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Nitric oxide and clustering of metabolic syndrome components in pediatrics

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Abstract This study was performed to determine the risk factor pattern of the metabolic syndrome (MetS) in association with serum nitric oxide metabolites (NO_x) in children and adolescents. The study included 851 children and adolescents, aged 4–19 years. The MetS was defined according to modified Adult treatment Panel III criteria. Cluster analysis was performed using principle components analysis with varimax orthogonal rotation to examine the risk factor pattern of the MetS. The prevalence of MetS was 10.8 and 10.0% in males and females, respectively. Age-and sex-adjusted odds ratio of having MetS was significantly higher in the upper quartile of NO_x compared to the lower quartile (2.2, 95% CI: 1.1–4.7, $p = 0.029$). In the whole population, three factors were identified including blood pressure/obesity, lipid/obesity, and glucose/NO_x. Stratifying for sex, again three factors were retained; however, in males NO_x was loaded in two factors. In conclusion, serum NO_x was associated and loaded with other MetS components in cluster analysis of metabolic risk factors.

Keywords Adolescent · Child · Metabolic syndrome · Nitric oxide · Obesity

Abbreviations

ATPIII	Adult Treatment Panel III
BP	Blood pressure
CI	Confidence interval
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eNOS	Endothelial nitric oxide synthase
FPG	Fasting plasma glucose
HDL-C	High-density lipoprotein cholesterol
iNOS	Inducible nitric oxide synthase
MetS	Metabolic syndrome
MONICA	*Moni*toring of trends and determinants in *ca*rdiovascular disease
mRNA	Messenger ribonucleic acid
MSA	Measure of sampling adequacy
NO	Nitric oxide
NO_x	Nitric oxide metabolites
SBP	Systolic blood pressure
TC	Total cholesterol
TG	Triglycerides
TLGS	Tehran Lipid and Glucose Study
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-to-hip ratio

Introduction

Metabolic syndrome (MetS) is the co-occurrence of multiple metabolic risk factors for type 2 diabetes, cardiovascular disease (CVD), and chronic kidney disease [1, 2]. The process begins early in life, long before clinical disease is evident and persists through childhood to adolescence/adulthood [3, 4]. The worldwide prevalence of MetS in pediatrics is increasing mostly due to the rising prevalence of childhood obesity [5, 6], and is high in Iran in both pediatric (10.1%) and adult (30.1%) subjects [7, 8]; where there is an alarming increase in the rate of overweight and obesity in Iranian children and adolescents [9]. Childhood

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MetS promotes the development of premature atherosclerosis, increases cardiovascular disease risk early in life [3], and increases the risk for MetS in adulthood [5]. In a follow-up study, Morrison et al. [10] have shown that pediatric MetS was a significant predictor of CVD, 25 years later in adult subjects. In addition, it has been shown that CVD risk factors continue to track from childhood to adulthood [11–13]. In general, MetS components include hypertension, glucose intolerance, obesity, and dyslipidemia [14]. Other variables such as markers of inflammation and fibrinolysis [15], hemostatic parameters [16], and hyperuricemia [17] have also been considered as components of MetS.

Nitric oxide (NO) plays important roles in biological systems [18]. Evidence points to associations between serum NO metabolites (NO_x) levels and clustering of MetS components in adult populations [19, 20]. Relations between serum NO_x levels and CVD risk factors, including type 1 diabetes, obesity, and lipid parameters in childhood have been reported previously [21–23]. A reciprocal relation between insulin resistance and endothelial dysfunction provides a pathophysiological mechanism connecting disorders of metabolic and cardiovascular homeostasis, typified by the MetS [24]. It has been shown that high production of NO leads to pathological changes in various physiological systems [25, 26], which can culminate in insulin resistance [27].

Given the increasing concern for the emergence of the MetS in pediatric populations, data on factors associated with childhood MetS facilitates an insight into the pathogenesis of this complex disorder [3]. To the best of our knowledge, so far, there is no study of young subjects where NO_x with metabolic variables has been included in the cluster analysis; therefore, the aim of the present study was to investigate the clustering of CVD risk factors and serum NO_x in children and adolescents using cluster analysis.

Subjects and methods

Study subjects

A cross-sectional study including 938 children and adolescents, aged 4–19 years, within the framework of the Tehran Lipid and Glucose Study (TLGS) was conducted between 2006 and 2007 [28]. The TLGS is an ongoing study aimed at determining the risk factors for non-communicable diseases among Tehran's urban population. The protocol of this study was based on the WHO-recommended model for field surveys of diabetes and other non-communicable diseases and the WHO-MONICA protocol for population surveys. A multistage stratified cluster random sampling technique was used to select 15,005 persons, aged over 3 years, from District 13 of Tehran (the capital of the Iran). All members of each family were invited for baseline measurements and are followed every 3 years. Rationales for choosing District 13 were high stability of the population residing in that district compared to the other districts and the representativeness of the age distribution for the overall population of Tehran [29]. After excluding those taking medications for thyroid disorders ($n = 15$), having dyslipidemia ($n = 2$), using diuretic drugs ($n = 1$), and subjects who had missing data on components of MetS ($n = 69$), overall 851 (409 boys, 442 girls) subjects were included in the analyses. The proposal of this study was approved by the Research Institute for Endocrine Sciences of Shahid Beheshti University of Medical Sciences. Informed written consent was obtained from both parents and adolescents aged ≥15 years; informed assent was obtained from all participants <15 years.

