# Defective Allele of the Neuronal Nitric Oxide Synthase Gene Increases Insulin Resistance During Acute Phase of Myocardial Infarction.

**Otávio T Nóbrega** ( otnobrega@gmail.com )
Universidade de Brasilia https://orcid.org/0000-0003-1775-7176
**Alessandra M. Campos-Staffico**
Universidade Estadual de Campinas
**Elayne Kelen Oliveira**
Universidade Estadual de Campinas
**Daniel B Munhoz**
Universidade Estadual de Campinas
**Filipe A. Moura**
Universidade Estadual de Campinas
**Luis Sérgio F. Carvalho**
Hospital de Base do Distrito Federal
**Audrey C. Tonet-Furioso**
Universidade Catolica de Brasilia
**Andrei C. Sposito**
Universidade Estadual de Campinas

**Short Report**

**Keywords:** nitric oxide, insulin, blood glucose, vasodilation, polymorphism, myocardial infarction.

**Posted Date:** March 11th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-285973/v1

# Abstract

**Background:** glycemia disorders are a strong predictor of mortality in ST-Elevation Myocardial Infarction (STEMI) patients. Disruption in nitric oxide (NO) production is associated with insulin-resistant states. We evaluated whether NO production in carriers of a defective allele of the neuronal nitric oxide synthase (nNOS or *NOS1*), whose *in vivo* expression is reduced by up to 50%, might influence the insulin response during acute phase of STEMI.

**Methods and Results:** Consecutive patients with STEMI (n = 354) underwent clinical evaluations and genotyping for the promoter variation rs41279104. Blood tests were performed at admission (D1) and after five days (D5) of in-hospital follow up, with the disposition index assessed in the period. Flow-mediated dilation (FMD) was assessed by reactive hyperemia on the 30$^{th}$ day. Homozygotes for the defective allele (A) showed lower glycemia and insulin sensitivity at D1 while showing the highest b-cells function and no changes in the circulating NO pool, what is compatible with hyperresponsive b-cells to counteract the inherent glucose-resistant state of AA patients. At D5, glycemic scores shifted to indicate greater insulin sensitivity among A homozygotes, paralleled by a slight yet poor increase in NO bioavailability than that among G carriers. All in all, defective homozygotes showed greater insulin resistance expressed by the disposition index at admission, which was compensated 5 days after STEMI even though FMD of A carriers was lower compared to G homozygotes.

**Conclusion:** a defective nNOS allele seems to elicit endocrine adaptation and to associate with insulin resistance during the acute phase of STEMI.


# 1. Introduction

Myocardial infarction (MI) is a stressful condition that predisposes individuals to a significant increase in blood glucose levels. Hyperglycemia prevails in up to 50% of all patients with ST-Elevation Myocardial Infarction (STEMI), though only 20 to 25% had previously been diagnosed with diabetes mellitus [1]. Mechanistically, insulin resistance causes proinflammatory and procoagulant states that can enhance myocardial injury from excessive coronary constriction, endothelial dysfunction and platelet aggregation [2]. Thus, hyperglycemia is an independent predictor of mortality in STEMI [3], with clinical and observational data confirming that normalization of blood glucose associate with improved outcomes and survival [4].

Nitric oxide (NO) is a key molecule during ischemic disorders due to its beneficial effects in controlling myocardial contractility, limiting cardiac remodeling after MI and promoting vasodilation [5]. However, NO production plays a central role not only in ischemic conditions but also in insulin metabolism. Pharmacological inhibition of murine NO production induced *in vivo* insulin resistance in skeletal and cardiac muscles and adipose tissue [6]. Similarly, disruptions of endothelial and neuronal nitric oxide synthase genes (*NOS3* and *NOS1*, respectively) have also caused insulin resistance in mice [7, 8]. In

humans, diminished NO production has been associated with insulin-resistant statuses, such as type 2 diabetes mellitus, obesity, hypertension and dyslipidemia [9-11].

Neuronal NOS (*NOS1* or nNOS) is a constitutive enzyme mainly expressed in the central nervous system that is also implicated in autonomic regulation of heart rhythm, microcirculation and contractility [12]. Given that a single nucleotide polymorphism (SNP) in the promoter region of *NOS1* (rs41279104) decreases its expression by 30% *in vitro* and by 50% *in vivo* [13, 14], we decided to investigate whether this surrogate for NO production might influence glycemic scores during the in-hospital acute phase of STEMI.

