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In vivo nitric oxide synthesis, insulin sensitivity, and asymmetric dimethylarginine in obese subjects without and with metabolic syndrome

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ABSTRACT

Metabolic syndrome (MetSyn) is associated with impaired endothelial function. Here the association between nitric oxide (NO) production and insulin sensitivity (Si) in obese subjects with and without MetSyn was evaluated. The relationship between NO production and asymmetric dimethylarginine (ADMA) was also explored. Seven healthy normal-weight subjects (male/female [M/F], 3/4; age, 27.4 ± 10.9 years; body mass index [BMI], 21.9 ± 2.2 kg/m^2), 7 obese subjects without MetSyn (M/F, 1/6; age, 48.0 ± 8.0 years; BMI, 34.5 ± 2.3 kg/m^2), and 7 with MetSyn (M/F, 3/4; age, 48.0 ± 10.7 years; BMI, 33.4 ± 2.9 kg/m^2) were recruited. Body composition and cardiometabolic functions (blood pressure, glucose, insulin, triglycerides, total cholesterol, high-density lipoprotein, ADMA) were measured. A frequent sampling intravenous glucose tolerance test was performed to measure Si. A novel stable isotopic method was used to measure in vivo rates of NO production. The NO production was lower in obese subjects with MetSyn compared with normal-weight subjects and obese subjects without MetSyn. Similarly, Si was significantly lower in obesity, both without and with MetSyn, compared with the control group. A significant direct association was found between NO synthesis and Si ($\rho = 0.47$, $P = .03$). Circulating levels of ADMA were significantly higher in the obese group with MetSyn. A nonsignificant negative trend between ADMA and NO synthesis was observed. The association between Si and NO production suggests a close mechanistic link between endothelial function and insulin signaling. The results may be highly informative for the development of controlled longitudinal interventions to improve endothelial and metabolic regulation.

1. Introduction

Obesity is a chronic metabolic disorder characterized by a multifactorial etiopathogenesis with systemic effects [1]. Obesity-related comorbidities are associated with an excessive accumulation of fat in adipose and in nonadipose tissues (ectopic deposition) [2] that increases the risk for cardiovascular and metabolic diseases [3].

The primary metabolic modification induced by excess adiposity is the development of insulin resistance [4], which is primarily linked to a postreceptorial defect in the insulin signaling pathway and to a compensatory hyperinsulinemia to maintain normal glucose levels [5,6]. Insulin is the key anabolic hormone promoting disposal and storage of nutrients, and it is rapidly secreted by the endocrine pancreas in response to food intake [7]. The efficiency of the insulin

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anabolic response is related to a concomitant increase in capillary recruitment and blood flow to regulate the transport and delivery of nutrients, substrates, and hormones to metabolizing tissues [8,9]. The elevation of postprandial blood flow is mainly induced by nitric oxide (NO) synthesized in response to the activation of the insulin signaling cascade on the endothelial cells. Therefore, insulin resistance is also associated with a reduction in NO production and impaired endothelial function [10-12].

Nitric oxide production can be selectively inhibited by guanidino-substituted analogues of arginine, including endogenously produced asymmetric dimethylarginine (ADMA). Asymmetric dimethylarginine is a naturally occurring amino acid acting as a competitive inhibitor of the NO synthase, and increased plasma ADMA concentrations have been reported in a number of metabolic disorders (chronic renal failure, type 2 diabetes mellitus, dyslipidemia, hypertension) associated with impaired NO synthesis [13].

The chronic impairment of the insulin signaling pathway and the onset of compensatory mechanisms may induce pathological modifications in different tissues and organs that characterize the insulin resistance syndrome or metabolic syndrome (MetSyn) [14]. The diagnosis of MetSyn is based on the presence of central obesity, dyslipidemia, hypertension, and glucose intolerance [15]. These conditions are associated with increased oxidative and inflammatory responses that may be linked to the impairment of endothelial function [16,17].

We have previously reported the difference in rates of NO production between normal-weight and obese subjects with and without MetSyn [18]. Here we tested the association between in vivo rates of NO production and insulin sensitivity (Si) in obese subjects with and without MetSyn. We also investigated the association between circulating levels of ADMA with rates of NO production and Si.