Clinical and anthropometric measurements

Subjects were interviewed by trained interviewers, using pretested questionnaires. Weight was measured, while subjects were minimally clothed without shoes, using digital scales (Seca 707) and recorded to the nearest 100 g. Height was measured in a standing position without shoes, while the shoulders were in a normal alignment. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2). Waist circumference (WC) was measured at the level of the umbilicus and hip circumference was measured over light clothing at the widest girth of the hip using an unstretched tape meter, without any pressure to body surface. Waist-to-hip ratio (WHR) was calculated as WC/hip circumference. To avoid inter-observer error, all measurements were taken by the same person. Two measurements of systolic and diastolic blood pressure (SBP and DBP) were performed after 15 min resting and the mean of the two measurements was considered the participant's blood pressure.

Laboratory measurement

Blood samples were taken after 12–14 h overnight fasting and centrifuged within 30–45 min of collection; all blood analyses were done at the TLGS research laboratory on the day of blood collection. Serum NO_x was measured by the Griess reaction as previously reported [30]. In brief, serum samples were deproteinized by adding zinc sulfate (15 mg/ml), followed by centrifugation at 10,000*g* for 10 min; a 100 µl of the supernatant was applied to a microplate well, and following addition of 100 µl vanadium (III) chloride (8 mg/ml) to each well, Griess reagents [50 µl sulfanilamide (2%) and 50 µl *N*-(1-Naphthyl) ethylendiamine dihydrochloride (0.1%)] were added. After 30 min incubation at 37°C, absorbance was read at 540 nm using the ELISA reader

(Sunrise, Tecan, Austria). Concentration of NO_x in serum samples was determined from the linear standard curve established by 0–100 μM sodium nitrate. Inter- and intra-assay coefficients of variation were 5.2 and 4.4% respectively; recovery of the assay was 93 ± 1.5%.

Plasma glucose was measured by the enzymatic colorimetric method using a glucose oxidase kit (Pars Azmoon Inc., Tehran, Iran); inter- and intra-assay coefficients of variation were both 2.2%. For lipid measurements, total cholesterol (TC) and triglycerides (TG) kits (Pars Azmoon Inc., Tehran, Iran) were used. TC and TG were assayed using the enzymatic colorimetric assay with cholesterol esterase and cholesterol oxidase, and glycerol phosphate oxidase, respectively. High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid. Inter- and intra-assay coefficients of variation were 2.0 and 0.5% for TC, and 1.6 and 0.6% for TG respectively. Analysis of samples was performed using a Selectra 2 auto-analyzer (Vital Scientific, Spankeren, Netherlands).

Definitions

There is no consensus definition for MetS in childhood, however, the majority of researchers agree that the pediatric definition requires the same risk factors as adults but the appropriate risk factor cut-offs for children remain to be determined [5, 31]. It has been suggested that extrapolation of adult definitions to children and adolescents, with appropriate adjustment of the thresholds for some variables is a reasonable approach to developing a pediatric definition [31]. In this study, the criteria used to establish the MetS were modified from Adult Treatment Pannel III (ATPIII) [14]. MetS was defined as the presence of ≥3 of the following: Fasting TG ≥ 1.13 mmol/l; HDL-C < 1.29 mmol/l (except in boys aged 15–19 years, in whom the cut-off was <1.16 mmol/l); WC ≥ age- and sex specific 90th percentile for this population; SBP/DBP ≥ 90th percentile for age, gender, and height from the National Heart, Lung, and Blood Institutes recommended cut points [32]; fasting plasma glucose (FPG) ≥ 5.6 mmol/l.

We used the International Obesity Task Force (IOTF) cut-off points for determining children's age- and gender specific body weight status and created three categories, normal weight, overweight, and obese [33]; IOTF provides international cut-off points for BMI for overweight and obesity by sex and age in childhood, defined to pass through BMI of 25 and 30 kg/m^2 at age 18 [33].

Statistical analyses

Data were analyzed with SPSS software (SPSS Inc., Chicago, IL, USA; Version 15) and expressed as mean ± SD, unless otherwise specified. Because values of serum NO_x and TG were skewed, they were log-transformed for the analyses and their geometric means [95% confidence interval (CI)] were presented. Two-sided p values less than 0.05 were considered significant. Student t-test was used for quantitative comparing variables between males and females and between lower and upper quartiles of serum NO_x. One-way analysis of variance was used to compare continuous variables between serum NO_x tertiles and multiple comparisons were done using the Tukey test if necessary. The relation between continuous and categorical variables was assessed using Pearson correlation coefficients and Chi-square test respectively. Logistic regression analysis was used for determining odds ratio and 95% CI of having MetS between lower and upper quartiles of NO_x.