# 2. Material And Methods

*2.1 Participants and Study Design*

The *Brazilian Heart Study* (BHS), an observational prospective cohort registered at ClinicalTrials.gov (NCT02062554) (detailed elsewhere [15]) enrolled 354 participants. Briefly, consecutive STEMI patients admitted to Coronary Care Unit at the Hospital de Base do Distrito Federal (Brasilia, Brazil) who met to these inclusion criteria were enrolled: (i) £ 24 hours from the onset of MI symptoms, (ii) ST-segment elevation ³ 1 mm (frontal plane) or 2 mm (horizontal) in contiguous leads, and (iii) myocardial necrosis evidenced by scores ³ the 99$^{th}$ percentile of the reference limit for CK-MB (25 U/L) and troponin I (0.04 ng/mL), followed by a decline of both.

A complete medical evaluation was performed upon admission (D1) with patients followed in-hospital until the fifth day (D5) after MI. Attending physicians decided on each medical treatment, including the choice of reperfusion therapy, without influence from the investigation team. Functional endothelial vascular reactivity was assessed 30 days after STEMI.

*2.2 Clinical Evaluation*

In the first 24 hours after the onset of symptoms, a standardized interview was performed to assess medical history, all drugs currently used, and lifestyle factors. To assist these assessments, blood samples were taken. Hypertension was defined either by use of antihypertensive drugs prior hospital admission or by repeatedly elevated blood pressure exceeding 140 over 90 mmHg during in-hospital stay. Diabetes was defined by use of hypoglycemic drugs and/or insulin, fasting blood glucose ≥ 126 mg/dL or glycated hemoglobin (Hb1Ac) ≥ 6.5%. Current smoking was defined by using 1 or more cigarette/day for more than 1 year before the coronary event. Former smoking was defined by smoking cessation for at least 6 months prior to hospitalization. Sedentary lifestyle was defined by not practicing physical activities for more than 30 minutes over at least 4 days a week. Drugs with vasodilation effects were defined by in-hospital use of calcium channel blockers (CCB), angiotensin receptor blockers (ARB) and/or angiotensin converting enzyme inhibitors (ACEi). Nitrates were not included by ruling out from analyses participants exposed to these drugs during hospitalization. Medical and biochemical assessments were

repeated as clinically needed during hospital stay, with glycemic scores being necessarily reassessed at D5.

*2.3 Biochemical Analyses*

Blood samples were drawn at admission and a second round of clinical biochemistry assays was also done after a 12-h overnight fast at D5. Samples in EDTA were centrifuged at 4500 rpm for 15 minutes at 5°C, and plasma was used in an automatic chemical analyzer to performe the following assessments, in duplicate: glucose, gycated hemoglobin A1c (HbA1c), insulin, C-peptide, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), creatinine, ultra-sensitive C-reactive protein (us-CRP), troponin and creatine kinase-myoglobin binding (CK-MB), with reagents from Roche Diagnostics (Mannheim, Germany), Bio-Rad Laboratories (Hercules, USA) or Dade Behring (Marburg, Germany). LDL cholesterol (LDL-C) was calculated by the Friedewald formula. Glomerular filtration rate (GFR) was estimated by the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation: eGFR (mL/min/1.73 $m^2$) = 141 x min (SCr x 0.0113/k, 1)$^a$ x max (SCr x 0.0113/k, 1)$^{-1.209}$ x $0.993^{Age}$ x 1.018 (if female) x 1.159 (if black), where SCr is serum creatinine, k is 0.7 for females and 0.9 for males, $^a$ is -0.329 form females and -0.411 for males.

*2.4 Glucose Homeostasis Model Assessment*

The Homeostasis Model Assessment (HOMA) Calculator, version 2.2.2, was used to assess insulin sensitivity (HOMA2S) based on plasma insulin and b-cell function (HOMA2B) based on plasma C-peptide [16] in all enrolled participants. The Disposition Index (DI) was calculated as (HOMA2B x HOMA2S)/100.