2. Methods

2.1. Subjects

Fourteen obese subjects (body mass index [BMI] range, 30-40 kg/m^2) and 7 normal-weight subjects (BMI range, 18.5-24.9 kg/m^2) were recruited. Obese subjects were divided into 2 groups according to the diagnosis of MetSyn (Obese-MetSyn and Obese NO-MetSyn). The 2 obese groups were matched for age and BMI; and they were compared with a younger, normal-weight group of healthy subjects. Participants were excluded if they had any medical condition or were taking any medications interfering with the study outcomes. A full list of the exclusion criteria is provided in the Online Supplementary Material. Each subject was required to give informed written consent before any measurement could be carried out. The study was approved by the Cambridgeshire 4 Ethics Committee.

2.2. Screening visit

Weight was measured to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Body mass index was calculated. Waist circumference was measured in triplicate according to a standardized protocol (midpoint between last rib and iliac crest on midaxillary line). Body fat was measured using a leg-to-leg bioimpedance analyzer (Tanita, Tokyo, Japan). Blood pressure was measured in triplicate using an automated sphygmomanometer (Dynamap, Critikon, Tampa, FL, USA) and using a larger cuff for obese subjects. Measurements were taken in a sitting position after the subject had rested for at least 30 minutes. Blood tests (full blood count, glucose, hemoglobin A_{1c}, lipid profile [high-density lipoprotein {HDL}, low-density lipoprotein {LDL}, triglycerides {TG}, total cholesterol], C-reactive protein [CRP], kidney function tests [urea, creatinine]) and urinalysis were performed after a 12-hour fast. The diagnosis of MetSyn was made according to the recent criteria proposed in a joint statement by the major diabetes, obesity, and heart organizations [19] that requires at least 3 of the following criteria to be fulfilled: waist circumference of at least 102 cm (male) or at least 88 cm (female); TG of at least 1.7 mmol/L; HDL less than 1.3 mmol/L (male) or less than 1.1 mmol/L (female); high blood pressure of at least 130/85 mm Hg; and fasting plasma glucose of at least 5.6 mmol/L.

2.3. Frequent sampling intravenous glucose tolerance test

Subjects arrived in the morning to the research unit after they had fasted for at least 12 hours and followed the low-nitrate (NO_3^-) diet for 24 hours. Three baseline blood samples were taken before the administration of an intravenous glucose dose (1.75 g of [6,6]-$^2H_2$-glucose with 19 g unlabeled glucose in the form of a 50% aqueous solution). Twenty minutes after administering glucose, 201 mU of Human Actrapid insulin (Novo Nordisk, Copenhagen, Denmark) was given. A total of 30 blood samples were taken at –10, –5, –1, 1, 2, 3, 4, 5, 6, 8, 10, 13, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 210, and 240 minutes. After the frequent sampling intravenous glucose tolerance test, subjects were served a very low NO_3^- meal as part of the oral nitrate test protocol. A description of the low NO_3^- meals and low NO_3^- diet has been described elsewhere [18].

2.4. Saliva oral nitrate test

After the low NO_3^- meal, each participant was instructed not to eat and to drink only low NO_3^- bottled water (Buxton, Nestlè UK, Croydon, UK) until the following morning (18-hour fasting period). Four hours after the meal, a predose saliva sample was collected; and a dose of 4 mg of labeled sodium nitrate ($Na^{15}NO_3^-$) was given as a drink in 100 mL of distilled water. Each subject was asked to provide another saliva sample 2 hours later. All subsequent saliva samples were collected into pre-prepared containers by the volunteer at home. The final saliva sample was collected 18 hours after the meal. The full protocol for the collection of saliva samples has been described elsewhere [18].

2.5. Mathematical modeling

2.5.1. Frequent sampling intravenous glucose tolerance test

The glucose and insulin pharmacological profile after an intravenous bolus of glucose and insulin was characterized by the minimal model as defined by Bergman et al [20]. The minimal model was solved to calculate the characteristic metabolic parameters such as Si and glucose effectiveness (Sg). Acute insulin response (AIR), an index of first-phase insulin

secretion, is the mean insulin concentration above basal during the first peak (from 2 to 10 minutes). The disposition index (DI) was used as an indicator of β-cell function normalized to Si, and it originates by plotting AIR against Si.

2.5.2. *Oral nitrate test protocol*

Briefly, the tracer disappearance from saliva of an oral dose of labeled NO_3^- was described by an exponential function for a single compartment. Data were described using a semilogarithmic plot, and the slope and intercept of the regression line were used to derive in vivo rates of NO synthesis as described elsewhere [18].

3. Materials

Trifluoroacetic anhydride, sodium hydroxide, and mesitylene were all obtained from Sigma-Aldrich (Poole, Dorset, UK). All the stable isotopically labeled compounds, ^{15}N-sodium nitrate (98%+), and [6,6]-$^2H_2$-glucose (98%+) were obtained from Cambridge Isotope Laboratories (Andover, MA). The Nitrate/-Nitrite Calorimetric Assay kits were purchased from Cayman Chemical (Ann Arbor, MI). ADMA assays were purchased from Immundiagnostik (Bensheim, Germany).

