doi.org/10.1016/j.cca.2014.03.030
7. Y. Wei, B. Wang, The expression levels of plasma dimethylglycine (DMG), human maternally expressed gene 3 (MEG3), and Apelin-12 in patients with acute myocardial infarction and their clinical significance. Ann. Palliat. Med. **10**, 2175–2183 (2021). https://doi.org/10.21037/apm-21-122
8. H.-J. Ba, H.-S. Chen, Z. Su, M.-L. Du, Q.-L. Chen, Y.-H. Li, H.-M. Ma, Associations between Serum Apelin-12 Levels and Obesity-Related Markers in Chinese Children. PLoS ONE **9**, e86577 (2014). https://doi.org/10.1371/journal.pone.0086577
9. C. Yin, H. Zhang, M. Zhang, Y. Xiao, Adropin and apelin-12 efficiently predict metabolic syndrome in obese children. Pediatr. Diabetes **21**, 1132–1139 (2020). https://doi.org/10.1111/pedi.13101
10. B. Karbek, N.C. Bozkurt, O. Topaloglu, M.S. Aslan, A. Gungunes, E. Cakal, T. Delibasi, Relationship of vaspin and apelin levels with insulin resistance and atherosclerosis in metabolic syndrome. Minerva Endocrinol. **39**, 99–105 (2014)
11. P.G. Colman, D.W. Thomas, P.Z. Zimmet, T.A. Welborn, P. Garcia-Webb, M.P. Moore, New classification and criteria for diagnosis of diabetes mellitus. The Australasian Working Party on Diagnostic Criteria for Diabetes Mellitus. N Z. Med. J. **112**, 139–141 (1999)
12. D.R. Review Team, LaBrecque, Z. Abbas, F. Anania, P. Ferenci, A.G. Khan, K.-L. Goh, S.S. Hamid, V. Isakov, M. Lizarzabal, M.M. Peñaranda, J.F.R. Ramos, S. Sarin, D. Stimac, A.B.R. Thomson, M. Umar, J. Krabshuis, A. LeMair, World Gastroenterology Organisation, World Gastroenterology Organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J. Clin. Gastroenterol. **48**, 467–473 (2014). https://doi.org/10.1097/MCG.0000000000000116
13. M. Kinoshita, K. Yokote, H. Arai, M. Iida, Y. Ishigaki, S. Ishibashi, S. Umemoto, G. Egusa, H. Ohmura, T. Okamura, S. Kihara, S. Koba, I. Saito, T. Shoji, H. Daida, K. Tsukamoto, J. Deguchi, S. Dohi, K. Dobashi, H. Hamaguchi, M. Hara, T. Hiro, S. Biro, Y. Fujioka, C. Maruyama, Y. Miyamoto, Y. Murakami, M. Yokode, H. Yoshida, H. Rakugi, A. Wakatsuki, S. Yamashita, Committee for Epidemiology and Clinical Management of Atherosclerosis, Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017. J. Atheroscler Thromb. **25**, 846–984 (2018). https://doi.org/10.5551/jat.GL2017
14. Q. Wang, X.-L. Zhou, G.-H. Mei, J. Wang, Z. Yu, Y. Hu, X. Guo, H. Zhang, X. Xu, C. He, J. Guo, Y. Tao, H. Zhou, Serum Heparanase: A New Clinical Biomarker Involved in Senile Metabolic Inflammatory Syndrome. Diabetes Metab. Syndr. Obes. Targets Ther. **14**, 3221–3228 (2021). https://doi.org/10.2147/DMSO.S291612
15. D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin

concentrations in man. Diabetologia **28**, 412–419 (1985). https://doi.org/10.1007/BF00280883

