Table 2. Comparison of atherosclerotic parameters between Japanese and Mongolian patients: separate populations by sex

Parameters	Men		Women	
	Japanese $n=71$	Mongolian $n=71$	Japanese $n=85$	Mongolian $n=85$
Age, years	56.9 ± 8.6	55.9 ± 8.8	58.4 ± 8.2	58.2 ± 7.9
Smoking, %	29.6	46.5**	8.2	9.4
BMI, kg/m^2	23.4 [20.9-25.5]	27.6 [24.9-31.2]**	24.0 [21.6-27.0]	27.3 [23.7-30.8]*
HR, bpm	64.0 [59.0-71.5]	68.0 [58.0-80.0]	65.0 [57.0-70.5]	67.0 [60.0-76.5]
SBP, mmHg	126.0 [118.5-136.5]	144.0 [134.0-160.0]**	130.1 ± 16.0	150.5 ± 27.9**
DBP, mmHg	80.0 [76.0-88.0]	93.0 [85.0-102.0]*	79.0 [74.0-89.0]	90.0 [80.0-101.5]**
PP, mmHg	45.0 [41.5-50.5]	51.0 [43.0-59.0]*	48.0 [41.0-55.0]	55.0 [45.5-67.0]*
T-Cho, md/dL	182.0 [154.5-193.5]	181.0 [162.0-194.0]	190.8 [175.2-205.7]	177.0 [159.5-196.0]**
Glucose, mg/dL	115.0 [101.5-147.5]	84.0 [78.0-107.0]**	100.0 [90.5-135.5]	82.0 [74.0-123.5]*
Insulin, μU/mL	5.2 [3.9-10.2]	8.2 [4.7-16.6]*	6.1 [3.7-8.8]	9.2 [5.9-14.5]**
HOMA-IR	1.62 [1.04-2.84]	1.81 [1.01-4.20]	1.60 [0.97-2.53]	2.18 [1.19-3.85]
IMT, mm	0.73 [0.60-0.95]	1.04 [0.71-1.39]*	0.67 [0.60-0.80]	0.79 [0.65-1.53]**
CRP, mg/dL	0.05 [0.03-0.13]	0.20 [0.09-0.49]**	0.05 [0.03-0.12]	0.19 [0.09-0.37]**
ABI	1.16 [1.12-1.21]	1.17 [1.12-1.22]	1.15 ± 0.06	1.13 ± 0.07*
CAVI	8.3 ± 1.1	8.9 ± 1.2*	8.0 ± 1.0	8.8 ± 1.2**
Disease composition				
HT, %	39.4	45.1	55.3	48.2
DM, %	33.8	32.4	23.5	29.4
HT plus DM, %	26.8	22.5	21.2	22.4

Age and CAVI in men and ABI in women were presented as the mean ± standard deviation, respectively. Other parameters are presented as the median [interquartile range]. BMI: body mass index, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, T-Cho: total cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, CRP: C-reactive protein, IMT: intima-media thickness, ABI: ankle-brachial index, CAVI: cardio-ankle vascular index, HT: hypertension, DM: diabetes mellitus. Significance level: *$p<0.05$, **$p<0.01$ (Comparison between Mongolian and Japanese patients by sex, smoking and disease composition: χ^2-test, other parameters: unpaired t-test after the log-transformation; smoking adjustment was performed).

A p-value <0.05 was considered significant.

Results

The clinical characteristics of the measured parameters in all patients are listed in **Table 1**. The levels of BMI, HR, SBP, DBP, PP, insulin, CRP, IMT and CAVI were significantly higher and the levels of T-Cho and glucose were significantly lower in Mongolian patients than Japanese patients. There was no significant difference between the percentage of drug-treated Mongolian and Japanese patients. **Table 2** shows the differences in atherosclerotic parameters by sex between Japanese and Mongolian patients. Except for the ABI and smoking status in women, no differences by sex were observed: the levels of BMI, SBP, DBP, PP, insulin, CRP, IMT and CAVI were significantly higher and T-Cho and insulin were significantly lower in Mongolian patients than in Japanese patients in both sexes.

Moreover, concerning the higher levels of CRP and CAVI in Mongolian patients than Japanese patients for both sexes (as shown in **Table 1**, **2**), we determined statistically significant differences (p-values in the general linear model) in CRP and CAVI between Japanese and Mongolian patients after adjusting for several variables, such as basic demographics, SBP, DBP and HOMA-IR. For example, the following models were tested in all patient groups and the respective sex-separated groups: Model I: adjusted for age, sex and smoking; Model II: adjusted for age, sex, smoking and BMI; Model III: adjusted for age, sex, smoking and HOMA-IR; Model IV: adjusted for age, sex, smoking, SBP and DBP; Model V: adjusted for age, sex, smoking, BMI, SBP, DBP and HOMA-IR. For CRP, p-values for differences in levels between Japanese and Mongolian patients were <0.01 (significant) in all models, regardless of sex. Also, for CAVI, p-values for differences between patients were <0.01 (0.03 in Model IV only) (significant) in all models, regardless of sex. Therefore, even with these adjustments, the results regarding the differences in CRP