Cluster analysis, a linear method of data reduction, was performed using principal components analysis. Cluster analysis aids in the interpretation of the underlying physiological and statistical structure of the MetS by reducing a number of intercorrelated variables to a smaller set of latent or underlying orthogonal (uncorrelated) independent factors [1]. The number of components was based on eigenvalue criteria (>1). To obtain a set of independent interpretable factors we selected varimax (orthogonal) rotation; this type of orthogonal rotation was used because the results of oblique rotation (oblimin rotation) showed correlations between factors below 0.3, supporting an assumption for independence between factors [34]. In addition, varimax rotation yields more interpretable clusters of factors and simplifies the interpretation of factors [35]. The resulting factor pattern was interpreted using factor loadings of ≥0.4 (and ≤−0.4). The analysis was initially conducted with a set of variables including BMI, WC, SBP, DBP, FPG, TG, HDL-C, and NO_x, following which we then re-ran the analysis within strata of sex, MetS, and BMI status.

We used the Kaiser–Meyer–Olkin measure of sampling adequacy and Barrtletts test of sphericity to examine the appropriateness of using cluster analysis. Kaiser's measure of sampling adequacy (MSA) was used to determine the usefulness of cluster analysis using our data and values of MSA <0.6 were considered unacceptable. Bartlett's test of sphericity is used to evaluate whether a correlation matrix is suitable for cluster analysis by testing the hypothesis that the matrix is an identity matrix; if a low probability is obtained and this hypothesis is rejected, it supports the use of cluster analysis as an appropriate procedure [36]. In the current study, all p values for Bartlett's test of sphericity were less than 0.001.

Results

This study included 851 children and adolescents (409 boys, 442 girls) aged 4–19 years; compared to boys, girls were

older (13.6 ± 4.4 vs. 12.8 ± 4.2 years, $p = 0.005$) and had lower values of WC (66.7 ± 11.2 vs. 71.6 ± 14.3 cm, $p < 0.001$), WHR (0.78 ± 0.07 vs. 0.88 ± 0.06, $p < 0.001$), SBP (97 ± 12 vs. 102 ± 12 mmHg, $p < 0.001$), DBP (62.6 ± 9.7 vs. 65.3 ± 10.5, $p < 0.001$), FPG (4.75 ± 0.38 vs. 4.85 ± 0.37, $p < 0.001$), and serum NO_x concentration (24.7 vs. 27.1 μmol/l, $p = 0.004$) and higher values of TC (4.10 ± 0.73 vs. 4.00 ± 0.71 mmol/l, $p = 0.044$).

The prevalence of MetS in our population was 10.3% and the prevalences of its components were: Low HDL-C 67.0%, high TG 33.7%, high BP 9.0%, high FPG 2.9%, and abdominal obesity 11.3%. In addition, while 41.5% of subjects exhibited at least 1 component of the MetS none had all 5 criteria. There was no difference between overall prevalence of MetS between boys and girls (10.8 vs. 10.0%, $p = 0.701$). Comparing the prevalence of MetS components between genders showed that, low HDL-C was more prevalent in girls, compared to boys (70.4 vs. 63.3%, $p = 0.034$) while high WC (12.2 vs. 10.3%), high BP (7.5 vs. 10.8%), high FPG (2.7 vs. 3.2%), and high TG (33.5 vs. 34.0%) were comparable in girls and boys, respectively. In addition, MetS was more prevalent in overweight (17.3%) and obese (61.9%) subjects as compared to normal weight ones (3.1%).

Table 1 shows clinical and biochemical characteristics of study subjects according to serum NO_x tertiles. As seen, subjects in third tertile of serum NO_x compared to first had significantly higher WHR and FPG but were younger. In addition, the number of overweight and obese subjects was higher in the third tertile of serum NO_x than in the first.

The number of subjects with MetS increased from 6.1% ($n = 12$) in the lower quartile of serum NO_x levels ($NO_x < 19$ μmol/l, $n = 198$) to 13.2% ($n = 29$) in the upper quartile of NO_x ($NO_x \geq 33.0$ μmol/l, $n = 219$) ($p = 0.014$). Age-and sex adjusted odds ratio of having MetS was significantly higher in the upper quartile of NO_x compared to the lower quartile (2.2, 95% CI: 1.1–4.7, $p = 0.029$). Figure 1 shows proportion of subjects with metabolic risk factors in the lower and upper quartiles of serum NO_x. The numbers of overweight and obese subjects and subjects with FPG ≥ 5.6 mmol/l were significantly higher in the upper versus lower quartiles of NO_x. In addition, a higher proportion of subjects with elevated BP was found in the upper versus the lower quartile of NO_x although it was marginally significant.

The Pearson correlation coefficients between anthropometric variables, metabolic factors, and serum NO_x in both genders are presented in Table 2. Serum NO_x was correlated with DBP and WHR in male and with WHR in female subjects. FPG was positively correlated with SBP and DBP in males but not in females. Considering lipid parameters in male subjects, TG and HDL-C had the strongest positive and negative correlation with WC respectively, while, in

Table 1 Clinical and biochemical characteristics of children and adolescents according to serum NO_x tertiles

