ORIGINAL ARTICLE

# Association Between Glutathione S-Transferase M1 Polymorphism and Urinary Sodium Excretion in a Brazilian Population

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**BACKGROUND**
Null genotypes of glutathione S-transferase (GST) exhibit the absence of enzymatic activity and are associated with increased cardiovascular risk. Recent reports have related both lower and higher urinary sodium excretion (USE) to higher cardiovascular risk. Here we investigate the impact of GSTM1 and GSTT1-null polymorphisms on USE in a Brazilian population.

**METHODS**
We cross-sectionally evaluated 1,308 subjects from the city of Vitoria, Brazil, based on clinical history, physical examination, anthropometry, analysis of laboratory parameters, measurement of USE, and GST polymorphisms genotyping.

**RESULTS**
The frequency of GST M1, T1, and double-deletion polymorphisms was 51%, 22%, and 11%, respectively. Individuals with the GSTM1-null genotype had lower USE than those with the non-null genotype (92.1 ± 52.3 vs. 102.8 ± 6 0.7 mEq/12 h; $P < 0.001$). Linear regression analysis adjusted for confounding factors revealed that the GSTM1-null genotype was independently associated with USE ($P = 0.001$). In addition, diastolic blood pressure and triglyceride levels were higher in GSTM1-null individuals than in non-null individuals in the highest tertile of USE. Finally, the presence of GSTT1-null or double-deleted genotypes did not influence USE or affect the interactions between USE and the variables studied.

**CONCLUSIONS**
Deletion of GSTM1 was associated with low USE and modulated the interaction between sodium intake and blood pressure in Brazilian subjects. These novel findings may provide a new unexplored link between sodium regulation and GST homeostasis.

*Keywords:* blood pressure; GST; hypertension; polymorphism; urinary sodium excretion.

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Experimental and clinical evidence indicates that oxidative stress plays an important pathophysiological role in the development of cardiovascular diseases.[1] Glutathione S-transferase (GST) comprises a complex family of stress-responsive detoxification proteins that can act on reactive compounds produced by reactive oxygen species.[2] The human GST is subdivided into 8 distinct classes codified by 16 genes,[3] and 2 common deletion polymorphisms of the *GSTM1* and *GSTT1* genes have been related to coronary heart disease and cardiovascular events,[4,5] albeit conflicting data have been reported.[6,7] Conversely, although GST-null polymorphisms have been associated with the development of hypertension, diabetes mellitus, and dyslipidemia, these associations do not fully explain the relationship between GST variants and increased cardiovascular risk.[8,9]

Urinary sodium excretion (USE), a putative marker of sodium intake, is clearly related to cardiovascular events. In this regard, recent reports demonstrated that both low and high USE are associated with increased cardiovascular risk.[10–12] Sodium homeostasis can be regulated by genetic factors, and several polymorphisms have been related to USE.[13–15] Moreover, genetic factors are reported to modulate the interaction between sodium intake and blood pressure.[13,16] For instance, polymorphisms of the cytochrome P450 3A (CYP3A), which is an enzyme involved in the metabolism of endogenous substances, xenobiotics, and reactive oxygen species, were implicated in renal sodium reabsorption and blood pressure regulation.[14] These data suggest that genetic variation in components of pathways that regulate oxidative stress may modulate USE and influence the interaction between USE and blood pressure regulation. Therefore, the aim of this study was to investigate the influence of the GSTM1 and GSTT1 polymorphisms on USE in a Brazilian population.

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## METHODS

### Study population

A cross-sectional study of risk factors for cardiovascular diseases was performed in the urban population of Vitoria, Brazil, using the World Health Organization Multinational MONItoring of trends and determinants in CArdiovascular disease (WHO-MONICA) project guidelines.[17] A sample of 1,308 individuals was evaluated for height, weight, smoking habits, blood pressure measurements, and use of medications. Subjects were classified as of European or of African descent according to a set of phenotypic characteristics (skin color, hair texture, shape of the nose, aspect of the lip, and jaw position).[18] Exclusion criteria were age < 65 years and use of any antihypertensive medications. The Institutional Review Board of the Espírito Santo Federal University approved the study protocol, and all participants read and signed informed consent.