*2.5 DNA Extraction and Analysis*

Genomic DNA was obtained from peripheral blood leukocytes. The rs41279104 *NOS1* SNP was genotyped using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method originally published elsewhere [17]. Briefly, the *GenBank* was search for the *NOS1* gene (NG_011991.2) and a pair of primers was designed to amplify a 150 bp-fragment with the SNP of interest (-84 G/A) (forward 5'-CTGACTGCCCTTGTCTCTCC-3' and reverse 5'-GCGACTGGGGTTTAATTGAC-3'). Each reaction (25 µL) was composed of 100 ng of DNA, 25 mM Tris-HCl pH 8.3, 75 mM KCl, 1.5 mM $MgCl_2$, 0.2 µM of each primer, 0.2 µM of each dNTP, 0.25 mg/mL of ovalbumin and 0.5 unit of Taq Polymerase (Phoneutria, Minas Gerais, Brazil). Amplification had an initial denaturation at 94ºC for 2 min, followed by 36 cycles of denaturation at 94ºC for 40 s, annealing at 63ºC for 45 s and extension at 72ºC for 50 s, followed by a final extension at 72ºC for 5 min. Enzymatic digestion was carried out at 37ºC overnight in 15 µL, using 0.1 U of Fnu4HI (New England BioLabs, USA) to yield two fragments (92 and 58 bp) visible under electrophoresis in 2.2% agarose gel.

*2.6 Measurement of total NO/nitrite/nitrate*

For quantification of total serum levels of nitric oxide (NO), the Griess diazotization reaction based on conversion of nitrate to nitrite ($NO^{2-}$) by nitrate reductase was employed, performed following the manufacturer's instructions (R&D Systems Inc., MN, USA), with readings done by colorimetric detection in a Biotek ELX 800 (DeMorellis, SP, Brazil) device, set at a 540 nm wavelength.

*2.7 Brachial artery reactivity*

Endothelial-dependent vasodilation was assessed by ischemia-induced reactive hyperemia on the 30$^{th}$ day post-STEMI, after a 12-hour overnight fasting and 24 hours after withdrawal of vasoactive drugs (described elsewhere [18]). Briefly, after 10 minutes of rest, longitudinal images of the brachial artery were taken by an experienced physician blinded to the patient's history, using ultrasound with a high-resolution linear transducer (model IE 33, 3–9 MHz transducer, Philips Medical Systems, MA, USA) synchronized with EKG monitoring, and participants in supine position. A blood pressure cuff placed on the forearm was inflated to 50 mmHg above the systolic pressure for 5 min, and then deflated. The flow-mediated dilation (FMD) was scanned for 2 min, and the change in diameter was calculated respective to the resting state.

*2.8 Statistical Analysis*

Assumptions for parametric models (linearity, normality of distribution and equal variance) were checked using histograms, normal probability plots and residual scatter plots by means of the Kolmogorov-Smirnov test. Normally distributed and skewed data were tested using ANOVA or Kruskal-Wallis, respectively, with a Tukey post-hoc test if necessary. ANCOVA was used to adjust the effect of the SNP rs41279104 on glycemic scores, NO levels and brachial artery reactivity for potential confounders. Changes between D1 and D5 were additionally adjusted for baseline levels to attenuate the effect of regression toward the mean. Categorical data were tested using the chi-square test. A two-sided p-value of $< 0.05$ was considered statistically significant, with analyses performed using SPSS for Mac version 25.0.

# 3. Results

There were no significant differences regarding age, gender, clinical traits, laboratory profile, anthropometry, and in-hospital drug treatment between genotypes (Table 1). More importantly, cases previously diagnosed with diabetes mellitus, arterial hypertension and other metabolic disorders were equally distributed across genotypic groups.

*3.1 Glucose metabolism and insulin response*

Homozygotes for the defective allele (A) showed lower glycemia and insulin sensitivity at D1 compared to carriers of the G allele (Table 2). Accordingly, the pancreatic function (HOMA2B) of defective homozygotes – whose blood glucose was the lowest throughout all the acute phase of STEMI – showed the highest b-cells function at D1, followed by a significant drop within 5 days contrasting to the positive

change observed among G carriers in the period (DD5-D1). This is compatible with hyperresponsive b-cells to counteract the reduced peripheral insulin sensitivity of AA patients at STEMI onset. Still among A homozygotes, this scenario radically shifts at D5 when HOMA2S and HOMA2B assume scores diametrically opposed to those at D1. Also, G carriers underwent lower drops in blood glucose by the end of the acute phase of MI (DD5-D1) than AA patients.

