Systemic nitric oxide metabolites and the chance of pre-diabetes regression to normoglycemia: A 9-year cohort study

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Introduction: Nitric oxide (NO) is a signaling gasotransmitter,1,2 which regulates whole-body glucose and insulin metabolism.3,4 The involvement of NO in regulating glucose and insulin homeostasis in humans is supported by genetic studies introducing endothelial NO synthase (eNOS)-gene polymorphisms [e.g., 27bp-VNTR (27-base pair-variable number of tandem repeats),5 G894T (Glu298Asp or reference single nucleotide polymorphism (rs)1799983 in exon 7),6 E298D (polymorphism in Exon7 with substitution of glutamic acid (E) at codon 298 by aspartic acid (D)), and intervening sequence 18 (IVS18) + A27C (Ala27Cys) polymorphism in intron 18] as strong predictors of insulin resistance (IR) and type 2 diabetes (T2D). A predominant eNOS gene polymorphism, 4b4a VNTR polymorphism, is related to a lower circulating NO concentration8 and a higher risk of T2D.9 Disrupted NO metabolism seems to contribute to the pathophysiology of IR and T2D,10 however, the association of systemic NO production with pre-diabetes (Pre-DM) [i.e., a common intermediate dysglycemia characterized by isolated impaired fasting glucose (iIFG), isolated impaired glucose tolerance (iIGT), or combined IFG-IGT]11 has not yet been fully clarified. Epidemiologic evidence implies

Abstract

Introduction: We aimed to track longitudinal changes of glycemic status in subjects with pre-diabetes (Pre-DM) in relation to their baseline levels of systemic nitric oxide (NO) production [i.e., measured as serum NO metabolites (NOx), crude and body weight (BW)-adjusted NOx to creatinine ratio (NOx-to-Cr)] over 9 years.

Methods: This cohort study included 541 middle-aged Iranian men and women with Pre-DM, recruited in 2006-2008 and followed up to 2015-2017. The colorimetric Griess method was used to measure serum NOx concentration. Multinomial logistic regression analyses estimated the odds ratios (OR) of Pre-DM regression and progression across tertiles (tertile 3 vs. tertile 1 and tertile 2) of serum NOx, crude, and BW-adjusted NOx-to-Cr ratio.

Results: Participants who regressed to normoglycemia (NG) had a higher BW-adjusted NOx-to-Cr ratio than those who developed type 2 diabetes (T2D) or those who remained Pre-DM (0.52±0.34 vs. 0.43±0.29 and 0.48±0.25, P=0.023). Higher BW-adjusted NOx-to-Cr increased chance of returning to NG (OR=2.05, 95% CI=0.98-4.32, P=0.058) and decreased levels of 2h-serum glucose over time (Ptime×group=0.025), as well as the decreased overall mean of fasting (106, 95% CI=103-109 vs. 110, 95% CI=108-112 mg/dL, P=0.008) and 2h-serum glucose (153, 95% CI=146-159 vs. 163, 95% CI=158-168 mg/dL, P=0.018).

Conclusion: A higher endogenous NO production (i.e., indirectly measured by BW- and Cr-adjusted serum NOx concentration) in Pre-DM subjects is associated with the chance of returning to NG.

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that serum NO metabolites (nitrate + nitrite=NOx), a surrogate of NO production,\textsuperscript{12} may improve predictive models of cardiometabolic outcomes.\textsuperscript{15-15} An increased NO production at early stages of dysglycemia has been reported\textsuperscript{16-18} that might be a compensatory mechanism for its function, a hypothesis needs to be elucidated within a longitudinal follow-up of Pre-DM subjects.

Here, we aimed to track changes in glycemic status [Pre-DM regression to normoglycemia (NG) or progression to T2D] in relation to baseline values of systemic NO production [measured as serum NOx, as well as both crude and body weight (BW)-adjusted NOx to creatinine ratio (NOx-to-Cr)] over 9 years, among middle-aged Pre-DM adults. The use of Cr- and BW-adjusted rather than the crude NOx concentration provides a more accurate estimation of whole-body NO production since serum nitrate is highly affected by renal function\textsuperscript{19} and has an extensive distribution volume (~28% of the BW).\textsuperscript{12} We also determined longitudinal changes and cumulative average of glycemic parameters over time across different levels of systemic NO production in Pre-DM subjects.