3.1. *Nitrate measurement*

3.1.2. *Concentration*

A 96-well commercially available kit was used to measure concentration using a diazotization reaction in urine and saliva samples. The measurements were performed without deviation from the manufacturer's instructions.

3.1.2. *Enrichment*

All urine and saliva samples were derivatized by nucleophilic substitution of mesitylene with trifluoroacetic anhydride as the catalyst to give a single product, nitromesitylene (1,3,5-trimethyl nitrobenzene). Gas chromatography–mass spectrometry was used to determine the level of enrichment. All the analyses were performed in triplicate using selected ion monitoring of the molecular ion (*M*) at *m*/z 165 and the *M* + 1 ion at *m*/z 166 representing the unlabeled and labeled nitromesitylene, respectively. The method has been previously described [21].

3.2. *Glucose enrichment measurement*

Plasma glucose enrichments were measured using a fluorinated methyl boronate derivative. These derivatized samples were then analyzed on gas chromatography–mass spectrometry for determination of isotopic composition. The common glucose derivative has a mass number of 240, and glucose isotope derivatives of [6,6]-$^2H_2$-glucose have a mass number of 242. The method has been previously described [22].

3.3. *Clinical biochemistry*

Blood tests (full blood count, glucose, insulin, hemoglobin A_{1c}, lipid profile [HDL, LDL, TG, and total cholesterol], CRP, kidney function tests [urea, creatinine]) were performed using validated methods. Homeostasis model assessment of insulin resistance (HOMA-R) was calculated [23]. Plasma ADMA levels were measured from plasma samples by competitive ELISA (enzyme-linked immunosorbent assay). The detection limit of the assay was 0.05 μmol/L.

3.4. *Statistical analysis*

Data are shown as mean, standard deviation (SD), and range of values (min-max). A formal sample size calculation was performed to calculate the number of subjects required to detect a statistically significant difference in rates of NO production between the 3 groups (Online Supplementary Material). The Kruskal-Wallis and the Mann-Whitney tests for independent samples were used to detect significant trends and differences between the 3 groups, respectively. Correlation analysis (Spearman rank) was used to assess the association between NO synthesis and relevant metabolic parameters. Analyses were adjusted for age using the rank transformation method [24]. The statistical analyses were carried out using SPSS 16 for Windows (SPSS, Chicago, IL). The significance level was set at .05.

4. Results

4.1. *Baseline characteristics*

The baseline characteristics of the study participants categorized according to the diagnosis of MetSyn are shown in Table 1. The control group was significantly younger and had a better overall metabolic profile than obese subjects. Obese subjects with MetSyn had significantly higher systolic blood pressure, TG, and total cholesterol compared with the control group. Both obese groups had significantly higher fasting plasma levels of CRP, insulin levels, and HOMA-R values than the control group. There were no significant differences in fasting plasma NO_3^- between the 3 groups after subjects had followed a 24-hour low NO_3^- diet.

4.2. *Glucose and insulin kinetics*

Normal-weight subjects were characterized by a lower glucose peak and by a more rapid return of glucose to baseline levels, whereas glucose kinetics were delayed in the obese groups. The areas under the curve for the glucose and insulin kinetics were statistically different between the 3 groups ($P < .001$). The Obese-MetSyn group had a higher insulin secretory response compared with the other 2 groups. However, the rate of disappearance of insulin circulating levels was more rapid in the control group (Online Supplementary Material).

The metabolic parameters derived from the minimal model analysis are described in Table 2. The control group had higher Si values compared with the 2 obese groups, which had similar Si values regardless of the MetSyn diagnosis (Fig. 1). One subject had a higher Si value, but the exclusion of this outlier did not modify any of the results.

