Water consumption as a source of error in the measurement of heart rate variability

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Abstract.

Heart rate variability (HRV) is a common measure of the autonomic modulation of the heart. To ensure accuracy, various external factors known to affect HRV such as medication use and concurrent illnesses are frequently controlled during measurement. While the influences of such confounds on accurate measurement of HRV are accepted, the influence of seemingly innocuous daily activities such as drinking and eating have received remarkably little attention. In fact, water consumption is known to provoke a powerful pressor stimulus that is buffered by changes in cardiovagal outflow yet it is sporadically controlled during experimentation. This paper examines the dose-dependent magnitude of the effect of water on HRV, the loss of HRV during a common attentional task after water consumption, and the typical scenario of water and mixed meal ingestion. Our findings show that water affects HRV in a dose-dependent manner, and exaggerates attentionally-mediated HRV reduction. This effect is antagonised by simultaneous mixed meal consumption, which strongly increases cardiac sympathetic activity. Water consumption during or previous to HRV measurement should be carefully controlled, if possible. Considering the diverse nature of experimental protocols and populations, we suggest a hierarchy of methods to control for water consumption in HRV research.

Keywords: Water, Food, Heart rate variability, Pre-ejection period

Highlights

- Establishes dose-dependency of water effects on HRV
- Demonstrates water-task interactions on HRV
- Establishes interactions between food and water consumption on HRV
- Suggests guidelines for controlling water consumption as a source of error in HRV

1. Introduction

Heart rate variability (HRV) analysis non-invasively assesses the outflow of the autonomic nervous system (ANS) through calculations performed on the beat-to-beat variability of the heart. It has broad applicability in the medical and behavioural sciences, and has been used variously as a correlate of myocardial infarction risk (Buccelletti et al. 2009), attention (Aasman et al. 1987), depression and anxiety (Gorman & Sloan 2000), and emotional regulation (Butler et al. 2006). To increase the utility of HRV methods, experimenters taking electrocardiographic (ECG) recordings seek to minimize both random and systematic sources of error to the calculation of interbeat intervals (IBIs). Hardware, software, and interpretative standards to achieve these ends have previously been outlined by the Task Force of the European Society of Cardiology (Camm et al. 1996). Likewise, there is a commonly understood list of confounds to HRV data collection: xanthine stimulants (e.g., coffee, tea, energy drinks), circadian rhythms, age, medication status, underlying medical conditions, etc. (see Heathers 2014; Tak et al. 2009 for a comprehensive discussion). Despite widespread recognition of the importance of such confounds, the influence of superficially innocuous daily activities such as eating and drinking have received surprisingly little attention.

1.1. Why consider water?

There are several reasons to explicitly consider water consumption in isolation as a confounding factor when using HRV measures. First, food consumption is already more commonly recognized as an experimental confound than water. An estimate from all forms of published HRV literature as of 2012 is that 43% of studies report control for food, and 23% for water (Heathers, 2014). This figure includes clinical studies conducted after total overnight fasting, thus the control within psychophysiological work in isolation can be assumed to be lower. Furthermore, even when food consumption is controlled (i.e., a standardized meal is provided), water may be provided *ad libitum* for participant comfort. Finally, the

question of how food consumption modifies ANS outflow is a more complicated one, and well beyond the scope of a single study – it may variously provoke changes in gastric distention, differ in macronutrient ratio, fiber content, caloric content, and so on. The influence of water consumption, by contrast, can be relatively easily investigated.

Early research noted water mediated pressor changes in rats (Hoffman et al. 1977). These changes were later identified in humans by Jordan and colleagues (1999), who observed profound haemodynamic changes after water consumption in adults with autonomic failure. This response is driven by the presence of hypo-osmotic fluids (i.e., water) in the stomach and/or portal vein (Lipp et al. 2005) which provokes a powerful pressor stimulus, immediately increasing blood pressure. In patients with autonomic denervation, this response is established within 10-15 minutes, lasts approximately 90 minutes, and peaks at approximately 30 mm Hg (Jordan et al. 2000; Jordan et al. 1999). By contrast, cardiovagal outflow in healthy subjects immediately increases to buffer these changes over the same time course. This change in autonomic outflow has been shown to clearly affect frequency-domain HRV (Routledge et al. 2002).