	Serum NO_x tertiles		
	1 ($NO_x < 21$ μmol/l, $n = 281$)	2 (21 μmol/l ≤ $NO_x < 30$ μmol/l, $n = 283$)	3 ($NO_x \geq 30$ μmol/l, $n = 287$)
Age (years)	13.8 ± 4.2	13.1 ± 4.3	12.7 ± 4.3*
BMI (kg/m^2)	20.3 ± 4.6	20.5 ± 8.9	20.5 ± 6.5
WC (cm)	68.6 ± 12.4	68.9 ± 13.7	69.5 ± 12.8
WHR	0.81 ± 0.08	0.83 ± 0.08*	0.84 ± 0.08*
SBP (mmHg)	100 ± 12.3	99.2 ± 13.4	100 ± 11.9
DBP (mmHg)	64.1 ± 10.2	63.7 ± 10.5	63.8 ± 9.8
FPG (mmol/l)	4.75 ± 0.35	4.78 ± 0.34	4.84 ± 0.42*
TG (mmol/l)	2.23 (2.12–2.35)	2.27 (2.16–2.38)	2.17 (2.05–2.29)
TC (mmol/l)	4.04 ± 0.71	4.06 ± 0.71	4.06 ± 0.74
HDL-C (mmol/l)	1.17 ± 0.24	1.18 ± 0.29	1.16 ± 0.26
Overweight and obese[a] (%)	23.5	27.2	32.4*

Data are mean ± SD and percent for continuous and categorical variables respectively, except for triglycerides reported as geometric mean (95% CI)

To convert the values for glucose to mg/dL, multiply by 18.01. To convert the values for TG to mg/dL, multiply by 88.57. To convert the values for TC and HDL-C to mg/dL, multiply by 38.67

BMI body mass index, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HDL-C* high-density lipoprotein cholesterol, *NO_x* total nitrite and nitrate, *SBP* systolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *WC* waist circumference, *WHR* waist-to-hip ratio

* $p < 0.05$ compared to first tertile by one-way analysis of variance

[a] Using International Obesity Task Force (IOTF) cut-off points

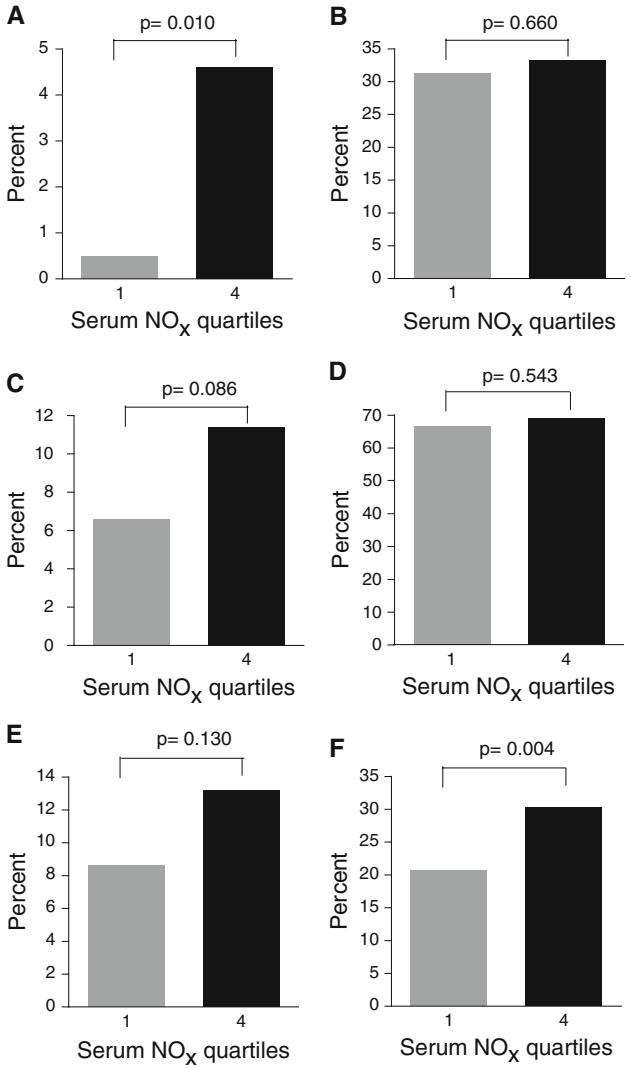

Fig. 1 Comparison of the proportion of subjects with high FPG (**a**), high TG (**b**), high BP (**c**), low HDL-C (**d**), and high WC (**e**) according to the ATP III-defined metabolic syndrome, and overweight and obese subjects (**f**) according to IOTF cut-points between lower ($NO_x < 19$ µmol/l, $n = 198$) and upper ($NO_x \geq 33.0$ µmol/l, $n = 219$) quartiles of serum NO_x

females TG had the strongest negative correlation with HDL-C.

Cluster analysis of metabolic variables and serum NO_x for the total population identified three dominant factors that explained 59.9% of the total variance (23.58, 23.19, and 13.14% for first, second and third factor, respectively). Factor loadings after varimax rotation showed that the first factor (blood pressure/obesity) correlated with SBP, DBP, WC, and BMI, the second factor (lipid/obesity) with TG, HDL-C, WC, and BMI, and the third factor (glucose/NO_x) with FPG and NO_x. Substitution of WHR instead of WC resulted in co-clustering of FPG, WHR, and NO_x in one factor in the total population (data not shown).