**Subjects and Methods**

**Study population**

Here, we used participants’ data from the Tehran Lipid and Glucose Study (TLGS), a community-based cohort study initiated in 1999 to investigate and prevent non-communicable diseases (NCDs).\textsuperscript{20} Men and women (age ≥21 years) participated in the third TLGS examination (2006-2008) with Pre-DM (n=541), who were assessed for serum NOx concentrations and had completed data, were recruited for the current study and followed up to 2015-2017. The median follow-up was 9.2 years (interquartile range= 7.8-10.2 years). Fig. 1 shows the flow of the study participants through the examinations. All study participants completed written informed consent.

**Measurements of variables**

Data collection has been reported elsewhere in detail.\textsuperscript{20,21} History of NCDs, medications (i.e., medications for dyslipidemia and hypertension, or use of NO-releasing drugs, including nitroglycerin, nitrates, and isosorbide dinitrate), lifestyle [smoking habits and physical activity (PA)] as well as measurements of BW, waist circumference (WC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were performed by the trained interviewers at 3-y follow-up intervals. Details of PA measurements were described elsewhere.\textsuperscript{22} PA level was described as metabolic equivalent (MET) and categorized at three levels of light, moderate, and high-PA (corresponding to MET<600, 600-1499, and ≥ 1500 minutes/week, respectively).\textsuperscript{23}

Biochemical measurements were described elsewhere in detail.\textsuperscript{24} Blood samples were obtained following a 12-14 h overnight fasting. Both fasting (FSG) and 2-hour (2h-SG) serum glucose, triglyceride (TG), and high-density lipoprotein cholesterol (HDLC) were measured at baseline and all subsequent examinations, using the enzymatic colorimetric methods (Pars Azmoon, Tehran, Iran).\textsuperscript{24} The standard oral glucose tolerance test (OGTT) was performed after glucose ingestion (82.5 g glucose monohydrate solution equivalent to 75 g anhydrous glucose; Cerestar EP, Spain) in participants who did not receive hypoglycemic medications. The Jaffe kinetic alkaline picrate method was used for measuring serum Cr concentrations. Intra- and inter-assay coefficients of variation (CV) were <5.0% for all measurements. As described elsewhere in detail,\textsuperscript{25} serum NOx concentration was measured using the modified Griess method.\textsuperscript{26,27}

**Definitions**

Pre-DM was defined as IFG (100 ≤ FSG <126 mg/dL), IGT (140 ≤ 2h-SG <200 mg/dL), or combined IFG-IGT.\textsuperscript{28}

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**Fig. 1. Flowchart of study. 2h-SG, 2 hour-serum glucose; Pre-DM, pre-diabetes; NG, normoglycemia; T2D, type 2 diabetes; iIFG, isolated impaired fasting glucose; iIGT, isolated impaired glucose tolerance; NOx, nitric oxide metabolites (nitrate + nitrite). The figure was created with BioRender.**
Incidence of NG was considered as the first occurrence of both normal fasting glucose (NFG; FSG <100 mg/dL) and normal glucose tolerance (NGT; 2h-SG <140 mg/dL). The incidence of T2D was considered as the first occurrence of FSG ≥126 mg/dL, 2h-SG ≥ 200 mg/dL, or the use of glucose-lowering drugs. Family history of T2D (FHD) was considered positive when having at least one parent/sibling with T2D.

**Statistical methods**

SPSS for Windows version 20 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. To compare the baseline characteristics of the study participants, analysis of variance and chi-square test were used for the continuous variables with normal distribution and categorical variables, respectively. Multinomial logistic regression analyses estimated the odds ratios (95% confidence intervals, CIs) for Pre-DM regression and progression across tertiles (tertile 3 vs. tertile 1 and tertile 2) of serum NOx (<35 and ≥ 35 µmol/L), serum NOx-to-Cr (<0.38 and ≥ 0.38), and BW-adjusted NOx-to-Cr ratio (<0.50 and ≥ 0.50). Potential covariates were chosen based on scientific and statistical evidence. A univariate analysis was performed to identify potential confounding variables, and P (P value for entry) <0.2 was considered inclusion criteria in the final multivariable models. Table S1 indicates the potential covariates and their OR (95% CIs) in the univariate multinomial logistic regression. Finally, two logistic models were conducted: age, sex, FSG, and 2h-SG were adjusted in model 1, and model 2 was additionally adjusted for FHD, SBP, and WC. No evidence of interaction between serum NOx and sex was observed in relation to the outcomes (PageNOx = 0.695 and 0.711, for NG and T2D, respectively); the analyses, therefore, were conducted overall.