Table 3. Comparison of atherosclerotic parameters between Japanese and Mongolian patients: separate populations of HT, DM and HT plus DM

Parameters	HT		DM		HT plus DM	
	Japanese $n=75$	Mongolian $n=73$	Japanese $n=44$	Mongolian $n=48$	Japanese $n=37$	Mongolian $n=35$
Age, years	57.8 ± 8.7	57.6 ± 8.2	57.2 ± 7.6	55.3 ± 8.0	58.1 ± 8.9	58.7 ± 9.2
Men, %	37.3	43.8	54.5	45.5	51.4	45.7
Smoking, %	16.0	23.3**	25.0	29.2**	13.5	28.6**
BMI, cm/kg^2	23.8 [22.4-26.1]	27.0 [24.1-30.9]**	21.9 [19.8-24.9]	26.9 [23.3-30.4]**	25.2 [22.6-29.3]	28.1 [26.1-32.6]
HR, bpm	64.0 [57.0-71.0]	65.0 [58.0-75.0]	63.0 [59.0-71.0]	68.5 [62.3-79.0]*	66.9 ± 10.7	69.3 ± 14.4
SBP, mmHg	132.0 ± 14.3	153.5 ± 18.3**	123.3 ± 15.1	127.0 ± 17.6	132.0 ± 12.7	167.0 ± 21.8**
DBP, mmHg	84.7 ± 10.1	95.6 ± 11.4**	76.1 ± 8.1	82.5 ± 12.5*	82.0 [75.5-86.5]	100.0 [89.5-107.0]*
PP, mmHg	46.0 [41.0-53.0]	57.0 [50.0-66.0]**	46.0 [40.3-51.0]	44.5 [37.3-50.8]	49.0 [44.0-56.0]	62.0 [48.0-83.0]**
IMT, mm	0.63 [0.57-0.80]	0.84 [0.69-1.43]*	0.73 [0.64-0.85]	0.81 [0.60-1.32]	0.83 [0.67-1.00]	1.11 [0.69-1.66]*
T-Cho, mg/dL	187.0 [172.0-199.3]	184.0 [160.0-196.5]	183.0 [170.5-195.0]	176.5 [156.8-192.5]	190.0 [173.0-205.7]	175.0 [165.0-196.0]
Glucose, mg/dL	95.0 [89.0-104.0]	78.0 [72.0-85.0]**	143.0 [111.3-186.3]	94.0 [80.3-169.5]*	139.0 [110.5-159.0]	128.0 [82.0-185.0]
Insulin, μU/mL	5.3 [4.0-8.9]	7.2 [5.7-14.0]*	6.0 [3.0-10.9]	8.7 [4.6-14.5]*	7.2 [4.5-12.2]	14.4 [9.7-18.0]*
HOMA-IR	1.23 [0.84-2.20]	1.49 [1.05-2.68]	1.94 [0.97-4.22]	2.37 [1.17-4.09]	2.49 [1.48-4.57]	4.63 [2.43-6.02]
CRP, mg/dL	0.06 [0.03-0.12]	0.18 [0.06-0.37]**	0.05 [0.03-0.12]	0.18 [0.08-0.34]**	0.05 [0.03-0.17]	0.29 [0.13-0.51]**
ABI	1.15 ± 0.07	1.13 ± 0.07	1.16 ± 0.07	1.18 ± 0.07	1.17 [1.13-1.24]	1.13 [1.07-1.20]
CAVI	8.0 ± 0.9	8.9 ± 1.1**	8.2 ± 1.0	8.4 ± 1.1	8.5 [7.5-9.4]	9.4 [8.7-10.2]*

Age, SBP, DBP, ABI and CAVI in HT and DM and age, SBP, DBP and PP in HT plus DM were presented as the mean ± standard deviation, respectively. Other parameters are presented as the median [interquartile range]. BMI: body mass index, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, T-Cho: total cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, CRP: C-reactive protein, IMT: intima-media thickness, ABI: ankle-brachial index, CAVI: cardio-ankle vascular index, HT: hypertension, DM: diabetes mellitus. Significance level: $^*p<0.05$, $^{**}p<0.01$ (Comparison between Mongolian and Japanese patients by disease; sex and smoking: χ^2-test, other parameters: unpaired t-test after the log-transformation; smoking adjustment was performed in each separate population).

and CAVI levels remained unchanged.