The factor patterns showed some differences in separate analyses between males and females although again three factors have been retained which explained 61.73 and 61.76% of the total variance in males and females, respectively. In male subjects, for the first factor SBP, WC, BMI, and TG had positive and HDL-C had negative loadings, while in female subjects SBP, DBP, WC, and BMI had positive loadings. For the second factor, in males SBP and DBP had positive loadings and NO_x had negative loadings, while in females, TG and HDL-C had strong positive and negative loadings respectively. In both genders, FPG and NO_x loaded into the third factor however, NO_x had negative loadings in male subjects but positive loadings in females. In female subjects, each variable was loaded only in one factor, while in male subjects SBP and NO_x were loaded in two factors.

Results of cluster analysis in strata of MetS are shown in Table 3. In subjects, with or without MetS, four factors have been retained; in subjects with MetS, NO_x was loaded with FPG while in subjects without MetS it constituted an independent factor.

In overweight/obese children, NO_x was loaded with BMI and FPG in the third factor accounting for 14.62% of total variance, while in normal weight subjects, NO_x had strong positive loadings in factor 4 as an independent factor (Table 4).

Table 2 Pearson correlation analysis of baseline variables among 409 male (above diagonal) and 442 female (below diagonal) subjects

	Male/female								
	SBP	DBP	FPG	TG	HDL-C	BMI	WC	WHR	NO_x
SBP		0.56‡	0.03	0.28‡	−0.24‡	0.27‡	0.53‡	0.10*	−0.07
DBP	0.44‡		0.06	0.18‡	−0.17‡	0.18‡	0.29‡†	0.01	−0.10*
FPG	0.12†	0.09*		0.16†	−0.02	−0.01	0.14†	0.11*	0.01
TG	0.12†	0.06	0.24‡		−0.41‡	0.26‡	0.51‡	0.33‡	−0.05
HDL-C	0.01	0.02	−0.06	−0.33‡		−0.24‡	−0.44‡	−0.17‡	−0.02
BMI	0.34‡	0.28‡	0.14†	0.22‡	−0.16†		0.49‡	0.23‡	−0.01
WC	0.36‡	0.28‡	0.15†	0.25‡	−0.19†	0.92‡		0.45‡	−0.05
WHR	−0.05	−0.09*	0.08	0.16†	−0.08*	−0.02	0.09*		0.1*
NO_x	0.06	0.06	0.04	0.04	−0.04	0.02	0.02	0.14†	

* $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$

Abbreviations same as in Table 1

Table 3 Factors loading for original variables with rotated factors among pediatric subjects within strata of metabolic syndrome

	Without metabolic syndrome ($n = 763$)				With metabolic syndrome ($n = 88$)			
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3	Factor 4
SBP (mmHg)	**0.830**	0.042	0.115	0.016	0.135	0.150	**0.742**	−0.221
DBP (mmHg)	**0.792**	−0.076	−0.020	−0.064	0.016	0.025	**0.813**	0.157
TG (mmol/l)	−0.009	**0.697**	0.297	−0.125	−0.011	**−0.839**	−0.139	0.142
HDL-C (mmol/l)	0.046	**−0.758**	0.085	−0.055	−0.129	**0.823**	0.015	0.174
FPG (mmol/l)	0.066	0.047	**0.945**	0.032	0.066	0.046	−0.325	**0.733**
WC (cm)	**0.599**	**0.568**	0.083	0.044	**0.880**	−0.209	0.151	−0.122
BMI (kg/m^2)	**0.465**	**0.507**	−0.220	0.064	**0.939**	0.057	−0.003	0.001
NO_x (μmol/l)	−0.019	−0.007	0.024	**0.989**	−0.187	−0.012	0.287	**0.696**
Variance explained (%)	23.72	20.65	13.21	12.61	21.62	18.16	18.02	14.50
Cumulative variance (%)	23.72	44.37	57.58	70.19	21.62	39.78	57.80	72.29

Abbreviations same as in Table 1

Loadings ≥0.4 (and ≤−0.4) are in bold font

Table 4 Factors loading for original variables with rotated factors among pediatric subjects within strata of obesity

	Normal weight ($n = 625$)				Overweight and obese ($n = 236$)		
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3
SBP (mmHg)	0.191	**0.826**	0.080	0.035	**0.805**	0.090	0.024
DBP (mmHg)	0.084	**0.805**	0.021	−0.029	**0.814**	−0.071	0.036
TG (mmol/l)	0.307	−0.080	**0.721**	−0.058	0.044	**0.799**	0.109
HDL-C (mmol/l)	**−0.575**	0.239	−0.294	−0.111	−0.023	**–0.836**	0.068
FPG (mmol/l)	–0.102	0.183	**0.792**	0.021	0.065	0.028	**0.703**
WC (cm)	**0.843**	0.348	0.051	–0.046	**0.568**	**0.473**	–0.139
BMI (kg/m^2)	**0.866**	0.279	–0.036	–0.068	0.262	0.003	**–0.590**
NO_x (μmol/l)	–0.015	0.005	–0.023	**0.992**	0.091	–0.018	**0.538**
% Variance explained	24.25	20.32	15.57	12.62	21.47	19.69	14.62
Cumulative variance (%)	24.25	44.57	60.14	72.76	21.47	41.17	55.78

Abbreviations same as in Table 1

Loadings ≥0.4 (and ≤−0.4) are in bold font

Discussion

This is the first study investigating the risk factor pattern of MetS and its association with serum NO_x concentrations in youth and results showed that NO_x was loaded with other metabolic components such as blood pressure, BMI, WHR, and FPG in cluster analysis. Three factors emerged in the analysis and the factor patterns showed some differences between boys and girls; in girls, NO_x was loaded in one factor along with FPG, while in boys it was loaded in two factors along with FPG and blood pressure. Serum NO_x levels constituted a single independent factor in normal weight subjects and in subjects without MetS.