Insulin secretion and insulin sensitivity are connected via a negative feedback loop, where pancreatic b-cells compensate changes in whole body sensitivity by an inversely proportional change in secretion (y = constant/x) [19]. The disposition index (DI) – the product of HOMA2B and HOMA2S – tends to remain roughly constant in healthy conditions, and deviation is observed when b-cell function is lost and/or insulin-mediated mechanisms to overcome insulin resistance fail, as was the case among A homozygotes at D1 (influenced by the very low scores of HOMA2S) followed by a recovery of the index at D5. Conversely, G carriers had higher indexes at admission, resulting in smaller changes in DI in the period (DD5-D1).

*3.2 NO/nitrite/nitrate levels and flow-mediated dilation*

No significant differences were observed in NO levels at STEMI onset (D1). However, defective homozygotes showed the lowest NO levels compared to GG and GA patients at D5 (Tukey post-hoc p-values at 0.038 and 0.042, respectively) and the smallest raise in time of NO levels among all genotypic groups. Also, FMD values obtained 30 days after STEMI show that G homozygotes reached higher dilation scores compared to heterozygotes and AA homozygotes (Tukey post-hoc p-values at 0.030 and 0.005, respectively).

# 4. Discussion

Insulin resistance has an undeniable relation to nitric oxide production, an assumption corroborated by epidemiological studies that associated type 2 diabetes mellitus, obesity, hypertension and dyslipidemia with reduced NO production [9-11]. Although this association is supported by a large body of evidence, the role of neuronal NOS in insulin resistance remains elusive. The present study explored this matter by evaluating a defective isoform of nNOS gene – whose expression *in vivo* is reduced by up to 50% – in actual clinical settings of a major, life-threatening vascular event. During this venture, we found both availability of and sensitivity to insulin vary differently between the genotypes and post-MI moment.

Insulin is a well-known vasodilator, and its vasodilatory effect is mediated by NO generation [20]. Therefore, the peak production of insulin at D1 observed herein appears to be a compensatory mechanism for the baseline resistant state among the defective homozygotes. It is possible that the unnoticed changes in NO levels at D1 could have occurred from the minor role of the nNOS isoform (compared to eNOS) to account for the systemic pool [21], and to the fact that insulin requires a relatively long time (4 h) to activate NOS so to contribute to the systemic availability [22]. Therefore, this phenomenon of a relatively high glucose-uptake among defective nNOS producers in the immediate hours after STEMI probably relied on a NO-independent mechanism based on hyperinsulinemia. A burst

in insulin secretion may compensate for a naturally reduced nNOS expression and activity in the paraventricular nucleus, where low NO production upregulates the sympathetic tone via catecholamines release and a-adrenergic activation [23, 24] that usually lead to an insulin-resistant state [25], what tends to be aggravated in a context of myocardial injury.

Interestingly, the observed pattern of high glucose uptake at D1 was sustained throughout the acute post-MI phase, mostly attributable to gains in peripheral insulin sensitivity with possible contribution by an increase in total NO bioavailability, but not solely for that since the NO reservoir was not enhanced to the extent reached among G carriers. As a matter of fact, glycemic scores in D5 and time-course changes (DD5-D1) among defective patients are likely to derive from a scenario of endothelial dysfunction objectively attested by NO shortage, with eNOS – the major isoform – unable to compensate for the endogenously defective nNOS. All in all, this scenario of a whole-body endocrine adaptation in glycemic balance is consistent with an impaired NO generation system among defective (A) homozygotes.

All in all, the interplay between glucose balance and alleles of nNOS during the acute phase of STEMI revealed that AA individuals had to compensate for the inherent inability to produce adequate amounts of NO compared to G carriers. Even so, defective homozygotes showed more severe insulin resistance on admission than other genotypes as expressed by the disposition index, which appeared to be compensated 5 days after STEMI. In the long run defective homozygotes were able to recover from endothelial dysfunction since their flow-mediated dilation was equal to that of heterozygotes ($p = 0.190$) and mildly lower in clinical terms to that of G homozygotes ($p = 0.005$).