Table 3 shows the differences in atherosclerotic parameters between Japanese and Mongolian patients by HT and DM. In the population of HT alone, the levels of BMI, SBP, DBP, PP, insulin, CRP, IMT and CAVI were significantly higher, and the levels of glucose were significantly lower in Mongolian patients than Japanese patients. In the population of DM alone, the levels of BMI, HR, DBP, insulin and CRP were significantly higher, and glucose was significantly lower in Mongolian patients than Japanese patients. The levels of SBP, PP and CAVI in Mongolian DM patients were relatively similar to those of Japanese DM patients. In the population of HT plus DM, the levels of SBP, DBP, PP, CRP, IMT and CAVI were significantly higher in Mongolian patients than Japanese patients.

Following the results of **Table 3**, **Table 4** shows the data obtained from a subanalysis of patients matched according to the SBP and DBP levels (1:1 ratio with ± 1 mm Hg in the respective Mongolian and Japanese patients) in the population of HT alone. Even in the current subanalysis, the differences in atherosclerotic parameters including CRP, IMT and CAVI between Mongolian and Japanese patients similarly remained.

Discussion

The present study is the first attempt to compare atherosclerotic parameters, including CAVI and CRP in particular, between populations of HT and DM patients in Mongolia and Japan. Notably, the overall levels of atherosclerotic parameters had higher tendencies, despite lower levels of T-Cho and glucose, in Mongolian patients than Japanese patients. Namely, the levels of BMI, HR, SBP, DBP, insulin, CRP, IMT and CAVI were significantly higher in Mongolian patients. While higher levels of several atherosclerotic parameters have been reported in Mongolian young and healthy people in comparison to similar Japanese people[5], the present study findings may be valuable, if consistent with the study on young people and if the higher mortality rates of CVD in Mongolian people are relevant to the higher levels of atherosclerotic parameters in comparison to Japanese people.

Table 4. Comparison of atherosclerotic parameters between Japanese and Mongolian patients: subanalysis with adjustments for blood pressure levels in hypertensive patients

Parameters	Japanese $n=36$	Mongolian $n=36$
Age, years	57.3±8.9	57.6±8.2
Men, %	44.4	47.2
Smoking, %	19.4	22.2
BMI, kg/m^2	24.5±3.8	26.7±4.4*
HR, bpm	66.5±8.5	68.0±13.0
SBP, mmHg	139.1±11.8	139.2±13.4
DBP, mmHg	87.4±8.6	87.5±8.9
PP, mmHg	51.6±8.4	49.5±11.0
IMT, mm	0.65 [0.60-0.73]	0.80 [0.69-1.30]*
T-Cho, mg/dL	184.9±22.0	183.5±35.1
Glucose, mg/dL	96.5 [90.0-104.5]	78.5 [72.3-84.8]**
Insulin, μU/mL	6.6 [3.9-8.9]	7.0 [5.5-13.0]*
HOMA-IR	1.42 [0.87-2.47]	1.46 [1.05-2.55]
CRP, mg/dL	0.05 [0.03-0.12]	0.20 [0.08-0.38]**
ABI	1.15±0.07	1.15±0.07
CAVI	8.0±1.0	8.9±1.1**

Age, BMI, HR, SBP, DBP, PP, T-Cho, ABI and CAVI were presented as the mean±standard deviation. Other parameters are presented as the median [interquartile range]. BMI: body mass index, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, T-Cho: total cholesterol, HOMA-IR: homeostasis model assessment of insulin resistance, CRP: C-reactive protein, IMT: intima-media thickness, ABI: ankle-brachial index, CAVI: cardio-ankle vascular index, HT: hypertension, DM: diabetes mellitus. Significance level: *$p<0.05$, **$p<0.01$ (sex and smoking: χ^2-test, other parameters: unpaired t-test after the log-transformation; smoking adjustment was performed).

CAVI is a newly developed index of arterial stiffness and its clinical significance in CVD has recently been established[6, 17]. Also, CRP has recently been identified to be a clinical useful atherosclerosis-related marker with accumulated data, for instance, showing up-regulated expression of endothelial adhesion molecules and inhibited expression of nitric oxide synthase in endothelial cells[18, 19]. Atherosclerotic profiles, including these new two markers, between Mongolian and Japanese patients were relatively similar in HT alone and HT plus DM. In DM, the obvious difference in CRP levels between Mongolian and Japanese patients was similar to that in HT and HT plus DM, while no marked difference in CAVI was observed. Although the reasons for this result in regard to the association with CAVI in DM were unclear, the non-significant but slightly younger age in Mongolians in comparison to Japanese DM patients might have partly affected the result. Regardless, it is important to disclose higher levels of atherosclerotic parameters such as CRP in patients with HT, DM and HT plus DM, and/or as CAVI in patients with HT and HT plus DM. Insulin resistance is generally regarded as one of the underlying mechanisms of these disorders[20]. Our Mongolian patients with both HT and DM presented higher levels of BMI and insulin, a suggestive parameter of greater insulin resistance, in comparison to the Japanese patients. Furthermore, an earlier work has reported greater levels of insulin resistance in Mongolian workers than Japanese workers[21]. However, interestingly, our study showed that significant differences in CRP and CAVI between Japanese and Mongolian patients were maintained, even after adjusting for factors related to insulin resistance (e.g., BMI, HOMA-IR) and/or other factors, such as blood pressure levels. One possible explanation for this may be the existence of differences in environmental factors. The enhanced effect of high altitude on not only blood pressure[22] but CAVI[5, 23] in Mongolia has been reported. The lowered effect of high altitude on plasma glucose has also been reported[24], and in fact our studied Mongolian DM patients had lower glucose levels. From the biological viewpoint in association with altitude, an increase in oxidative stress (via reactive oxygen metabolism) in Mongolian people compared with Japanese people[25, 26] has been reported, and this may cause differences in atherosclerotic parameters[27]. Additionally, genetic factors could have played roles in the present results. Whereas the genetic background of Mongolian and Japanese people is generally thought to be similar, different frequencies of some gene polymorphisms have been suggested[28-30]. These factors are thought to merit further investigation to elucidate such differences between Mongolian and Japanese patients, while more unmeasured factors might remain hidden.