Brachial blood pressure was measured using a standard mercury sphygmomanometer after 5 minutes rest in the sitting position. Systolic and diastolic blood pressures were calculated from 3 readings with a minimum interval of 5 minutes. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg. Blood glucose, total cholesterol, lipoprotein fractions, and triglycerides were assayed by standard techniques in 12-hour fasting blood samples. Diabetes mellitus was defined as a fasting glucose ≥ 126 mg/dL and/or use of hypoglycemic drugs.[19] No participant reported the presence of type 1 diabetes mellitus. Body mass index was calculated as body weight divided by height squared (kg/m$^2$). Height was measured in centimeters and weight in kilograms using a calibrated balance.

Urine was collected from participants during a 12-hour period (from 7:00 PM to 7:00 AM) before the clinic visit. Sodium and potassium concentrations were measured in the 12-hour urine by flame photometry. All measurements were performed in the same laboratory, and the same commercial kits were used in all investigations. Previous data have shown that 12-hour urine collected at night can be used as a reliable tool to estimate 24-hour excretion of sodium and potassium.[20]

### DNA extraction and GSTM1 and GSTT1 genotyping

Genomic DNA was extracted from leukocytes in samples of whole blood following a standard salting-out procedure. The polymerase chain reaction conditions for determining GSTM1 and GSTT1 genotypes were the same as those reported previously by Maciel *et al.*[21] Amplification of the beta actin gene was used as an internal control and produced a 350-bp product. Successful amplification by beta actin–specific primers confirmed the proper function of the polymerase chain reaction. Quality control for these assays was assessed by randomly selecting 40 samples to be regenotyped by 2 independent technicians.

### Statistical analysis

Data were analyzed using SPSS 15.0. Descriptive statistical results are given as means ± standard deviation. All continuous variables presented normal distribution as assessed by the Kolmogorov–Smirnov test. The differences in genotype distributions and categorical variables were tested using $\chi^2$ test. Unpaired $t$ test was used to compare continuous variables and to evaluate differences in 12-hour USE between genotype groups. General linear model analysis was used to assess the differences in selected variables after adjustment for relevant covariates. Bivariate correlations between variables were examined using Pearson correlation coefficient for normally distributed data and Spearman rank correlation coefficient for nonnormally distributed data. Linear regression analysis was used to evaluate the independent predictors of USE. Variables that exhibited significant correlation at bivariate analysis were included as independent variables in linear regression analyses. A test for linear trend across the tertiles of USE (based on logistic regression) was performed for the GSTM1 polymorphism. Significance was accepted if $P < 0.05$.

## RESULTS

The frequencies of genotypes as well as the clinical and laboratory characteristics of the enrolled participants according to the GST polymorphisms are presented in Table 1. Among the 1,308 subjects, 51% were GSTM1 null, 22 % were GSTT1 null, and 11% exhibited the double-deleted genotype. Subjects with the GSTM1-null genotype exhibited lower USE (92.1 ± 52.3 vs. 102.8 ± 60.7 mEq/12 h; $P < 0.001$) had a higher prevalence of European descent (46% vs. 37%; $P < 0.001$) than individuals with the GSTM1–non-null genotype. After adjustment for ethnicity, subjects with GSTM1-null genotype still exhibited significantly lower USE ($P = 0.001$). In this regard, further analysis revealed that individuals of both European and African descent with the GSTM1 genotype exhibited lower USE (90.3 ± 50.4 vs. 103.0 ± 64.5 mEq/12 h; $P = 0.017$; 93.3 ± 53.6 vs. 102.8 ± 58.9 mEq/12 h; $P = 0.019$, respectively). Conversely, subjects with GSTT1-null or double-deleted genotypes presented no differences in clinical, anthropometric, and biochemical variables except for higher creatinine levels. However, after adjustment for gender, such differences in creatinine levels became not statistically significant.

