Title page

Title

Influence of fat intake and BMI on the association of rs1799983 *NOS3* polymorphism with blood pressure levels in an Iberian population

Authors

Leticia Goni [a,b]

Marta Cuervo [a,b,c,d]

Fermín I. Milagro [a,b,c]

J. Alfredo Martínez [a,b,c,d]

[a]Department of Nutrition, Food Sciences and Physiology, University of Navarra, Pamplona, Spain

[b]Centre for Nutrition Research, University of Navarra, Pamplona, Spain

[c]CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain

[d]Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain

Corresponding author

J. Alfredo Martínez

E-mail address: jalfmtz@unav.es

Tel: (34) 948 425 665

Fax: (34) 948 425 740

Department of Nutrition, Food Sciences and Physiology

University of Navarra

Irunlarrea, 1 31008 Pamplona

Navarra, Spain

Abstract

Purpose There is controversy about the effect of the rs1799983 *Nitric Oxide Synthase* (*NOS3*) genetic variant on hypertension and Blood Pressure (BP) levels. The aims of the current study were to examine whether rs1799983 affects BP levels and to identify potential interactions between this polymorphism and other non-genetic risk factors.

Methods A total of 705 subjects were examined for anthropometric and body composition measurements, BP, dietary habits and physical activity. Oral epithelial cells were collected for the identification of rs1799983 using Luminex® 100/200TM System.

*Result*s After adjusted for covariates, TT genotype showed a 2.30 fold higher predisposition of hypertension than GG genotype subjects. According to BP levels, for each risk allele diastolic blood pressure (DBP) increased in 1.99 mmHg. Significant interactions between rs1799983 and Saturated Fatty Acids (SFA) and Monounsaturated Fatty Acids (MUFA) were found. Moreover, an interaction with body weight status was observed. Among overweight individuals, T allele carriers showed higher DBP than GG genotype.

Conclusion The present study evidenced that rs1799983 *NOS3* polymorphism could be associated with hypertension and DBP among Southern Europeans, being this association influenced by dietary fat (SFA and MUFA) and Body Mass Index (BMI).

Keywords

Hypertension; blood pressure; *NOS3;* saturated fatty acids; monounsaturated fatty acids; obesity

Introduction

Hypertension contributes to the burden of heart disease, stroke, kidney failure and premature mortality [1]. In fact, hypertension complications account for 9.4 million deaths worldwide every year [2]. Key risk factors include age, race, endocrine and metabolic disorders, lifestyle behaviors and genetics, among others [1].

In this context, familial and twin studies have estimated the heritable component of Blood Pressure (BP) to be about 30%-60% [3]. Moreover, Genome Wide Association Studies (GWAS) have identified a large number of polymorphisms associated with BP or hypertension, which are located in or near genes involved in the renin-angiotensin-aldosterone system, related to enzymes and receptors of the mineral-and glucocorticoid pathways and associated with proteins implicated in the structure and/or regulation of vascular tone [4].

Among them, the Nitric Oxide Synthase (*NOS3*) gene is regarded as one of the putative candidate gene for BP regulation and hypertension, since it is involved in the production of Nitric Oxide (NO) which has vasodilator effects (i.e. inhibiting vascular smooth muscle contraction) [5]. Indeed, it has been observed in an animal model that the disruption of *NOS3* gene led to hypertension, while in humans the inhibition of NOS3 elevated BP [5, 6]. Between *NOS3* genetic variants, the rs1799983 is the most recognized polymorphism related not only to BP and hypertension, but also to coronary artery and vascular diseases, myocardial infarction, metabolic syndrome and type 2 diabetes [7]. Unfortunately, the results of studies seeking associations of rs1799983 and BP or hypertension have not always been consistent in different populations, which might be due to gene-environmental risk factors interactions [8]. However, to our knowledge, there are few reports on the modulation of environmental factors such as excess body weight or diet, two risk factors widely associated with increased BP levels and hypertension, on the association between rs1799983 and BP or hypertension [9, 10].

Therefore, the aims of the present research were to examine the potential association between the rs1799983 *NOS3* genetic variant and BP levels and hypertension, and to investigate the possible influence of non-genetic risk factors on that association.

Methods

Study population

The present study encompassed men and women of Caucasian ancestry who voluntarily attended community pharmacies located in 7 regions of Spain (Barcelona, Zaragoza, La Coruña, Pontevedra, Madrid, Granada and Málaga). Genotype information of 718 individuals was available. Of these 13 subjects were excluded with missing values for dietary intake, physical activity, anthropometric measurements and/or blood pressure levels. Therefore, the screened group included 705 individuals with a mean age of 50.2±13.2 y.o. Of the total population 22.3% (n 157) were male and 77.7% (n 548) were female.

