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Insulin Resistance and Carotid Intima-Media Thickness Mediate the Association between Resting-State Heart Rate Variability and Executive Function: A Path Modelling Study

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Highlights

1. HRV is associated with executive function, but causal pathways remain to be examined
2. Available evidence provides a framework on which potential mechanisms are explored
3. Insulin resistance and atherosclerosis mediated the HRV-cognition relationship in seriatim
4. Results support a regulatory role of vagal function over downstream processes

Abstract

BACKGROUND: Research has linked high-frequency heart rate variability (HF-HRV) to cognitive function. The present study adopts a modern path modelling approach to understand potential causal pathways that may underpin this relationship.

METHODS: Here we examine the association between resting-state HF-HRV and executive function in a large sample of civil servants from Brazil (N=8,114) recruited for the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). HF-HRV was calculated from 10-minute resting-state electrocardiograms. Executive function was assessed using the trail-making test (version B).

RESULTS AND CONCLUSIONS: Insulin resistance (a marker of type 2 diabetes mellitus) and carotid intima-media thickness (subclinical atherosclerosis) mediated the relationship between HRV and executive function in seriatim. A limitation of the present study is its cross-sectional design; therefore, conclusions must be confirmed in longitudinal study. Nevertheless, findings support that possibility that HRV provides a 'spark' that initiates a cascade of adverse downstream effects that subsequently leads to cognitive impairment.

Keywords: heart rate variability, HRV, cognition, executive function, Trail Making Test, TMT, Homeostasis Model Assessment Index for Insulin Resistance, HOMA-IR, carotid intima-media thickness, IMT, path modelling, mediation analyses

Introduction

An increasing body of research highlights important links between cardiac function and cognition, resonating with Aristotelian thinking (Gross, 1995) on the functional role of the heart. Vagal nerve stimulation in humans has been shown to influence higher-order cognitive processing including executive function (Sackeim et al., 2001; Vonck et al., 2014). Other studies have demonstrated that reduced high-frequency HRV (HF-HRV) – an index of cardiac vagal function (Cacioppo, Tassinary, & Berntson, 2007) – is associated with a 6.7-fold increase in odds for cognitive impairment in older women (Kim et al., 2006), and that experimental modulation of HRV improves prefrontal cognitive function in both young (Hansen, Thayer, Johnsen, Sollers, & Stenvik, 2004) and older (Albinet, Boucard, Bouquet, & Audiffren, 2010) participants. Furthermore, recent epidemiological studies have shown that poor cardiovascular health is associated with future cognitive impairment (Reis et al., 2013; Thacker et al., 2014). Therefore, efforts to improve cardiovascular health – consistent with the American Heart Association strategic goals for 2020 and beyond (Lloyd-Jones et al., 2010) – may have important implications for cognitive outcomes later in life, including delaying onset of dementia. Here we examine the association between resting-state HF-HRV – an important marker of cardiac health – and executive function in civil servants recruited in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) (Aquino et al., 2012; Schmidt et al., 2014) using a modern path modelling approach (Hayes, 2013).

Despite an increasing body of evidence linking HRV to cognitive function, the underlying pathways mediating this relationship remain unclear. Despite this, the available evidence provides a solid

framework on which potential mechanisms may be explored. Vagal nerve function – commonly indexed by HF-HRV – plays an important role in regulating the inflammatory reflex (Tracey, 2002a; Tracey & Pavlov, 2012), a neural mechanism involved in metabolic homeostasis and rapid control of innate immune responses, just as it controls heart rate and other vital functions. In this regard, reduced resting-state HRV may reflect a poorly functioning anti-inflammatory reflex (Jarczok, Koenig, Mauss, Fischer, & Thayer, 2014; Kemp & Quintana, 2013; Tracey & Pavlov, 2012), leading to chronic inflammation and the development of insulin resistance (Donath & Shoelson, 2011; Hotamisligil, 2006), a syndrome that subsequently contributes to progressive atherosclerosis (DeFronzo, 2010) and cognitive impairment (Zhong et al., 2012). Decreased vagal nerve function leads to impaired hepatic vagal nerve signalling and baroreflex sensitivity that is causally associated with insulin resistance (Miller, Sims, Canavan, Hsu, & Ujhelyi, 1999; Ribeiro, Lautt, Legare, & Macedo, 2005; Tracey & Pavlov, 2012). Insulin resistance is a hallmark of type 2 diabetes mellitus and is strongly associated with a cluster of metabolic and cardiovascular risk factors including dyslipidaemia, hypertension, obesity, glucose intolerance and endothelial dysfunction (DeFronzo, 2010; Kashyap & Defronzo, 2007), and these components may actually contribute to predementia syndromes and the evolution of dementia (Panza et al., 2012).

Chronic inflammation also plays an important role in the pathophysiology of metabolic risk factors (i.e. the metabolic syndrome) (Haffner, 2006). Longitudinal studies have demonstrated that the metabolic syndrome leads to cognitive decline over a 3-year period in an elderly Latino population, and these findings were especially pronounced in individuals with high serum levels of inflammation (median high-sensitivity C-reactive protein level $\geq$ 3.2mg/L) (Yaffe et al., 2007). Insulin resistance also has proatherogenic effects at the level of the arterial wall, that subsequently lead to cellular events critical for plaque progression (Reddy, Singh, Bangit, & Batsell, 2010; Tabas, Tall, & Accili, 2010) (see also: Karrowni et al., 2013). Inflammation also contributes to the

development of atherosclerosis (Ross, 1993; 1999), further suggesting an important role for reduced vagal function in the accumulation of lipids and hardening of the arterial wall.