Bivariate correlation analysis between clinical/laboratory variables and USE was performed in order to determine potential confounding factors in the relationship between USE and the GSTM1 polymorphism. This analysis revealed that USE also exhibited significant correlation coefficients with urinary potassium excretion (r = 0.495; $P < 0.001$), diastolic blood pressure (r = 0.177; $P < 0.001$), male gender (r = 0.176; $P < 0.001$), hypertension (r = 0.166; $P < 0.001$), body mass index (r = 0.139; $P < 0.001$), systolic blood pressure (r = 0.128; $P < 0.001$), triglycerides (r = 0.126; $P < 0.001$), uric acid (r = 0.097; $P < 0.001$), creatinine (r = 0.065; $P = 0.019$), glycemia (r = 0.064; $P = 0.022$), and smoking (r = 0.062; $P = 0.026$). To evaluate whether the GSTM1 polymorphism was independently related to USE, linear regression analysis was performed. The GSTM1-null allele was independently related to USE in a model that also included urinary potassium excretion, male gender, diastolic blood pressure, systolic blood pressure, hypertension, body

**Table 1.** Clinical features of studied subjects according to GST M1, T1, and double-deleted polymorphisms

| | GSTM1 | | GSTT1 | | Double-deleted | |
|---|---|---|---|---|---|---|
| **Characteristics** | **Null** | **Non-null** | **Null** | **Non-null** | **Null** | **Non-null** |
| N (%) | 668 (51) | 640 (49) | 283 (22) | 1,025 (78) | 143 (11) | 1,165 (89) |
| Clinical | | | | | | |
| Age, years | 43.8 ± 10.3 | 42.8 ± 10.7 | 44.2 ± 11.0 | 43.1 ± 10.3 | 44.1 ± 10.8 | 43.3 ± 10.5 |
| Gender (male/female) | 316/352 | 316/324 | 150/133 | 482/543 | 75/68 | 557/608 |
| Body mass index, kg/m$^2$ | 25.9 ± 4.8 | 25.5 ± 4.3 | 25.6 ± 4.4 | 25.7 ± 4.7 | 25.7 ± 4.3 | 25.7 ± 4.7 |
| Systolic blood pressure, mm Hg | 123.9 ± 19.8 | 124.1 ± 19.1 | 124.0 ± 20.6 | 124.0 ± 19.2 | 123.5 ± 22.2 | 124.1 ± 19.1 |
| Diastolic blood pressure, mm Hg | 82.7 ± 13.1 | 81.7 ± 12.9 | 82.9 ± 13.2 | 82.1 ± 12.9 | 83.5 ± 13.7 | 82.1 ± 12.9 |
| Smoking, n (%) | 143 (21) | 164 (26) | 68 (24) | 239 (23) | 30 (21) | 277 (24) |
| Hypertension, n (%) | 243 (36) | 218 (34) | 107 (38) | 354 (34) | 57 (40) | 404 (35) |
| Diabetes mellitus, n (%) | 104 (15) | 112 (17) | 44 (15) | 172 (17) | 20 (14) | 196 (17) |
| Ethnicity (European descent) | 309 (46) | 235 (37)*** | 112 (40) | 432 (42) | 65 (45) | 479 (41) |
| Serum analysis | | | | | | |
| Glycemia, mg/dL | 101.5 ± 26.8 | 101.8 ± 27.5 | 101.3 ± 27.7 | 101.7 ± 27.0 | 99.1 ± 20.7 | 102.0 ± 27.8 |
| Uric acid, mg/dL | 4.8 ± 3.6 | 5.2 ± 6.7 | 4.7 ± 1.4 | 5.1 ± 5.9 | 4.6 ± 1.3 | 5.0 ± 5.6 |
| Creatinine, mg/dL | 0.97 ± 0.18 | 0.96 ± 0.19 | 0.99 ± 0.19 | 0.96 ± 0.19* | 0.99 ± 0.18 | 0.96 ± 0.19* |
| Triglycerides, mg/dL | 132.6 ± 99.2 | 123.2 ± 90.7 | 133.1 ± 91.2 | 126.6 ± 96.3 | 136.6 ± 88.8 | 126.9 ± 95.9 |
| High-density-lipoprotein cholesterol, mg/dL | 51.4 ± 32.5 | 50.7 ± 34.2 | 50.5 ± 33.8 | 51.2 ± 33.2 | 48.5 ± 29.5 | 51.3 ± 33.7 |
| Low-density-lipoprotein cholesterol, mg/dL | 139.7 ± 39.1 | 139.2 ± 39.8 | 141.7 ± 40.2 | 138.8 ± 39.2 | 144.5 ± 41.2 | 138.8 ± 39.2 |
| 12-hour urinary analysis | | | | | | |
| Sodium, mEq | 92.1 ± 52.3 | 102.8 ± 60.7*** | 97.1 ± 54.7 | 97.4 ± 57.4 | 95.7 ± 48.6 | 97.5 ± 57.7 |
| Potassium, mEq | 22.3 ± 15.2 | 23.6 ± 15.2 | 23.0 ± 15.1 | 22.9 ± 15.3 | 22.3 ± 12.4 | 23.0 ± 15.5 |
| Urinary sodium to urinary creatinine ratio, mEq/mg | 1.27 ± 3.32 | 1.45 ± 1.44** | 1.32 ± 1.37 | 1.37 ± 2.82 | 1.35 ± 1.23 | 1.36 ± 2.70 |