16. J. Boucher, B. Masri, D. Daviaud, S. Gesta, C. Guigné, A. Mazzucotelli, I. Castan-Laurell, I. Tack, B. Knibiehler, C. Carpéné, Y. Audigier, J.-S. Saulnier-Blache, P. Valet, Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology **146**, 1764–1771 (2005). https://doi.org/10.1210/en.2004-1427
17. C. Bertrand, P. Valet, I. Castan-Laurell (2015) Apelin and energy metabolism. Front. Physiol. 6:. https://doi.org/10.3389/fphys.2015.00115
18. C. Li, H. Cheng, B.K. Adhikari, S. Wang, N. Yang, W. Liu, J. Sun, Y. Wang, The Role of Apelin–APJ System in Diabetes and Obesity. Front. Endocrinol. **13**, 820002 (2022). https://doi.org/10.3389/fendo.2022.820002
19. L. Li, G. Yang, Q. Li, Y. Tang, M. Yang, H. Yang, K. Li, Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Exp. Clin. Endocrinol. Diabetes Off J. Ger. Soc. Endocrinol. Ger. Diabetes Assoc. **114**, 544–548 (2006). https://doi.org/10.1055/s-2006-948309
20. D. Sacks, B. Baxter, B.C.V. Campbell, J.S. Carpenter, C. Cognard, D. Dippel, M. Eesa, U. Fischer, K. Hausegger, J.A. Hirsch, M. Shazam Hussain, O. Jansen, M.V. Jayaraman, A.A. Khalessi, B.W. Kluck, S. Lavine, P.M. Meyers, S. Ramee, D.A. Rüfenacht, C.M. Schirmer, D. Vorwerk, Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke. Int. J. Stroke **13**, 612–632 (2018). https://doi.org/10.1177/1747493018778713
21. H. Antushevich, M. Wójcik, Review: Apelin in disease. Clin. Chim. Acta **483**, 241–248 (2018). https://doi.org/10.1016/j.cca.2018.05.012
22. M.V. Heinonen, D.E. Laaksonen, T. Karhu, L. Karhunen, T. Laitinen, S. Kainulainen, A. Rissanen, L. Niskanen, K.H. Herzig, Effect of diet-induced weight loss on plasma apelin and cytokine levels in individuals with the metabolic syndrome. Nutr. Metab. Cardiovasc. Dis. **19**, 626–633 (2009). https://doi.org/10.1016/j.numecd.2008.12.008
23. X. Zhou, Q. Wang, G. Mei, J. Wang, Z. Yu, Y. Hu, X. Guo, H. Zhang, X. Xu, C. He, J. Guo, Y. Tao, H. Zhou, Serum Heparanase: A New Clinical Biomarker Involved in Senile Metabolic Inflammatory Syndrome. Diabetes Metab. Syndr. Obes. Targets Ther. Volume **14**, 3221–3228 (2021). https://doi.org/10.2147/DMSO.S291612
24. L. Ma, Y. Xu, Y. Zhang, T. Ji, Y. Li, Lower Levels of Circulating Adiponectin in Elderly Patients with Metabolic Inflammatory Syndrome: A Cross-Sectional Study. Diabetes Metab. Syndr. Obes. Targets Ther. **13**, 591–596 (2020). https://doi.org/10.2147/DMSO.S242397
25. N.R. Sproston, J.J. Ashworth, Role of C-Reactive Protein at Sites of Inflammation and Infection. Front. Immunol. **9**, 754 (2018). https://doi.org/10.3389/fimmu.2018.00754
26. H. Fang, R.L. Judd, Adiponectin Regulation and Function, in *Comprehensive Physiology*, 1st edn., ed. by R. Terjung (Wiley, 2018), pp. 1031–1063
27. A.A. Ghadge, A.A. Khaire, A.A. Kuvalekar, Adiponectin: A potential therapeutic target for metabolic syndrome. Cytokine Growth Factor Rev **39**, 151–158 (2018).