Carotid intima-media thickness (IMT) is a non-invasive surrogate measurement for subclinical atherosclerosis (Touboul et al., 2012), associated with cognitive impairment and cognitive test performance ten years later (Zhong et al., 2012). In that study (Zhong et al., 2012), larger IMT was associated with longer time to complete the Trail-Making Test (part B) – a commonly employed measure of executive function employed in the present study – after multiple adjustments. The authors (Zhong et al., 2012) proposed three possible mechanisms for the association between carotid atherosclerosis and cognitive function including: 1) unstable plaque peeling off, causing cerebral emboli, stroke and vascular dementia, 2) silent stroke damaging the brain and causing cognitive impairment, and 3) chronic cerebral hypo-perfusion and ischemia, increasing the vulnerability of neurons and subsequently impacting on cognitive performance. Interestingly, it has recently been shown that low resting HRV is associated with decreased resting brain perfusion as measured by pulsed arterial spin labelling (Allen, Jennings, Gianaros, Thayer, & Manuck, 2015). We suggest here that the adverse, downstream effects of reduced HRV may include insulin resistance subsequently leading to increased atherosclerosis, which will contribute to cognitive impairment. This is the model we sought to test in the present study.

We hypothesised that insulin resistance and atherosclerosis would mediate the relationship between HRV and executive function in a relatively large cohort of individuals from Brazil. We further sought to determine whether a single serial pathway of multiple mediators, or multiple indirect pathways underpinned this relationship. For instance, it is possible that reduced HRV directly contributes to atherosclerosis, beyond any contribution from insulin resistance. It is also possible that insulin resistance itself contributes directly to impairment in cognitive function (McCrimmon, Ryan, & Frier, 2012; Talbot et al., 2012). While we expected an important role of insulin resistance

and atherosclerosis as mediating factors between HRV and executive function, we sought to determine, which particular pathways best explain this association. To our knowledge this is the first study to explore the relationship between cardiac function and executive function using path modelling.

Methods

Participants

ELSA-Brasil is a cohort of 15,105 civil servants aged 35 to 74 enrolled between August 2008 and December 2010 at six cities (Belo Horizonte, Porto Alegre, Río de Janeiro, Salvador, São Paulo and Vitória). Exclusion criteria for the ELSA-Brasil study included 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 centre's metropolitan area. ELSA-Brasil is an ongoing cohort study designed to investigate the development and progression of chronic diseases including cardiovascular diseases, diabetes and dementia (Aquino et al., 2012; Passos, Caramelli, Benseñor, Giatti, & Barreto, 2014; Schmidt et al., 2014). The ethics committees of the participating universities approved the research protocol. All participants provided written informed consent after a complete description of the study.

Data from participants completing the Trail Making Test (TMT, version B) – a measure of executive function – was available for 13,142 participants after excluding participants (n= 1,963) with a history of stroke or those using medications known to interfere with cognition (neuroleptics, anticonvulsants, anticholinesterase or antiparkinsonian agents), as well as those participants who were not able to complete the test within 5-minutes. Analyses were conducted on cases with complete data (N=8,114). (See section on Participant Characteristics for further information).

Procedure

Participants were asked to abstain from caffeine, alcohol and physical activity 12 hours prior to assessments, described below. (See also Aquino et al., 2012; Schmidt et al., 2014).

Measures

Heart rate variability (HRV): Ten-minute, resting-state electrocardiogram (ECG) was recorded from participants in the supine position during spontaneous breathing without task demands. The ECG was always collected in the morning (8:00 to 12:00h) in a temperature-controlled room (21-24°C). Lead II ECG signals were recorded at 250 Hz with a digital electrocardiograph (Micromed, Brazil), consistent with international standards for the collection of heart rate variability (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). (see also R. J. Ellis, Zhu, Koenig, Thayer, & Wang, 2015). Wincardio (4.4a) software generated the R-R interval series from a lead associated with the highest ECG R-wave amplitude (usually D2). We have described the artefact detection and spectral analytic techniques previously (Dantas et al., 2012; Kemp et al., 2014a). Briefly, the R-R series was automatically preprocessed to remove ectopic beats and artifact, and linear interpolation was employed to replace any removed beats. Power spectral analysis was carried out by autoregressive modeling, estimated by the Yule Walker method, using the recursive algorithm of Levinson-Durbin. The high frequency (HF; 0.15–0.40 Hz) component was estimated, expressed in absolute units and then log-transformed as a normalisation strategy. The root mean square of successive differences (RMSSD), a commonly reported time-domain measure of heart rate variability was also extracted from participant data for sensitivity analysis. While RMSSD is highly correlated with HF-HRV (Goedhart, Van Der Sluis, & Houtveen, 2007), the former may be less affected by changes in breathing frequency (Penttilä et al., 2001; Saboul, Pialoux, & Hautier, 2013). While HRV is generally accepted to index cardiac vagal function (Cacioppo et al., 2007), there are several caveats to its interpretation (e.g. Berntson et al., 1997; Grossman & Kollai, 1993).