*$P$ <0.05, **$P$ < 0.01, and ***$P$ ≤ 0.001 compared to the respective null polymorphism.

mass index, triglycerides, uric acid, creatinine, glycemia, and smoking as independent variables (Table 2).

Further linear regression analyses were performed for each ethnogeographic group. In this regard, the GSTM1-null allele was independently related to USE in individuals of European descent ($\beta$ = 12.806 ± 4.700; $P$ = 0.007) in a model that also included urinary potassium excretion, male gender, diastolic blood pressure, systolic blood pressure, hypertension, body mass index, triglycerides, and age as independent variables. This was also the case in individuals of African descent ($\beta$ = 7.176 ± 3.257; $P$ = 0.028) in a model that also included urinary potassium excretion, male gender, diastolic blood pressure, systolic blood pressure, hypertension, body mass index, triglycerides, and creatinine as independent variables.

The next step was to evaluate the frequencies of the GSTM1 polymorphism and the clinical and laboratory features of all studied subjects according to the tertiles of USE (Table 3). There was a significant linear trend across USE tertiles for the GSTM1 polymorphism ($P$ = 0.021). In addition, increased diastolic blood pressure (87.1 ± 13.8 vs. 84.3 ± 13.5 mm Hg; $P$ = 0.037) and triglycerides (156.4 ± 118.5 vs. 105.2 ± 33.7 mg/dL; $P$ = 0.023) levels were detected in GSTM1-null individuals in comparison with GSTM1–non-null individuals in the highest tertile of USE.

## DISCUSSION

Previous studies have demonstrated that genetic polymorphisms may be associated with USE[13–15] and that genetic factors may modulate the interaction between sodium intake and cardiovascular risk factors, such as blood pressure levels.[13,16] Here, the analysis of a Brazilian general population showed that deletion of the GSTM1 gene was associated with lower USE and that increased diastolic blood pressure and triglyceride levels were detected in GSTM1-null individuals in comparison with GSTM1–non-null individuals in the highest tertile of USE, suggesting that the interaction between USE and cardiovascular risk factors may be modulated by the GSTM1 polymorphism. To our knowledge, this is the first study to show that a GST polymorphism may be related to USE, which may provide a new unexplored link between sodium regulation and GST homeostasis.