The recruited subjects were specifically asked if they would be willing to take part anonymously in the research study. Only those who gave written informed consent for participation were enrolled. All procedures were in accordance with the guidelines laid down in the Declaration of Helsinki. Patient data were codified to guarantee anonymity. The Research Ethics Committee of the University of Navarra provided confirmation of fulfillment of the ethical standards affecting this research (ref. 2410/2014).

Anthropometric measurements, habitual dietary intake and physical activity were collected by trained nutritionists as described elsewhere [11]. Briefly, weight, height, waist circumference and body fat mass were measured with a digital scale (Tanita BF-522W, Tanita Corporation, Tokyo, Japan), a portable stadiometer (Leicester Tanita), inextensible tape measure and bioelectrical impedance (Tanita BF-522W), respectively. Dietary intake was determined using a food frequency questionnaire in which basic foods were classified into 19 food groups, where 4 responses were possible (daily, weekly, monthly or never). Physical activity was collected by a short 24h physical activity questionnaire [12].

Blood pressure

BP was measured using the following standardized protocol with a validated automatic device (MIT Elite Plus, OMRON Healthcare, Hoofddorp, Netherland) and appropriately sized cuff [13]. Measurements were carried out in the non-dominant arm, with the elbow at the level of the right atrium and with the subject in a sitting position. Systolic BP (SBP) and Diastolic BP (DBP) were taken two times, separate of at least 10 minutes. The last measurement was used in the analysis, discarding the first one. If in the second reading the SBP or DBP were ≥140 mmHg or ≥90 mmHg, respectively, was performed a third measurement.

BP was considered as a categorized variable (hypertension vs normotension) and as a continuous variable. In order to dichotomize variables, individuals with a SBP ≥140 mmHg or DBP ≥90 mmHg or with declared diagnostic hypertension were defined as "hypertensive". On the other hand, as a continuous variable, BP was treated after adjustment of the BP treatment. In this sense, according to previous studies and based on the known average treatment effects, fixed increment of 15 mmHg SBP and 10 DBP were added to the pressures of individuals with diagnostic hypertension [14-17].

DNA isolation and genotyping

Genomic DNA was isolated from oral epithelial cells (collected by ORAcollect DNA®, DNAGenotek, Kanata, Canada) by QIAcube using QiAmp DNA Mini QIAcube Kit (Qiagen, Hilden, Germany), following the manufacturer's standard protocol. rs1799983 *NOS3* genetic variant was genotyped by Luminex® 100/200TM System (Luminex Corporation, Austin, Texas), which is based on the principles of xMAP® Technology. This method uncompressed polystyrene microspheres internally dyed with various ratios of spectrally distinct fluorochromes, that are detected by a flow cytometry-based instrument [18].

Statistical analysis

Examination of Hardy Weinberg Equilibrium (HWE) was assessed using the χ^2 test and allele frequencies were estimated. Quantitative variables are expressed as means (SD), and differences among genotype groups were analyzed using general linear models of analysis of covariance (ANCOVA) with age, gender, energy intake and physical activity as covariates. Multiple logistic regression (categorical variable) and multiple linear regression models (continuous variables), adjusted for covariates (model A adjusted for non-modifiable factors and model B adjusted for model A plus modifiable factors), were applied to investigate the association of the *NOS3* polymorphism with hypertension and SBP/DBP, respectively. Interactions between rs1799983 (as a dummy variable) and weight status or dietary fatty acid intake on BP were investigated using likelihood ratio test, once adjusted for potential confounders including gender, age, physical activity, energy intake, smoking status and alcohol consumption. All statistical procedures were performed using STATA/SE version 12.0 (StataCorp, College Station, Tex., USA). Tests were considered statistically significant at p value <0.05.

Results

Minor allele frequency (MAF) (T=36.9) and genotypic frequencies (GG=40.1, GT=46.0, TT=13.9) for rs1799983 were in accordance with the reference data for Caucasian populations (HapMap CEU). Moreover, the distribution of the polymorphism was in Hardy Weinberg Equilibrium (HWE) ($p>0.05$). Baseline characteristics of the population have been described according to rs1799983 *NOS3* genotype subgroups (Table 1). There were no statistically significant differences based on genotype for anthropometrical, physical activity and dietary variables. Of the 705 individuals, 22.3% (n 157) self-declared hypertension. Moreover, about 30.6 % of the individuals self-declared that they suffered one or more metabolic disorders: 3.3 % type 2 diabetes, 28.6 % different lipid metabolism impairments and 3.1 % cardiovascular disease.