The present study had strength in that all measurements were performed using the same methodology and at the same central facility, which is necessary in an international comparative study; however, there were a few potential limitations. The cross-sectional design did not address the cause-and-effect. In the population of HT and DM, information on the duration and degree of disease and the precise medication was not fully examined. Differences in medical circumstances can also induce differences in atherosclerotic parameters between the two populations. Different medication strategies (e.g., greater usage of angiotensin-converting enzyme inhibitors in Mongolian HT patients and angiotensin receptor blockers in Japanese HT patients) might result in some differences. Actually, blood hemoglobin A1c, a long-term glyce-

mic parameter, is not usually measured in Mongolia, in contrast to Japan, and this might effect the disease management between countries (although Mongolian and Japanese patients do not seem to have a large different background because of the diagnostic criteria for DM by WHO, as mentioned above). The absence of detailed information is thought to be a great limitation; therefore, more sophisticated research is called for in any future surveys.

In summary, the current study found, on the whole, more atherosclerotic traits, including CAVI and CRP, in addition to BMI, HR, SBP, DBP, PP and insulin, in Mongolian patients with HT and DM than Japanese patients. This suggests that Mongolian patients may be at higher risk for CVD than Japanese patients. Further studies are needed to clarify the mechanisms of higher levels of atherosclerotic parameters in Mongolian patients.

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Original Article

Plasma NOx Concentrations in Glucose Intolerance and Type 2 Diabetes: a Case-control Study in a Vietnamese Population

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Aim: The Vietnamese develop type 2 diabetes (T2D) and metabolic syndrome (MS) at a lower BMI than other ethnicities. Thus, biomarkers that identify subjects at an increased risk of T2D independently of obesity are being sought. Recent studies show that circulating NO metabolites (NOx) are increased in T2D. We investigated whether plasma NOx levels predict insulin resistance and glucose intolerance before the development of T2D, independently of obesity.
Methods: The current study was derived from a population-based study in HCMC, Vietnam, which was designed to investigate the prevalence of MS and T2D in a population aged 30-69 years. Four hundred and twenty-two subjects were recruited from the study and were stratified into 4 age- and gender-matched groups according to a glucose tolerance test {normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and T2D}.
Results: Plasma NOx concentrations were significantly increased in T2D but not in IFG or IGT compared with NGT. Multiregression analysis showed that plasma NOx levels were inversely correlated with BMI in T2D whereas no association was found between plasma NOx levels and BMI in non-diabetic subjects. Moreover, there was no correlation between plasma NOx levels and homeostasis model assessment-insulin resistance (HOMA-IR) in both diabetic and non-diabetic subjects.
Conclusion: Plasma NOx levels did not predict glucose intolerance or insulin resistance before the development of T2D and the increase in plasma NOx levels in T2D was not caused by adiposity. Thus, plasma NOx is not a useful marker for the prediction of high-risk subjects for T2D among Vietnamese.

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Key words; Case-control study, Insulin resistance, Adiposity

Abbreviations; BMI: body mass index, eNOS: endothelial nitric oxide synthase, HDL-C: high-density lipoprotein cholesterol, HOMA-IR: homeostasis model of assessment of insulin resistance, hsCRP: high sensitive C-reactive protein, IFG: impaired fasting glucose, IGT: impaired glucose tolerance, iNOS: inducible nitric oxide synthase, MS: metabolic syndrome, NGT: normal glucose tolerance, NO: nitric oxide, NOx: nitrite and nitrate combined, OGTT: oral glucose tolerance test, T2D: type 2 diabetes

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Introduction

Obesity is a major predictor of type 2 diabetes (T2D); however, similar to other Asians, Vietnamese are known to develop metabolic syndrome and T2D

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at a lower body mass index (BMI) than Whites[1-3]. Moreover, abdominal obesity, dyslipidemia and hypertension despite having a normal BMI are more common among Vietnamese (unpublished observation), suggesting the widespread presence of insulin resistance without being overweight or obese. Therefore, it is hoped to identify circulating biomarkers that can predict high-risk subjects for developing T2D independently of obesity. Thus far, C-reactive protein (CRP) in association with TNF-α receptor 2 and IL-6 has been shown to predict T2D independently of BMI and other indices of obesity in some studies[4-8]; however, others have shown that the association between CRP and T2D is largely due to obesity[9-12].