To determine longitudinal changes of glycemic parameters (FSG and 2h-SG concentrations) and to estimate their cumulative average across different levels (tertile 3 vs. tertile 1 and tertile 2) of serum NOx, crude, and BW-adjusted NOx-to-Cr ratio, repeated-measures ANOVA was used.

**Results**

Over 9 years, Pre-DM regression and progression rates were 39.4% and 37.5%, respectively. Mean serum NOx and Cr concentrations were 33.1±17.8 and 95.3±14 µmol/L at baseline.

Table 1 shows the baseline characteristics of the study participants. Participants who regressed to NG were significantly younger and had lower BW, WC, FSG, 2h-SG, and serum Cr than those who progressed to T2D. They also had a higher BW-adjusted NOx-to-Cr ratio than those who developed T2D or remained Pre-DM (0.52±0.34 vs. 0.43±0.25 and 0.48±0.29, P = 0.023). The frequency of using NO-releasing drugs was significantly different between groups (7.2%, 1.4%, and 3.4% in those who remained Pre-DM, regressed to NG, and progressed to T2D, respectively).

Table 2 displays serum NOx concentrations and crude and BW-adjusted NOx-to-Cr ratio across Pre-DM phenotypes (iIFG, iIGT, and IFG-IGT) at baseline. No significant difference was found between Pre-DM phenotypes for serum NOx and other NOx-based values.

As indicated in Table 3, adjusted (age, sex, FSG, 2h-SG)-OR for regression to NG among the participants with serum NOx-to-Cr ≥ 0.38 was 1.79 (95% CI = 0.91-3.53), compared with people who had a ratio lower than 0.38. After further adjusting for FHD, SBP, WC, and PA, the association was strengthened but remained non-significant (OR =1.85, 95% CI =0.89-3.82). The Pre-DM subjects with BW-adjusted NOx-to-Cr ≥ 0.5 had a higher chance of returning to NG, independent of the known potential confounders (OR = 2.05, 95% CI = 0.98-4.32, P = 0.058).

Using repeated measurement ANOVA (Table 4), we also found that higher levels of BW-adjusted NOx-to-Cr ≥ 0.5 were associated with repeated lower levels of 2h-SG over time (Ptime×group =0.025) and a lower overall mean of both FSG (106, 95% CI =103-109 vs. 110, 95% CI =108-112 mg/dL, P =0.008) and 2h-SG (153, 95% CI=146-159 vs. 163, 95% CI =158-168 mg/dL, P =0.018). Neither overall means nor trends of FSG and 2-SG changes over time differed between lower and higher levels of serum NOx and NOx-to-Cr ratio (data not shown).

**Discussion**

Following some preliminary observations about the potential compensatory overproduction of NO at the initial stages of dysglycemia, we followed longitudinal changes in the glycemic status of Pre-DM subjects based on their baseline systemic NO levels. A 9-year follow-up of Pre-DM subjects showed that the chance of returning to NG increased by 2-fold in those with higher baseline levels of whole-body NO production (indicated as adjusted NOx-to-Cr ratio ≥ 0.5). The Pre-DM subjects with a higher systemic NO production also experienced a better longitudinal postprandial glucose tolerance (i.e., measured as repeated 2h-SG levels during the study with 3-year intervals). This glycemic parameter mainly denotes peripheral insulin sensitivity and is related to endothelial function.

Pre-DM is now affecting about 30% of the middle-aged adults worldwide. Although over 70% of Pre-DM subjects will progress to T2D with an annual rate of 5-10% per year, the chance of NG is predicted to be 33-59% within 1-5 years. Several biological (e.g., age, sex, FHD, obesity, hypertension, serum glucose, and lipid levels) and lifestyle (dietary factors, smoking, and PA) determinants may potentially predict the chance of Pre-DM regression and progression.
early, and a reduced production is observed in established T2D. Both human and animal studies reported an increased NO production in the pre-DM state, while in established T2D, a diminished NO production was observed.

Both fractional and absolute rates of NO production from L-arginine are diminished in T2D compared to NG (19.3±3.9% vs. 22.9±4.5% per day, and 320 vs. 890 μmol per day, respectively). The rate of NO synthesis from plasma L-arginine turnover was reduced by 50%, resulting in a 16% diminished rate of whole-body NO production in T2D compared to NG. The impaired NO production in T2D may be explained by decreased eNOS activity and expression, decreased eNOS sensitivity to its agonists, elevated arginase activity, and L-arginine deficiency.