Logistic regression models to estimate odds ratios of self-declared hypertension for individuals with the rs1799983 polymorphism were analyzed (Fig. 1). Once adjusted for covariates, those subjects with the TT genotype showed a statistically significant 2.30 fold higher predisposition of hypertension (95%CI 1.18-4.44; p 0.014) than those subjects with the GG genotype. However, individuals with the GT genotype did not show a higher risk of hypertension than those with the GG genotype (95%CI 0.82-2.09; p 0.266). Additive and recessive model showed a statistically significant 1.46 (95%CI 1.06-2.01; p 0.019) fold per risk allele and 1.98 (95%CI 1.08-3.63; p 0.028) fold higher predisposition of hypertension, respectively. There were no a higher risk of hypertension when the dominant model was applied (OR 1.48 95% CI 0.95-2.30; p 0.084). When the hypertension was defined according to blood pressure measurements of the study, once adjusted for hypertensive treatment, no statistically significant differences were observed.

Results from linear regression analyses showed an association between rs1799983 and DBP. Nevertheless, no relationship with SBP was found (Table 2). Carriers of the T allele had significantly greater values of DBP and DBP corrected according to treated hypertensive subjects after adjusted for non-modifiable risk factors of hypertension and both non-modifiable and modifiable risk factors of hypertension. Specifically each additional risk allele was associated with a 1.99 mmHg increase in DBP, when DBP was adjusted according to treated hypertensive subjects and after statistical analysis was adjusted for gender, age, smoking status, alcohol consumption and physical activity. Moreover, GT and TT individuals showed a 2.55 mmHg higher DBP than GG individuals after adjusted for non-modifiable and

modifiable covariates. Upon stratifying data by gender, similar results were found among men (data not shown).

After data were adjusted for potential confounder variables, Saturated Fatty Acids (SFA) and Monounsaturated Fatty Acids (MUFA) modified the effect of the rs1799983 *NOS3* genetic variant on DBP (Fig. 2). The influence of Body Mass Index (BMI) on the association between rs1799983 and DBP was also analyzed (Fig. 3). When the analysis was carried out according to the three genotype groups no significant interactions were found. In contrast, when GT and TT genotype were clustered, a significant interaction was found. Among normal weight individuals no significant differences were found in DBP between GG genotype subjects and GT and TT genotype subjects. However, among overweight individuals GT and TT genotype subjects showed higher DBP than TT genotype subjects.

Discussion

The current study provides evidence that rs1799983 *NOS3* genetic variant is associated with an increased odds of hypertension and a high to normal DBP in Southern Europeans. Interestingly, an interaction between BMI and dietary fat intake (SFA and MUFA), with the rs1799983 polymorphism to influence DBP levels was observed.

In the last few years, increased attention was paid to this *NOS3* polymorphism since it is directly involved in BP regulation through NO levels [5]. A meta-analysis involving 45,287 subjects identified that the T allele of the rs1799983 polymorphism was associated with hypertension predisposition with an odds ratio of 1.20 (p 0.015) [8]. However, when the population was stratified for ethnicity, no significance was reached for Whites (OR 0.99, p 0.828). It should be highlighted that most of the studies in European population, included in the meta-analysis, were carried out in North and Central Europe. In line of our results, a study in American white women revealed a hazard ratio of hypertension for rs1799983 *NOS3* genetic variant of 1.05 (p 0.03) [19]. Afterwards reports linked the rs1799983 not only to hypertension, but also to left ventricular hypertrophy, coronary artery disease and venous thromboembolism, among others [20-22]. In the rs1799983 polymorphism a guanine/thymine substitution at exon 7 leads to a glutamate/aspartate substitution at position 298. Since this genetic variant alters the primary structure of the protein and could alter one or more functional properties of the enzyme, several mechanistic studies have been carried out [23-

27]. Different reports have revealed that in the presence of a T instead of a G at nucleotide position 894, *NOS3* encodes a protein which leads to a higher susceptibility to cleavage into a 100k-Da fragment [23, 24]. Thus, the cleaved fragment could decrease NOS3 activity. However, other studies concluded that this finding might be a technical artifact [25, 26]. On the other hand, it has been confirmed that T allele carriers have less NOS3 bound to caveolin-1, which is a protein essential for its activation and therefore to endothelial cell NO production [27].

To our knowledge, two previous studies have suggested an influence of rs1799983 *NOS3* genetic variant on DBP [28, 29]. The research by Kimura et al., in African-derived Brazilian population, revealed a single two locus effect between rs1799983 and rs1801058, *NOS3* and *GKR4* variants, respectively, on DBP [28]. Lately, Nunes et al. showed that GG genotype subjects presented lower exercise DBP than T allele carriers, among Brazilian women [29]. Nevertheless, Seidlerová et al. failed to confirm in Europeans the relationship between rs1799983 and any arterial properties [30]. It should be mentioned that Delgado-Lista et al. reported that after a meal rich in SFA the microvascular endothelial function, which is a subclinical condition found in most patients with hypertension, was lower among TG and GG genotype subjects than TT genotype subjects of a Spanish population [31]. In one hand, this finding suggests a possible ethnic influence on genetic modulation of BP levels responses. On the other hand, as other authors have previously suggested gene-environmental interactions should be analyzed [8].