Recently, several studies indicated that circulating levels of the metabolites of nitric oxide (NOx) are associated with T2D. A population-based study conducted by Zahedi *et al.* demonstrated that serum NOx concentrations were significantly elevated in subjects with metabolic syndrome and T2D compared to their corresponding controls[13]; however, there is a contradicting report showing that increased plasma NO levels were observed in T2D but not in non-diabetic subjects with the presence of insulin resistance[14]. Thus, although these studies unequivocally demonstrated that circulating NOx levels are increased in T2D subjects, it is still controversial whether circulating NOx levels can predict high-risk subjects before the development of T2D.

This study was undertaken to investigate whether plasma NOx levels predict high-risk subjects for T2D independently of obesity using a population in which obesity is rare and yet the incidence of T2D is markedly increasing.

Methods

Study design and measurements

The present study is derived from a population-based study in HCMC, Vietnam. Subjects aged 30-69 years attended a health check up and underwent anthropometric and clinical measurements (weight, height, waist circumference and blood pressure), sampling of venous blood at 5:00-7:00 a.m. after overnight fasting and 75-g oral glucose tolerance test (OGTT). Blood pressure was measured in the sitting position after 5 min of rest. BMI was determined as weight in kilograms divided by the square of the height in meters (kg/m^2). Fasting serum was separated from coagulated whole blood and insulin, high sensitive C-reactive protein (hsCRP), total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by Diag Center International (Lab Group International-Division Vietnam) on the same day as blood sampling. The TG/HDL-C ratio was calculated as a surrogate marker for insulin resistance[15-16]. The homeostasis model of assessment of the insulin resistance (HOMA-IR) score was calculated as fasting insulin (μU/mL) multiplied by fasting glucose (mmol/L) divided by 22.5[17]. Plasma specimens were separated from non-coagulated whole blood and were frozen at −70℃ until NOx measurements.

75-g OGTT

Fasting and 2-h postload glucose levels of 75-g OGTT were measured. The criteria of the 1999 World Health Organization were used to stratify subjects into 4 groups: normal glucose tolerance (NGT); impaired fasting glucose (IFG); impaired glucose tolerance (IGT) and type 2 diabetes (T2D)[18]: for T2D, fasting ≥ 7.0 mmol/L (126 mg/dL), 2-h ≥ 11.1 mmol/L (200 mg/dL); for IGT, fasting <7.0 mmol/L (126 mg/dL) and 2-h ≥ 7.8 mmol/L (140 mg/dL) and < 11.1 mmol/L (200 mg/dL); and for IFG ≥ 6.1 mmol/L (110 mg/dL) and <7.0 mmol/L (<126 mg/dL) and if measured, 2-h <7.8 mmol/L (140 mg/dL). A total of 422 subjects were recruited from the list of the main study in such a way that each OGTT-stratified group was matched for gender and age group.

The study was approved by the Institutional Review Board of the Health Services of Ho Chi Minh City and all participants signed informed consent.

Measurements of plasma NOx

The concentrations of plasma NO_2^- (nitrite) and NO_3^- (nitrate), stable metabolites of NO, were measured by the Griess reaction using an HPLC-Griess system (NOx Analyzer ENO-10; EiCom Instrument, Kyoto, Japan) as previously described[19]. Briefly, 30 μL plasma is mixed with a equal volume of methanol and centrifuged at 15,000 rpm for 15 min to precipitate protein. Ten microliters each of supernatants are injected into the NOx Analyzer using an automatic injector (Gilson, Middleton, WN, USA). In the analyzer, nitrite and nitrate are separated on a reverse-phase separation column. Nitrite is then mixed with the Griess reagent to form a purple azo dye in the reaction coil, whose absorbance is measured at 540 nm by a flow-through spectrophotometer. The absorbance reaches a peak with a retention time of approximately 4.5 min. Nitrate is reduced to nitrite with a cadmium reduced copper column, which subsequently reacts with the Griess reagent. This peak arrives with a retention time of approximately 8 min. The area under the absorption curve is compared with that of a standard

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Table 1. Characteristics of 4 OGTT-stratified groups