The high prevalence of MetS found in this study [10.3% (95% CI: 8.3–12.4)] was comparable with findings of another report from this population among 10–19 years old adolescents [10.1% (9.0–11.1)] [7]. In other populations, MetS prevalences were 9.2% in American adolescents, aged 12–19 years, and 11.5% in Canadian children and adolescents aged 9–16 years [5, 37]. However, the prevalence of MetS in our study is higher than most reports including 4.2% for 3–18 year-old Finnish children and adolescents, and 6.5% for 10–18 year-old Mexican children and adolescents [5]. As regards the precise causes of our higher prevalence this could be the result of changes in the diet of the Iranian population, a steep reduction in physical activity [7], or the alarming rise in prevalence of pediatric obesity in Middle East countries, including Iran [9]. Difference in definitions of MetS could also to some extent explain such a high prevalence.

Co-clustering of NO_x with other metabolic components is consistent with other studies indicating an association between some metabolic factors and serum NO_x; a positive association between plasma NO_x level and blood pressure has been observed in normotensive African Americans [38]. In addition, it has been suggested that measurement of

serum NO_x levels may help to monitor the state and severity of hypertension [38]. In our study, NO_x constituted a factor with FPG and WHR in the total population and with FPG and BMI in overweight and obese subjects. These findings are in agreement with the hypothesis that NO is the missing link in obesity-induced insulin resistance [27]. In addition, it has been shown that serum NO_x concentrations are about 14-fold higher in obese as compared to extremely lean adolescents [39]. Partly in agreement with this, the number of overweight and obese subjects in our study was about 60% higher in the upper versus the lower quartile of serum NO_x. Adipose tissue may be a potential source of NO production because both eNOS and iNOS have been found in this tissue [39].

In the current study, serum NO_x was associated with the glucose factor. Reports on the effect of diabetes on NO metabolism are controversial. Hyperglycemia leads to glucotoxicity that causes insulin resistance and endothelial dysfunction [24]. It has been demonstrated that NO production increases in diabetes [20, 40] which may be due to over-expression of iNOS mRNA in the pancreatic islets [41]. In addition, it has been reported that NO donors cause β cell dysfunction and damage [41].

A review of literature shows that 13 studies have used exploratory [31, 42, 43], and one study used confirmatory factor analysis [44] for MetS in youth; none included serum NO_x as a variable. The number of factors that has been retained ranges between 1 and 5 and most studies found 3 and 4 factors, a finding in agreement with our results. We found an inverse factor loading for NO_x in the second and third factors in boys, a pattern similar to that reported previously for glucose by others, although its etiology is unclear [4, 37]. In addition, in male subjects, NO_x was loaded in the second and third factors with a factor loading >0.4 and in the first factor, the factor loading was close to 0.3, which could play a unifying role for this variable. It should be noted that boys had significantly higher NO_x values compared to girls. Nevertheless, these results should be interpreted with caution because we did not measure insulin which has previously been suggested to have a unifying role [45]. However, NO_x did have this role in the presence of obesity which also seems to act as a linking variable [37].

In the current study, in subjects without MetS and those who had normal weight, NO_x constituted a separate factor, while in subjects with MetS and those who were overweight or obese, it was loaded with FPG and/or BMI. In agreement with our results, different patterns of a metabolic variable clustering have previously been reported for both MetS versus non-MetS [42] and obese versus non-obese [46] children and adolescents.

Our results showed that the number of subjects with MetS was twice higher in the upper quartile of NO_x compared to the lower quartile; also, the risk for having MetS was higher in the upper quartile of NO_x. These results indicate an association between serum NO_x and MetS in youth, one that has previously been reported in adults [19, 20]. NO is biosynthesized in several cell types. Although it is not possible to identify the sites responsible for NO synthesis by measuring only serum NO_x, it has been suggested however, that vascular endothelium is the major source of total NO synthesis and that serum NO_x levels are useful for the rough evaluation of basal NO generation by endothelial cells [47]. Therefore, it may be speculated that increased serum NO_x in MetS, seen in this study, could be due to eNOS inhibition and iNOS overexpression as previously proposed [20]. Cytotoxic and pathologic effects of excess NO have been documented [41, 48] and recently it has been shown that NO_x acts as a marker for prediction of survival rate of the elderly [49], indicating a positive association between high NO_x levels and a high risk for adverse health outcomes [19].

This study has some limitations, which need to be considered in the interpretation of the results. First, the diet of the subjects was not recorded; it has been suggested that NO_x derived from the diet is a contributor to plasma NO_x concentration in healthy subjects [50]; however, the blood samples of subjects were obtained after overnight fasting and it has been shown that dietary nitrate is cleared from the plasma pool within 12 h of a meal [51]. Second, the findings presented in this study are based on cross-sectional data, and therefore they cannot directly contribute to an understanding of the temporal relationship between serum NO_x values and MetS. Finally, some of the gender-related differences observed in this study might be related to the age differences between males and females; although the number of subjects was approximately equal in males and females under 15 years, there was higher percent of 15–19 year-old females compared to males (59 vs. 40%). This limitation might be partly explained by our observation that attendance of the older male subjects with the family is less compared with females.