In addition to high sodium intake the scientific literature has established a link between dietary fat and increased risk of hypertension [9]. In this sense, we found gene-diet interactions between rs1799983 *NOS3* genetic variant and SFA and MUFA intakes and the effect on DBP. This outcome diverges from the results of Kingah et al. that did not find any interaction between the *NOS3* genetic variant and dietary fat (total fat, SFA, MUFA and polyunsaturated fatty acids -PUFA-) [32]. Nevertheless, our observations are consistent with the results of Pereira et al. that suggested a possible gene-diet interaction between rs1799983 and a diet rich in SFA and cholesterol to influence BP levels [33]. However, the mechanisms by which dietary fat modulates the effect of *NOS3* genetic variant on BP levels are unknown and can at best only be speculated. In line of our results it has been reported in a molecular model that a diet rich in olive oil (high MUFA diet) increased the expression of the NOS3 enzyme. In contrast, a diet rich in SFA decreased the expression of NOS3 [34]. Thus, we

hypothesized that carriers of the T allele presented lower effect to dietary fat due to a reduced function of NOS3. To establish the mechanism by which dietary fat intake influences BP depending on the NOS3 genotype, more studies are needed.

Interestingly this study has found a novel interaction between BMI and rs1799983 on the modulation of DBP levels. This observation is consistent with the results of Abdel-Aziz et al., who described that the association of obesity with TT genotype of rs1799983 polymorphism increased the risk to develop premature coronary artery disease in Egyptians [11]. Moreover, previous studies have identified the influence of obesity on the relationship between different polymorphisms and BP levels or hypertension among adults and children [35-37]. Initial epidemiological studies suggesting a relationship between obesity and BP levels have been supported with the understanding of potential mechanisms involved in both conditions, such as vascular and systemic insulin resistance, dysfuntion of the sympathetic nervous system and the renin-angiotensin-aldosterone system, among other pathogenetic factors [10, 38]. On the other hand, NOS3 protein content was shown to be significantly lower in overweight and obese people and inversely associated with body fat mass [39, 40]. Moreover, oxidative stress, a well-known process implicated in obesity, has been related to NOS3 response. In this regard, a previous study in an animal model suggested that a reduced NO production and an increased production of Reactive Oxygen Species (ROS) may contribute to hypertension in obese rats [41]. Therefore, such scientific evidence suggests that our result may be a genuine interaction rather than a random chance finding.

The present study bears some strengths and limitations that need to be mentioned. First, this study included only subjects of Caucasian ancestry, so our findings may not be generalizable to other ethnic populations. Second, the study site could be considered as a random effect. However, when the statistical analysis was carried out adjusted for the covariate region the analysis gave similar results (data not shown). Third, due to the relatively small sample size and the use of a nominal significance threshold p value less than 0.05, the findings may need to be replicated in other population. Nevertheless, it was large enough to provide us adequate statistical power and the associations and interactions have reached statistical significance in this context of limited sample size. Fourth, we did not exclude subjects with diagnosed hypertension. However, the adjustments of SBP and DBP according to diagnostic hypertension avoided the possibility that a biased selection might result from selecting only individuals without hypertension. This correction has been widely adopted for different

authors [14-17]. Fifth, non-genetic factors could influence BP levels. In this sense, multivariate adjustments support that the effects are independent of some common traditional hypertension risk factors, including age, BMI, physical activity, smoking status and alcohol consumption. However, sodium intake was not taken into account, although it would be of interest to be included in the analysis, but unfortunately data were not available. Finally, as a cross-sectional study, to get definitive clinical conclusions about dietary intake may be elusive.

The present research represents a pilot effort to explore the role of gene-environment interactions in BP levels. The investigation of genetic factors and their interactions with the environment may improve the selection of more individualized effective treatment of hypertension.

Acknowledgements

We thank all the seekers of the Nutrigenetic Service who voluntarily offered their data. The authors are grateful to the 5 nutritionists for data collection and to Amaia Ibañez for excellent technical assistance. The authors thank the Linea Especial (University of Navarra; LE/97) and to the Spanish Ministry of Economy and Competitiveness (AGL2013-45554-R project) for financial support, CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn)/RETICS schedules (Instituto de Salud Carlos III, Madrid, Spain) for assistance in this study and the support from CINFA (Olloki, Spain) concerning the genetic tools and general logistic. The pre-doctoral research grant to Leticia Goñi from the Asociación de Amigos Universidad de Navarra is gratefully acknowledged.

Ethical standards

The Research Ethics Committee of the University of Navarra provided confirmation of fulfillment of the ethical standards affecting this research (ref. 2410/2014). Therefore, the survey was in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments.