Trail Making Test (TMT; major outcome measure of cognitive performance): We focus on the TMT (version B) given work highlighting a relationship between HRV and executive function (Thayer, Hansen, Saus-Rose, & Johnsen, 2009), as well as a more recent study (Zhong et al., 2012) that reported higher IMT at baseline to be associated with longer time to complete this task at a 10-year follow-up assessment. The TMT (version B) is a widely used test of executive functions including cognitive flexibility, attention, concentration and psychomotor speed (Bowie & Harvey, 2006). Methodological detail relating to all cognitive testing in the ELSA study has been previously described (Passos et al., 2014). The TMT task requires participants to draw lines connecting letters and numbers in an order that alternated between increasing numeric value and alphabetic order (1, A, 2, B, 3, C etc). Participants must complete the task as quickly as they can without lifting the pencil point from the page. The total time (in seconds) to complete the Trails Test B is then recorded.

Homeostasis Model Assessment Index for Insulin Resistance (HOMA-IR, first mediator): Blood samples were collected after a 12-hour fast. Serum glucose levels were determined using the Hexokinase method (ADVIA Chemistry; Siemens, Deerfield, Illinois). Fasting insulin levels were measured using the immunoenzymatic assay (ELISA) (Siemens). The homeostasis model assessment (HOMA) index for insulin resistance was then calculated using the following formula: (Glucose (mg/dl) * Insulin (mcUI/mL))/405. This method (originally described by D. R. Matthews et al., 1985) is highly correlated with insulin resistance as measured by the euglycemic-hyperinsulinemic clamp method. It is also a relatively simple, non-invasive alternative that makes it particularly suitable for epidemiological studies (Hrebícek, Janout, Malincíková, Horáková, & Cízek, 2002) such as ELSA-Brasil.

Carotid intima-media thickness (IMT, second mediator): The technique for IMT measurement in the ELSA-Brasil study has been described previously (Mill, Pinto, Griep, & Goulart, 2013; Santos, Bittencourt, et al., n.d.; Santos, Goulart, et al., n.d.). The same protocol was performed in all centers using a Toshiba (Aplio XG™) with a 7.5 MHz linear transducer. IMT was measured in the outer wall of a pre-defined carotid segment of 1 cm in length from 1 cm below carotid bifurcation, during three cardiac cycles. All participating centers obtained the carotid images during three cardiac cycles and sent these acquisitions to the centralized reading center in São Paulo. The validity of acquired images was established by clearly visualising: (1) the anatomic guides for the common carotid arteries, (2) interfaces between the lumen and the vessel far wall and, (3) the interfaces between the media and the adventitia layers of the far vessel wall. The average of the maximum values of right and left common carotid artery IMT was used in analysis.

Covariates

Covariates included demographic characteristics, age, sex, education (less than high school vs other), ethnicity (black vs other), physical activity (sedentary vs other, determined using scoring guidelines for the International Physical Activity Questionnaire) (Craig et al., 2003), the severity of depression and anxiety (as measured by the total score from the Clinical Interview Schedule-Revised, CIS-R) (Lewis, Pelosi, Araya, & Dunn, 1992; Nunes, de Mello Alves, Chor, Schmidt, & Duncan, 2012), smoking (current vs other), and use of other cardiovascular and psychoactive medications not previously excluded (see section on Participants) (yes vs no).[1] Less "than high school level of education, 'black' ethnicity, low levels of physical activity, smoking and use of medications may be associated with altered levels of HRV, heightened cardiovascular risk and impaired cognitive performance (especially in older cohorts), and may therefore confound unadjusted associations between variables of interest. Depression and anxiety are also associated

[1] We would like to note here that we also ran additional analysis, which separated this single medication covariate into either cardiovascular and psychoactive medications (ATC classification C versus N: http://www.whocc.no/atc_ddd_index/), and findings do not differ from those that we report in the body of our manuscript. (See also our third footnote).

with our variables of interest (Kemp et al., 2014a; Kemp, Quintana, Felmingham, Matthews, & Jelinek, 2012; Kemp, Quintana, Quinn, Hopkinson, & Harris, 2014b), and as preliminary modelling indicated that depression did not moderate (or mediate) pathways between HRV and executive function, a measure of the severity of depression and anxiety was also added as a covariate.

Statistical Methods

Descriptive information is presented in Table 1 for all participants with complete data (N=8,114), as well as by tertile of high-frequency HRV (HF-HRV) to aid interpretation. Path modelling was then conducted to examine the direct effects (i.e. HF-HRV on executive function) and indirect (mediating) effects (i.e. relationships between HRV and executive function via the mediators, HOMA-IR and IMT) to test our hypotheses. A series of mediation models are presented, which quantify the direct and indirect pathways through which an antecedent (predictor or independent) variable transmits its effect on a consequent (dependent or outcome) variable. Mediation modelling is a suitable analytic approach for testing predicted directional relationships in cross-sectional datasets (Hayes, 2013). Our conceptual model – in which HRV transmits its effects on cognitive function through insulin resistance (our first mediator) followed by IMT (second mediator)– is presented in Figure 1. Results for an alternative model – in which insulin resistance and IMT transmit their effects on HRV, which subsequently impacts on cognitive function – is presented in the supplementary information.