	NGT (n=120)	IFG (n=111)	IGT (n=101)	T2D (n=90)	All (n=422)
Gender, n (%)					
Men	57 (13.5)	51 (12.1)	47 (11.1)	47 (11.1)	201 (47.9)
Women	63 (14.9)	60 (14.2)	54 (12.8)	43 (10.2)	220 (52.1)
Age (yrs)	50.9 ± 11.2	50.1 ± 10.8	53.1 ± 10.0	57.7 ± 8.0***	52.7 ± 10.6
BMI (kg/m^2)	22.7 ± 2.9	23.4 ± 3.5	24.2 ± 4.1**	23.8 ± 3.8*	23.5 ± 3.6
Waist circumference (cm)	78.4 ± 9.4	80.4 ± 9.5	82.9 ± 10.2**	83.1 ± 9.2***	81.0 ± 9.7
Systolic BP (mmHg)	118 ± 18	123 ± 22	127 ± 20**	132 ± 20***	125 ± 20
Diastolic BP (mmHg)	73 ± 10	75 ± 12	78 ± 11**	76 ± 10*	75 ± 11
Fasting glucose (mmol/L)	5.1 (0.4)	5.7 (0.3)	5.5 (0.7)	7.0 (2.7)	5.6 (0.8)
Fasting insulin (μU/mL)	6.4 (6.0)	7.6 (6.3)*	9.0 (8.4)***	10.2 (10.2)***	8.1 (7.3)
HOMA-IR	1.5 (1.4)	1.9 (1.6)*	2.3 (2.0)***	3.1 (2.8)***	2.0 (2.0)
Total cholesterol (mg/dL)	210 ± 40	203 ± 39	210 ± 47	212 ± 55	208 ± 45
HDL-C (mg/dL)	55 ± 14	54 ± 15	52 ± 12	49 ± 13**	53 ± 14
Non HDL-C (mg/dL)	155 ± 41	149 ± 40	157 ± 46	163 ± 54	156 ± 45
Triglyceride (mg/dL)	137 (101)	138 (119)	168 (114)	204 (160)***	156 (128)
TG: HDL-C ratio	2.6 (2.8)	2.6 (3.8)	3.2 (3.3)	4.4 (4.1)***	3.1 (3.5)
hsCRP (mg/L)	1.0 (2.0)	1.0 (2.0)	2.0 (2.5)	2.0 (3.0)**	1.0 (2.0)
NOx (μmol/L)	26.4 (17.6)	26.4 (16.4)	23.5 (21.0)	35.7 (31.3)**	26.5 (21.5)

BMI, body mass index; BP: blood pressure; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment of insulin resistance; hsCRP, high sensitive C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; NOx, nitrite and nitrate combined; OGTT: oral glucose tolerance test; T2D, type 2 diabetes; TG: triglyceride.
Average age, BMI, waist circumference, body fat percentage, total cholesterol, HDL-C and non HDL-C are expressed as the mean ± SD and differences among groups were examined by one-way ANOVA and t-test. Triglyceride, TG: HDL-C ratio, fasting glucose, fasting insulin, HOMA-IR, hsCRP and NOx are presented as the median followed by interquartile range in parentheses and differences among groups were examined by the non-parametric Mann-Whitney test. P values refer to differences as determined by t tests or the Mann-Whitney test. *P<0.05, **P<0.01, ***P<0.001 compared with NGT group.

solution containing sodium nitrite and sodium nitrate (Wako Pure Chemical Industries Inc.) to determine plasma nitrite and nitrate concentrations. Plasma NOx concentration was obtained by summing nitrite and nitrate concentrations.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 14.0 (SPSS, Chicago, IL). Categorical variables were presented as percentages with 95% confidence intervals, and differences between subgroups were examined using Pearson chi-squared tests. Normally distributed continuous variables were presented as the mean and standard deviation and differences among groups were examined by one-way ANOVA and t-test. Skewed continuous variables (TG, fasting glucose, fasting insulin, HOMA-IR, and NOx) were presented as the median and interquartile range and differences among groups were examined by the non-parametric Kruskal-Wallis and Mann-Whitney tests. Spearman correlation analysis was used to assess the univariate correlation between NOx and potential predictors. Skewed variables were logarithmically converted before correlation analysis. Gender, age and covariates that were positively correlated to NOx from univariate correlation analysis were selected for forward stepwise multiple linear regression analysis to determine factors independently associated with NOx. All statistical tests used a significance level of P<0.05.

Results

Characteristics of 4 OGTT-stratified groups

Characteristics of 4 OGTT-stratified groups are shown in **Table 1**. Four groups were similar in gender. Subjects with T2D were older than those with NGT and IFG (P<0.001). Both IGT and T2D groups had significantly greater BMI (P<0.01 and P<0.05, respectively) and waist circumference (P<0.01 and P<0.001, respectively) than NGT group. Both systolic and diastolic blood pressure were significantly higher in both IGT (P<0.01 for both systolic and diastolic blood pressure) and T2D (P<0.001 and P<0.05, respectively) groups than NGT group.