Conflict of interest

The authors declare that they have no conflict of interest

References

1. World Health Organization (WHO) (2013) A global brief on hypertension. World Health Organization, Geneva

2. Lim SS, Vos T, Flaxman AD, et al (2012) A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2224-2260

3. Zhao Q, Kelly TN, Li C, He J (2013) Progress and future aspects in genetics of human hypertension. Curr Hypertens Rep 15:676-686

4. Butler MG (2010) Genetics of hypertension. Current status. J Med Liban 58:175-178

5. Gamboa A, Shibao C, Diedrich A, Choi L, Pohar B, Jordan J, Paranjape S, Farley G, Biaggioni I (2007) Contribution of endothelial nitric oxide to blood pressure in humans. Hypertension 49:170-177

6. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC (1995) Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 377:239-242

7. Vecoli C (2014) Endothelial nitric oxide synthase gene polymorphisms in cardiovascular disease. Vitam Horm 96:387-406

8. Niu W, Qi Y (2011) An updated meta-analysis of endothelial nitric oxide synthase gene: three well-characterized polymorphisms with hypertension. PLoS One 6:e24266

9. Hall WL (2009) Dietary saturated and unsaturated fats as determinants of blood pressure and vascular function. Nutr Res Rev 22:18-38

10. DeMarco VG, Aroor AR, Sowers JR (2014) The pathophysiology of hypertension in patients with obesity. Nat Rev Endocrinol 10:364-376

11. Abdel-Aziz TA, Mohamed RH (2013) Association of endothelial nitric oxide synthase gene polymorphisms with classical risk factors in development of premature coronary artery disease. Mol Biol Rep 40:3065-3071

12. Panel on Macronutrients, Panel on the Definition of Dietary Fiber, Subcommittee on Upper Reference Levels of Nutrients, Subcommittee on Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2005) Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). The National Academies Press, Washington, D.C.

13. Grim CE, Grim CM (2009) The Omron Elite 7300W home blood pressure monitor passes the European Society of Hypertension International Validation Protocol for women and men. Blood Press Monit 14:87-90

14. Fava C, Sjogren M, Montagnana M, Danese E, Almgren P, Engstrom G, Nilsson P, Hedblad B, Guidi GC, Minuz P, Melander O (2013) Prediction of blood pressure changes over time and incidence of hypertension by a genetic risk score in Swedes. Hypertension 61:319-326

15. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, et al (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature 478:103-109

16. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Kottgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM (2009) Genome-wide association study of blood pressure and hypertension. Nat Genet 41:677-687

17. Newton-Cheh C, Johnson T, Gateva V, et al (2009) Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet 41:666-676

18. Dunbar SA (2006) Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. Clin Chim Acta 363:71-82

19. Conen D, Cheng S, Steiner LL, Buring JE, Ridker PM, Zee RY (2009) Association of 77 polymorphisms in 52 candidate genes with blood pressure progression and incident hypertension: the Women's Genome Health Study. J Hypertens 27:476-483

20. Rai H, Parveen F, Kumar S, Kapoor A, Sinha N (2014) Association of endothelial nitric oxide synthase gene polymorphisms with coronary artery disease: an updated meta-analysis and systematic review. PLoS One 9:e113363

21. Xin Y, Song X, Xue H, Liu Z, Wang X, Wang H, Sun K, Bai Y, Liu J, Hui R (2009) A common variant of the eNOS gene (E298D) is an independent risk factor for left ventricular hypertrophy in human essential hypertension. Clin Sci (Lond) 117:67-73

22. Qin J, Dai J, Xu Z, Chen D, Qin J, Shi D, Teng H, Jiang Q (2013) Genetic polymorphism of NOS3 with susceptibility to deep vein thrombosis after orthopedic surgery: a case-control study in Chinese Han population. PLoS One 8:e70033

23. Persu A, Stoenoiu MS, Messiaen T, Davila S, Robino C, El-Khattabi O, Mourad M, Horie S, Feron O, Balligand JL, Wattiez R, Pirson Y, Chauveau D, Lens XM, Devuyst O (2002) Modifier effect of ENOS in autosomal dominant polycystic kidney disease. Hum Mol Genet 11:229-241

24. Tesauro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J (2000) Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. Proc Natl Acad Sci U S A 97:2832-2835

25. Fairchild TA, Fulton D, Fontana JT, Gratton JP, McCabe TJ, Sessa WC (2001) Acidic hydrolysis as a mechanism for the cleavage of the Glu(298)-->Asp variant of human endothelial nitric-oxide synthase. J Biol Chem 276:26674-26679

26. McDonald DM, Alp NJ, Channon KM (2004) Functional comparison of the endothelial nitric oxide synthase Glu298Asp polymorphic variants in human endothelial cells. Pharmacogenetics 14:831-839