Modelling involved ordinary least squares path analysis using the add-on PROCESS tool (Hayes, 2013) in SPSS (version 21) (Hayes, 2013). The path modelling approach (Hayes, 2013) that we employ here has three major advantages over the traditional approach to testing mediation (Baron & Kenny, 1986). These are: 1) the capacity to determine whether there is a significant indirect effect, quantify this effect and determine whether distinct indirect pathways significantly differ from each other, 2) the application of a nonparametric bootstrapping mediation method, which doesn't make

assumptions regarding the distribution of the regression coefficients of the direct effect, and 3) the application of a more powerful approach to conducting inferential statistics (see Hayes, 2013).

Results from path modelling were derived from 10,000 bootstrapped samples; unstandardized parameter estimates (*B*), heteroscedastic-consistent standard errors and bias-corrected 95% confidence intervals (95% CI) determined the significance of direct and indirect associations (Hayes, 2013). Consistent with recommendations for the reporting of findings from observational studies in epidemiology (Vandenbroucke et al., 2007), unadjusted and adjusted findings are reported. Two effect size measures for the total indirect effect are reported (as described in Hayes, 2013). These include the ratio of the indirect effect to the total (and direct) effect reflecting the proportion of the total (and direct) effect occurring indirectly. Additional analyses on two randomly selected subsamples (~50%) were then conducted to determine the robustness of findings in the entire cohort (i.e. could findings be replicated in two randomly selected subsamples from the entire sample?) in order to establish the extent to which findings are replicable. These results are reported in the supplementary information.

It should be noted here that it is not the point of modern path modelling to determine the best fitting model given the data, in the same way that structural equation modelling is sometimes practiced (Hayes, 2013). Instead, the goal is to estimate and interpret the effects that are predicted on the basis of the model proposed in the context of the extant literature.

Results

Participant Characteristics

The potential impact of missing data was first examined by comparing the characteristics of participants with and without missing data. Analyses was carried out on a total of 8,114 participants after excluding participants that did not have data available for any of the additional variables

entered into analyses, including covariates (n= 5,028). Carotid IMT assessments and HRV data were available for 10,943 and 13,796 participants, respectively, due to local data collection issues. An additional 504 participants were excluded from HRV analyses due to the presence of ectopic beats. Other measures on which data was missing included the Homeostasis Model Assessment Index for Insulin Resistance (HOMA-IR) (n=19), race (n=184), activity level (n=223), and severity of depression (n=11). Relative to cases completing the Trail Making Test (N=13,142 after exclusions; see description of participants in Methods section), cases included in analyses (N=8,114) were slightly younger (51.17 years versus 51.66 years; p=0.002, Cohen's *d* = 0.05), were more likely to be female (St. Residual = 1.6, χ^2=14.43, p<0.001, r=0.03), white (St. Residual = 3.8, χ^2=86.94, p<0.001, r=0.08), have had less than a high school level of education (St. Residual = 1.7, χ^2=8.52, p=0.004, r=0.03). Included cases were also less likely to have diabetes mellitus (St. Residual = -1.7, χ^2=9.68, p= 0.002, r=0.03), and to be using medications (St. Residual = -2.2, χ^2=20.35, p<0.001, r= 0.04). However, these differences are very small according to effect size measures (Cohen's d and r), and are unlikely to have biased results to any meaningful extent.

Table 1 provides information relating to the characteristics of the participants with complete data (N=8,114). This table also presents group characteristics by HF-HRV tertiles to aid interpretation of characteristics according to HF-HRV, our focal predictor [high tertile, M = 6.60, SD = 0.54; middle tertile, M = 5.43, SD = 0.28; low tertile, M = 4.10, SD = 0.71]. Relative to those in the highest HF-HRV tertile, those in the lowest tertile were associated with older age, fewer women, more participants with reported 'white' ethnicity and few participants with 'brown' or 'black' ethnicity, less education, fewer participants engaged in high levels of physical activity, fewer current smokers, higher level of insulin resistance, more participants with diabetes, greater carotid IMT, more cases with 'hard' coronary heart disease (CHD) (i.e. history of myocardial infarction or coronary revascularisation) and higher heart rate. Participants in the lowest HF-HRV tertile also

displayed a slower time to complete the trail-making test, a finding associated with small effect size (Cohen's d = 0.13).

Multiple Mediation Modelling

Results from our unadjusted serial mediation model revealed significant direct (*B* = -0.0165, 95% CI: -0.0246, -0.0085) and (total) indirect effects (*B* = -0.0095, 95% CI: -0.0117, -0.0074) for HRV on time taken to complete the trails test (Table 2, Fig 2). Two significant indirect pathways were identified: the first included paths a1 → d21 → b2 (see Fig 1) (*B* = -0.0027, 95% CI: -0.0032, -0.0022), while the second included paths a2 → b2 (*B* = -0.0070, 95% CI: -0.0088, -0.0054) (Fig 2). The ratio of the indirect effect to the total effect, a measure of effect size, is 0.3644, indicating that 36% of the effect of HRV on executive function occurs indirectly. (The indirect effect is 57% of the size of the direct effect.)