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Table 2. Spearman univariate correlations (r) between plasma NOx concentrations and demographic, anthropometric and biochemical variables in 4 OGTT-stratified groups

	NGT ($n=120$)	IFG ($n=111$)	IGT ($n=101$)	T2D ($n=90$)
Gender	−0.218*	−0.240*	−0.149	−0.176
Age	0.024	0.050	0.097	0.002
BMI	0.079	−0.038	−0.079	−0.247*
Waist circumference	0.088	0.008	0.077	−0.183
Systolic blood pressure	0.079	0.141	0.018	−0.099
Diastolic blood pressure	0.018	0.150	0.008	−0.251*
Fasting glucose	0.172	0.215*	0.125	0.191
Fasting insulin	−0.076	−0.113	−0.050	−0.174
HOMA-IR	−0.057	−0.098	−0.019	−0.099
Total cholesterol	0.090	−0.101	−0.080	−0.071
Triglyceride	0.265**	0.124	0.133	0.144
HDL-C	−0.141	−0.158	−0.239*	−0.208*
Non HDL-C	0.104	−0.056	−0.017	−0.018
TG: HDL-C ratio	0.258**	0.133	0.159	0.198
hsCRP	−0.027	−0.155	−0.001	−0.016

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment of insulin resistance; hsCRP, high sensitive C-reactive protein; NOx, nitrite and nitrate combined; TG: triglyceride.
r indicates Spearman correlation coefficient between plasma NOx concentration and each variable. *$P<0.05$, **$P<0.01$

While no significant difference was found in the concentration of serum total cholesterol or non HDL-C among the 4 groups, T2D group had lower HDL-C ($P<0.01$) and higher serum triglyceride ($P<0.001$), resulting in a higher TG/HDL-C ratio ($P<0.001$) than NGT. This higher TG/HDL-C ratio was not observed in IFG or IGT group compared with NGT group.

Fasting serum insulin levels were higher in IFG, IGT and T2D groups ($P<0.05$, $P<0.001$ and $P<0.001$, respectively) compared with NGT group. HOMA-IR was significantly greater in IFG, IGT and T2D groups ($P<0.05$, $P<0.001$ and $P<0.001$, respectively) compared with NGT group.

Serum hsCRP and plasma NOx concentrations were higher in T2D ($P<0.01$) but not in IFG or IGT group compared with NGT group.

Univariate correlation analysis to determine factors associated with plasma NOx concentrations in 4 OGTT-stratified groups

To identify variables that are associated with plasma NOx concentrations, univariate correlation analysis was performed separately in each OGTT-stratified group. The Spearman correlation coefficients of variables in the association with plasma NOx concentration are shown in **Table 2**. Among subjects with T2D, BMI ($r=-0.247$, $P<0.05$), HDL-C ($r=-0.208$, $P<0.05$) and diastolic blood pressure ($r=-0.251$, $P<0.05$) were inversely correlated with plasma NOx concentrations whereas no significant association was found between plasma NOx concentrations and HOMA-IR.

Although plasma NOx levels were similar among 3 non-diabetic groups (NGT, IFG and IGT), factors associated with plasma NOx were different. Among NGT subjects, plasma NOx levels were positively correlated with male sex ($r=-0.218$, $P<0.05$), serum TG levels ($r=0.265$, $P<0.01$) and TG: HDL-C ratios ($r=0.258$, $P<0.01$). Among IFG subjects, plasma NOx levels were positively correlated with male sex ($r=-0.240$, $P<0.05$) and fasting glucose levels ($r=0.215$, $P<0.05$). Among IGT subjects, plasma NOx levels were inversely correlated with serum HDL-C levels ($r=-0.239$, $P<0.05$); however, no significant association was found between plasma NOx concentrations and HOMA-IR or fasting insulin when the analysis was performed on any OGTT-stratified group as well as on 3 non-diabetic groups combined (data not shown).

Multiple regression analysis to determine independent predictors of higher plasma NOx concentrations in 4 OGTT-stratified groups

Forward stepwise multiple linear regression analysis was performed to determine independent deter-

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Table 3. Predictors of log NOx determined by multiple regression analysis in 4 OGTT-stratified groups

	Predictors	B-coefficient	p	Adjusted R^2 value
NGT				0.093 ($P<0.001$)
	Log TG	0.317	<0.001	
	Variables entered in the model: gender, age, log triglyceride			
IFG				0.044 ($P=0.015$)
	Gender (1=men, 2=women)	−0.230	0.015	
	Variables entered in the model: gender, age, log fasting glucose			
IGT				0.049 ($P=0.015$)
	HDL-C	−0.241	0.015	
	Variables entered in the model: gender, age, HDL-C			
T2D				0.093 ($P=0.048$)
	Diastolic blood pressure	−0.239	0.022	
	BMI	−0.206	0.048	
	Variables entered in the model: gender, age, BMI, HDL-C, diastolic blood pressure			

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol.
R^2 is the squared multiple correlation coefficient that indicates the coefficient of determination of the regression.