4.3. *NO production*

The assessment of NO production was performed using the oral nitrate test protocol method [18]. Obese subjects with

Table 1 – Baseline characteristics of the control group and the 2 obese groups categorized according to the diagnosis of MetSyn

	Controls	Obese NO-MetSyn	Obese-MetSyn	*P* value
n	7	7	7	
M/F	3/4	1/6	3/4	
Age (y)	27.4 (10.9)	48.0 (8.0)	48.0 (10.7)	**.01** [a, b]
Weight (kg)	65.2 (16.2)	95.7 (11.8)	103.9 (10.7)	**.001** [a, b]
Height (cm)	171.0 (14.0)	166.4 (9.8)	176.1 (5.4)	.24
BMI (kg/m^2)	21.9 (2.2)	34.5 (2.3)	33.4 (2.9)	**.001** [a, b]
Waist circumference (cm)	78.0 (10.7)	109.9 (8.5)	111.3 (5.1)	**.001** [a, b]
Fat mass (kg)	12.7 (4.8)	39.9 (5.0)	40.9 (9.8)	**.001** [a, b]
Fat-free mass (kg)	52.5 (15.3)	55.8 (11.7)	63.0 (11.1)	.22
Systolic blood pressure (mm Hg)	106.7 (8.3)	120.3 (13.1)	130.1 (11.6)	**.01** [b]
Diastolic blood pressure (mm Hg)	64.4 (10.2)	74.2 (10.2)	76.0 (7.7)	.16
Glucose (mmol/L)	4.3 (0.4)	4.7 (0.4)	4.9 (0.5)	.20
Insulin (pmol/L)	28.8 (6.8)	48.8 (18.1)	56.5 (12.5)	**.004** [a, b]
HOMA-R	0.92 (0.28)	1.71 (0.74)	1.98 (0.36)	**.005** [a, b]
TG (mmol/L)	0.8 (0.3)	1.1 (0.3)	1.8 (0.4)	**.006** [b]
HDL (mmol/L)	1.5 (0.3)	1.5 (0.1)	1.2 (0.1)	.07
Total cholesterol (mmol/L)	4.4 (0.7)	5.9 (1.2)	6.1 (1.4)	**.05** [b]
Creatinine (mmol/L)	74.7 (11.9)	63.0 (6.8)	78.7 (17.4)	.09
Urea (mmol/L)	4.0 (1.3)	4.5 (0.6)	5.2 (0.1)	.16
CRP (mg/L)	0.98 (0.63)	3.37 (1.66)	2.97 (1.90)	**.01** [a, b]
Urinary nitrate (mmol/L)	0.50 (0.51)	0.32 (0.23)	0.32 (0.22)	.48
Salivary nitrate (mmol/L)	0.25 (0.21)	0.27 (0.12)	0.48 (0.21)	.48

Means (SDs) are shown. Significant results are shown in bold. The Kruskal-Wallis test was used to test for differences across the 3 groups (trends). The Mann-Whitney test was used in a post hoc analysis to compare the individual groups. Fasting glucose and insulin plasma levels are used to calculate HOMA-R [23].
Letters indicate statistical significance between groups ($P < .05$):
[a] Control vs Obese NO-MetSyn.
[b] Control vs Obese MetSyn.

MetSyn had a significantly lower fractional rate of NO_3^- elimination (K_{UP}) compared with the control group, whereas the rate was not different between the 2 obese groups ($P = .08$). The rate of NO synthesis instead was significantly lower in the obese group with MetSyn compared with the other 2 groups (controls and obese without MetSyn), and the difference was not modified by the adjustment of the rate of NO synthesis for body weight (Table 2, Fig. 1). A wider distribution of the rates of NO synthesis was observed in the control group, whereas a narrower distribution and lower values were observed in the obese group with MetSyn (Fig. 1). The rates of NO production remained significantly different after adjusting the analysis for age ($P = .006$).

4.4. *NO and metabolic health*

The association between NO synthesis and the components of the MetSyn is described in Fig. 2. Fasting glucose and diastolic blood pressure were not significantly associated with rates of NO production, whereas a nonsignificant, negative trend was

Table 2 – Si and NO synthesis in healthy subjects and obese subjects with and without MetSyn

	Controls	Obese NO-MetSyn	Obese-MetSyn	*P* value
Si (pmol·h^{-1}·L^{-1})	0.012 (0.008)	0.0057 (0.0026)	0.0052 (0.0031)	**.05** [a, b]
Sg (h^{-1})	0.37 (0.15)	0.41 (0.06)	0.36 (0.12)	.31
AIR (pmol)	214.5 (109.7)	180.9 (77.1)	255.8 (128.8)	.50
DI	2.14 (0.89)	0.94 (0.44)	1.38 (1.12)	**.06** [a]
NO synthesis (mmol·h^{-1})	0.039 (0.017)	0.047 (0.019)	0.022 (0.012)	**.03** [b, c]
NO synthesis (μmol·kg^{-1}·h^{-1}) [d]	0.63 (0.29)	0.49 (0.22)	0.21 (0.13)	**.009** [b, c]

Means (SDs) are shown. Significant results are shown in bold. The Kruskal-Wallis test was used to test for differences across the 3 groups (trends). The Mann-Whitney test was used in a post hoc analysis to compare the individual groups. The model parameters have been estimated by modeling the kinetics of labeled glucose (hot model).
Letters indicate statistical significance between groups ($P < .05$):
[a] Control vs Obese NO-MetSyn.
[b] Control vs Obese MetSyn.
[c] Obese MetSyn vs Obese NO-MetSyn.
[d] Rate of NO synthesis is adjusted for body weight (kilograms).