27. Joshi MS, Mineo C, Shaul PW, Bauer JA (2007) Biochemical consequences of the NOS3 Glu298Asp variation in human endothelium: altered caveolar localization and impaired response to shear. FASEB J 21:2655-2663

28. Kimura L, Angeli CB, Auricchio MT, Fernandes GR, Pereira AC, Vicente JP, Pereira TV, Mingroni-Netto RC (2012) Multilocus family-based association analysis of seven candidate polymorphisms with essential hypertension in an african-derived semi-isolated brazilian population. Int J Hypertens 2012:859219

29. Nunes RA, Barroso LP, Pereira Ada C, Krieger JE, Mansur AJ (2014) Gender-related associations of genetic polymorphisms of alpha-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor with treadmill exercise test responses. Open Heart 1:e000132-2014-000132.

30. Seidlerova J, Filipovsky J, Mayer O,Jr, Kucerova A, Pesta M (2015) Association between endothelial NO synthase polymorphisms and arterial properties in the general population. Nitric Oxide 44:47-51

31. Delgado-Lista J, Garcia-Rios A, Perez-Martinez P, Fuentes F, Jimenez-Gomez Y, Gomez-Luna MJ, Parnell LD, Marin C, Lai CQ, Perez-Jimenez F, Ordovas JM, Lopez-Miranda J (2011) Gene variations of nitric oxide synthase regulate the effects of a saturated fat rich meal on endothelial function. Clin Nutr 30:234-238

32. Kingah PL, Luu HN, Volcik KA, Morrison AC, Nettleton JA, Boerwinkle E (2010) Association of NOS3 Glu298Asp SNP with hypertension and possible effect modification of dietary fat intake in the ARIC study. Hypertens Res 33:165-169

33. Pereira TV, Rudnicki M, Cheung BM, Baum L, Yamada Y, Oliveira PS, Pereira AC, Krieger JE (2007) Three endothelial nitric oxide (NOS3) gene polymorphisms in hypertensive and normotensive individuals: meta-analysis of 53 studies reveals evidence of publication bias. J Hypertens 25:1763-1774

34. Martins MA, Catta-Preta M, Mandarim-de-Lacerda CA, Aguila MB, Brunini TC, Mendes-Ribeiro AC (2010) High fat diets modulate nitric oxide biosynthesis and antioxidant defence in red blood cells from C57BL/6 mice. Arch Biochem Biophys 499:56-61

35. Grove ML, Morrison A, Folsom AR, Boerwinkle E, Hoelscher DM, Bray MS (2007) Gene-environment interaction and the GNB3 gene in the Atherosclerosis Risk in Communities study. Int J Obes (Lond) 31:919-926

36. Xi B, Zhao X, Chandak GR, Shen Y, Cheng H, Hou D, Wang X, Mi J (2013) Influence of obesity on association between genetic variants identified by genome-wide association studies and hypertension risk in Chinese children. Am J Hypertens 26:990-996

37. Marteau JB, Sass C, Pfister M, Lambert D, Noyer-Weidner M, Visvikis S (2004) The Leu554Phe polymorphism in the E-selectin gene is associated with blood pressure in overweight people. J Hypertens 22:305-311

38. Silva DA, Petroski EL, Peres MA (2013) Accuracy and measures of association of anthropometric indexes of obesity to identify the presence of hypertension in adults: a population-based study in Southern Brazil. Eur J Nutr 52:237-246

39. Hickner RC, Kemeny G, Stallings HW, Manning SM, McIver KL (2006) Relationship between body composition and skeletal muscle eNOS. Int J Obes (Lond) 30:308-312

40. Kraus RM, Houmard JA, Kraus WE, Tanner CJ, Pierce JR, Choi MD, Hickner RC (2012) Obesity, insulin resistance, and skeletal muscle nitric oxide synthase. J Appl Physiol (1985) 113:758-765

41. da Cunha NV, Pinge-Filho P, Panis C, Silva BR, Pernomian L, Grando MD, Cecchini R, Bendhack LM, Martins-Pinge MC (2014) Decreased endothelial nitric oxide, systemic oxidative stress, and increased sympathetic modulation contribute to hypertension in obese rats. Am J Physiol Heart Circ Physiol 306:H1472-80