Results from adjusted analyses (Table 3; Fig 3) provides evidence for a serial pathway of multiple mediators including HOMA-IR (insulin resistance) followed by IMT (carotid intima-media thickness) after controlling for a host of potential confounding factors in all of the regressions that comprised the mediation analysis. This finding indicates that the association between HRV and time to complete the trails test is mediated by HOMA-IR and IMT (a_1 → d_{21} → b_2; full sample effect = -0.0003, 95% CI: -0.0005, -0.0002) (Table 3, Fig 3).[2,3] Additional analyses (reported in supplementary information; supplementary tables 1 and 2, and supplementary figures 1 and 2)

[2] In these analyses, we did not control for certain variables, such as dyslipidemia and hypertension, considered to lie on the theoretical pathway between HRV and executive function. These factors are generally considered to be central features of the 'insulin resistance syndrome' (DeFronzo, 2010; Kashyap & Defronzo, 2007), the basic cause of which is insulin resistance, a variable included in the analysis as the first major mediator. However, further analyses were run which included these variables, and our main finding of a single mediating pathway via HOMA-IR and IMT was found to be robust to the inclusion of these additional covariates into the model (full sample effect = -0.0002, 95% CI: -0.0004, -0.0001).

[3] We also ran a further analysis, which included a cardiovascular medication covariate (ATC classification C) and a psychoactive medication covariate (ATC classification N), and findings indicating a single mediating pathway via HOMA-IR and IMT do not change (full sample effect = -0.0003, 95% CI: -0.0005, -0.0002).

revealed that the mediating pathway via HOMA-IR and IMT is replicable in two randomly selected subsamples highlighting the robustness of the findings observed in the entire cohort (Table 3, Fig 3). While the second mediating pathway via IMT (a2 → b2) was also significant for the full sample (*B* = 0.0005, 95% CI: 0.0001, 0.0010) (Fig 2, Fig 3), it could not be replicated in additional analyses on the two randomly selected subsamples. Notably, while the single serial-mediating pathway was robust to control for potential confounding factors, the total and direct effects between HRV and trail-making test were no longer significant. It is worth noting here the impact that adjusting for covariates have on path b2 in particular (compare Fig 2, which reports unadjusted findings versus Fig 3, which reports adjusted findings). Following adjustment, paths a1 (which refers to the negative relationship between HRV and insulin resistance) and b2 (which refers to the positive relationship between IMT and slowing on the Trail Making Task) sum to near zero. This alteration in path coefficients is likely to have contributed to the loss of the significant total effect observed in Figure 2, and in part, helps to better understand the small effect associated with the overall singular mediating pathway.

Our alternative model determined whether insulin resistance and IMT transmit their effects on HRV, which subsequently impacts on performance in the TMT taking into account all covariates. Results provide evidence against this alternative explanation for the relationship between HRV and TMT in our cohort (supplementary Fig 3). No paths involving HRV as the second mediator were significant. Therefore, this model fails to link HRV to performance on the trail-making task after controlling for covariates, unlike the results reported in Table 3 and Fig 3. Finally, additional sensitivity analyses were conducted. The first on RMSSD as the measure of HRV replicated the findings reported above on HF-HRV (see supplementary information: Table 4 and Figure 4). The second on heart rate instead of HRV (see supplementary figure 5) failed to replicate the findings observed for HRV.

Discussion

The present study sought to better understand the relationship between HRV and performance on an executive function task through modelling of specific variables that may lie on the causal pathway. Key findings from the present study were that 1) participants with high HRV performed better on the trail-making test – a measure of executive function – than those with low HRV, a finding associated with a small effect size (Cohen's d = 0.13) and approximately a third of this effect was observed to occur indirectly, 2) there is a single, serial multiple-mediator pathway linking HRV and executive function by insulin resistance and subclinical atherosclerosis and these findings were robust to the adjustment of potential confounding variables, and 3) the validity of this single, serial multiple-mediator pathway was supported in four different sensitivity analyses (reported in the supplementary information). These additional analyses include the 1) replication of findings in two randomly selected subsamples from the entire cohort reinforcing the confidence in our findings (reported in supplementary tables and figures 1 and 2), 2) failure to support an alternative model in which insulin resistance and IMT drive alterations in HRV, which subsequently affects performance on the trail-making test (supplementary table and figure 3), 3) replication of findings using RMSSD, an alternative, commonly-reported measure of HRV that may be less affected by alterations in respiration (supplementary table and figure 4), and 4) the failure to replicate findings when HRV was replaced by heart rate (supplementary Figure 5). Finally, it is also worth noting here that although effects are small, the finding is replicable in two randomly selected subsamples (as reported above, and presented in supplementary information). In summary, our study makes a novel contribution to the literature on the relation between cardiac and cognitive function, identifying a single mediating pathway of multiple mediators that contribute seriatim to variance in cognitive function.