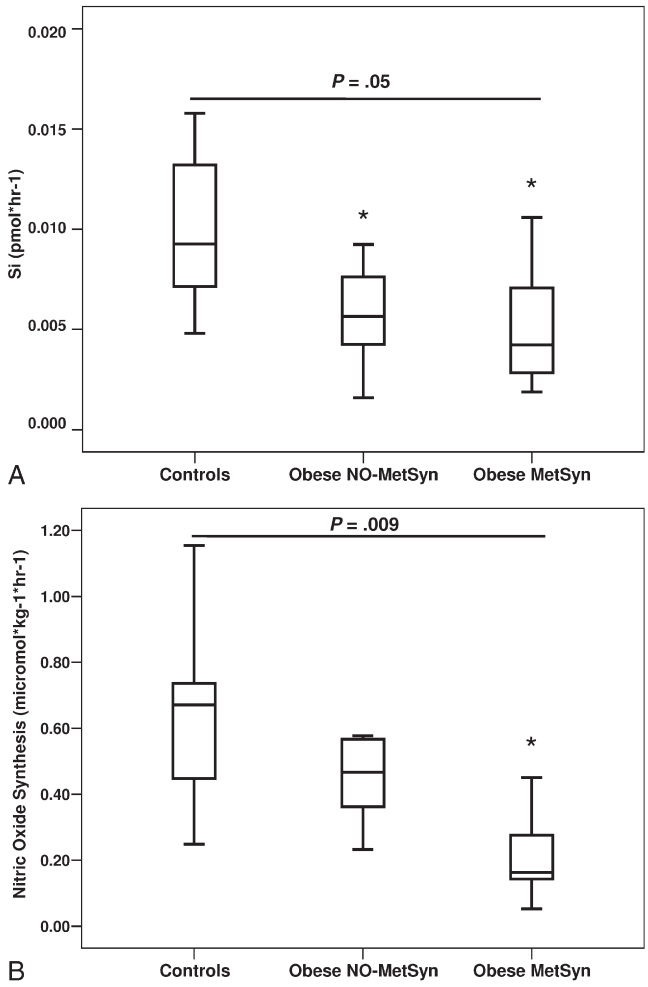

Fig. 1 – Insulin sensitivity (A) and in vivo rates NO synthesis (B) in controls and in obese subjects with and without MetSyn. *Significantly different from the control group.

observed for waist circumference. Nitric oxide synthesis significantly decreased with the increase in systolic blood pressure and TG. Plasma levels of HDL were directly associated with NO synthesis. The association between the rates of NO production and CRP was not significant (ρ = –0.26, P = .24). A significant correlation between NO synthesis and Si was found (ρ = 0.47, P = .03) (Fig. 3), and the relationship became marginally not significant after the adjustment for age (ρ = 0.39, P = .09). Three outliers were clearly identifiable in the scatter plot (Fig. 3), two with lower values of Si and high NO production and one with higher Si. The exclusion of these 3 subjects improved substantially the linearity of the association (N = 18, ρ = 0.71, P = .001), which was not modified by the adjustment for age (N = 18, ρ = 0.69, P = .002). The association of NO with Sg, AIR, and DI was not statistically significant.

4.5. *ADMA, NO production, and insulin resistance*

Circulating levels of ADMA were significantly higher in the obese group with MetSyn compared with normal-weight and obese subjects without MetSyn (Fig. 4A). The mechanistic link between ADMA and NO production pointed toward the existence of an inverse linear association. A nonsignificant negative trend between ADMA and NO synthesis was observed (Fig. 4B). A closer examination of the distribution of the data indicated a wider distribution of the data in the control and obese subjects without MetSyn and a narrower and more clustered distribution (high ADMA levels–low NO synthesis) in obese subjects with MetSyn. There was a significant association between ADMA and age, fasting glucose, and insulin levels; and a marginal, nonsignificant trend with HOMA-R (ρ = 0.42, P = .05) and Si (ρ = –0.31, P = .15) was observed (Online Supplementary Material).