USE is a variable that is usually considered a marker of sodium intake in clinical studies.[22] Therefore our findings may point toward the GSTM1-null polymorphism as a

**Table 2.** Linear regression analysis for urinary sodium excretion

| Variable | $\beta \pm$ Standard error of $\beta$ | *P* | $R^2$ |
|---|---|---|---|
| Dependent: urinary sodium excretion | | | 0.307 |
| Urinary potassium excretion | 1.764 ± 0.089 | < 0.00001 | |
| Hypertension | 12.862 ± 2.984 | < 0.00001 | |
| Male gender | 9.720 ± 2.762 | < 0.00001 | |
| GSTM1 genotype (0 = null; 1 = non-null) | 8.616 ± 2.684 | 0.001 | |
| Smoking | 8.513 ± 3.167 | 0.006 | |
| Body mass index | 0.780 ± 0.308 | 0.011 | |

Only variables that exhibited significant association with urinary sodium excretion are presented. The model also included systolic blood pressure, diastolic blood pressure, triglycerides, uric acid, creatinine, and glycemia as independent variables.

**Table 3.** Features of studied subjects according to the tertiles of urinary sodium excretion and GSTM1 genotypes

| Characteristics | Low (< 67mEq/12h) | | Medium (67–111 mEq/12h) | | High (> 111 mEq/12h) | |
|---|---|---|---|---|---|---|
| GSTM1 genotype | Null | Non-null | Null | Non-null | Null | Non-null |
| N (%) | 239 (55) | 192 (45) | 214 (51) | 212 (49) | 210 (47) | 231 (53) |
| Clinical | | | | | | |
| Age, years | 43.3 ± 10.5 | 41.4 ± 10.6 | 43.9 ± 10.3 | 42.5 ± 10.6 | 44.4 ± 10.2 | 44.2 ± 10.5 |
| Gender (male/female) | 84/155 | 84/108 | 99/115 | 95/117 | 131/79 | 136/95 |
| Body mass index, kg/m$^2$ | 25.3 ± 5.2 | 24.9 ± 4.1 | 25.5 ± 4.2 | 25.0 ± 4.4 | 27.1 ± 5.0 | 26.4 ± 4.3 |
| Systolic blood pressure, mmHg | 120.9 ± 22.4 | 122.5 ± 18.0 | 123.8 ± 17.5 | 121.7 ± 18.1 | 127.8 ± 18.5 | 127.3 ± 20.4 |
| Diastolic blood pressure, mmHg | 79.6 ± 13.5 | 79.8 ± 12.3 | 82.0 ± 10.8 | 80.4 ± 12.3 | 87.1 ± 13.8 | 84.3 ± 13.5* |
| Smoking, n (%) | 48 (20) | 51 (27) | 50 (23) | 44 (21) | 45 (21) | 68 (29) |
| Hypertension, n (%) | 63 (26) | 55 (29) | 73 (34) | 61 (29) | 105 (50) | 99 (43) |
| Diabetes mellitus, n (%) | 35 (15) | 32 (17) | 30 (14) | 34 (16) | 37 (18) | 45 (19) |
| Ethnicity (European descent) | 117 (49) | 73 (38)* | 95 (44) | 79 (37) | 93 (44) | 81 (35) |
| Serum analysis | | | | | | |
| Glycemia, mg/dl | 99.2 ± 20.6 | 100.8 ± 28.3 | 103.2 ± 32.0 | 99.9 ± 16.4 | 102.2 ± 27.4 | 105.2 ± 33.7 |
| Uric acid, mg/dL | 4.5 ± 1.4 | 4.7 ± 1.4 | 4.5 ± 1.5 | 4.6 ± 1.5 | 5.3 ± 5.9 | 4.9 ± 1.5 |
| Creatinine, mg/dL | 0.94 ± 0.18 | 0.96 ± 0.19 | 0.96 ± 0.18 | 0.95 ± 0.18 | 0.99 ± 0.19 | 0.99 ± 0.20 |
| Triglycerides, mg/dL | 116.3 ± 74.8 | 119.9 ± 100.1 | 127.7 ± 98.8 | 115.7 ± 79.3 | 156.4 ± 118.5 | 105.2 ± 33.7* |
| High-density lipoprotein cholesterol, mg/dL | 49.5 ± 21.6 | 55.2 ± 42.1 | 51.3 ± 30.9 | 47.8 ± 24.3 | 53.6 ± 43.0 | 49.7 ± 34.8 |
| Low-density lipoprotein cholesterol, mg/dL | 141.4 ± 38.2 | 138.7 ± 39.3 | 137.9 ± 38.6 | 140.7 ± 43.4 | 139.5 ± 40.7 | 137.9 ± 36.6 |