A compensatory NO overproduction (reported up to 2-fold compared to controls) during the initial stages of impaired glucose and insulin homeostasis is mainly explained by hyperglycemia- and hyperinsulinemia-induced upregulation of NOS expression and activity.

Table 1. Baseline characteristics of the study participants according to the outcomes (n=541)

<table>
<thead>
<tr>
<th></th>
<th>Remained Pre-DM (n=125)</th>
<th>Regressed to NG (n=213)</th>
<th>Progressed to T2D (n=203)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57.4±13.3</td>
<td>50.5±13.7b</td>
<td>55.5±13.7</td>
</tr>
<tr>
<td>Men (%)</td>
<td>48.0</td>
<td>39.0</td>
<td>46.3</td>
</tr>
<tr>
<td>FHD (%)</td>
<td>18.4</td>
<td>22.0</td>
<td>29.4</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid-lowering (%)</td>
<td>6.3</td>
<td>5.4</td>
<td>7.7</td>
</tr>
<tr>
<td>BP-lowering (%)</td>
<td>8.9</td>
<td>7.0</td>
<td>9.0</td>
</tr>
<tr>
<td>NO-releasing drugs</td>
<td>7.2</td>
<td>1.4</td>
<td>3.4</td>
</tr>
<tr>
<td>TNG (%)</td>
<td>4.8</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Nitrocontin (%)</td>
<td>0.8</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>ISDN (%)</td>
<td>1.6</td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>22.4</td>
<td>25.4</td>
<td>20.7</td>
</tr>
<tr>
<td>Physical activity (MET-h/week)</td>
<td>32.1±55.5</td>
<td>39.6±61.8</td>
<td>42.1±61.8</td>
</tr>
<tr>
<td>Light (%)</td>
<td>53.3</td>
<td>62.5</td>
<td>60.4</td>
</tr>
<tr>
<td>Moderate (%)</td>
<td>23.0</td>
<td>23.1</td>
<td>23.1</td>
</tr>
<tr>
<td>High (%)</td>
<td>23.7</td>
<td>14.0</td>
<td>16.5</td>
</tr>
<tr>
<td>BW (kg)</td>
<td>74.6±12.1b</td>
<td>74.3±14.2b</td>
<td>79.1±14.4</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>98.3±11.1</td>
<td>95.2±12.5b</td>
<td>101±11.0</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>125±17.8</td>
<td>122±19.8</td>
<td>128±20.1</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>75.6±9.6</td>
<td>76.4±9.2</td>
<td>76.9±11.6</td>
</tr>
<tr>
<td>FSG (mg/dL)</td>
<td>102±7.9b</td>
<td>97.7±8.8b</td>
<td>105±9.5</td>
</tr>
<tr>
<td>2h-SG (mg/dL)</td>
<td>135±30.0b</td>
<td>130±29.3b</td>
<td>150±31.0</td>
</tr>
<tr>
<td>TG to HDL-C ratio</td>
<td>4.8±3.4</td>
<td>4.6±3.9</td>
<td>5.2±3.8</td>
</tr>
<tr>
<td>Serum NOx (μmol/L)</td>
<td>33.1±18.2</td>
<td>33.7±18.6</td>
<td>32.4±16.8</td>
</tr>
<tr>
<td>Serum Cr (μmol/L)</td>
<td>96.2±11.3</td>
<td>91.0±13.6</td>
<td>97.1±15.5</td>
</tr>
<tr>
<td>NOx-to-Cr ratio</td>
<td>0.35±0.19</td>
<td>0.37±0.22</td>
<td>0.33±0.18</td>
</tr>
<tr>
<td>BW-adjusted NOx-to-Cr ratio</td>
<td>0.48±0.29</td>
<td>0.52±0.14</td>
<td>0.43±0.25</td>
</tr>
</tbody>
</table>

Data are mean ± SD (unless stated otherwise). Physical activity levels (Light <10, moderate 10-25, and high ≥25 MET-h/week).

* Significant difference between groups (chi-square test was used, P=0.020).