5. Discussion

The study showed for the first time a significant association between rates of NO synthesis measured using stable isotopes and Si in subjects characterized by a different metabolic load. A nonsignificant trend was observed between ADMA circulating levels and NO production rates. A significant association was observed between systolic blood pressure, TG, and rates of NO synthesis.

Forte et al [25] describe a similar association between systolic blood pressure and NO production measured using stable isotopes in hypertensive subjects. The infusion of TG in rats caused insulin resistance and impaired physiologic insulin-mediated capillary recruitment in muscle, supporting a causal association between NO production and TG levels [26]. However, these findings were not replicated in another study because the relationship between ^{15}N-nitrate excretion and age, blood pressure (systolic or diastolic), plasma cholesterol, TG, or glucose levels was not significant [27]. Flow-mediated dilation (FMD) was comparable between controls and subjects with MetSyn [28,29], whereas a significant inverse association between FMD and MetSyn criteria was found in the Framingham Offspring cohort [30].

Avogaro et al [31] reported a lower rate of NO synthesis measured using stable isotopes in patients with type 2 diabetes mellitus diabetes (T2D) compared with healthy controls. A reduction in NO synthesis in T2D, assessed by flow-mediated vasodilation, was reported in another study that linked the impaired vascular function to a higher inflammatory status (higher CRP levels) in T2D patients [32].

The relationship between glucose metabolic clearance rate, assessed using a hyperinsulinemic-euglycemic clamp, and FMD was investigated in subjects with different levels of insulin resistance (insulin-resistant first-grade relative of T2D [FDR], insulin-sensitive FDR, and T2D). The study showed that FMD was significantly lower in insulin-resistant FDR and T2D compared with controls. In multiple regression analysis, low metabolic clearance rate was significantly correlated with FMD independent of age, sex, smoking, BMI, percentage body fat, serum insulin, and lipids [33]. The association of endothelial-dependent FMD with Si, again measured by hyperinsulinemic-euglycemic clamp, was investigated in older-aged men (>60 years) with MetSyn; but the relationship between FMD and insulin-mediated glucose uptake was not significant [29]. The association of FMD with the HOMA index in subjects with MetSyn was investigated in 2 large representative studies. The results were again discrepant, as the