While these changes are significant, contemporary psychophysiological studies usually do not report the explicit control of water intake previous to or during an experiment, unless included under the rubric of total fasting. During normal activities of daily living, experimental participants will often have consumed water within the window affected by the osmopressor response (approximately two hours; Jordan et. al., 2000), generally between a standard glass (200mL, approx. 7 oz.) and a commercial bottle (600mL, approx. 20oz.). Thus, failing to control for water intake may be a significant oversight.

1.2. Aims of the present study

1.2.1. Dose dependency of water in isolation

To address these poorly understood issues, the current study has three precise aims. First, we seek to establish whether there is a dose-dependent effect on HRV associated with common levels of water

consumption. Jordan et al. (2000) reported a clear increase in blood pressure after 480mL vs. 240mL of water in patients with autonomic failure, although this difference was not analysed. As already noted, in healthy people these changes are buffered by the autonomic nervous system; 500mL of water consumption raises parasympathetic activity, lowering HR period and increasing HF-HRV (Routledge et al. 2002). Thus, we may expect a broad increase from baseline in HRV power as measured by HF-HRV.

1.2.2. Interaction between water consumption and tasks

Second, we aim to address the possibility that there is an interaction between water consumption and task related HRV changes. One of the well-established findings within the psychophysiology of attention shows focused, attentionally demanding tasks provoke a reliable and robust decrease in LF HRV power (Althaus et al. 1998; Heathers 2013; Meijman 1997; Middleton et al. 1999; Nickel & Nachreiner 2003). As the LF spectrum is indicative of gain of the baroreflex (Goldstein et al. 2011), it has been previously assumed that decreased baroreflex sensitivity is involved in the task response (Mulder & Mulder 1981). However, the details of this process remain unclear (Fairclough & Mulder 2012). If water consumption affects basal HRV, it is possible that an attentional change due to task will occur normally but at an inflated level. Alternatively, an interaction may exist such that a task effect is exaggerated by falling from a water-inflated baseline. In this latter scenario, water consumption could prove to be the source of an additional form of within-subject error by increasing the apparent task effect.

Crucially, the aforementioned relationship between HRV measurement affected due to water consumption and task-mediated reduction in HRV occurs in light of a separate body of literature that directly relates impaired hydration status to task performance (Lieberman 2007; Ritz & Berrut 2005; Secher & Ritz 2012). While the effect of water on cognition is equivocal, it has been observed to improve performance on tasks which involve psychomotor speed or visual attention both in children (Benton & Burgess 2009) and adults (Edmonds et al. 2013). This body of work frames drinking in terms of the

presence/absence of a degree of dehydration (rather than the presence/absence of drinking water), and has so far been investigated independently of similar visual attention tasks that affect LF HRV.

1.2.3. Water consumption with food

The most common and essential activities of daily living involve eating and drinking, often simultaneously. When uninstructed, participants may present at experimental sessions having recently eaten and consumed a variable quantity of liquids. Thus, we seek to establish a dose-dependent effect on HRV associated with water consumption during eating. Food consumption has a well-established but complex relationship to various measures of sympathetic outflow. Early work in microneurography (Berne et al. 1989; Fagius & Berne 1994) revealed a clear increase in peroneal muscle sympathetic nerve activity to various meal types. Various, increases in whole body and renal (but not cardiac) noradrenaline spillover have been observed (Cox et al. 1995). Likewise, a number of investigators have shown a decrease in IBI subsequent to eating (Hayano et al. 1990; Kelbaek et al. 1989; Kuwahara et al. 2010; Lipsitz et al. 1993; Lu et al. 1999; Uijtdehaage et al. 1994). However, the pattern of results within HRV is mixed: no directional effects on LF or HF power (Dionne et al. 2002); an equivocal increase in LF power but no change in HF power (Lipsitz et al. 1993); an equivocal loss of HF power in fasting as compared to high energy/high fat and moderate energy/high carbohydrate meals (Kuwahara et al. 2010); and a substantial reduction in respiratory sinus arrhythmia post meals in supine but not standing participants (Hayano et al. 1990). Water consumption also significantly increases sympathetic outflow, as measured by muscle sympathetic nerve activity (Scott et al. 2001), and urinary norepinephrine (Jordan et al. 2000). Likewise, the pressor response provoked by water is abolished by the β -blocker metoprolol (a selective β_1 -antagonist, Boschmann et al. 2003).