Important limitations need to be acknowledged when interpreting our findings. Firstly, the study exclusively enrolled STEMI patients with a great extent of myocardial injury, what prevents extrapolations to other acute coronary syndromes. Secondly, the observational design precludes any causality inference. Thirdly, the sample size was limited – STEMI is an uncommon, lethal coronary disease – and the possibility of selection bias cannot be ruled out. These limitations may favor over/underestimations on the magnitude of the associations found and may also influence the lack of statistical significance in part of the analyses. However, the uniqueness of the study population and its consistency with biological processes at stake in the acute phase of MI make it relevant and plausible. Moreover, our data do not seem biased by an unleveled distribution of diabetic cases or by consumption of pharmaceutical products across genotypic groups.

# 5. Conclusion

The decline in NO production mediated by the defective nNOS polymorphic gene is associated with insulin resistance during acute phase of STEMI.

# Declarations

Funding: Research supported with grants # 471016/2011-0 (CNPq) and # 193.000.032-2012 (FAPDF), with a stipend to AC Tonet-Furioso (CAPES, FinanceCode 001) and fellowships for productivity in

research to AC Sposito and to OT Nóbrega (CNPq).

Conflicts of interest: The authors declare not having conflicts of interest.

Authors' contributions: AC Tonet-Furioso: genotyped the subjects. AM Campos-Staffico, EK Oliveira, DB Munhoz and FA Moura substantially contributed to the interpretation of results and drafted the original manuscript. LSF Carvalho executed the statistical analyses and the medical component of the study. AC Sposito supervised the medical component and the statistical analyses. AC Sposito and OT Nóbrega designed and coordinated the study and prepared the final version of the manuscript.

Data availability: The data can be made available upon a reasonable request.

Ethics approval: The study was carried out in accordance with The Declaration of Helsinki, being approved by the local Ethics Committee (code 083/06).

Consent to participate: All participants were enrolled after an informed consent was signed by the patient or a next-of-kin.

Consent for publication: All authors reviewed the final version of the manuscript and concur with its submission in the present format.

# References

1. Wahab NN, Cowden EA, Pearce NJ, Gardner MJ, Merry H, Cox JL et al (2002) Is blood glucose an independent predictor of mortality in acute myocardial infarction in the thrombolytic era? J Am Coll Cardiol 40(10):1748–1754. doi:10.1016/s0735-1097(02)02483-x
2. Kim JA, Montagnani M, Koh KK, Quon MJ (2006) Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 113(15):1888–1904. doi:10.1161/CIRCULATIONAHA.105.563213
3. Zeller M, Steg PG, Ravisy J, Laurent Y, Janin-Manificat L, L'Huillier I et al (2005) Prevalence and impact of metabolic syndrome on hospital outcomes in acute myocardial infarction. Arch Intern Med 165(10):1192–1198. doi:10.1001/archinte.165.10.1192
4. Kosiborod M, Inzucchi SE, Krumholz HM, Masoudi FA, Goyal A, Xiao L et al (2009) Glucose normalization and outcomes in patients with acute myocardial infarction. Arch Intern Med 169(5):438–446. doi:10.1001/archinternmed.2008.593
5. Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westerhof N et al (2007) Nitric oxide and cardiac function. Life Sci 81(10):779–793. doi:10.1016/j.lfs.2007.07.019
6. Roy D, Perreault M, Marette A (1998) Insulin stimulation of glucose uptake in skeletal muscles and adipose tissues in vivo is NO dependent. Am J Physiol 274(4):E692–E699. doi:10.1152/ajpendo.1998.274.4.E692