Table 2. Nitric oxide (NO)-related parameters across Pre-DM phenotypes

<table>
<thead>
<tr>
<th></th>
<th>iIFG</th>
<th>IGT</th>
<th>Combined IFG-IGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum NOx (μmol/L)</td>
<td>32.9±17.4</td>
<td>32.9±18.7</td>
<td>33.5±17.6</td>
</tr>
<tr>
<td>Serum NOx-to-Cr ratio</td>
<td>0.34±0.19</td>
<td>0.36±0.22</td>
<td>0.35±0.19</td>
</tr>
<tr>
<td>BW-adjusted NOx-to-Cr ratio</td>
<td>0.46±0.28</td>
<td>0.52±0.34</td>
<td>0.48±0.29</td>
</tr>
</tbody>
</table>

Data are mean ±SD

BW, body weight; Cr, creatinine; iIFG, isolated impaired fasting glucose; IGT, isolated impaired glucose tolerance; NOx, serum nitric oxide metabolites (nitrate+nitrite).
Moreover, NO
1.45 (0.80-2.63)
1.30 (0.63-2.69)
102±9.4
115±30.4
0.84 (0.52-1.36)
1.79 (0.91-3.53)
0.95 (0.59-1.53)
14 hours of fasting, significantly reducing the contribution
of ingested nitrate in the circulation.
49 Some points should be considered when interpreting
this study’s findings. First, since two independent
pathways (i.e., L-arginine-NOS and nitrate-nitrite
pathways) are involved in endogenous NO production,
the interpretation of serum NOx values might be complex.
Serum nitrate is valid as a surrogate of NOS activity if
its non-NOS sources (i.e., ingested nitrate from food,
water, and medications containing NO prodrugs, inhaled
nitrogen oxides, originated nitrate from gut microbiota,
and potential contamination of serum samples with
nitrogen oxides during measurements) are excluded.55
Second, because serum NOx concentration is affected by
renal function, using its Cr-corrected values is considered
a more accurate indicator of NOS activity than the crude
NOx levels.55 Three, due to its relatively large distribution
volume (28% of BW) and low clearance rate (30±2 mL/
in/l.73 m²), extensive, significant, and long-term changes
in NO synthesis are required to affect circulating nitrate
significantly;62 serum NOx, therefore, may represent
the long-lasting status of whole-body NO production.
Fourth, serum NOx could not be considered equal to NO
availability in the body because increased serum NOx may
coop-er with a decreased biologically active form of NO
and vice versa.56
This investigation is the first attempt to address the
potential association of whole-body NO production
with the transition of Pre-DM to T2D and NG within
a population-based setting. However, the study had
some limitations. Our limited sample size may diminish
the findings’ generalizability. Because of the distinct
pathophysiology of IGT and IFG,61 the observed
association may differ for these subgroups; however, we did
not address the hypothesis due to a relatively low sample
size and insufficient power. Here, systemic NO production
was estimated using the measurement of NO metabolites
instead of direct evaluation. Due to the low levels and short
half-life of circulating, measurement of its metabolites
(nitrate+nitrite), rather than its direct measurement, is
a common and practical approach in population-based
studies.55 After overnight fasting, NOx measurement
may reflect systemic NO production in subjects without
renal dysfunction since the half-life of oral nitrate is about
5 hours.59 In our study, blood sampling was taken after 12-
14 hours of fasting, significantly reducing the contribution
of ingested nitrate in the circulation.

### Table 3. The odds ratio (95% CI) of pre-diabetes (Pre-DM) regression to normoglycemia (NG) and progression to type 2 diabetes (T2D) in relation to nitric oxide (NO)-related parameters

<table>
<thead>
<tr>
<th></th>
<th>Regressed to NG</th>
<th>Progressed to T2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum NOx (≥ 35 µmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.07 (0.67-1.71)</td>
<td>0.95 (0.59-1.53)</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.02 (0.62-1.66)</td>
<td>0.99 (0.60-1.63)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.45 (0.80-2.63)</td>
<td>1.33 (0.72-2.46)</td>
</tr>
<tr>
<td>Serum NOx-to-Cr (≥ 0.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.30 (0.82-2.08)</td>
<td>0.87 (0.54-1.41)</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.79 (0.91-3.53)</td>
<td>1.22 (0.62-2.39)</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.85 (0.89-3.82)</td>
<td>1.28 (0.63-2.56)</td>
</tr>
<tr>
<td>BW-adjusted NOx-to-Cr (≥ 0.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>1.46 (0.92-2.33)</td>
<td>0.84 (0.52-1.36)</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.85 (0.93-3.66)</td>
<td>1.13 (0.57-2.22)</td>
</tr>
<tr>
<td>Model 2</td>
<td>2.05 (0.98-4.32)</td>
<td>1.30 (0.63-2.69)</td>
</tr>
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</table>

Data are odds ratios (ORs) and 95% confidence intervals (95% CI) (odds values refer to T2 vs. T1 and T3).