https://doi.org/10.1016/j.cytogfr.2018.01.004

28. J.-Y. Kim, S.V. Ahn, J.-H. Yoon, S.-B. Koh, J. Yoon, B.-S. Yoo, S.-H. Lee, J.-K. Park, K.-H. Choe, E. Guallar, Prospective Study of Serum Adiponectin and Incident Metabolic Syndrome. Diabetes Care **36**, 1547–1553 (2013). https://doi.org/10.2337/dc12-0223
29. A.E. Achari, S.K. Jain, Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int. J. Mol. Sci. **18**, 1321 (2017). https://doi.org/10.3390/ijms18061321
30. S.E. Kahn, R.L. Hull, K.M. Utzschneider, Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature **444**, 840–846 (2006). https://doi.org/10.1038/nature05482
31. M. Gaggini, M. Morelli, E. Buzzigoli, R.A. DeFronzo, E. Bugianesi, A. Gastaldelli, Non-Alcoholic Fatty Liver Disease (NAFLD) and Its Connection with Insulin Resistance, Dyslipidemia, Atherosclerosis and Coronary Heart Disease. Nutrients **5**, 1544–1560 (2013). https://doi.org/10.3390/nu5051544
32. A. Di Pino, R.A. DeFronzo, Insulin Resistance and Atherosclerosis: Implications for Insulin-Sensitizing Agents. Endocr. Rev. **40**, 1447–1467 (2019). https://doi.org/10.1210/er.2018-00141
33. M. Kanbay, T. Jensen, Y. Solak, M. Le, C. Roncal-Jimenez, C. Rivard, M.A. Lanaspa, T. Nakagawa, R.J. Johnson, Uric acid in metabolic syndrome: From an innocent bystander to a central player. Eur. J. Intern. Med. **29**, 3–8 (2016). https://doi.org/10.1016/j.ejim.2015.11.026

Figures

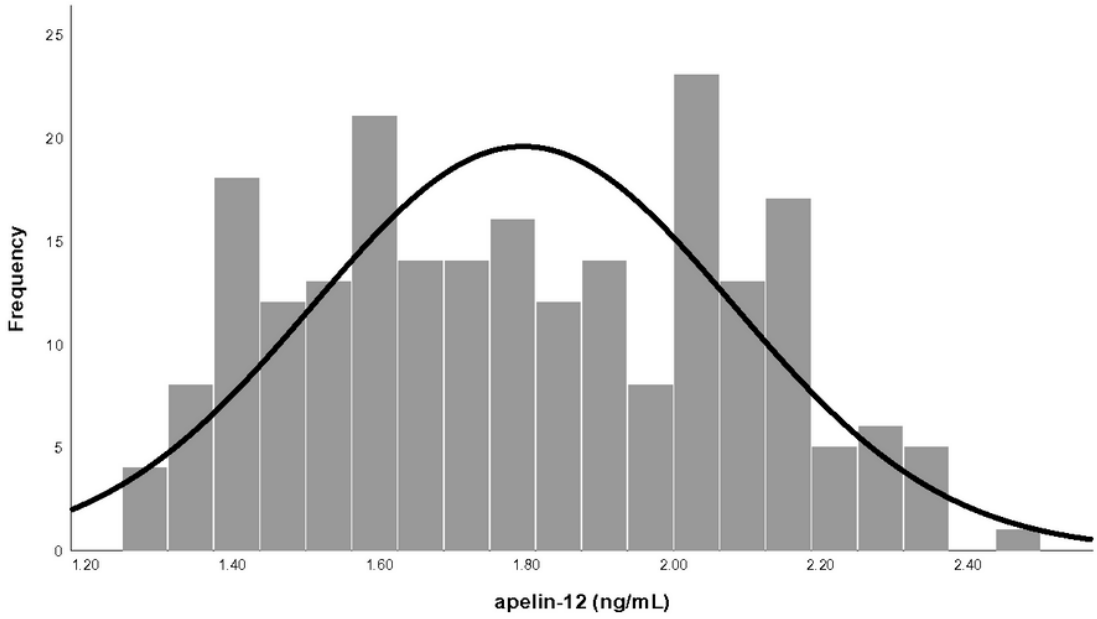

Figure 1

Distribution of serum apelin-12 in the participants

(created by SPSS)