1.3. Measuring sympathetic outflow

HRV in isolation is widely regarded as an inadequate measure of sympathetic outflow to the heart (Billman 2011; Goldstein et al. 2011; Heathers 2014; Reyes del Paso et al. 2013). Instead, another cardiac timing interval – pre-ejection period (PEP) – is commonly used (e.g., Goedhart et al. 2006; Licht et al. 2010). PEP is the time from the electrical initiation of myocardial contraction (i.e., the Q-wave of the ECG) to the beginning of the ejection of blood from the heart, at the opening of the aortic valve. Thus, it represents the electromechanical delay from the initiation to the action of the heart beat. Changes in cardiac contractility are well established as primarily mediated by the adrenergic signal to the heart (Ahmed et al. 1972; de Champlain et al. 1980; Harris et al. 1967). It is unknown how water consumption level will variously modify the expected changes in SNS outflow as measured by PEP, and how water consumption vs. food will interact to modify HRV.

2. Experiment 1

In Experiment 1, participants consumed a measured dose of water then completed a simple reaction time task (RTT) in order to address the first two aims; to establish a dose-dependent effect of water consumption on HRV, and to address the possibility of an interaction between water and task-related changes in HRV. It was hypothesised that water would induce a dose-dependent increase in HF-HRV. No directional hypothesis was posed for the interaction between water and task-related effects on HRV.

2.1. Method

The current study was undertaken and reported in accordance with the Guidelines for Reporting on Articles on Psychiatry and Heart rate variability (GRAPH; Quintana et al. 2016) which provides a standardized set of criteria for reporting HRV studies in the biobehavioral sciences (see Supplementary material for checklist)

2.1.1. Participants

An *a priori* power analyses was conducted to estimate the number of participants required to achieve statistical power of 80% for a repeated-measures ANOVA assessing the main effect of water dose (four conditions). As we expected a large effect size, it was calculated that 10 participants would be required to achieve a statistical power of 80% ($\alpha = .05$). Thus, 10 healthy participants (7 female) aged 18 – 24yrs (mean=19.2; SD=1.9) were recruited to the study in exchange for undergraduate course credit.

Participants reported no history of any chronic, cardiovascular, or autonomic diseases, took no regular medication and were casual or non-exercisers (less than 4 hrs per week) and non-smokers. Participants

reported having ingested no medication, alcohol or recreational drugs for 24 hours, no xanthine-based beverages (e.g., coffee, tea, energy drinks) for 4 hours, and no food or water for 2 hours previous to all experimental sessions. Participants emptied their bladders prior to testing. Participants provided informed consent, and the study was approved by the Sydney University Human Research Ethics Committee (2013/572).

2.1.2. Design and procedure

Participants were assigned a random experimental schedule of 4 sessions, which differed only in the amount of water ingested. Dose of water consumed (Dose, 4 levels, 0mL, 200mL, 400mL, 600mL) was manipulated within-subjects. Sessions were held at identical times 2 to 7 days apart, and all sessions commenced between 9am and 1pm to minimize circadian differences.

At each session ECG data was recorded for 3 time-points (Baseline, Water, RTT). Participants rested for an initial 10 minutes while screening was completed and electrodes attached, after which a 5-minute ECG baseline (time 1, Baseline) was recorded, followed by a 5-minute period during which the assigned amount of water (0, 200ml, 400ml, or 600ml) was drunk as fast as was comfortable. ECG measurement continued for a further 15 min, the time in which a significant osmopressor response is typically established (Jordan et al. 1999; McHugh et al. 2010; Routledge et al. 2002), the final 5 min of which formed the water-baseline (time 2, Water), after which subjects completed the 6-minute simple reaction time task (time 3, RTT). The laboratory temperature was constant at 24°C and subjects sat upright and still throughout. As water temperature affects the osmopressor response (Girona et al. 2014), all water was served at 4°C.