Tables

Table 1 Characteristics of the population by *NOS3* rs1799983 genotype

	Total mean (SD)	GG mean (SD)	GT mean (SD)	TT mean (SD)	p-value
n (%)	705	283 (40.1)	324 (46.0)	98 (13.9)	0.733[a]
Women (%)	548 (77.7)	211 (29.9)	260 (36.9)	77 (10.9)	-
Age (years)[b]	50.2 (13.2)	50.6 (13.1)	50.7 (13.1)	47.7 (13.1)	0.121
Anthropometric measurements					
Height (cm)[b]	163.0 (8.7)	163.0 (6.6)	163.0 (6.6)	163.2 (6.6)	0.886
Weight (kg)[c]	78.3 (16.7)	77.9 (13.6)	79.3 (13.6)	75.9 (13.7)	0.083
BMI[c]	29.5 (5.8)	29.3 (5.0)	29.8 (5.0)	28.7 (5.1)	0.155
BFM (%)[c]	34.6 (10.2)	34.6 (7.4)	34.7 (7.4)	34.2 (7.4)	0.861
Waist circumference (cm)[c]	96.5 (15.2)	95.8 (12.1)	97.4 (12.1)	95.2 (12.1)	0.157
Physical activity [c]					
Physical activity level[c]	1.23 (0.03)	1.23 (0.03)	1.23 (0.03)	1.24 (0.03)	0.153
Baseline dietary intake					
Energy (kcal/day)[d]	2,149 (430)	2,138 (391)	2,150 (392)	2,144 (393)	0.791
Carbohydrate (g/day)[e]	197.8 (71.4)	197.5 (53.3)	197.8 (53.3)	198.6 (53.4)	0.986
Protein (g/day)[e]	92.9 (24.8)	93.2 (17.0)	92.7 (17.0)	92.8 (17.0)	0.962
Fat (g/day)[e]	95.1 (22.4)	95.4 (16.6)	95.2 (16.7)	94.4 (16.7)	0.895
MUFA (g/day)[e]	46.6 (11.8)	46.9 (9.9)	46.5 (9.9)	45.9 (9.9)	0.641
PUFA (g/day)[e]	14.1 (3.7)	14.1 (2.6)	14.1 (2.6)	14.0 (2.6)	0.889
SFA (g/day)[e]	19.1 (5.4)	19.0 (4.0)	19.3 (4.0)	19.1 (4.0)	0.681

BMI, Body mass index; BFM, Body fat mass; %E, Percentage of energy; MUFA; Monounsaturated fatty acids; Polyunsaturated fatty acids; SFA, Saturated fatty acids

[a] Hardy Weinberg Equilibrium

[b] Adjusted for gender

[c] Adjusted for gender, age and energy intake

[d] Adjusted for gender, age and physical activity

[e] Adjusted for gender, age, energy intake and physical activity

Table 2 Association of the rs1799983 *NOS3* genetic variant with Systolic and Diastolic BP among Southern Europeans[a]

		Model 1		Model 2	
	Genotype	B (95% CI)	p	B (95% CI)	p
SBP					
	Additive effect of T allele	1.06 (-0.95-3.07)	0.303	1.25 (-0.69-3.19)	0.205
	GT vs GG	0.59 (-2.39-3.58)	0.696	-0.00 (-2.87-2.86)	0.998
	TT vs GG	2.43 (-1.87-6.73)	0.267	3.38 (-0.76-7.53)	0.110
	GT + TT vs GG	1.02 (-1.80-3.84)	0.478	0.78 (-1.93-3.49)	0.573
	TT vs GG + GT	2.11 (-1.88-6.11)	0.299	3.38 (-0.47-7.24)	0.085
DBP					
	Additive effect of T allele	1.81 (0.31-3.32)	0.018	1.99 (0.61-3.36)	0.005
	GT vs GG	2.87 (0.63-5.10)	0.012	2.16 (0.13-4.20)	0.037
	TT vs GG	2.90 (-0.32-6.12)	0.077	3.86 (0.91-6.80)	0.010
	GT + TT vs GG	2.88 (0.77-5.00)	0.008	2.55 (0.63-4.47)	0.009
	TT vs GG + GT	1.37 (-1.63-4.38)	0.370	2.71 (-0.04-5.45)	0.053

SBP, Systolic blood pressure; DBP, Diastolic blood pressure; 95% CI, 95% confidence interval

[a] SBP + 15mmHg and DBP + 10mmHg to treated hypertensive subjects

Model 1 adjusted for gender and age

Model 2 adjusted for gender, age, BMI, physical activity, smoking status and alcohol consumption

Figures

Figure 1. Risk of hypertension according to rs1799983 genotype

Adjusted for gender, age, physical activity, energy intake, smoking status and alcohol consumption