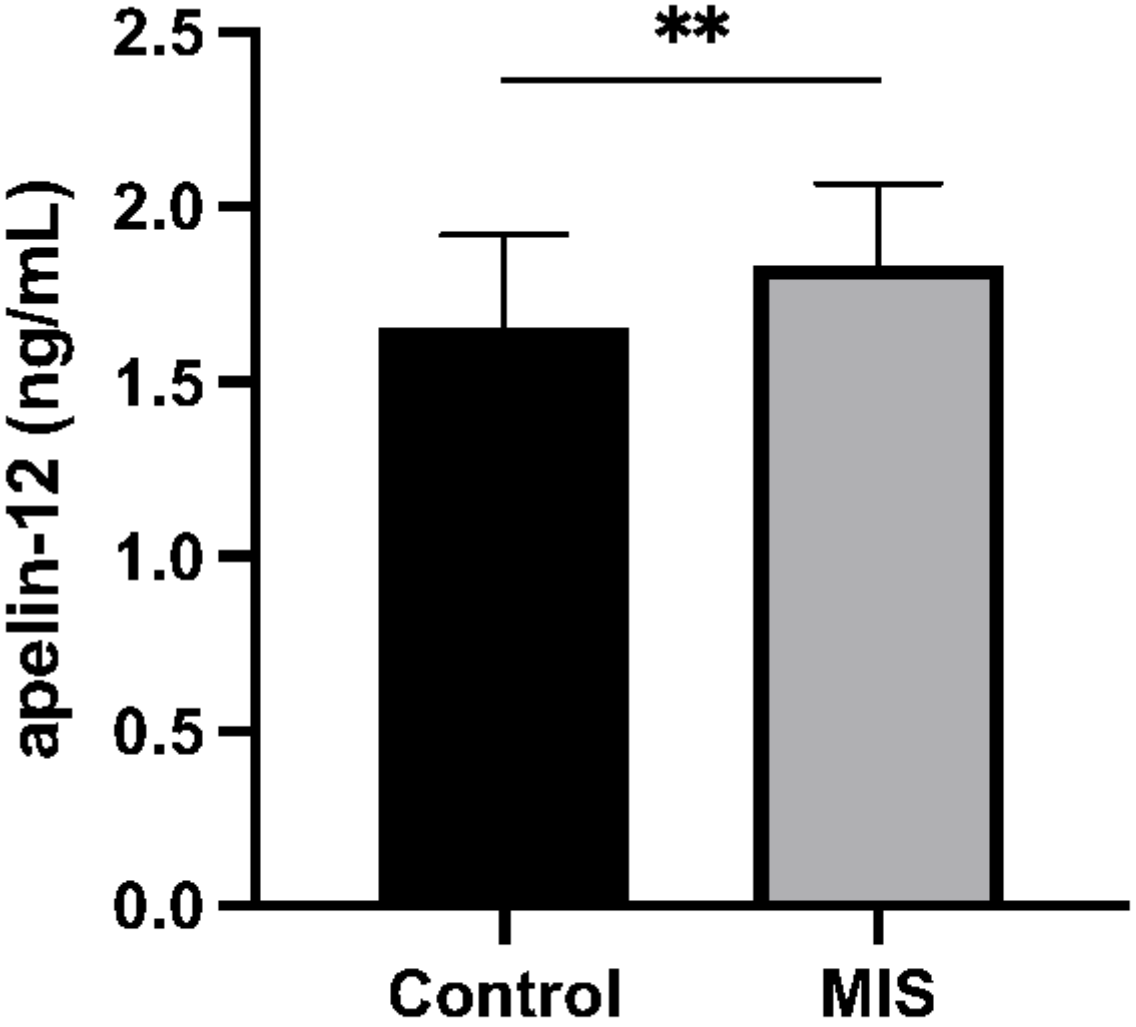

Figure 2

Serum apelin-12 in the control and MIS group. Data are expressed as median (IQR), *P*<0.01(**)

(created by Prism)

