Race and Resting State Heart Rate Variability in Brazilian Civil Servants and the Mediating Effects of Discrimination: An ELSA-Brasil Cohort Study

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Abstract

Objectives: African Americans are characterized by higher heart rate variability (HRV), a finding ostensibly associated with beneficial health outcomes. However, these findings are at odds with other evidence that ‘Blacks’ present with worse cardiovascular outcomes. Here we examine associations in a large cohort from the ELSA-Brasil study and determined whether these effects are mediated by discrimination.

Methods: Three groups were compared based on self-declared race: 'Black' (n=2,020, 'Brown' (n=3,502), and 'Whites' (n=6,467). Perceived discrimination was measured using a modified version of the Everyday Discrimination Scale. Resting-state HRV was extracted from 10-minute resting-state electrocardiograms. Racial differences in HRV were determined by regression analyses weighted by propensity scores, which controlled for potentially confounding variables including age, sex, education, and other health-related information. Non-linear mediation analysis quantified the average total effect, comprising direct (race – HRV) and indirect (race – discrimination – HRV) pathways.

Results: ‘Blacks’ displayed higher HRV relative to ‘Browns’ (Cohen’s *d* = 0.20) and ‘Whites’ (Cohen’s *d* = 0.31). ‘Browns’ also displayed a small, but significantly higher HRV relative to ‘Whites’ (Cohen’s *d* = 0.14). Discrimination indirectly contributed to the effects of race on HRV.

Conclusions: This large cohort from the Brazilian population shows that HRV is greatest in 'Blacks' followed by 'Browns' relative to 'Whites.' The presence of higher HRV in 'Blacks' and 'Browns' may reflect a sustained compensatory psychophysiological response to the adverse effects of discrimination. Additional research is needed to determine the health consequences of these differences in HRV across racial and ethnic groups.

Keywords: race, heart rate variability, HRV, propensity score weighting, mediation analysis, discrimination

Abbreviations: ANOVA: analysis of variance; CIS-R: Clinical Interview Schedule-Revised; CHD: coronary heart disease; CVD: cardiovascular disease; ELSA-Brasil: The Brazilian Longitudinal Study of Adult Health; HF-HRV: high frequency heart rate variability; HRV: heart rate variability

Introduction

Non-communicable diseases including neuropsychiatric disorders and cardiovascular disease (CVD) are leading global burdens of disease [1, 2]. These diseases are responsible for 63% of all deaths worldwide, and 80% of these occur in low-and middle-income countries [3]. In Brazil, an upper-middle-income country, morbidity and mortality due to non-communicable diseases are greatest in the most socioeconomically disadvantaged groups, and especially 'Blacks' [4-8]. A potential candidate marker for future ill-health is heart rate variability (HRV), an index of parasympathetic (vagal) function that may underpin psychophysiological flexibility, psychological wellbeing, health and longevity [9-11]. Reduced HRV is observed in the mood and anxiety disorders [12-17], and is associated with a 32-45% increased risk of a first cardiovascular event, even in populations without known CVD [18]. The vagus nerve plays an important regulatory role over a variety of allostatic systems including the hypothalamic-pituitary-adrenal axis [19], inflammatory processes [20], and glucose regulation [21, 22] [see also: 23-25]. Consequently, tonic vagal dysregulation – indexed using resting-state HRV – may lead to ill-health from a host of conditions and diseases including CVD [9, 10, 26, 27].

It is unexpected therefore that vagally-mediated HRV in African Americans is *higher* – not lower – than HRV in 'Whites' [28]. These findings, based on a meta-analysis of 17 studies published between 1995 and 2013, were associated with a large effect size (Hedges g = 0.93, 95% CI = 0.25-1.62). Higher HRV in African Americans represents a paradox because African Americans are also characterized by a high prevalence of cardiovascular morbidity and mortality [29, 30]. One potential explanation for increased HRV in 'Blacks' is a sustained, compensatory psychophysiological response to the adverse effects of discrimination, which include a worsening of blood pressure, cholesterol, body mass index and self-assessed general health [31, 32]. Consistent with this possibility, African

Americans display higher resting levels of systolic blood pressure and total peripheral resistance in combination with greater HRV in comparison to European Americans [33]. For the present study, we hypothesized that 'Blacks' and 'Browns' would display increased resting state HRV relative to 'Whites' in a large cohort from the Brazilian population. We also determined whether these racial differences in HRV would be mediated by discrimination consistent with a compensatory response.

Methods

Participants: ELSA-Brasil is a cohort of 15,105 civil servants aged 35-74 years enrolled between August 2008 and December 2010 at 6 different sites in Brazil (Belo Horizonte, Porto Alegre, Rio de Janeiro, Salvador, Sao Paulo and Vitoria). The ethics committees of the participating universities approved the research protocol. All participants provided written informed consent after a complete description of the study. Exclusion criteria comprised current or recent pregnancy (within 4 months of first interview), intention to quit working at the institution in the near future, severe cognitive or communication impairment, and if retired, residence outside of a study center's metropolitan area. The study design and sampling procedures of ELSA-Brasil have been reported previously [34, 35] [36]. The present study focused on participants who self-reported their race including 6,467 'Whites', 3,502 'Browns' and 2,020 'Blacks', consistent with the classification adopted by the 2010 National Brazilian Census. Racial measurement in the Brazilian census has always referred to phenotype (skin colour), not ancestry (origin), and the census has always included a term for the admixed population ("*Pardo"* or "Brown"), unlike the U.S. [37] Self-declared race is a construct with sociopolitical significance that may capture unmeasured factors impacting on health outcomes. While we do not focus on health disparities here per se, our outcome measure (HRV) is medically important and may mediate downstream changes in a variety of allostatic systems. A total of 11,989 participants were available

from the ELSA-Brasil cohort after dropping a relatively small number of participants who refused to self-report their race (n=184), participants who self-reported Asian (n=374) or indigenous ancestry (n=157), participants without available HRV exams (n=1813, including 504 participants with ectopic beats, reflecting either extra or skipped beats on the ECG trace) and those participants missing data on other variables used in analysis (n= 653).

Electrocardiogram Assessment

Resting-state data from the electrocardiogram was recorded for ten-minutes during spontaneous breathing without task demands, in the supine position. The electrocardiogram was always collected in the morning (8:00 to 12:00h) in a temperature-controlled room (21-24°C) and was sampled at 250 Hz with a digital electrocardiograph (Micromed, Brazil), consistent with international standards for the collection of HRV [38] [see also 39]. Wincardio (4.4a) software generated the R-R interval series from a selected lead (D2), which is associated with higher R-wave amplitude. Data was then processed to obtain high frequency (HF-HRV) using the autoregressive method as described elsewhere [40]. The HF-HRV (0.15–0.40 Hz) component was expressed in absolute units, and then log-transformed data as a normalization strategy [41]. The focus of our study is on HF-HRV, a specific marker of vagal function [42].