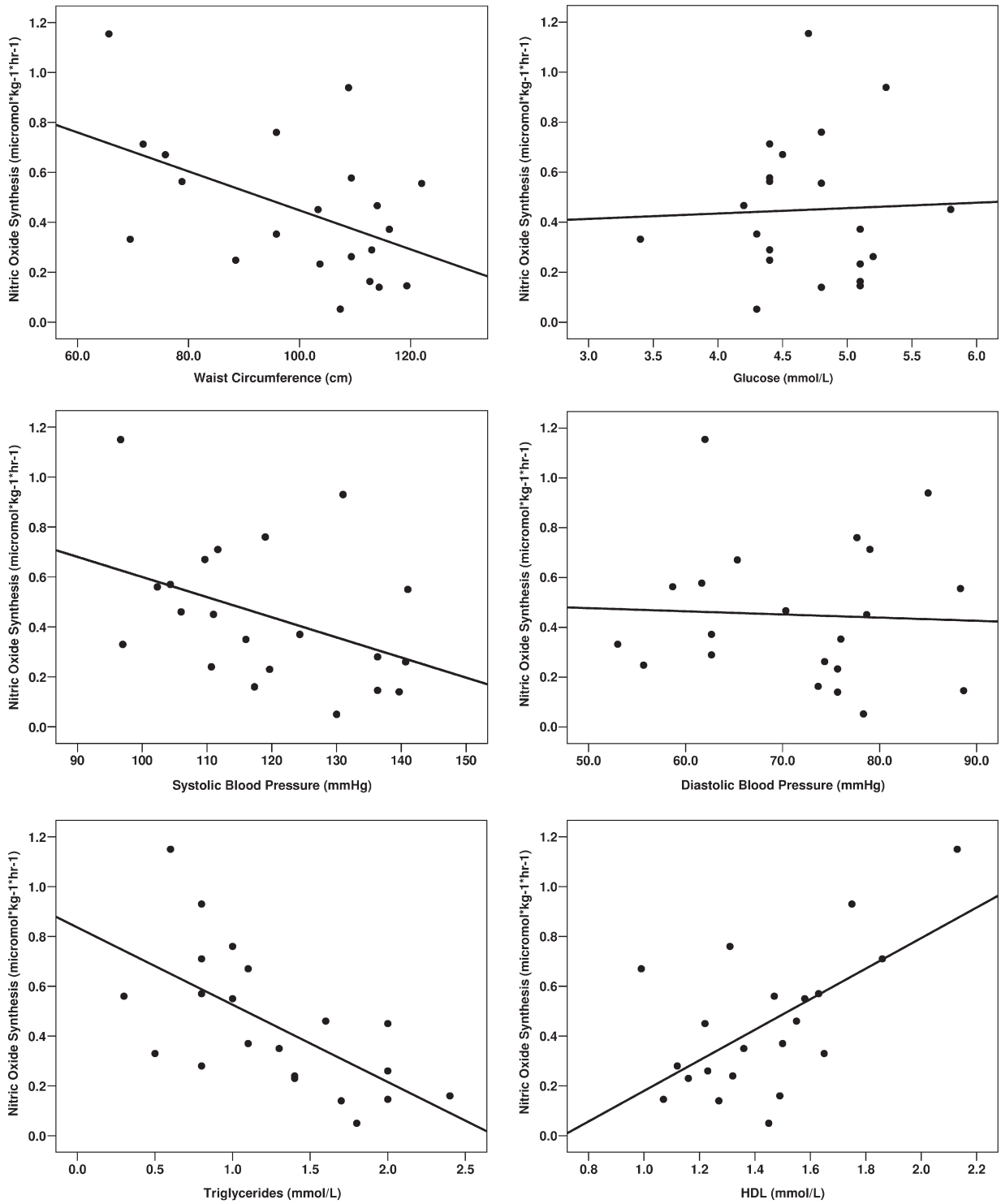

Fig. 2 – Scatter plots of NO synthesis with the individual components of the MetSyn in 21 adult subjects. Spearman rank correlation was used to test the strength of the associations. The ρ coefficients and *P* values for each analysis were as follows: waist circumference (ρ = –0.35, *P* = .11), fasting glucose (ρ = 0.05, *P* = .81), systolic blood pressure (ρ = –0.44, *P* = .04), diastolic blood pressure (ρ = –0.11, *P* = .61), TG (ρ = –0.67, *P* = .001), and HDL (ρ = 0.49, *P* = .02).

association was significant in one study [34] but not in the other [28].

Asymmetric dimethylarginine causes a reduction in NO synthesis by inhibiting the NO synthases [35]. The ADMA levels were higher in obese subjects with MetSyn. However, the relationship between ADMA and NO production was not linear because ADMA levels in controls and obese subjects without MetSyn were comparable. Conflicting results on the association between ADMA and NO synthesis have been reported, which may have been related to the different

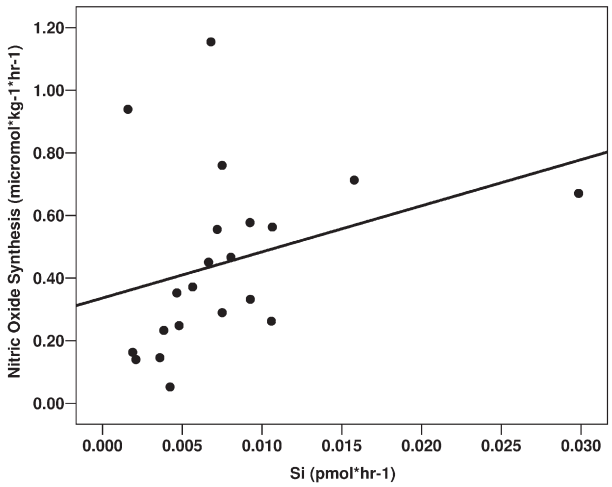

Fig. 3 – Scatter plot of Si and NO synthesis in 21 adult subjects. Spearman rank correlation was used to test the strength of the associations (ρ = 0.47, P = .03). The exclusion of the 3 identifiable outliers determines a correlation of 0.71 (P = .001).