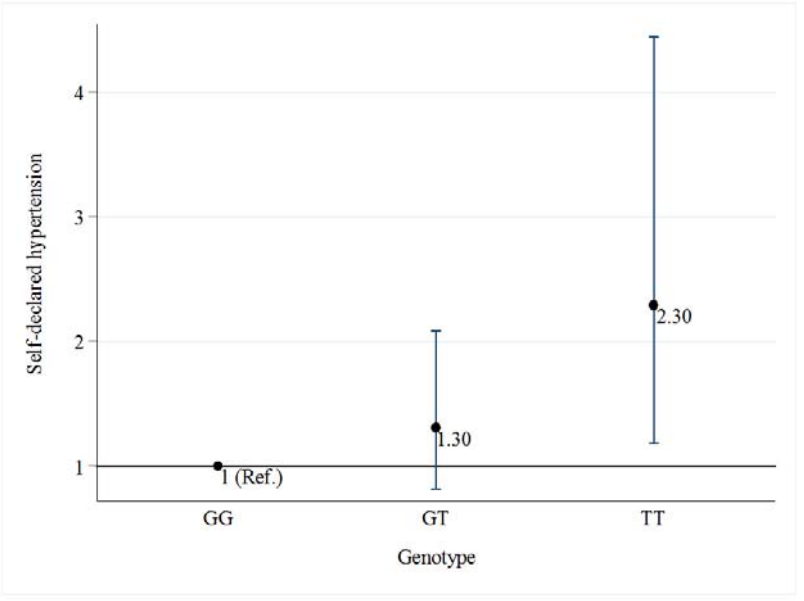
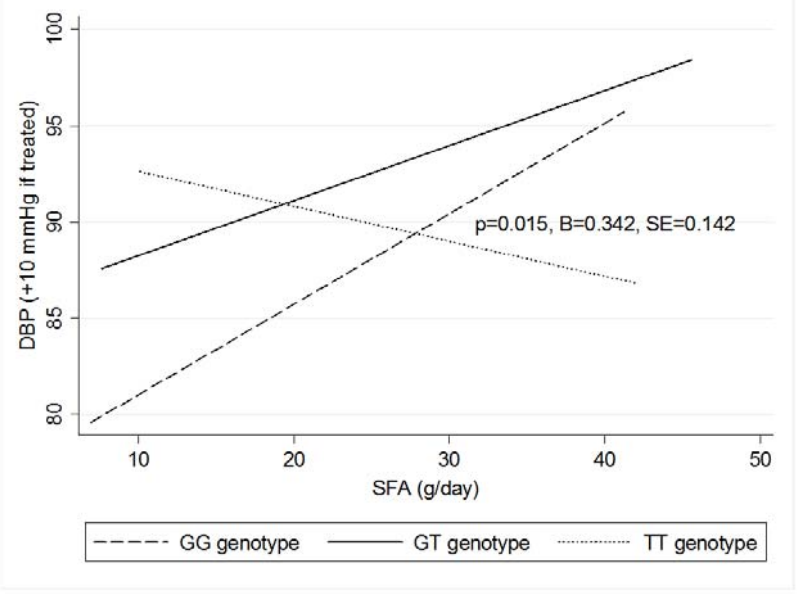


Figure 2. Interaction between *NOS3* and SFA (a) and MUFA (b) on DBP

DBP, Diastolic blood pressure; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids

Adjusted for gender, age, physical activity, energy intake, smoking status and alcohol consumption

(a)



(b)

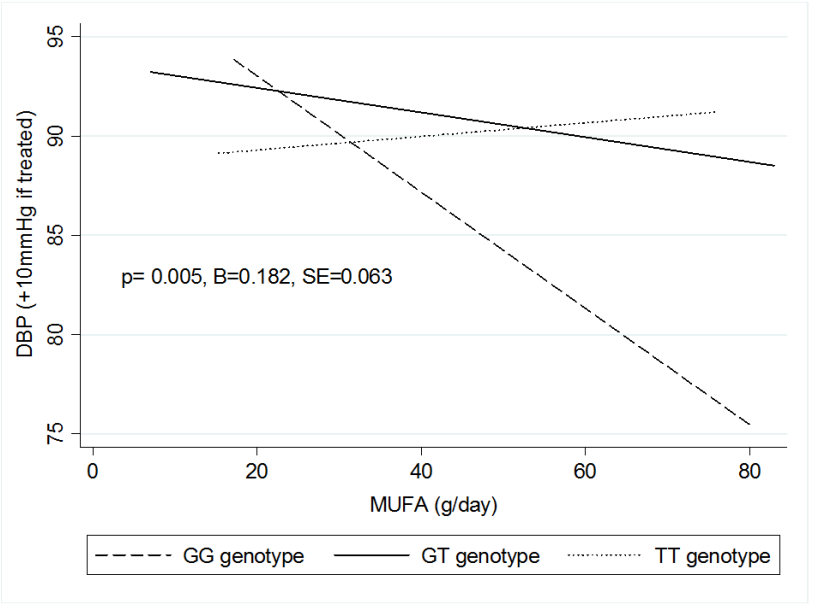


Figure 3. Interaction between *NOS3* and obesity status on DBP

Adjusted for gender, age, physical activity, energy intake, smoking status and alcohol consumption