Discrimination Questionnaire

Perceived discrimination was determined based on a modified version of the Everyday Discrimination Scale [43] (see also: http://scholar.harvard.edu/davidrwilliams/book/export/html/32495). This measure captures unfair treatment on 5 common experiences including: 1) the workplace such as being fired or not recommended for promotion (employment item), 2) difficulty in renting property or living within the community (housing item), 3) unfair accusation, being searched or

assaulted by the police (interactions with police item), 4) receiving poorer service within a public place such as a bank, shop, hospital or government office (discrimination in public places item), or 5) being (unfairly) discouraged within school or college (education item). For each situation, participants were asked whether they had experienced discrimination and responded on an ordinal scale. Available responses were ‘no’, ‘yes, one time’ and ‘yes, more than one time’. Participants were then given a score of 0 if they did not experience discrimination, 1 if they reported experiencing any of these forms of discrimination once only, and a score of 2 if they had experienced any of these forms of discrimination on more than one occasion. Test-retest reliability (n=92, 2 weeks apart) [44] relating to overall discrimination is associated with a kappa coefficient of 0.85 (95%CI 0.72 – 0.98).

Covariates

The present study sought to provide an important extension to the literature on the association between self-reported race and HRV. While the decision over what variables should be used as covariates is controversial [see 45], our decision was driven by the need to set the distributions of certain variables as equal across racial groups [46], allowing us to draw conclusions that the outcome – heart rate variability – is affected by race, rather than some other common, potentially confounding variable.

Age, sex and education (less than high school, high school or college education) are commonly employed covariates. Each of these variables statistically differ by race (see participant characteristics) highlighting some degree of structural confounding, and are known to impact on HRV. Health related information also differs by race and therefore represents possible alternate pathways to alterations in HRV. Health related variables that were controlled in our study included smoking status (current versus past or never),

physical activity (measured using the International Physical Activity Questionnaire and categorized according to low activity versus moderate or high activity as determined using scoring guidelines: http://www.ipaq.ki.se/scoring.pdf), body mass index (weight in kilograms divided by height in meters squared, kg/m2) and medication use (including antidepressants, antihypertensive medications, and lipid lowering medications identified on the basis of pill bottle review). Additional health information on which groups were controlled included a continuous measure of psychiatric symptom severity determined using the Portuguese version [47] of the Clinical Interview Schedule-Revised (CIS-R) [47], hypertension (yes versus no; defined as systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥ 90mm Hg, or use of antihypertensive medications), diabetes mellitus (yes versus no; defined as self-reported or fasting blood glucose ≥ 126mg/dL or 2-hour oral glucose tolerance test ≥ 200mg/dL or glycated haemoglobin ≥ 6.5%), dyslipidemia (yes versus no; defined as LDL-cholesterol ≥ 130mg/dL or use of lipid lowering medication), 'hard' coronary heart disease (including myocardial infarction and coronary revascularization) (yes versus no), and use of antidepressant medication (yes versus no).

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics Version 21 and R version 3.0.1. Participant characteristics (Table 1) were examined using one-way analyses of variance (ANOVA) for contrasts involving continuous dependent measures, and χ^2 statistics for categorical variables in IBM SPSS Statistics Version 21. Tukey's HSD is reported to correct for multiple comparisons and aid interpretation of ANOVA's, while post hoc analyses were inspected to determine those groups that statistically differed from 'Whites' in χ^2 statistics. Propensity score weighted analysis and subsequent mediation analysis were conducted using R. These procedures are further described below and the R-code is provided in online supplementary information [see also 48, 49].

Propensity score weighted analysis was used to control for potentially confounding variables (see above section on covariates) after which racial differences in HRV were determined. Propensity score analyses have several advantages over traditional regression-based analyses [48, 50]. Firstly, all covariates are summarized as a single propensity score providing an important dimension reduction tool for evaluating differences on the independent variable (e.g. race). Secondly, a formal model is explicitly specified that is not conflated with the modeling approach (i.e. the analysis on which conclusions are drawn). Thirdly, the propensity score is a function only of covariates, not outcomes (e.g. HRV), therefore repeated analyses attempting to balance covariate distributions across groups (e.g. race) do not bias estimates of the outcome (e.g. HRV). Fourthly, propensity score methods avoid extrapolating beyond the observed data unlike traditional parametric modeling when groups (e.g. race) are disparate on covariates.

Weighted regression analyses were conducted using the 'twang' and 'survey' packages in the R statistical environment. Effective sample sizes (reported as 'n') were obtained from propensity score weighting, reflecting the numbers of participants with similar features on covariates in each of the groups. These effective sample sizes are smaller than the numbers of participants included in analyses as they capture the adverse impact of increased variance on precision and power [48]. Omnibus F-tests of statistical significance were conducted followed by t-tests to determine between group differences using the *regTermTest* and *svydesign* functions of the *survey* package [51]. The figure displays group means and 95% confidence intervals after propensity score weighting, extracted using the *svyby* function in the same package. Cohen's *d* effect sizes were calculated using an online calculator (available here:

http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-SMD8.php; based on [52]). A sensitivity analysis was then conducted to rule out any possibility that increased HRV in 'Blacks' is due to survival bias and under-representation of more severe disease due to premature cardiovascular death. Therefore sensitivity analysis involved re-doing our main analysis on younger individuals, as defined by those participants under the median age of the entire cohort.

Following the propensity score weighted analysis, mediation analysis was conducted to determine whether discrimination mediates the effects of race on HRV, as proposed previously [28]. While mediation modeling has typically been conducted within a linear structural equation modeling framework, linear models cannot be used on ordinal data such as the discrimination variable in the present study. We employed the 'mediation' package [49] for mediation analyses, as this package provides a robust and flexible option for modeling non-linearity. Mediation allows the average total ($\bar{\tau}$) effect to be decomposed into average direct ($\bar{\zeta}$) and average indirect ($\bar{\delta}$) effects, and for hypotheses relating to the mediating effects of discrimination to be tested statistically. Direct, indirect and total effects are reported along with their uncertainty estimates based on 1000 nonparametric bootstrap resamples. Like that for our propensity score analyses (above), we also conducted sensitivity analysis to determine whether mediation could be replicated in the younger cohort, and in a cohort in which potential confounding variables will be less of a concern, especially ill-health and medication. Further sensitivity analyses were conducted on heart rate to determine whether discrimination mediated any relationship between race and this variable (see supplementary information).

Results

Participant Characteristics

Participant characteristics are presented in Table 1. Unadjusted analyses on the impact of race on HRV indicated that all groups significantly differed from each other, such that 'Blacks' display higher HF-HRV than 'Browns', and 'Browns' display higher HF-HRV than 'Whites'. (See also the Table in supplementary information, which reports on unadjusted correlations between each participant characteristic and HRV measures). Propensity score weighting analyses (reported below) confirmed these unadjusted findings after controlling for potentially confounding variables. 'Blacks' also displayed a higher level of repeated discrimination, followed by 'Browns' relative to 'Whites'. This finding provides the foundation on which results for mediation analyses is interpreted.

INSERT TABLE 1 HERE

Inspection of participant characteristics (Table 1) indicates that 'Blacks' and 'Browns' were slightly younger, and displayed more psychiatric symptoms (relative to 'Whites'). None of the averaged CIS-R scores for race-based groupings reached the threshold required for diagnostic status (threshold score = 12; [47]). 'Blacks' (relative to 'Whites') were also heavier, more likely to be female, to have a lower level of education, to be physically inactive, to be current smokers, and to have a higher prevalence of hypertension, diabetes mellitus, and dyslipidemia. 'Blacks' (relative to 'Whites' and 'Browns') were also less likely to be using antidepressants. 'Browns' (relative to 'Whites') were more likely to have a lower level of education, to be physically inactive, to be current smokers, to have hypertension, diabetes mellitus, dyslipidemia and 'hard' CHD. Like 'Blacks', they were also less likely to be using antidepressants relative to 'Whites'. These findings highlight the need for propensity score weighting, which allowed for differences on these covariates to be equalized across racial groupings.