7. Duplain H, Burcelin R, Sartori C, Cook S, Egli M, Lepori M et al (2001) Insulin resistance, hyperlipidemia, and hypertension in mice lacking endothelial nitric oxide synthase. Circulation 104(3):342–345. doi:10.1161/01.cir.104.3.342
8. Shankar RR, Wu Y, Shen HQ, Zhu JS, Baron AD (2000) Mice with gene disruption of both endothelial and neuronal nitric oxide synthase exhibit insulin resistance. Diabetes 49(5):684–687. doi:10.2337/diabetes.49.5.684
9. Gilligan DM, Guetta V, Panza JA, Garcia CE, Quyyumi AA, Cannon RO 3 (1994) Selective loss of microvascular endothelial function in human hypercholesterolemia. Circulation 90(1):35–41. doi:10.1161/01.cir.90.1.35. rd. ; ) .
10. Zeiher AM, Drexler H, Wollschlager H, Just H (1991) Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 83(2):391–401. doi:10.1161/01.cir.83.2.391
11. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD (1996) Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 97(11):2601–2610. doi:10.1172/JCI118709
12. Zhang YH, Jin CZ, Jang JH, Wang Y (2014) Molecular mechanisms of neuronal nitric oxide synthase in cardiac function and pathophysiology. J Physiol 592(15):3189–3200. doi:10.1113/jphysiol.2013.270306
13. Saur D, Vanderwinden JM, Seidler B, Schmid RM, De Laet MH, Allescher HD (2004) Single-nucleotide promoter polymorphism alters transcription of neuronal nitric oxide synthase exon 1c in infantile hypertrophic pyloric stenosis. Proc Natl Acad Sci U S A 101(6):1662–1667. doi:10.1073/pnas.0305473101
14. Cui H, Nishiguchi N, Yanagi M, Fukutake M, Mouri K, Kitamura N et al (2010) A putative cis-acting polymorphism in the NOS1 gene is associated with schizophrenia and NOS1 immunoreactivity in the postmortem brain. Schizophr Res 121(1–3):172–178. doi:10.1016/j.schres.2010.05.003
15. Machado-Silva W, Alfinito-Kreis R, Carvalho LS, Quinaglia ESJC, Almeida OL, Brito CJ et al (2015) Endothelial nitric oxide synthase genotypes modulate peripheral vasodilatory properties after myocardial infarction. Gene 568(2):165–169. doi:10.1016/j.gene.2015.05.042
16. Caumo A, Perseghin G, Brunani A, Luzi L (2006) New insights on the simultaneous assessment of insulin sensitivity and beta-cell function with the HOMA2 method. Diabetes Care 29(12):2733–2734. doi:10.2337/dc06-0070
17. Reif A, Herterich S, Strobel A, Ehlis AC, Saur D, Jacob CP et al (2006) A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function. Mol Psychiatry 11(3):286–300. doi:10.1038/sj.mp.4001779
18. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA et al (2002) Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 39(2):257–265

19. Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW et al (1993) Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 42(11):1663–1672. doi:10.2337/diab.42.11.1663
20. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD (1994) Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest 94(3):1172–1179. doi:10.1172/JCI117433
21. Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012;33(7):829 – 37, 37a-37d. doi: 10.1093/eurheartj/ehr304
22. Kashyap SR, Roman LJ, Lamont J, Masters BS, Bajaj M, Suraamornkul S et al (2005) Insulin resistance is associated with impaired nitric oxide synthase activity in skeletal muscle of type 2 diabetic subjects. J Clin Endocrinol Metab 90(2):1100–1105. doi:10.1210/jc.2004-0745
23. Turini P, Thalmann S, Jayet PY, Cook S, Mathieu C, Burcelin R et al. Insulin resistance in mice lacking neuronal nitric oxide synthase is related to an alpha-adrenergic mechanism. Swiss Med Wkly. 2007;137(49–50):700-4. doi: 2007/49/smw-11950
24. Lu QB, Feng XM, Tong N, Sun HJ, Ding L, Wang YJ et al (2015) Neuronal and Endothelial Nitric Oxide Synthases in the Paraventricular Nucleus Modulate Sympathetic Overdrive in Insulin-Resistant Rats. PLoS One 10(10):e0140762. doi:10.1371/journal.pone.0140762
25. Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G (2001) Sympathetic nervous system and insulin resistance: from obesity to diabetes. Am J Hypertens 14(11 Pt 2):304S–304S9S. doi:10.1016/s0895-7061(01)02236-1

# Tables

Table 1
Baseline data.