We suggest that our findings are practically significant for at least four reasons. (See P. D. Ellis, 2010 for a detailed discussion of the concept of practical significance). First, research findings are

consistent with our proposed hypotheses based on the available literature (i.e. the "theoretical plausibility" rationale). There is now a large body of theoretical and experimental evidence that causally links dysregulation in the anti-inflammatory reflex – indexed by reductions in HRV – to insulin resistance (Donath & Shoelson, 2011; Hotamisligil, 2006; Tracey & Pavlov, 2012), a syndrome associated with progressive atherosclerosis (Beckman, Creager, & Libby, 2002; DeFronzo, 2010), which is itself associated with cognitive impairment, including longer time to complete the Trail-Making Test (Zhong et al., 2012). Second, while the relationships we observed here are small in size, small effects may trigger adverse downstream effects that accumulate over time to become large effects (i.e. the "small sparks start big fires" rationale). Vagal nerve dysfunction is known to lead to chronic inflammation (Jarczok et al., 2014; Tracey, 2002b; Tracey & Pavlov, 2012), which may subsequently lead to a variety of conditions including psychiatric disorders (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008) and cardiovascular disease (Hansson, 2005), conditions known to be associated with cognitive impairment (Caspi et al., 2014; Morris & Cuthbert, 2012; Snyder, 2013; Thacker et al., 2014). We suggest that HRV may be considered the 'spark' that initiates a cascade of adverse downstream effects subsequently leading to cognitive impairment. Third, we provide new evidence for a regulatory role of vagal function over a variety of downstream processes that subsequently impact on cognition (i.e. the "contribution to the literature" rationale). These findings were further strengthened by replication in two randomly selected subsamples, as well as evidence against the alternative explanation that insulin resistance (HOMA) and IMT transmit their effects on HRV, which subsequently impact on cognitive function. Although we acknowledge the limitations associated with testing reverse causality (Wiedermann & Eye, 2015), our proposed model is in line with a significant body of research indicating that low HRV precedes other risk factors (Jarczok et al., 2014; Thayer & Sternberg, 2006; Thayer, Yamamoto, & Brosschot, 2010; Tracey & Pavlov, 2012). Fourth, we provide evidence for a single pathway that mediates executive dysfunction; no additional pathways

were observed to mediate the effect of HRV on executive function (i.e. the "elimination of competing explanations" rationale).

Our study has a number of strengths including the application of a modern path modelling approach to understand the relationship between HRV and executive function in a relatively large cohort, the confirmation of initial findings on two randomly selected subsamples, demonstrating the validity of the findings reported here, and a comparison of alternative paths within the same model as well as comparison against an alternative model, reinforcing confidence in our findings. We also demonstrated that the findings were replicable when replacing HF-HRV with RMSSD, a time-domain measure of HRV that may be less susceptible to the effects of respiration (Penttilä et al., 2001; Saboul et al., 2013).

It is also important to acknowledge a number of limitations associated with our study. First, the reliability of short-term measurements of HRV has been questioned (Sandercock, Bromley, & Brodie, 2005) highlighting the importance of the conditions during which data are collected. However, reliable and valid measures of HRV can be obtained from shorter recordings under standardized and controlled conditions (Anonymous, 1996). We have also demonstrated that the protocol on which this study is based shows good reproducibility (Dantas et al., 2010). Second, we did not collect data on respiration rate or tidal volume, which may influence estimates of HRV. While researchers have suggested several approaches to controlling for these factors (e.g. Berntson et al., 1997; Grossman & Taylor, 2007), the question over whether they should be controlled remains a divisive one in the field of psychophysiology (Denver, Reed, & Porges, 2007; Porges, 2007; Ritz, 2009; Thayer, Loerbroks, & Sternberg, 2011). Third, a weakness of our study is the cross-sectional design. Therefore, we only present evidence of covariation between the variables in our model consistent with available evidence for a mediating relationship by insulin resistance and atherosclerosis. While mediation modelling on cross-sectional datasets is an accepted analytic

approach for testing predicted directional relationships (Hayes, 2013), longitudinal studies are of course needed to confirm the findings we report here. Finally, findings were based on complete case analysis after dropping participants according to listwise deletion procedures given limitations of the software used. The task of integrating missing data imputation procedures with mediation analysis is complicated by the need to combine results according to Rubin's rules to account for missing-data uncertainty (Rubin, 2009; Schafer, 1997), and the development of such tools (i.e. those that allow for data imputation followed by mediation modelling) is still in their infancy. While complete case analysis is standard practice in the estimation of mediation models, they may be characterised by biased parameter estimates and loss of power. However, only small differences were observed on participant characteristics between those cases included and excluded in analyses, and findings were consistent with hypotheses and the available literature minimising the importance of any bias that may have impacted on parameter estimates. Loss of statistical power is also unlikely to be a major issue given the large sample on which findings are based.

In summary, the present study provides new evidence for a single, serial mediating pathway that may link HRV reductions to cognitive impairment, reinforcing a potential regulatory role of vagal function – indexed by HRV – over a variety of downstream processes that subsequently impact on cognition. While we do not claim that the pathway we observe here is the only pathway that might mediate the relationship between HRV and executive function, we do suggest that our findings are likely to be one of the pathways underpinning the relationship between HRV and executive function, and that the alternative pathways we examined may be less important. Future analysis exploring more complex models – including for example, whether mediators of the relationship between HRV and cognitive function are moderated – would be welcomed. Simple health behaviours including weight loss, smoking cessation, physical exercise, mediation and use of positive emotions are all associated with increased HRV, and may therefore provide opportunities to short circuit the deleterious effects of associated with chronic vagal impairment.