Propensity Score Analysis

Here we examined the impact of race on resting state HRV after propensity score weighting. Effective sample sizes were as follows: 'Whites', n=5,747; 'Browns', n=3,139; and 'Blacks', n=1,314. Significant effects were observed for HF-HRV [F(2,11986)=67.23, p= < 0.001]. 'Blacks' displayed higher vagally mediated HF-HRV (M = 5.60, 95% CI: 5.54, 5.66) relative to 'Whites' (p < 0.001, Cohen's *d* = 0.31; M = 5.23, 95% CI: 5.20, 5.26). In addition, 'Blacks' displayed higher vagally mediated HF-HRV relative to 'Browns' (p < 0.001, Cohen's *d* = 0.20; M = 5.39, 95% CI: 5.35, 5.44), while 'Browns' also displayed higher HF-HRV (M = 5.39, 95% CI: 5.35, 5.44) than 'Whites' (HF-HRV, p < 0.001, Cohen's *d* = 0.14). (See Fig 1).

INSERT FIG 1 HERE

An additional sensitivity analysis was conducted on participants aged less than the median of the entire cohort (median age = 51.00 years) to rule out the possibility that greater HRV observed in 'Blacks' and 'Browns' relative to 'Whites' is due to survival bias. Effective sample sizes were as follows: 'Whites', n=2,959; 'Browns', n=1,807; and 'Blacks', n=792. Again, significant effects were observed for HF-HRV [F(2,6126)=32.20, p<0.001]. 'Blacks' displayed higher vagally mediated HF-HRV (M = 5.82, 95% CI: 5.75, 5.90) relative to 'Whites' (p < 0.001, Cohen's *d* = 0.29; M = 5.50, 95% CI: 5.45, 5.54). In addition, 'Blacks' displayed higher vagally mediated HF-HRV relative to 'Browns' (p < 0.001, Cohen's *d* = 0.16; M = 5.65, 95% CI: 5.60, 5.70). Finally, 'Browns' also displayed higher HF-HRV (M = 5.65, 95% CI: 5.60, 5.70) than 'Whites' (p<0.001, Cohen's *d* = 0.14). In summary, analyses revealed that the three groups differed from each other on HRV such that 'Blacks' displayed the highest HRV followed by 'Browns', and 'Whites', the group that displayed the lowest values.

Mediation Analysis

Here we examined whether the relationship between race and HRV is mediated by discrimination. Results focusing on 'Whites' and 'Blacks' revealed significant total ($\bar{\tau}$= 0.3590, 95% CI: 0.2980, 0.4147, p<0.01), direct ($\bar{\zeta}$ = 0.3521, 95% CI: 0.2900, 0.4086, p<0.01) and indirect effects (δ = 0.0069, 95% CI: 0.0010, 0.0143, p=0.02). Analysis on 'Whites' and 'Browns' also revealed significant total ($\bar{\tau}$ = 0.1778, 95% CI: 0.1492, 0.2063, p<0.01) and direct effects ($\bar{\zeta}$ = 0.1751, 95% CI: 0.1462, 0.2034, p<0.01), but no indirect effect (δ = 0.0027, 95% CI: -0.0006, 0.0057, p= 0.10). An analysis was also conducted on 'Browns' and 'Blacks'; results indicated significant total ($\bar{\tau}$ = 0.1820, 95% CI: 0.1521, 0.2078, p<0.01) and direct ($\bar{\zeta}$ = 0.1767, 95% CI: 0.1475, 0.2028, p<0.01) effects, as well as an indirect effect at trend levels (δ = 0.0053, 95% CI: 0.0001, 0.0100, p= 0.05).

We then conducted a sensitivity analysis to determine whether mediation could be replicated in the younger cohort. Results from this sensitivity analysis focusing on 'Whites' and 'Blacks' revealed significant total ($\bar{\tau}$= 0.3347, 95% CI: 0.2572, 0.4090, p<0.01) and direct ($\bar{\zeta}$ = 0.3234, 95% CI: 0.2487, 0.3973, p<0.01) and an indirect effect at trend levels (δ = 0.0113, 95% CI: -0.0001, 0.0203, p= 0.06). Analysis on 'Whites' and 'Browns' also revealed significant total ($\bar{\tau}$ = 0.1623, 95% CI: 0.1256, 0.1981, p<0.01) and direct effects ($\bar{\zeta}$ = 0.1593, 95% CI: 0.1230, 0.1943, p<0.01), but no indirect effect (δ = 0.0030, 95% CI: -0.0016, 0.0080, p=0.21). The sensitivity analysis on 'Browns' and 'Blacks' indicated significant total ($\bar{\tau}$ = 0.1704, 95% CI: 0.1357, 0.2063, p<0.01), direct ($\bar{\zeta}$ = 0.1629, 95% CI: 0.1281, 0.1992, p<0.01) and indirect effects (δ = 0.0075, 95% CI: 0.0008, 0.0152, p= 0.03). In summary, mediation analysis revealed a small effect of discrimination in regards to the proportion of the mediated effect. Although small, this mediating effect of

discrimination significantly contributes to the race-related increase in HRV. Finally, additional sensitivity analysis (reported in supplementary information) revealed no significant mediating effect of discrimination for the relationship between race and heart rate.

Discussion

Recently published meta-analytic findings [28] indicate that African Americans display higher HRV relative to 'Whites', despite increased risk for cardiovascular morbidity and mortality among 'Blacks'. The present study examined whether these findings extend to a large cohort from the Brazilian population, which may be less prone to residual confounding due to substantial racial admixture [53]. We also examined whether these findings are mediated by discrimination consistent with a compensatory response to the adverse effects of discrimination including hypertension [32] (see also Table 1). We observed that HRV is higher in 'Blacks' *and* 'Browns', relative to 'Whites', and that this finding is unlikely to be a consequence of survival bias as indicated by the sensitivity analysis on younger participants. Therefore, as per findings in African Americans [28], our findings in a Brazilian sample are a conundrum given the increased risk for morbidity and mortality that is most prominent in 'Blacks'. We also present the first evidence – to our knowledge – that racial differences in HRV may be underpinned by the experience of repeated discrimination.

Although discrimination is strongly associated with adverse physiological effects including hypertension [31, 32] (see also table 1), our findings also show that it may be associated with a sustained, compensatory psychophysiological response (see below). Although the parasympathetic (vagal) and sympathetic nervous systems (PNS and SNS) are typically conceptualized as two opposing components, this is not correct. Vagal activity – indexed

by HRV – may be co-activated or co-deactivated [54, 55]. While co-activation of the PNS and SNS activity may help to mitigate the deleterious effects of increased SNS activity [56], sympathetic-parasympathetic cardiac deactivation may reflect passive sensory intake [57]. Higher HRV in 'Blacks' may therefore reflect a compensatory response to the adverse physiological responses associated with perceived discrimination, which may include increased SNS activity. It is important to note that while 'Blacks' may display increased HRV, these alterations do not ameliorate other adverse physiological effects of discrimination [31, 32], which contribute to higher rates of morbidity and mortality caused by cardiovascular disease. An earlier study [33] demonstrated that African Americans – relative to European Americans – display greater HRV, in combination with higher resting levels of systolic blood pressure and total peripheral resistance (TPR). TPR was also increased and its recovery, delayed when anger was inhibited [33], and TPR may contribute to elevations in blood pressure and increased risk of morbidity and mortality [58, 59]. These and other differences in vascular activity may therefore underpin the increased risk for CVD in 'Blacks' versus 'Whites'.