minants of plasma NOx concentrations among 4 OGTT-stratified groups (**Table 3**). Gender, age and all other covariates that were found to positively correlate with plasma NOx concentrations by univariate correlation analysis were taken into multiple regression models. In T2D subjects, independent predictors of plasma NOx levels were diastolic blood pressure (B-coefficent = −0.239, $P=0.022$) and BMI (B-coefficent = −0.206, $P=0.048$) with low but significant adjusted R^2 of 0.093. The independent predictors of plasma NOx levels were different among 3 non-diabetic groups despite their similar NOx levels. In each group, a single determinant of plasma NOx concentrations was found with a quite low coefficient of determination of the regression (adjusted R^2 values varied from 0.044 to 0.093). Serum triglyceride levels were the determinant (B-coefficent =0.317, $P<0.001$) that accounted for 9.3% variations of plasma NOx concentrations in NGT subjects. Male gender was the determinant (B-coefficent = −0.230, $P=0.015$) that accounted for only 4.4% variations of plasma NOx levels in IFG subjects while serum HDL-C levels were the determinant (B-coefficent = −0.241, $P=0.015$) that accounted for only 4.9% variations of plasma NOx levels in IGT subjects.

Discussion

The current study clearly showed that plasma NOx levels were elevated in subjects with T2D compared with NGT among Vietnamese. This finding was in accordance with previous reports by others on Iranian, Taiwanese and Japanese populations[13, 14, 20]; however, plasma NOx levels in IFG and IGT were neither significantly increased nor decreased compared with NGT. Furthermore, no correlation was found between plasma NOx concentrations and HOMA-IR in both diabetic and non-diabetic subjects. Thus, plasma NOx levels predict neither insulin resistance nor glucose intolerance before the development of T2D.

Several experimental studies using mouse models have shown that adipose tissue is a potential source of NO[21-22] and the overproduction of NO by inducible NO synthase (iNOS) is involved in the development of insulin resistance in both genetic and diet-induced obesity[23-26]. However, the current study revealed that plasma NOx concentrations were not positively associated with obesity in T2D subjects but were instead inversely correlated with BMI, suggesting that the increase in plasma NOx levels in T2D was not caused by adiposity. Moreover, we found no association between plasma NOx levels and BMI or waist circumference in non-diabetic subjects where plasma NOx levels were not increased.

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In the current study, we could not pinpoint why plasma NOx levels were increased in T2D, since the only variables that were significantly associated with higher plasma NOx levels in multiple regression analysis were lower BMI and lower diastolic blood pressure. The inverse association of diastolic blood pressure with plasma NOx levels was most likely due to the consequence of the effect of NO on blood pressure, since excess NO has been shown to decrease blood pressure in T2D[27]. There was a borderline positive correlation between fasting glucose and NOx concentrations ($r=0.191$, $P=0.071$) in T2D subjects (see **Table 2**); therefore, the inverse association between plasma NOx levels and BMI might indicate that higher plasma NOx levels in T2D are associated with more severe diabetes. In an animal model, the upregulation of iNOS mRNA was found in the pancreatic islets of Zucker diabetic rats, which further led to β cell destruction and impaired insulin secretion. Both nicotinamide and aminoguanidine, which lower NO production, ameliorated β cell destruction and hyperglycemia in this model[28]. The finding suggests that increased NO could exacerbate T2D by further decreasing insulin secretion. Thus, although plasma NOx did not predict high-risk subjects for T2D, it might predict the outcome of T2D.

Although plasma NOx levels in IFG and IGT were neither significantly increased nor decreased compared with those in NGT, predictors of plasma NOx levels were different among 3 non-diabetic groups. While the serum TG level was an independent predictor of the plasma NOx level in NGT, it was not in IGT; instead, the serum HDL-C level was an independent predictor in this group. We do not have enough evidence to explain exactly why serum TG and HDL-C levels were correlated with plasma NOx levels in NGT and IGT, respectively; however, it is clear that the association of plasma NOx levels with serum TG or HDL-C levels was not due to insulin resistance, since no correlation of plasma NOx levels with HOMA-IR was found in any of 3 non-diabetic groups. There was an association between plasma NOx levels and the TG: HDL-C ratio, which is considered to be a surrogate marker for insulin resistance, in NGT. However, the fact that plasma NOx levels did not correlate with HOMA-IR in NGT indicates that the association between plasma NOx levels and the TG: HDL-C ratio in this case was not due to insulin resistance. Although the exact mechanism regulating plasma NOx levels has not been well understood, plasma NOx levels can be affected by the expression levels and activity of eNOS and iNOS. The divergence in the predictors of plasma NOx levels in 3 non-diabetic groups suggests that the expression levels and activity of eNOS and iNOS may be different in the 3 non-diabetic groups even though plasma NOx levels were similar.

Conclusion

Plasma NOx concentrations were significantly increased in T2D but not in subjects with glucose intolerance before the development of T2D. Plasma NOx levels were not associated with adiposity or insulin resistance in both diabetic and non-diabetic subjects. Thus, plasma NOx is not a useful marker to predict high-risk subjects for T2D in the Vietnamese population.

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