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Figure Captions

Fig 1: The conceptual serial mediation model for the relationship between HRV and executive function (time to complete trail making test) allowing for comparison of multiple independent paths.

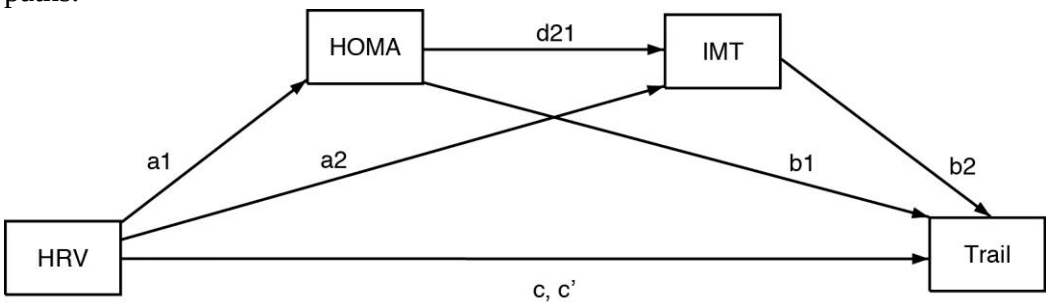

Fig 2: Path coefficients for unadjusted mediation model for entire sample with complete data (N=8,114). Results provide evidence for indirect paths between HRV and the trail-making test HOMA-IR and IMT (a1→d21→b2; bolded path 1) and via IMT (a2→ b2; bolded path 2). See also Table 2. * = p<0.05, ** = p<0.01, *** = p<0.001

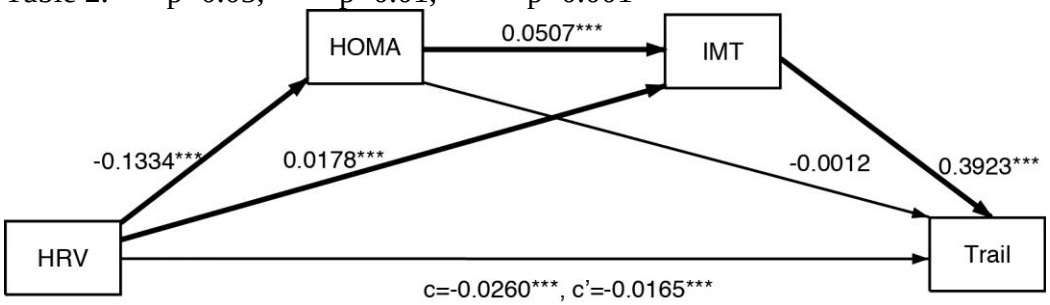

Fig 3: Path coefficients for adjusted mediation model for entire sample with complete data (N=8,114) after adjusting for covariates including age, gender, level of education, ethnicity, physical activity, depression severity, smoking status and medication use. Results provide evidence for an indirect path between HRV and the trail-making test via HOMA-IR and IMT (a1→d21→b2; bolded path). This path significantly differs from alternate path via a2 (dotted line) and b2. See also Table 2. * = p<0.05, ** = p<0.01, *** = p<0.001

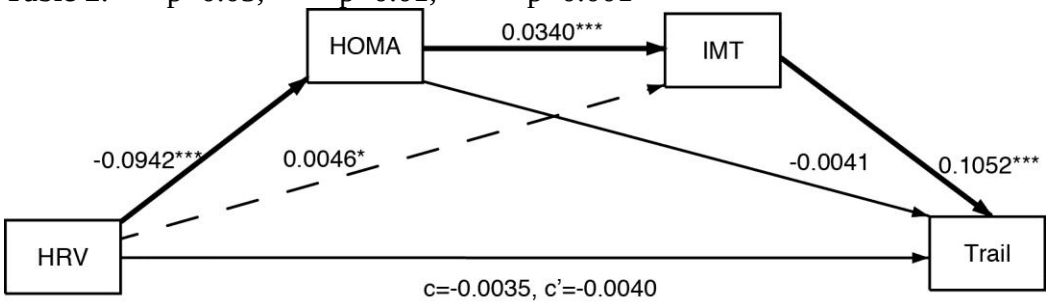

Table 1: Participant characteristics of those participants that underwent ECG and IMT assessments in the ELSA-

Characteristics are reported by overall sample with complete data (N=8,114) and tertiles on heart rate variability

(Pearson's r or point biserial correlation) is reported for the correlation between each characteristic and HF-HRV

of the relationships.