A notable feature of HRV is that it reflects psychological (e.g. emotion regulation) as well as physiological processes (e.g. control over allostatic systems), providing a structural link between mental and physical health [9]. While emotion regulation is a complex and multi-dimensional construct that may also include rumination, we consider resting-state HRV to index capacity for emotion regulation reflecting effective prefrontal control of visceromotor, neuroendocrine, and behavioral responses, critical for goal-directed behavior, adaptability and health [11, 60, 61]. In this regard, higher HRV in 'Blacks' may reflect a heightened, yet forced capacity to regulate emotions associated with day-to-day stressors and discrimination against 'Blacks' and 'Browns'. Some prior studies support this possibility. Firstly, 'Blacks' may be conditioned from an early age to inhibit their expression of anger in

an effort to reduce or avoid aggression [33], a consequence of early conditioning of avoidance learning in a social context [62, 63]. While inhibition of anger may be associated with more rapid blood pressure recovery, it is also associated with delayed TPR recovery, which may increase risk from hypertension-related death and disability [33]. Although we did not focus on hypertension here, it is notable that in our own sample that 'Blacks' display greater frequency of hypertension, followed by 'Browns' and 'Whites' (table 1). Furthermore, earlier research has demonstrated that racism-related vigilance represents an important source of chronic stress that contributes to this higher prevalence in hypertension [32]. Secondly, 'Blacks' are associated with *a lower lifetime risk of mood and anxiety disorders*, and that associated protective factors likely originate in childhood (prior to age 10) [64], as does learning to regulate emotions, according to the avoidance-learning hypothesis [33, 62, 63].

Our study has several strengths including the investigation of HRV race-related differences in a large cohort characterized by substantial genetic admixture [53], the application of robust statistical techniques including propensity score weighted regression analyses and non-linear mediation analysis on which results are based, and confirmation of findings when only focusing on the younger cohort. We also demonstrate that although heart rate was decreased in 'Blacks' relative to 'Browns' and 'Whites', discrimination did not mediate the relationship between race and heart rate (see supplementary information), highlighting the specificity of the HRV findings that we report here. It is important to recognize here that resting-state HRV and the HF-HRV component in particular – unlike heart rate – is a relatively pure indicator of vagal activity [11, 65].

Several limitations of our work should also be noted. We did not collect data on respiratory rate or depth, which may influence estimates of HRV. However, the question over whether

they should be controlled – especially during resting state recordings – remains a divisive one in the field of psychophysiology [27, 66-68]. The use of a self-report measure of discrimination may also be considered a limitation, and we do not have any measure of emotion regulation, the ability to avoid anger or the frequency of anger episodes. In the future, researchers may wish to consider the possibility of using a variant of the implicit association test for assessing the unconscious experience of discrimination [69, 70]. Another limitation is the difficulty in quantifying the degree to which mediation analysis was robust to the possibility of unobserved confounding. Therefore, we adopt a balanced approach to inferring causation, presenting a theoretical set of relationships within a cross-sectional dataset and interpret findings within the context of the available literature. Studies with longitudinal designs are necessary to further examine the pathways through which HRV is increased in 'Blacks' and 'Browns', as well as the downstream mechanisms that may underpin increased risks for cardiovascular morbidity in these individuals.

We must also acknowledge that the proportion of variance explained by discrimination in our mediation analysis was small (the mediating effect was only 2-3% of the total effect). This small effect is perhaps unsurprising considering that 62% of the sample reported no experience of discrimination and that race-associated differences in HRV is likely to be multi-determinable. As suggested previously, it is possible that the conscious report of no discrimination in persons with fewer socioeconomic resources reflects a conscious decision not to report discrimination, or even internalized oppression that is not consciously perceived [69].

In conclusion, we demonstrate that 'Blacks' and 'Browns' display higher HRV relative to 'Whites', findings that are partly mediated by discrimination. The race-based differences we observed in the present study may reflect a compensatory response to the adverse

physiological effects of discrimination. They may also reflect other factors including gene-environment interactions and psychosocial issues on which further study is required. Future work in this area is necessary to further clarify the differences underpinning the observed race-related differences in HRV, as well as the mechanisms linked to race-related health disparities. Our findings therefore lay an important foundation for future work that seeks to better understand the relationship between race and HRV, and the differential pathways to CVD.

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References

1. GBD 2013 Mortality and Causes of Death Collaborators (2014) Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 385:117–171. doi: 10.1016/S0140-6736(14)61682-2

2. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, Burstein R, Murray CJL, Vos T (2013) Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet 382:1575–1586. doi: 10.1016/S0140-6736(13)61611-6

3. Bloom DE, Cafiero E, Jané-Llopis E, Abrahams-Gessel S, Bloom LR, Fathima S, Feigl AB, Gaziano T, Hamandi A, Mowafi M, O'Farrell D, Ozaltin E, Pandya A, Prettner K, Rosenberg L, Seligman B, Stein AZ, Weinstein C, Weiss J (2012) The Global Economic Burden of Noncommunicable Diseases. PGDA Working Papers

4. Chiavegatto Filho ADP, Beltrán-Sánchez H, Kawachi I (2014) Racial disparities in life expectancy in Brazil: challenges from a multiracial society. Am J Public Health 104:2156–2162. doi: 10.2105/AJPH.2013.301565

5. Filho AC, Laurenti R (2013) Disparidades étnico-raciais em saúde autoavaliada: análise multinível de 2.697 indivíduos residentes em 145 municípios brasileiros. Cad Saude Publica

6. Lotufo PA, Fernandes TG, Bando DH, Alencar AP, Benseñor IM (2013) Income and heart disease mortality trends in Sao Paulo, Brazil, 1996 to 2010. Int J Cardiol 167:2820–2823. doi: 10.1016/j.ijcard.2012.07.006

7. Lotufo PA, Pereira AC, Vasconcellos PS, Santos IS, Mill JG, Benseñor IM (2015) Resistant Hypertension: Risk Factors, Subclinical Atherosclerosis, and Comorbidities Among Adults—The Brazilian Longitudinal Study of Adult Health (ELSA- Brasil). The Journal of Clinical Hypertension 17:74–80. doi: 10.1111/jch.12433

8. Chor D, Pinho Ribeiro AL, Sá Carvalho M, Duncan BB, Andrade Lotufo P, Araújo Nobre A, Aquino EMLL de, Schmidt MI, Griep RH, Molina MDCB, Barreto SM, Passos VM de A, Bensenor IJM, Matos SMA, Mill JG (2015) Prevalence, Awareness, Treatment and Influence of Socioeconomic Variables on Control of High Blood Pressure: Results of the ELSA-Brasil Study. PLoS ONE 10:e0127382. doi: 10.1371/journal.pone.0127382

9. Kemp AH, Quintana DS (2013) The relationship between mental and physical

health: insights from the study of heart rate variability. Int J Psychophysiol 89:288–296. doi: 10.1016/j.ijpsycho.2013.06.018