Multinomial logistic regression was used.
Model 1, adjusted for age, sex, fasting serum glucose (FSG), and 2-hour serum glucose (2h-SG).
Model 2, additionally adjusted for family history of T2D (FHD), systolic blood pressure (SBP), waist circumference (WC) and physical activity (PA).
BW, body weight; Cr, creatinine; NOx, nitric oxide metabolites (nitrate+nitrite).

These compensatory mechanisms may lead to adaptation and relative normalization of whole-body NO production to maintain its function in handling glucose and insulin homeostasis. NO regulates insulin secretion49 and potentiates skeletal muscle insulin sensitivity and glucose uptake by increasing blood flow-dependent glucose delivery, increasing expression and membrane translocation of glucose transporter 4, and increasing transendothelial insulin transport.50-52 Moreover, NO facilitates insulin-dependent and -independent glucose uptake in adipose tissue.53,54

Some points should be considered when interpreting the study’s findings. First, since two independent pathways (i.e., L-arginine-NOS and nitrate-nitrite pathways) are involved in endogenous NO production, the interpretation of serum NOx values might be complex. Serum nitrate is valid as a surrogate of NOS activity if

### Table 4. Mean concentrations of glycemic parameters in subjects with lower and higher values of body weight (BW)-adjusted NOx-to-creatinine (Cr) ratio over 9 years of follow-up

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>FSG (mg/dL)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;0.50</td>
<td>102±9.4</td>
<td>110±21.9</td>
<td>115±30.4</td>
<td>115±33.8</td>
<td>110 (108-112)</td>
<td>0.147</td>
</tr>
<tr>
<td>≥0.50</td>
<td>100±9.9</td>
<td>105±14.2</td>
<td>108±21.7</td>
<td>109±25.0</td>
<td>106 (103-109)†</td>
<td></td>
</tr>
<tr>
<td>2h-SG (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.50</td>
<td>138±31.9</td>
<td>153±54.7</td>
<td>173±69.1</td>
<td>188±77.0</td>
<td>163 (158-168)</td>
<td>0.025</td>
</tr>
<tr>
<td>≥0.50</td>
<td>139±30.3</td>
<td>142±45.6</td>
<td>155±59.9</td>
<td>175±64.5</td>
<td>153 (146-159)**</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD. The repeated measures analysis of variance (ANOVA) was used. *P=0.008 †P=0.018 for the overall mean difference between categories. 2h-SG, 2-hour serum glucose; FSG, fasting serum glucose; NOx, nitric oxide metabolites (nitrate+nitrite).
NOx-to-Cr ratio ≥ 0.5) is associated with the chance of Pre-DM remission by 2-fold. This observation suggests that serum NOx concentration (a rapid, inexpensive, and easily measured biomarker of NO production) may be used alongside other factors to predict the future state of Pre-DM subjects. However, our findings were derived from a relatively low-sample size population and needed to be approved by further larger sample-size prospective cohorts. The association of endogenous NO production and Pre-DM regression and progression must be distinguished across Pre-DM phenotypes (iIFG, iIGT, and combined IFG-IGT) to provide helpful evidence for designing individualized therapeutic strategies in Pre-DM. Future investigations must also address whether dietary approaches and therapeutic agents targeting NO production may affect Pre-DM regression and progression.

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Competing Interests
The authors have no conflict of interest.

Ethical Statement
The study protocol was evaluated and approved by the ethics research council of the Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Ethics code: IR.SBMU.ENDOCRINE.REC.1401.112). Written informed consent was obtained from all TLGS participants.

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Supplementary file 1
Supplementary file 1 contains Table S1.

References

Research Highlights
What is the current knowledge?
✓ Impaired nitric oxide (NO) metabolism contributes to the development of type 2 diabetes.
✓ Increased NO production was reported in the initial stages of dysglycemia and insulin resistance.
✓ The association of systemic NO production with changes in glycemic status in pre-diabetes (Pre-DM) subjects is unclear.

What is new here?
✓ Higher levels of NO production in Pre-DM subjects may increase the chance of returning to normoglycemia.


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