One final point, in our study the number of subjects using medications for thyroid disorders seems to be relatively high, which nevertheless might be explained by a previous report that estimated incidence of congenital hypothyroidism in Iran is over three times as high as the worldwide incidence [52].

In conclusion, serum NO_x was associated with MetS in children and adolescents in Tehran; in addition, NO_x was loaded with other MetS components, especially fasting glucose in the cluster analysis of metabolic risk factors and it may have a unifying role in the clustering of MetS components, at least in male subjects, a role which needs further investigation.

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References

1. Meigs JB. Invited commentary: insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. Am J Epidemiol. 2000;152:908–11. Discussion 12.
2. Pladevall M, Singal B, Williams LK, et al. A single factor underlies the metabolic syndrome: a confirmatory factor analysis. Diabetes Care. 2006;29:113–22.
3. Kohen-Avramoglu R, Theriault A, Adeli K. Emergence of the metabolic syndrome in childhood: an epidemiological overview and mechanistic link to dyslipidemia. Clin Biochem. 2003;36: 413–20.
4. Goodman E, Dolan LM, Morrison JA, Daniels SR. Factor analysis of clustered cardiovascular risks in adolescence: obesity is the predominant correlate of risk among youth. Circulation. 2005; 111:1970–7.
5. De Ferranti SD, Osganian SK. Epidemiology of paediatric metabolic syndrome and type 2 diabetes mellitus. Diabetes Vasc Dis Res. 2007;4:285–96.
6. Sen Y, Kandemir N, Alikasifoglu A, Gonc N, Ozon A. Prevalence and risk factors of metabolic syndrome in obese children and adolescents: the role of the severity of obesity. Eur J Pediatr. 2008;167:1183–9.
7. Esmaillzadeh A, Mirmiran P, Azadbakht L, Etemadi A, Azizi F. High prevalence of the metabolic syndrome in Iranian adolescents. Obesity (Silver Spring). 2006;14:377–82.
8. Azizi F, Salehi P, Etemadi A, Zahedi-Asl S. Prevalence of metabolic syndrome in an urban population: Tehran Lipid and Glucose Study. Diabetes Res Clin Pract. 2003;61:29–37.
9. Azizi F, Mirmiran P, Sherafat-Kazemzadeh R. Pediatric obesity: an impending catastrophe. Arch Iran Med. 2008;11:242–5.
10. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. Pediatrics. 2007;120:340–5.
11. Bao W, Srinivasan SR, Wattigney WA, Berenson GS. Persistence of multiple cardiovascular risk clustering related to syndrome X from childhood to young adulthood. The Bogalusa Heart Study. Arch Intern Med. 1994;154:1842–7.
12. Li S, Chen W, Srinivasan SR, et al. Childhood cardiovascular risk factors and carotid vascular changes in adulthood: the Bogalusa Heart Study. JAMA. 2003;290:2271–6.
13. Webber LS, Srinivasan SR, Wattigney WA, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. Am J Epidemiol. 1991; 133:884–99.
14. Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol In adults (Adult Treatment Panel III). JAMA. 2001;285:2486–97.
15. Hanley AJ, Festa A, D'Agostino RB Jr, et al. Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. Diabetes. 2004;53:1773–81.
16. Sakkinen PA, Wahl P, Cushman M, Lewis MR, Tracy RP. Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. Am J Epidemiol. 2000;152:897–907.
17. Reimann M, Schutte AE, Malan L, Huisman HW, Malan NT. Hyperuricaemia is an independent factor for the metabolic syndrome in a sub-Saharan African population: a factor analysis. Atherosclerosis. 2008;197:638–45.
18. Yoon S, Moon J, Shin C, Kim E, Jo SA, Jo I. Smoking status-dependent association of the 27-bp repeat polymorphism in intron 4 of endothelial nitric oxide synthase gene with plasma nitric oxide concentrations. Clin Chim Acta. 2002;324:113–20.
19. Ueyama J, Kondo T, Imai R, et al. Association of serum NO_x level with clustering of metabolic syndrome components in middle-aged and elderly general populations in Japan. Environ Health Prev Med. 2008;13:36–42.
20. Zahedi Asl S, Ghasemi A, Azizi F. Serum nitric oxide metabolites in subjects with metabolic syndrome. Clin Biochem. 2008;41:1342–7.
21. Choi JW. Enhanced nitric oxide production is closely associated with serum lipid concentrations in adolescents. Clin Chim Acta. 2004;347:151–6.
22. Gruber HJ, Mayer C, Mangge H, Fauler G, Grandits N, Wilders-Truschnig M. Obesity reduces the bioavailability of nitric oxide in juveniles. Int J Obes (Lond). 2008;32:826–31.
23. Lo HC, Lin SC, Wang YM. The relationship among serum cytokines, chemokine, nitric oxide, and leptin in children with type 1 diabetes mellitus. Clin Biochem. 2004;37:666–72.
24. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation. 2006;113:1888–904.
25. Perreault M, Marette A. Targeted disruption of inducible nitric oxide synthase protects against obesity-linked insulin resistance in muscle. Nat Med. 2001;7:1138–43.
26. Colasanti M, Suzuki H. The dual personality of NO. Trends Pharmacol Sci. 2000;21:249–52.
27. Dallaire P, Marette A. Obesity-linked insulin resistance: is nitric oxide the missing link? Can J Diabetes. 2004;28:59–66.
28. Azizi F, Rahmani M, Emami H, Madjid M. Tehran Lipid and Glucose Study: rationale and design. CVD Prev. 2000;3:242–7.
29. Azizi F, Rahmani M, Emami H, et al. Cardiovascular risk factors in an Iranian urban population: Tehran lipid and glucose study (phase 1). Soz Praventivmed. 2002;47:408–26.
30. Ghasemi A, Zahedi Asl S, Mehrabi Y, Saadat N, Azizi F. Serum nitric oxide metabolite levels in a general healthy population: relation to sex and age. Life Sci. 2008;83:326–31.
31. Ford ES, Li C. Defining the metabolic syndrome in children and adolescents: will the real definition please stand up? J Pediatr. 2008;152:160–4.
32. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004; 114: 555–76.
33. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. Bmj. 2000;320:1240–3.
34. Pallant J. SPSS survival manual, a step by step guide to data analysis using SPSS for windows. Sydney: McGraw Hill; 2007.
35. Field A. Discovering statistics using SPSS. London: SAGE publications Ltd; 2009.
36. Dixon JK. Explaratory factor analysis. In: Munro BH, editor. Statistical methods for health care research. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 321–50.