10. Thayer J, Yamamoto SS, Brosschot JF (2010) The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol 141:122–131. doi: 10.1016/j.ijcard.2009.09.543

11. Thayer J, Hansen AL, Saus-Rose E, Johnsen BH (2009) Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. Ann Behav Med 37:141–153. doi: 10.1007/s12160-009-9101-z

12. Brunoni AR, Kemp AH, Dantas EM, Goulart AC, Nunes MA, Boggio PS, Mill JG, Lotufo PA, Fregni F, Benseñor IM (2013) Heart rate variability is a trait marker of major depressive disorder: evidence from the sertraline vs. electric current therapy to treat depression clinical study. Int J Neuropsychopharm 16:1937–1949. doi: 10.1017/S1461145713000497

13. Chalmers JA, Quintana DS, Abbott MJ-A, Kemp AH (2014) Anxiety disorders are associated with reduced heart rate variability: A meta-analysis. Front Psychiatry 5:80. doi: 10.3389/fpsyt.2014.00080

14. Kemp AH, Quintana DS, Gray MA, Felmingham KL, Brown K, Gatt JM (2010) Impact of depression and antidepressant treatment on heart rate variability: A review and meta-analysis. Biol Psychiatry 67:1067–1074. doi: 10.1016/j.biopsych.2009.12.012

15. Kemp AH, Quintana DS, Felmingham KL, Matthews S, Jelinek HF (2012) Depression, Comorbid Anxiety Disorders, and Heart Rate Variability in Physically Healthy, Unmedicated Patients: Implications for Cardiovascular Risk. PLoS ONE 7:e30777. doi: 10.1371/journal.pone.0030777.t002

16. Kemp AH, Brunoni AR, Santos IS, Nunes MA, Dantas EM, Carvalho de Figueiredo R, Pereira AC, Ribeiro ALP, Mill JG, Andreão RV, Thayer J, Benseñor IM, Lotufo PA (2014) Effects of depression, anxiety, comorbidity, and antidepressants on resting-state heart rate and its variability: an ELSA-Brasil cohort baseline study. American Journal of Psychiatry 171:1328–1334. doi: 10.1176/appi.ajp.2014.13121605

17. Kemp AH, Quintana DS, Quinn CR, Hopkinson P, Harris AWF (2014) Major depressive disorder with melancholia displays robust alterations in resting state heart rate and its variability: implications for future morbidity and mortality. Frontiers in Psychology 5:1387. doi: 10.3389/fpsyg.2014.01387

18. Hillebrand S, Gast KB, de Mutsert R, Swenne CA, Jukema JW, Middeldorp S, Rosendaal FR, Dekkers OM (2013) Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. Europace 15:742–749. doi: 10.1093/europace/eus341

19. Porges SW (2011) The Polyvagal Theory: Neurophysiological Foundations of Emotions, Attachment, Communication, and Self-regulation, 1st ed. W. W. Norton & Company, New York

20. Huston JM, Tracey KJ (2010) The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. J Intern Med

269:45–53. doi: 10.1111/j.1365-2796.2010.02321.x

21. Pocai A, Obici S, Schwartz GJ, Rossetti L (2005) A brain-liver circuit regulates glucose homeostasis. Cell Metab 1:53–61. doi: 10.1016/j.cmet.2004.11.001

22. Wang P, Caspi L, Lam C, Chari M, Li X, Light PE (2008) Upper intestinal lipids trigger a gut–brain–liver axis to regulate glucose production. Nature

23. Thayer J, Sternberg E (2006) Beyond heart rate variability: Vagal regulation of allostatic systems. Ann N Y Acad Sci 1088:361–372. doi: 10.1196/annals.1366.014

24. Thayer J (2009) Vagal tone and the inflammatory reflex. Cleve Clin J Med 76 Suppl 2:S23–6. doi: 10.3949/ccjm.76.s2.05

25. Thayer J, Sternberg EM (2010) Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun 24:1223–1228. doi: 10.1016/j.bbi.2010.07.247

26. Thayer J, Lane RD (2007) The role of vagal function in the risk for cardiovascular disease and mortality. Biol Psychol 74:224–242. doi: 10.1016/j.biopsycho.2005.11.013

27. Thayer J, Loerbroks A, Sternberg EM (2011) Inflammation and cardiorespiratory control: the role of the vagus nerve. Respir Physiol Neurobiol 178:387–394. doi: 10.1016/j.resp.2011.05.016

28. Hill LK, Hu DD, Koenig J, Sollers JJ, Kapuku G, Wang X, Snieder H, Thayer J (2015) Ethnic differences in resting heart rate variability: a systematic review and meta-analysis. Psychosom Med 77:16–25. doi: 10.1097/PSY.0000000000000133

29. Sharma S, Malarcher AM, Giles WH, Myers G (2004) Racial, ethnic and socioeconomic disparities in the clustering of cardiovascular disease risk factors. Ethn Dis 14:43–48.

30. Mensah GA, Mokdad AH, Ford ES, Greenlund KJ, Croft JB (2005) State of disparities in cardiovascular health in the United States. Circulation 111:1233–1241. doi: 10.1161/01.CIR.0000158136.76824.04

31. Johnston DW, Lordan G (2012) Discrimination makes me sick! An examination of the discrimination-health relationship. J Health Econ 31:99–111. doi: 10.1016/j.jhealeco.2011.12.002

32. Hicken MT, Lee H, Morenoff J, House JS, Williams DR (2013) Racial/Ethnic Disparities in Hypertension Prevalence: Reconsidering the Role of Chronic Stress. http://dxdoiorg/102105/AJPH2013301395. doi: 10.2105/AJPH.2013.301395

33. Dorr N, Brosschot JF, Sollers JJ, Thayer J (2007) Damned if you do, damned if you don't: the differential effect of expression and inhibition of anger on cardiovascular recovery in black and white males. Int J Psychophysiol 66:125–134. doi: 10.1016/j.ijpsycho.2007.03.022

34. Schmidt MI, Duncan BB, Mill JG, Lotufo PA, Chor D, Barreto SM, Aquino EM, Passos VMA, Matos SM, Molina MDCB, Carvalho MS, Benseñor IM (2014) Cohort profile: Longitudinal Study of Adult Health (ELSA-Brasil). International journal of

epidemiology. doi: 10.1093/ije/dyu027

35. Aquino EML, Barreto SM, Benseñor IM, Carvalho MS, Chor D, Duncan BB, Lotufo PA, Mill JG, Molina MDC, Mota ELA, Azeredo Passos VM, Schmidt MI, Szklo M (2012) Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): Objectives and design. Am J Epidemiol 175:315–324. doi: 10.1093/aje/kwr294

36. Mill JG, Pinto K, Griep RH, Goulart A (2013) Medical assessments and measurements in ELSA-Brasil. Rev Saúde Pública 47:54–62. doi: 10.1590/S0034-8910.2013047003851

37. Travassos C, Williams DR (2004) The concept and measurement of race and their relationship to public health: a review focused on Brazil and the United States. Cad Saude Publica 20:660–678.

38. (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 93:1043–1065.