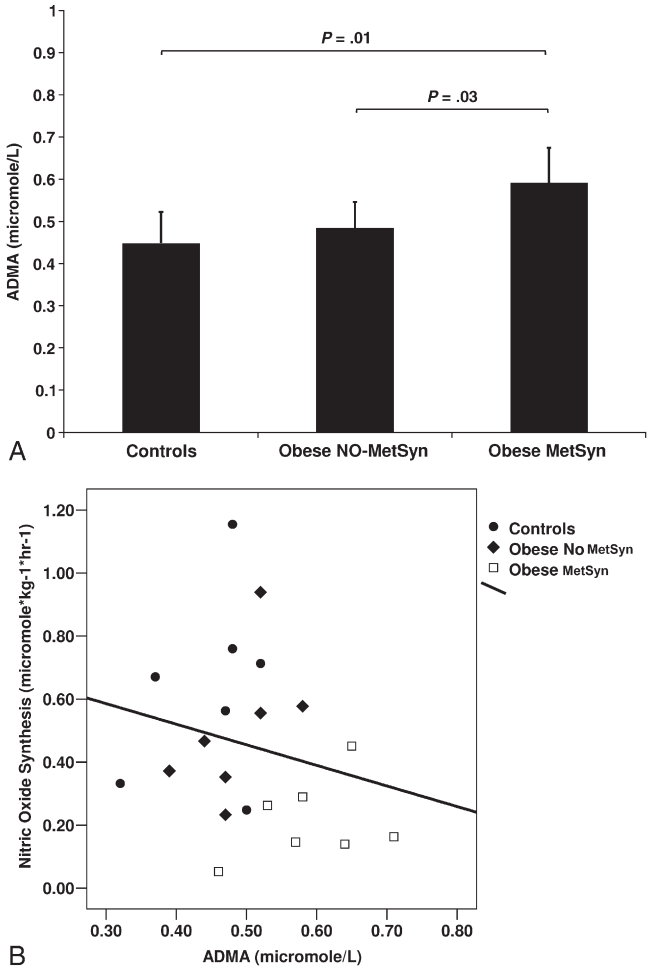

Fig. 4 – Mean plasma ADMA concentrations in healthy normal-weight subjects (n = 7) and in obese subjects without (n = 7) and with MetSyn (n = 7). The Mann-Whitney test was used to compare groups. Error bars are 1 SD (A). Scatter plot of plasma ADMA levels and NO synthesis in 21 adult subjects. The data points on the graph were categorized as controls and obese subjects without and with MetSyn. Spearman rank correlation was used to test the strength of the associations (ρ = –0.18, P = .42) (B).

methods used for the assessment of NO synthesis. The majority of the studies measured endothelial-dependent changes in blood flow or arterial diameter [36-44], and only one study used stable isotope tracers [36]. The stable isotopic study reported a significant reduction in NO synthesis in subjects with hypercholesterolemia (LDL >4.1 mmol/L) that was not explained by differences in ADMA levels [36]. On the contrary, higher levels of ADMA have been consistently reported in metabolic disorders such as T2D, obesity, MetSyn, hypertension, chronic heart failure, end-stage renal disease, dyslipidemia, and hyperhomocysteinemia [39,42,45-49]; and significant associations between ADMA levels and measures of vascular reactivity and atherosclerotic risk (intima media thickness) have been observed [50,51]. In addition, various studies have used indirect biomarkers (cyclic guanosine monophosphate [cGMP], nitrate + nitrite) to estimate whole-body NO production. Cui et al [52] reported that the number of components of MetSyn was inversely correlated with urinary cGMP excretion. Gomez et al [53] found that, compared with healthy subjects, patients with MetSyn had lower concentrations of plasma nitrite (measured using the Griess reaction) and cGMP. Contrasting results were reported in 2 studies, as nitrate + nitrite levels, measured using the Griess reaction, were significantly higher in overweight and obese subjects [54] and in obese subjects with MetSyn and T2D [55] compared with lean healthy subjects.

The use of dynamic test, stable isotopic methodologies, and mathematical modeling represents important features of this study because they provide greater accuracy and validate the biological plausibility of the association between rates of NO production with Si and cardiometabolic outcomes. One of the main limitations of the study is the cross-sectional design that cannot establish the causality and direction of the relationship between Si and NO production. In addition, a significantly younger control group may have confounded the association. We purposely chose this group as the reference population to remove potential age-related changes in hormonal and cardiometabolic functions to evaluate the deviation of NO production measured in obese, insulin-resistant individuals from optimal, physiological rates. The analyses were adjusted for age to account for the potential confounding effect.

6. Conclusions

Obese subjects with MetSyn had a decreased rate of NO synthesis compared with obese subjects without MetSyn and lean, healthy subjects. The causality and the direction of the relationship between NO production and Si were not established, but the methodologies and the results reported in this study could be used to design longitudinal studies to provide evidence on the mechanistic relationship between endothelial and metabolic functions. For example, Si could be

modified by dietary or pharmacological interventions such as weight loss, low glycemic index, or insulin-sensitizing agents; and the effects on rates of NO production could be measured. The longitudinal paradigm could also be reversed by the adoption of dietary (inorganic nitrate, arginine) and pharmacological interventions (statins, NO donors) influencing vascular reactivity and then evaluation of their effects on metabolic functions.

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Conflict of Interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.metabol.2011.10.003.

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