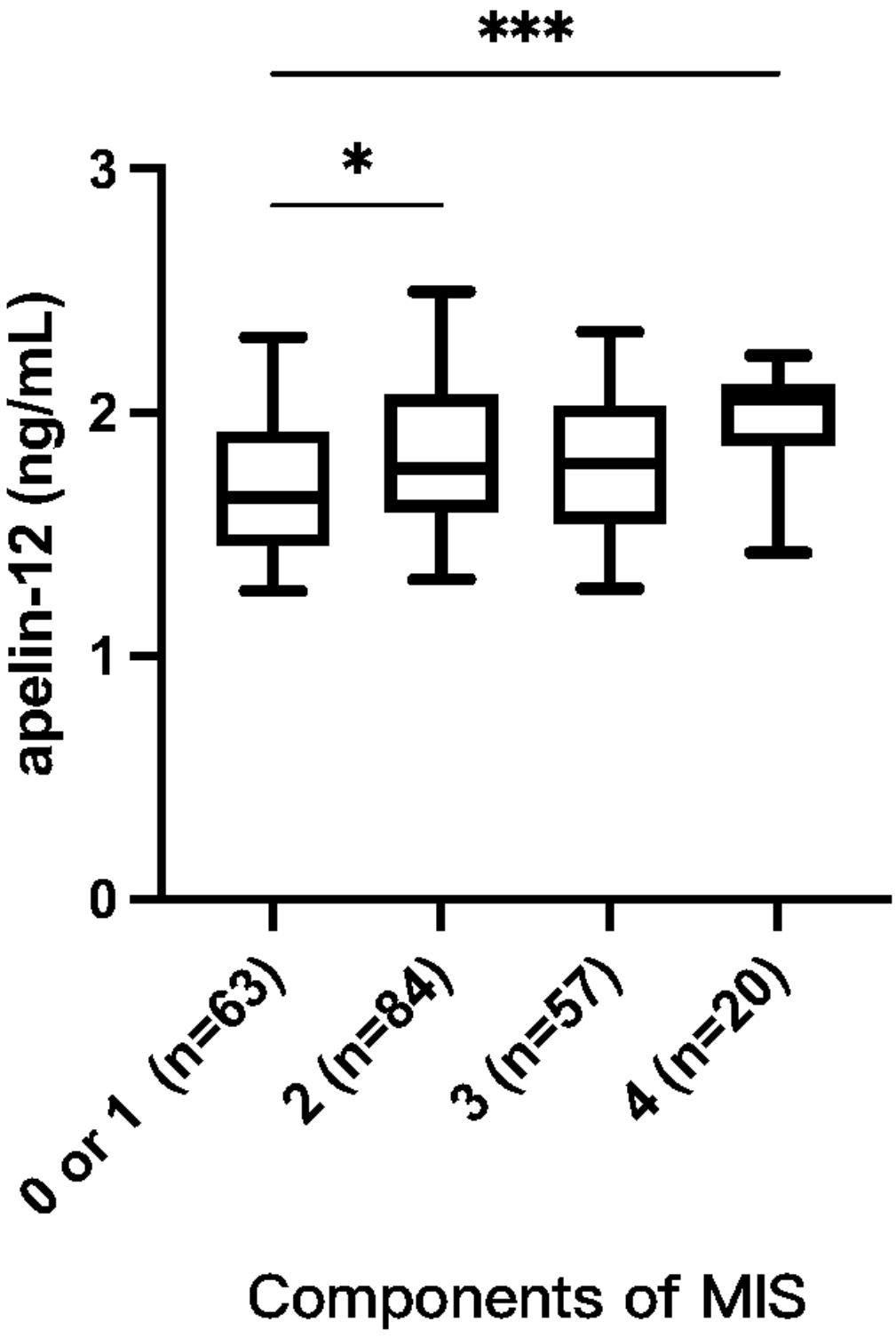

Figure 3

Serum apelin-12 in participants with 0/1, 2, 3 and 4 components. Data are expressed as median (IQR). *P*<0.05(*), *P*<0.001(***)

(created by Prism)