39. Ellis RJ, Zhu B, Koenig J, Thayer J, Wang Y (2015) A careful look at ECG sampling frequency and R-peak interpolation on short-term measures of heart rate variability. Physiol Meas 36:1827–1852. doi: 10.1088/0967-3334/36/9/1827

40. Dantas EM, Sant'Anna ML, Andreão RV, Gonçalves CP, Morra EA, Baldo MP, Rodrigues SL, Mill JG (2012) Spectral analysis of heart rate variability with the autoregressive method: what model order to choose? Comput Biol Med 42:164–170. doi: 10.1016/j.compbiomed.2011.11.004

41. Ellis RJ, Thayer J, Sollers Iii JJ, Edelstein EA (2008) Data transforms for spectral analyses of heart rate variability. Biomed Sci Instrum 44:392–397.

42. Cacioppo JT, Tassinary LG, Berntson G (2007) Handbook of psychophysiology. Cambridge University Press

43. Williams DR, Yu Y, Jackson JS, Anderson NB (1997) Racial Differences in Physical and Mental Health Socio-economic Status, Stress and Discrimination. J Health Psychol 2:335–351. doi: 10.1177/135910539700200305

44. Faerstein E, Chor D, Werneck GL, Lopes C de S, Kaplan G (2014) Race and perceived racism, education, and hypertension among Brazilian civil servants: the Pró-Saúde Study. Rev Bras Epidemiol 17 Suppl 2:81–87.

45. Hernán MA, Robins JM (2016) Causal Inference. Chapman & Hall/CRC, Boca Raton

46. VanderWeele TJ, Robinson WR (2014) On the causal interpretation of race in regressions adjusting for confounding and mediating variables. Epidemiology 25:473–484. doi: 10.1097/EDE.0000000000000105

47. Lewis G, Pelosi AJ, Araya R, Dunn G (1992) Measuring psychiatric disorder in the community: a standardized assessment for use by lay interviewers. Psychol Med 22:465–486.

48. McCaffrey D, Griffin BA, Almirall D, Slaughter ME, Ramchand R, Burgette LF (2013)

A tutorial on propensity score estimation for multiple treatments using generalized boosted models. Stat Med 32:3388–3414. doi: 10.1002/sim.5753

49. Imai K, Keele L, Tingley D (2010) A general approach to causal mediation analysis. Psychol Methods 15:309–334. doi: 10.1037/a0020761

50. Ridgeway G (2006) Assessing the effect of race bias in post-traffic stop outcomes using propensity scores. Journal of Quantitative Criminology 22:1–29.

51. Lumley T (2004) Analysis of complex survey samples. Journal of Statistical Software 9:1–19.

52. Lipsey MW, Wilson DB (2001) Practical meta-analysis. Sage Publications, Inc

53. Giolo SR, Soler JMP, Greenway SC, Almeida MAA, de Andrade M, Seidman JG, Seidman CE, Krieger JE, Pereira AC (2012) Brazilian urban population genetic structure reveals a high degree of admixture. Eur J Hum Genet 20:111–116. doi: 10.1038/ejhg.2011.144

54. Berntson GG, Cacioppo JT, Quigley KS (1991) Autonomic determinism: The modes of autonomic control, the doctrine of autonomic space, and the laws of autonomic constraint. Psychol Rev 98:459–487. doi: 10.1037/0033-295X.98.4.459

55. Berntson GG, Cacioppo JT, Quigley KS (1993) Cardiac psychophysiology and autonomic space in humans: empirical perspectives and conceptual implications. Psychol Bull 114:296–322.

56. Norman GJ, Berntson GG, Cacioppo JT, Morris JS, Malarkey WB, Devries AC (2011) Oxytocin increases autonomic cardiac control: moderation by loneliness. Biol Psychol 86:174–180. doi: 10.1016/j.biopsycho.2010.11.006

57. Kreibig SD (2010) Autonomic nervous system activity in emotion: A review. Biol Psychol 84:14–41. doi: 10.1016/j.biopsycho.2010.03.010

58. Fagard RH, Pardaens K, Staessen JA, Thijs L (1996) Prognostic value of invasive hemodynamic measurements at rest and during exercise in hypertensive men. Hypertension 28:31–36.

59. Mensah GA, Pappas TW, Koren MJ, Ulin RJ, Laragh JH, Devereux RB (1993) Comparison of classification of the severity of hypertension by blood pressure level and by World Health Organization criteria in the prediction of concurrent cardiac abnormalities and subsequent complications in essential hypertension. J Hypertens 11:1429–1440.

60. Geisler FCM, Vennewald N, Kubiak T, Weber H (2010) The impact of heart rate variability on subjective well-being is mediated by emotion regulation. Personality and Individual Differences 49:723–728. doi: 10.1016/j.paid.2010.06.015

61. Smith TW, Cribbet MR, Nealey-Moore JB, Uchino BN, Williams PG, MacKenzie J, Thayer J (2011) Matters of the variable heart: Respiratory sinus arrhythmia response to marital interaction and associations with marital quality. Journal of Personality and Social Psychology 100:103–119. doi: 10.1037/a0021136

62. Hokanson JE (1970) Psychophysiological evaluation of the catharsis hypothesis. In: Megargee EI, Hokanson JE (eds) The Dynamics of Aggression. New York, pp 74–86

63. Hokanson JE, Edelman R (1966) Effects of three social responses on vascular processes. Journal of Personality and Social Psychology 3:442–447. doi: 10.1037/h0023053

64. Breslau J, Aguilar-Gaxiola S, Kendler KS, Su M, Williams D, Kessler RC (2006) Specifying race-ethnic differences in risk for psychiatric disorder in a USA national sample. Psychol Med 36:57–68. doi: 10.1017/S0033291705006161

65. Saul JP (1990) Beat-To-Beat Variations of Heart Rate Reflect Modulation of Cardiac Autonomic Outflow. Physiology 5:32–37.

66. Denver JW, Reed SF, Porges SW (2007) Methodological issues in the quantification of respiratory sinus arrhythmia. Biol Psychol 74:286–294. doi: 10.1016/j.biopsycho.2005.09.005

67. Porges SW (2007) The polyvagal perspective. Biol Psychol 74:116–143. doi: 10.1016/j.biopsycho.2006.06.009

68. Ritz T (2009) Studying noninvasive indices of vagal control: The need for respiratory control and the problem of target specificity. Biol Psychol 80:158–168. doi: 10.1016/j.biopsycho.2008.08.003

69. Krieger N, Carney D, Lancaster K, Waterman PD, Kosheleva A, Banaji M (2010) Combining explicit and implicit measures of racial discrimination in health research. Am J Public Health 100:1485–1492. doi: 10.2105/AJPH.2009.159517

70. Krieger N (2012) Methods for the scientific study of discrimination and health: an ecosocial approach. Am J Public Health 102:936–944. doi: 10.2105/AJPH.2011.300544

Figure Captions

Fig 1: HF-HRV (±95% confidence interval) by group. ‘Blacks’ display significantly higher values than ‘Browns’ who display significantly higher values than ‘Whites’.