37. Lambert M, Paradis G, O'Loughlin J, Delvin EE, Hanley JA, Levy E. Insulin resistance syndrome in a representative sample of children and adolescents from Quebec, Canada. Int J Obes Relat Metab Disord. 2004;28:833–41.
38. Higashino H, Miya H, Mukai H, Miya Y. Serum nitric oxide metabolite (NO*x*) levels in hypertensive patients at rest: a comparison of age, gender, blood pressure and complications using normotensive controls. Clin Exp Pharmacol Physiol. 2007;34: 725–31.
39. Choi JW, Pai SH, Kim SK, Ito M, Park CS, Cha YN. Increases in nitric oxide concentrations correlate strongly with body fat in obese humans. Clin Chem. 2001;47:1106–9.
40. Ding Y, Vaziri ND, Coulson R, Kamanna VS, Roh DD. Effects of simulated hyperglycemia, insulin, and glucagon on endothelial nitric oxide synthase expression. Am J Physiol Endocrinol Metab. 2000;279:E11–7.
41. Shimabukuro M, Ohneda M, Lee Y, Unger RH. Role of nitric oxide in obesity-induced beta cell disease. J Clin Invest. 1997; 100:290–5.
42. Kelishadi R, Ardalan G, Adeli K, et al. Factor analysis of cardiovascular risk clustering in pediatric metabolic syndrome: CASPIAN study. Ann Nutr Metab. 2007;51:208–15.
43. Ghosh A. Factor analysis of risk variables associated with metabolic syndrome in Asian Indian adolescents. Am J Hum Biol. 2007;19:34–40.
44. Li C, Ford ES. Is there a single underlying factor for the metabolic syndrome in adolescents? A confirmatory factor analysis. Diabetes Care. 2007;30:1556–61.
45. Chen W, Srinivasan SR, Elkasabany A, Berenson GS. Cardiovascular risk factors clustering features of insulin resistance syndrome (Syndrome X) in a biracial (Black–White) population of children, adolescents, and young adults: the Bogalusa Heart Study. Am J Epidemiol. 1999;150:667–74.
46. Moreno LA, Pineda I, Rodriguez G, et al. Leptin and metabolic syndrome in obese and non-obese children. Horm Metab Res. 2002;34:394–9.
47. Tanaka S, Yashiro A, Nakashima Y, Nanri H, Ikeda M, Kuroiwa A. Plasma nitrite/nitrate level is inversely correlated with plasma low-density lipoprotein cholesterol level. Clin Cardiol. 1997;20: 361–5.
48. Curcelli EC, Muller SS, Novelli Filho JL. Beneficial effects of diclofenac therapy on serum lipids, oxidized low-density lipoprotein and antioxidant defenses in rats. Life Sci. 2008;82: 892–8.
49. Osawa M, Hayashi T, Nomura H, et al. Nitric oxide (NO) is a new clinical biomarker of survival in the elderly patients and its efficacy might be nearly equal to albumin. Nitric Oxide. 2007;16: 157–63.
50. Himeno M, Ishibashi T, Nakano S, et al. A practical procedure for achieving a steady state of NO_x concentration in plasma: with special reference to the NO_x content of Japanese daily food. Tohoku J Exp Med. 2003;199:95–110.
51. Node K, Kitakaze M, Yoshikawa H, Kosaka H, Hori M. Reduced plasma concentrations of nitrogen oxide in individuals with essential hypertension. Hypertension. 1997;30:405–8.
52. Ordookhani A, Mirmiran P, Najafi R, Hedayati M, Azizi F. Congenital hypothyroidism in Iran. Indian J Pediatr. 2003;70: 625–8.