| Characteristics | rs41279104 polymorphism | | | p-value |
|---|---|---|---|---|
| | GG | GA | AA | |
| Participants, n (%) | 208 (59) | 114 (32) | 32 (9) | |
| Age, years | 59 ± 11 | 61 ± 11 | 59 ± 9 | 0.450 |
| Gender: male, n (%) | 160 (77) | 85 (74) | 24 (75) | 0.885 |
| Diabetes mellitus, n (%) | 39 (19) | 26 (23) | 6 (19) | 0.635 |
| Hypertension, n (%) | 123 (59) | 70 (61) | 20 (62) | 0.845 |
| Previous MI, n (%) | 19 (9) | 15 (13) | 3 (9) | 0.492 |
| Previous stroke, n (%) | 9 (4) | 5 (4) | 4 (12) | 0.140 |
| Current smoking, n (%) | 77 (37) | 42 (37) | 10 (31) | 0.794 |
| Former smoking, n (%) | 64 (31) | 31(27) | 12 (38) | 0.491 |
| Sedentary lifestyle, n (%) | 116 (56) | 61 (54) | 21 (66) | 0.525 |
| In-hospital drug treatment | | | | |
| Simvastatin, n (%) | 131 (63) | 69 (61) | 15 (47) | 0.253 |
| Calcium channel blockers n (%) | 7 (3) | 4 (4) | 1 (3) | 0.992 |
| Beta-blockers, n (%) | 127 (61) | 65 (57) | 20 (62) | 0.714 |
| Angiotensin receptor blockers, n (%) | 7 (3) | 3 (3) | 0 (0) | 0.583 |
| ACEi, n (%) | 111 (53) | 60 (53) | 13 (41) | 0.551 |
| Morphine, n (%) | 72 (35) | 39 (34) | 9 (28) | 0.841 |
| Unfractionated heparin, n (%) | 54 (26) | 20 (18) | 6 (19) | 0.249 |
| Low molecular weight heparin, n (%) | 124 (60) | 70 (61) | 14 (44) | 0.213 |
| Aspirin, n (%) | 186 (89) | 98 (85) | 24 (75) | 0.162 |
| Clopidogrel, n (%) | 128 (62) | 67 (59) | 18 (56) | 0.986 |
| Insulin, n (%) | 13 (6) | 8 (7) | 0 (0) | 0.412 |

[a]MI: Myocardial infarction; ACEi: Angiotensin converting enzyme inhibitors; CK-MB: creatinine kinase-MB; hs-CRP: high-sensitivity C-reactive protein; HbA1c: Glycated hemoglobin; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol. Data expressed as mean ± standard deviation, absolute count (within-group proportion) or median [interquartile range].

| Characteristics | rs41279104 polymorphism | | | p-value |
|---|---|---|---|---|
| | GG | GA | AA | |
| Coronary reperfusion therapy | | | | |
| Thrombolytic drugs, n (%) | 145 (70) | 70 (61) | 22 (69) | 0.488 |
| Percutaneous angioplasty, n (%) | 80 (38) | 56 (49) | 16 (50) | 0.065 |
| Surgical revascularization, n (%) | 16 (8) | 9 (8) | 5 (16) | 0.304 |
| Peak of CK-MB, U/L | 163 ± 235 | 188 ± 219 | 242 ± 537 | 0.152 |
| Troponin, ng/mL | 1.74 ± 11.2 | 1.90 ± 15.1 | 0.99 ± 17.8 | 0.975 |
| hs-CRP, mg/L | 0.60 [1.06] | 0.60 [1.11] | 0.57 [1.18] | 0.855 |
| HbA1c, % | 5.9 ± 0.9 | 6.1 ± 1.4 | 6.1 ± 0.8 | 0.162 |
| HDL-C, mg/dL | 37 ± 13 | 36 ± 14 | 39 ± 18 | 0.494 |
| LDL-C, mg/dL | 118 ± 57 | 125 ± 57 | 124 ± 58 | 0.314 |
| Triglycerides, (mg/dL) | 122 [113] | 140 [109] | 107 [93] | 0.448 |
| Glomerular filtration rate, mL/min | 97 ± 25 | 97 ± 25 | 102 ± 21 | 0.077 |
| Systolic blood pressure, mmHg | 130 ± 38 | 130 ± 40 | 130 ± 57 | 0.915 |
| Diastolic blood pressure, mmHg | 80 ± 30 | 80 ± 30 | 90 ± 30 | 0.365 |
| Body Mass Index, kg/m$^2$ | 26.7 ± 5.0 | 25.6 ± 5.1 | 27.7 ± 7.7 | 0.280 |
| Waist circumference, cm<br>Female<br>Male | 96 ± 14<br>96 ± 14 | 93 ± 10<br>95 ± 13 | 93 ± 24<br>100 ± 18 | 0.527<br>0.227 |