Characteristics		Heart rate variability			Effect size
Group	Overall	High	Middle	Low	
Sample size	N=8,114[4]	(n=2,703)	(n=2,704)	(n=2,702)	
Age, mean (SD), y	51.17 (8.81)	48.53 (8.22)	50.83 (8.43) *	54.15 (8.87)*	-0.28
Women, no., %	4,571 (56.3)	1,663 (61.5)	1,546 (57.2) *	1,358 (50.3) *	+0.10
Ethnicity, no., %					
Whites	4,705 (58.0)	1,409 (52.1)	1,582 (58.5) *	1,711 (63.3)*	-0.10
Browns	2,000 (24.6)	733 (27.1)	651 (24.1) *	615 (22.8) *	+0.04
Blacks	1,098 (13.5)	468 (17.3)	374 (13.8) *	255 (9.4) *	+0.10
Education, no., %					
<High school	672 (8.3)	192 (7.1)	208 (7.7)	272 (10.1) *	-0.05
High School	2,843 (35.0)	1,026 (38.0)	950 (35.1) *	865 (32.0) *	+0.06
College	4,599 (56.7)	1,485 (54.9)	1,546 (57.2)	1,565 (57.9)	-0.03
Physical activity, no., %					
Low level	6,198 (76.4)	2,052 (75.9)	2,042 (75.5)	2,100 (77.7)	-0.02
Moderate level	1,141 (14.1)	356 (13.2)	394 (14.6)	391 (14.5)	-0.01
High level	775 (9.6)	295 (10.9)	268 (9.9)	211 (7.8) *	+0.05
Depression Severity, mean (SD)	8.03 (7.80)	8.58 (8.11)	7.85 (7.72)*	7.65 (7.53)*	+0.05
Current smoker, yes, %	1,034 (12.7)	398 (14.7)	316 (11.7)	319 (11.8)*	+0.03
HOMA-IR[1], mean (SD)	2.34 (2.72)	1.98 (1.98)	2.23 (2.40)*	2.81 (3.50)*	-0.15
Diabetes, yes, %	1,373 (16.9)	323 (11.9)	385 (14.2) *	665 (24.6) *	-0.17
IMT[2], mean (SD), mm	0.75 (0.16)	0.72 (0.15)	0.74 (0.16)*	0.77 (0.17)*	-0.14
Hard CHD events, yes %	165 (2.0)	36 (1.3)	43 (1.6)	86 (3.2) *	-0.05
Medication Use[3], yes, %	2,874 (35.4)	794 (29.4)	882 (32.6) *	1,198 (44.3) *	-0.14
HR Average[3], mean (SD), BPM	66.80 (8.98)	62.78 (7.54)	66.21 (7.93)*	71.40 (9.21)*	-0.47
TMT[4], mean (SD), seconds	108.64 (50.98)	105.57 (50.38)	107.98 (50.36)	112.41 (52.20)*	-0.07

[1]HOMA-IR: homeostasis model assessment – insulin resistance; [2]IMT: carotid intima-media thickness, average
Use: any medication including lipid reductor, antihypertensives, diuretics, angiotensin II receptor blocker; [4]Sam
participants with complete data; [5]Tertile cut-offs for (ln)HF-HRV: low <4.92, middle >4.92 and <5.91, and high
tertile

Table 2: Results for the unadjusted serial mediation model on the association between HRV and executive funct
entire sample with complete data (N=8,114). Regression coefficients (unstandardized), standard errors, and mod
consequent are provided. See also Fig 2.

		Consequent									
		M_1(HOMA-IR)			M_2(IMT)			Y (TMT)			
Antecedent		Coeff.	SE	p	Coeff.	SE	p	Coeff.	SE	p	
X (HRV)	a_1	-0.1334	0.0091	<0.0001	a_2	-0.0178	0.0019	<0.0001	c'	-0.0165	0.0041	0.0001
M_1(HOMA-IR)					d_{21}	0.0507	0.0024	<0.0001	b_1	-0.0012	0.0052	0.8232
M_2(IMT)									b_2	0.3923	0.0240	<0.0001
Constant		1.1589	0.0498	<0.0001		-0.2416	0.0110	<0.0001		4.8051	0.0235	<0.0001
Model Summary		R^2=0.0272				R^2=0.0718				R^2=0.0389		
		F(1, 8112)=215.034, p<0.0001				F(2,8111)=310.15, p<0.0001				F(3,8110)=108.74, p<0.0001		

Table 3: Results for serial mediation model on the association between HRV and executive function (trail makin covariates[1] for the entire sample with complete data (N=8,114). Regression coefficients (unstandardized), standa information for each consequent are provided. See also Fig 3.

		Consequent										
		M_1(HOMA-IR)				M_2(IMT)				Y (TMT)		
Antecedent		Coeff.	SE	p		Coeff.	SE	p		Coeff.	SE	p
X (HRV)	a_1	-0.0942	0.0092	<0.001	a_2	0.0046	0.0017	0.0083	c’	-0.0040	0.0038	0.2962
M_1(HOMA-IR)					d_{21}	0.0340	0.0022	<0.0001	b_1	-0.0041	0.0049	0.4031
M_2(IMT)									b_2	0.1052	0.0254	<0.0001
Constant		0.6852	0.0926	<0.0001		-0.8727	0.0173	<0.0001		3.9947	0.0448	<0.0001
Model Summary		R^2=0.1015				R^2=0.3082				R^2=0.2071		
		F(9, 8104)=101.71, p<0.0001				F(10,8103)=361.45, p<0.0001				F(11,8102)=210.38, p<0.0001		

[1] Covariates included age, gender, level of education, ethnicity, physical activity, depression severity, smoking s