Figure

Click here to download Figure Figure.jpg

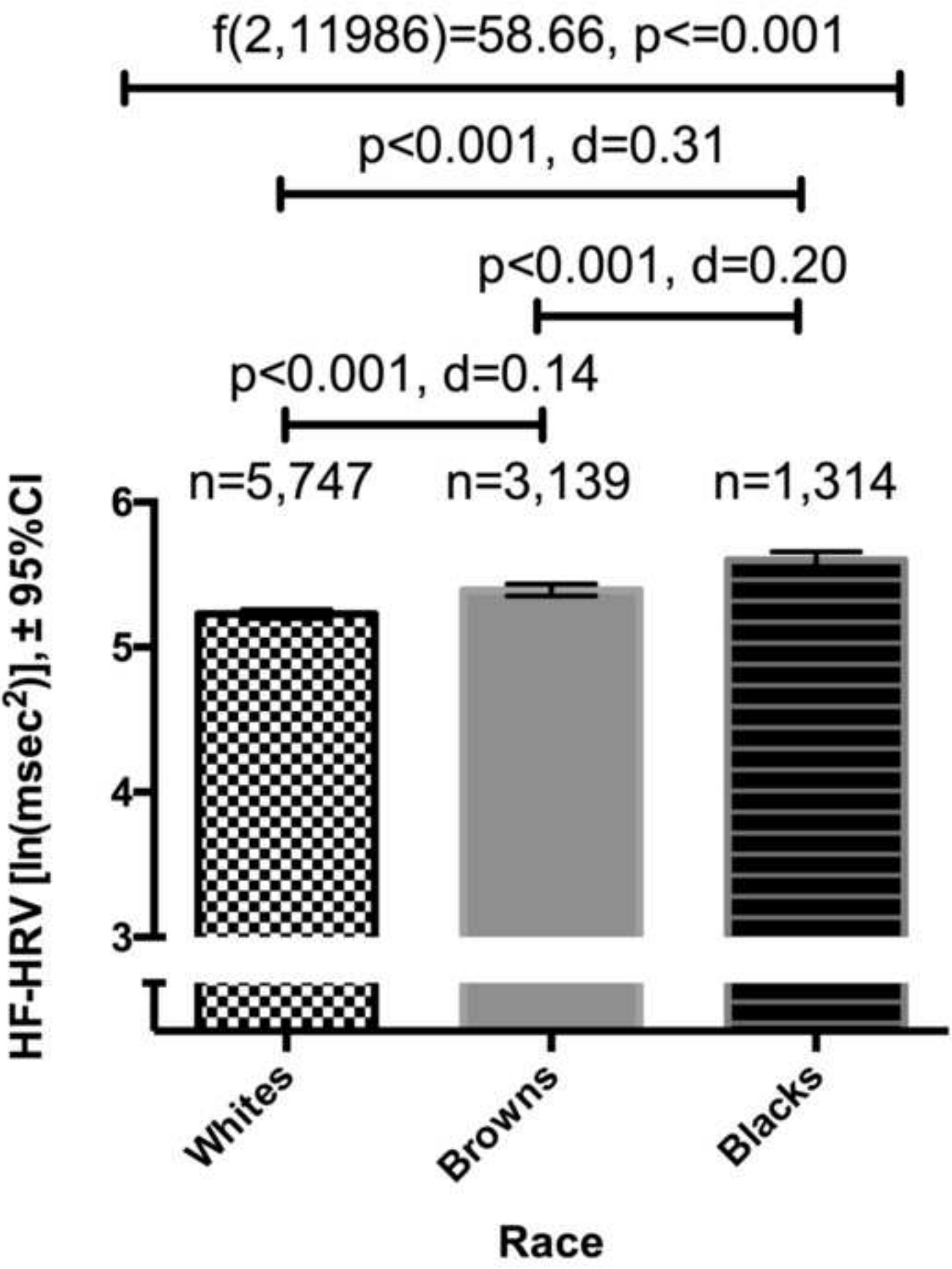

Table: Unadjusted correlations (Pearson's r or point biserial) between participant characteristics and HRV measures (HF-HRV, LF-HRV) for entire sample (N=11,989) to provide information about the size of the relationships.

Characteristics, mean (SD) or N (%)	HF-HRV	LF-HRV
Demographic variables		
Age	-0.270	-0.301
Female	0.091	-0.150
Education		
Less than high-school	-0.059	-0.094
High School	0.043	-0.001
Lifestyle characteristics		
Physical Inactivity	-0.023	-0.059
Current Smoker	0.035	-0.001
Body mass index (kg/m^2)	-0.036	-0.080
Discrimination[3]	0.081	0.036
Health characteristics		
CIS-R Score[1]	0.030	-0.040
Hypertension	-0.140	-0.184
Diabetes Mellitus	-0.163	-0.171
Dyslipidemia	-0.086	-0.073
Hard CHD[2]	-0.058	-0.079
Antidepressant Use	-0.102	-0.110
Heart rate measures		
Heart rate (BPM) [4]	-0.476	-0.366

HF-HRV [ln(msec^2)]	1	0.628
LF-HRV [ln(msec^2)]	0.628	1

[1] CIS-R: Clinical Interview Schedule-Revised. [2] Hard CHD: Hard coronary heart disease includes myocardial infarction and coronary revascularization. [3] Experience of major discrimination in any of the 5 major experiences on more than one occasion. This combined measure of discrimination is used in the mediation model. [4] BPM: beats per minute

Supplementary Information

Propensity Score Analysis on Heart Rate

In addition to the impact of race on resting state HF-HRV (reported in the manuscript), we examined impact on heart rate. Effective sample sizes were as follows: 'Whites', n=5,747; 'Browns', n=3,139; and 'Blacks', n=1,314. Significant effects were also observed for heart rate [f(2,11986)=6.72, p=0.001] such that 'Blacks' displayed a slightly reduced HR [M= 66.25, 95% CI: 65.77, 66.73] relative to 'Whites' (p<0.001, Cohen's *d* = 0.11) (M = 67.25, 95% CI: 67.01, 67.49), as well as 'Browns' (p=0.011, Cohen's *d* = 0.08) (M = 67.01, 95% CI: 66.68, 67.33). However, no difference between 'Browns' and 'Whites' was observed. Notably effect sizes are smaller than those that are reported in the manuscript for HF-HRV.

Mediation Analysis

Here we examined whether the relationship between race and heart rate is mediated by discrimination, to determine whether a similar mediating effect is obtained to that which we report in our manuscript. Results focusing on 'Whites' and 'Blacks' revealed significant total ($\bar{\tau}$= -1.2135, 95% CI: -1.6577, -0.7471, p<0.01) and direct effects ($\bar{\zeta}$ = -1.1626, 95% CI: -1.1694, -1.6199, p<0.01), but no indirect effect (δ = -0.0441, 95% CI: -0.1012, 0.0109, p=0.10). Analysis on 'Whites' and 'Browns' also revealed significant total ($\bar{\tau}$ = -0.606393, 95% CI: -0.854218, -0.380643, p<0.01) and direct effects ($\bar{\zeta}$ = -0.586562, 95% CI: -0.825550, -0.356875, p<0.01), but again no indirect effect was observed (δ = -0.019831, 95% CI: -0.047160, 0.000594, p= 0.06). An analysis was also conducted on 'Browns' and 'Blacks'; again results indicated significant total ($\bar{\tau}$ = -0.60155, 95% CI: -0.84210, -0.39233, p<0.01) and direct effects ($\bar{\zeta}$ = -0.58348, 95% CI: -0.81342, -0.36749, p<0.01), but no indirect effect (δ = -0.01807, 95% CI: -0.06092, 0.01680, p= 0.29). Therefore, unlike the