*$P < 0.05$ compared to the respective null polymorphism.

potential marker of lower salt intake in Brazilian individuals. On the other hand, changes in USE may also be a reflex of alterations in sodium reabsorption in the renal tubules.[23] Individuals who are null for GSTM1 and GSTT1 are considered to have the absence of enzymatic activity of these genes,[24] which may result in increased oxidative stress.[3] Oxidative stress is known to enhance sodium reabsorption in renal tubules, thus leading to reduced USE.[23] Since GSTM1-null individuals are considered to exhibit a lower capacity of defense against oxidative stress[25] and the GSTM isoform is expressed in the kidneys,[26] it can be hypothetized that the lower USE in these subjects was a consequence of enhanced renal sodium reabsorption. On the other hand, the higher sodium retention in GSTM1-null individuals could explain the higher diastolic blood pressure levels[13,16] of these individuals at the highest tertile of USE. In agreement with this assumption, a report from another group revealed that functional variations (loss of function) of the CYP3A5 gene, which also codifies a protein that detoxifies reactive oxygen species, is also associated with higher blood pressure levels in subjects with higher urinary sodium.[14] It was also noteworthy that increased triglyceride levels were detected in GSTM1-null individuals in comparison with GSTM1–non-null individuals in the highest tertile of USE. This finding suggests that there might be interactions among GSTM1, sodium excretion, and metabolic parameters that

could provide a potential explanation to the direct association between USE and triglyceride levels previously reported by other groups.[27,28] However, the precise mechanisms underlying the interaction among USE, GSTM1 polymorphism, blood pressure and triglycerides are unclear, and further studies are required to address this issue.

Several studies have also investigated the association of GSTM or GSTT variants with the development and expression of phenotypes that may modulate cardiovascular risk; however, the results are conflicting. Polimanti *et al.*[8] showed that only the GSTT1-null phenotype was significantly associated with hypertension. Capoluongo *et al.*[29] reported that only GSTM1-null variants were significantly associated with hypertension in very old subjects. Oniki *et al.*[30] reported that GSTA1*B allele carriers were associated with hypertension, while Bid *et al.*[9] showed the association of a combined effect of GSTM1, T1, and P1 genotypes in a representative cohort of patients with type 2 diabetes mellitus. Although the genetic distribution of GST polymorphisms in our population resembled those reported in European and African populations,[31] our data did not show an association between GSTM1 or GSTT1 gene variants with hypertension or diabetes mellitus. The reasons for such divergences are not apparent. However, it is possible that differences in the design of the studies as well as variation in clinical features, genetic background, and ethnicity among the studied populations played a role in this regard. In addition, such conflicting results are likely to exist because multiple longstanding risk factors may confound the possible effect of the studied polymorphisms on polygenic diseases such as hypertension and diabetes mellitus.

Some limitations to our study need to be addressed. First, we cannot exclude the possibility that xenobiotics or environmental agents (e.g., toxins in cigarette smoke) could have influenced our results. However, we diminished this potential bias by considering the presence of smoking in multivariate models. Second, we did not assess the reproducibility of USE. However, although a single 12-hour urine collection might be insufficient to fully characterize an individual's sodium excretion, it does reproducibly reflect the average excretion of groups of subjects.[32] In addition, in order to diminish the influence of external factors on USE in our analysis, we only included individuals who were not using antihypertensive medications in our protocol. Third, we cannot exclude the possibility that an allele at another locus, in strong linkage disequilibrium with the GSTM1 allele, could account for our observed associations. In addition, although individuals included in the present study were derived from the same source population, the residual bias from population stratification cannot be excluded.

In conclusion, the present study provided novel evidence that the GSTM1-null genotype is associated with lower USE. Furthermore, our results reveal that the GSTM1-null variant may modulate the interaction between USE and blood pressure/triglyceride levels. However, further studies in other populations are warranted to confirm the current evidence.

## DISCLOSURE

The authors declared no conflict of interest.

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