[a]MI: Myocardial infarction; ACEi: Angiotensin converting enzyme inhibitors; CK-MB: creatinine kinase-MB; hs-CRP: high-sensitivity C-reactive protein; HbA1c: Glycated hemoglobin; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol. Data expressed as mean ± standard deviation, absolute count (within-group proportion) or median [interquartile range].

Table 2

Comparative analysis of glycemic characteristics across nNOS genotypes.

| Characteristics | rs41279104 polymorphism | | | ANCOVA* |
|---|---|---|---|---|
| | GG | GA | AA | |
| Blood glucose, mg/dL | | | | |
| D1 | 126.5 ± 52.3 | 127.0 ± 68.0 | 120.0 ± 29.5 | < 0.001 |
| D5 | 105.0 ± 35.8 | 118.0 ± 49.5 | 103.0 ± 37.8 | < 0.001 |
| ΔD5-D1 | -15.0 | -10.0 | -16.5 | < 0.001 |
| HOMA2B, % | | | | |
| D1 | 111.6 ± 80.9 | 107.5 ± 86.6 | 169.0 ± 125.8 | < 0.001 |
| D5 | 129.1 ± 84.0 | 119.3 ± 96.3 | 122.6 ± 83.8 | < 0.001 |
| ΔD5-D1 | + 12.4 | + 9.7 | -6.9 | < 0.001 |
| HOMA2S, % | | | | |
| D1 | 35.9 ± 50.3 | 38.2 ± 58.4 | 26.2 ± 41.8 | 0.017 |
| D5 | 64.2 ± 70.5 | 53.7 ± 82.2 | 70.4 ± 96.3 | 0.026 |
| ΔD5-D1 | + 23.4 | + 11.9 | + 39.2 | < 0.001 |
| Disposition Index, % | | | | |
| D1 | 43.5 ± 59.2 | 46.9 ± 57.6 | 37.0 ± 66.1 | < 0.001 |
| D5 | 82.7 ± 99.8 | 61.1 ± 75.0 | 80.6 ± 99.4 | < 0.001 |
| ΔD5-D1 | + 29.5 | + 12.5 | + 25.5 | < 0.001 |

[a]D1: first day of hospitalization; D5: fifth day of hospitalization; ΔD5-D1: change between fifth and first day of myocardial infarction; HOMA2S: The Homeostasis Model Assessment of Insulin Sensitivity; HOMA2B: The Homeostasis Model Assessment of Beta Cell Function. *Adjusted for age, gender, diabetes mellitus, peak of CK-MB, in-hospital use of statin, morphine and insulin. All ΔD5-D1 were also adjusted for basal values (D1).

Table 3
Comparative analysis of plasma NO levels and flow-mediated dilation (FMD) across nNOS genotypes.

| Characteristics | rs41279104 polymorphism | | | ANCOVA* |
|---|---|---|---|---|
| | GG | GA | AA | |
| NO levels, μmol/L | | | | |
| D1 | 17.0 ± 10.8 | 17.5 ± 9.9 | 19.1 ± 9.7 | 0.874 |
| D5 | 23.8 ± 14.2 | 25.5 ± 17.5 | 21.7 ± 36.5 | 0.039 |
| ΔD5-D1 | + 4.7 | + 6.3 | + 3.7 | 0.012 |
| FMD at 30th day, (%) | 6.70 [6.96] | 6.08 [7.09] | 6.30 [8.88] | 0.009 |

[a] D1: first day of hospitalization; D5: fifth day of hospitalization; DD5-D1: change between fifth and first day of myocardial infarction; FMD: Flow Mediated Dilation. *Adjusted for age, gender, diabetes mellitus, hypertension, peak of CK-MB and in-hospital use of statin and drugs with vasodilation effects. All DD5-D1 were also adjusted for basal values (D1). Data expressed as mean ± standard deviation or median [interquartile range].