findings reported in the manuscript for HF-HRV, discrimination did not mediate the observed relationship between race and heart rate.

```
#########################

# Multiple propensity score matching for ethnicity - ETNIA variable: branca,
parda, preta

#code adapted from: Burgette et al., Propensity scores for multiple treatments:
#A tutorial for the mnps function in the twang package

#########################

library(twang)
set.seed(1)

# reads in CSV datafile
mydata <- read.table('/…/N = 11989.csv', header=TRUE, sep=",")

#convert ETNIA to factor variable
mydata$ETNIA <- factor(mydata$ETNIA, labels=c('branca','parda','preta'))

# propensity score model of treatment assignment
mnps.mydata.ATE <- mnps(ETNIA ~ AGEPART + RCTA8 + lessthanhs + high_school +
Smoker + nofisica + IMCA01 + A_ESCORETOTAL + A_HAS2 + A_DM + DISLIP + HARDCHD +
anyAdep, data = mydata, estimand = "ATE", verbose = FALSE, stop.method =
c("es.mean", "ks.mean"), n.trees = 20000)

# simple summary of balance
means.table(mnps.mydata.ATE, digits = 3)

# More extensive balance information
bal.table(mnps.mydata.ATE)

# Summary
summary(mnps.mydata.ATE)

##################

# Analysis: Whites are set as controls

##################

require(survey)
mydata$w <- get.weights(mnps.mydata.ATE, stop.method = "es.mean")
design.mnps <- svydesign(ids=~1, weights=~w, data=mydata)

# perform the propensity score-adjusted regression - heart rate with Whites as
ref
glm1 <- svyglm(DERAP_VFCCL_BPMmed_deitado ~ as.factor(ETNIA), design =
design.mnps)
summary(glm1)

# clean printout of svyglm
require(arm)
display(glm1, digits=2, detail=TRUE)

#substitute for anova - for df
regTermTest(glm1, ~ as.factor(ETNIA))

# means for groups
```

```
svyby(~DERAP_VFCCL_BPMmed_deitado, ~ ETNIA, svymean, design=design.mnps,
vartype=c("se","ci","var"), na.rm=TRUE)

# perform the propensity score-adjusted regression - lnHFms2 with Whites as ref
glm1 <- svyglm(lnHFms2 ~ as.factor(ETNIA), design = design.mnps)
summary(glm1)

# clean printout of svyglm
require(arm)
display(glm1, digits=2, detail=TRUE)

#substitute for anova - for df
regTermTest(glm1, ~ as.factor(ETNIA))

# means for groups
svyby(~lnHFms2, ~ ETNIA, svymean, design=design.mnps,
vartype=c("se","ci","var"), na.rm=TRUE)

##################

# Analysis: Browns as controls

##################

# perform the propensity score-adjusted regression - heart rate with Browns as
ref
design.mnps$variables$ETNIA <- relevel(design.mnps$variables$ETNIA,ref="parda")
glm1 <- svyglm(DERAP_VFCCL_BPMmed_deitado ~ as.factor(ETNIA), design =
design.mnps)
summary(glm1)

# clean printout of svyglm
require(arm)
display(glm1, digits=2, detail=TRUE)

#substitute for anova - for df
regTermTest(glm1, ~ as.factor(ETNIA))

# means for groups
svyby(~DERAP_VFCCL_BPMmed_deitado, ~ ETNIA, svymean, design=design.mnps,
vartype=c("se","ci","var"), na.rm=TRUE)

# perform the propensity score-adjusted regression - lnHFms2 with Browns as ref
glm1 <- svyglm(lnHFms2 ~ as.factor(ETNIA), design = design.mnps)
summary(glm1)

# clean printout of svyglm
require(arm)
display(glm1, digits=2, detail=TRUE)

#substitute for anova - for df
regTermTest(glm1, ~ as.factor(ETNIA))

# means for groups
svyby(~lnHFms2, ~ ETNIA, svymean, design=design.mnps,
vartype=c("se","ci","var"), na.rm=TRUE)

##################
```

```
# Analysis: Blacks as controls

##################

# perform the propensity score-adjusted regression - heart rate with Blacks as
ref
design.mnps$variables$ETNIA <- relevel(design.mnps$variables$ETNIA,ref="preta")
glm1 <- svyglm(DERAP_VFCCL_BPMmed_deitado ~ as.factor(ETNIA), design =
design.mnps)
summary(glm1)

# clean printout of svyglm
require(arm)
display(glm1, digits=2, detail=TRUE)

#substitute for anova - for df
regTermTest(glm1, ~ as.factor(ETNIA))

# means for groups
svyby(~DERAP_VFCCL_BPMmed_deitado, ~ ETNIA, svymean, design=design.mnps,
vartype=c("se","ci","var"), na.rm=TRUE)

# perform the propensity score-adjusted regression - lnHFms2 with Blacks as ref
glm1 <- svyglm(lnHFms2 ~ as.factor(ETNIA), design = design.mnps)
summary(glm1)

# clean printout of svyglm
require(arm)
display(glm1, digits=2, detail=TRUE)

#substitute for anova - for df
regTermTest(glm1, ~ as.factor(ETNIA))

# means for groups
svyby(~lnHFms2, ~ ETNIA, svymean, design=design.mnps,
vartype=c("se","ci","var"), na.rm=TRUE)


#########################

# MEDIATION with multiple covariates

#code adapted from: Imai et al., Chapter 8: Causal Mediation Analysis Using R

#########################

library("mediation")

# reads in CSV datafile
mydata <- read.table("/…/N = 11989.csv", header=TRUE, sep=",")

#convert dis_3grp to factor variable
mydata$dis_3grp <- factor(mydata$dis_3grp)

# Fit the mediator and outcome models using polr() and lm() functions
# Note that the mediator here is fitted with an ordered probit model, polr(), as
it is an ordered variable
```

```
med.fit <- polr(dis_3grp ~ ETNIA + AGEPART + RCTA8 + lessthanhs + high_school  +
Smoker + nofisica + IMCA01 + A_ESCORETOTAL  + A_HAS2 + A_DM + DISLIP + HARDCHD +
anyAdep, data = mydata, method = "probit", Hess = TRUE)

out.fit <- lm(lnHFms2 ~ dis_3grp + ETNIA + AGEPART + RCTA8 + lessthanhs +
high_school + Smoker + nofisica + IMCA01 + A_ESCORETOTAL + A_HAS2 + A_DM +
DISLIP + HARDCHD + anyAdep, data = mydata)

# Analysis will now take output objects from these models, med.fit and out.fit,
and use them as inputs for the main function, mediate().
# This mediate() function estimates the causal mediation effects, direct
effects, and total effect along with their uncertainty estimates.

# med.out refers to comparison between Blacks versus Whites
med.out <- mediate(med.fit, out.fit, control.value = 1, treat.value = 3, sims =
1000, boot = TRUE, treat = "ETNIA", mediator = "dis_3grp")

# med.out2 refers to comparison between Browns versus Whites
med.out2 <- mediate(med.fit, out.fit, control.value = 1, treat.value = 2, sims =
1000, boot = TRUE, treat = "ETNIA", mediator = "dis_3grp")
# med.out3 refers to comparison between Blacks versus Browns
med.out3 <- mediate(med.fit, out.fit, control.value = 2, treat.value = 3, sims =
1000, boot = TRUE, treat = "ETNIA", mediator = "dis_3grp")

# The summary() function prints out the results of the analysis in tabular form:

summary(med.out)
summary(med.out2)
summary(med.out3)

# Note that sensitivity analysis is not able to be conducted when the polr() is
used
```