2.1.3. Simple serial reaction time

Participants were required to attend to a central fixation cross, and depress a key as fast as possible on the presentation of an asterisk. Inter-trial interval was random from 1200ms to 2500ms. Participants were encouraged to maintain the fastest level of response possible throughout the RTT. Pilot data (n = 3) showed a reduction in LF HRV levels during RTT performance, and no participant reported task difficulties, or showed any significant lapses of attention.

2.1.4. Water

Water was drawn from a commercial filter. A 100mL sample was boiled dry in a rotary evaporator to 0.3mg of total dissolved solids (i.e., 3mg/L) which was confirmed as hypo-osmotic. All water was delivered through a straw from a sterile container at 4°C, forming a reasonable analogue of commercially available bottled water.

2.1.5. Data acquisition and analysis

An ECG signal in Lead II configuration was sampled at 1000Hz (Powerlab 8/35, AD Instruments, Bella Vista, Australia) using commercial ECG electrodes (MLA1010), and inter-beat intervals (IBIs) were calculated off-line (HRV Module, LabChart 7, AD Instruments, Bella Vista, Australia). Ectopic beats (i.e., extrasystolic heart beats with a successive compensatory pause) were visually identified and replaced with the interpolated adjacent IBIs. All signals consisted of >99.5% NN beats.

2.1.6. Time and frequency domain analysis

Frequency domain analysis of LF and HF frequency spectra were performed in Kubios (Version 2.0, University of Kuopio, Kuopio, Finland). For Fast Fourier Transform (FFT) spectrum estimation, the RR series was cubic-spline interpolated and resampled at 4hz, with a window width of 256s and 50% overlap. Baseline spectral values in ms² were log-transformed as frequency spectral values are not normally

distributed (e.g. Kobayashi et al. 2012). Low-frequency (LF) and high-frequency (HF) HRV measures were recorded over standard frequency bands (LF = 0.04-0.15Hz; HF = 0.15-0.40Hz).

2.1.7. Statistical analysis

Changes over time from baseline due to water consumption were measured by a two-way repeated-measures ANOVA [2*4; i.e., time (baseline, water) vs. dose (0mL, 200mL, 400mL, 600mL)]. Changes due to water consumption and RTT interactions (post-consumption) were measured by two-way repeated-measures ANOVA [2*4; i.e., time (water, RTT) vs. dose (0mL, 200mL, 400mL, 600mL)]. Corresponding generalized eta squared (η_G^2) and Bayes Factors (BF_{10}) were also calculated for each main effect and interaction. The following priors were used for Bayes Factor calculation: r scale fixed effects = 0.5, r scale random effects = 1). For η_G^2 values, 0.01, 0.06, and 0.14 were considered small, medium, and large effect sizes, respectively (Cohen 1988; Olejnik & Algina 2003). For BF_{10} values, 3, 10, and 30 were considered moderate, strong, and very strong evidence, respectively (Lee & Wagenmakers 2014) In the event of interaction effect evidence (defined as $p < .1$), follow up tests of simple main effects were performed to assess changes over time for each dose.

As noted earlier, water consumption prior to experimental sessions may be highly variable between participants. Thus, additional ANOVAs were performed in which the three water dose levels were averaged into an aggregate water condition, to represent the potential state of an experimental participant who had consumed water, but an unknown amount [2*4; i.e., time (baseline, water) vs. dose (0mL, 200mL, 400mL, 600mL)]. In the event of any evidence of interaction effects ($p < .1$), follow up tests of simple main effects were performed to assess changes over time for each dose.

No comparisons were significant on Mauchly's Test of Sphericity, thus the degrees of freedom for within-subjects effects were not modified. The level of statistical significance was set at $p = 0.05$. All

statistical processing was performed in the R statistical environment (version 3.3.2). Analysis scripts and data are available at <https://osf.io/uqztv/>.

2.2. Results

2.2.1. Osmopressor effect of water dose

Descriptive statistics are presented in Table 1. Water consumption did not appear to have a strong dose-dependent effect on LF power (Table 2; Figure 1A). While a main effect for dose was observed, this appeared to be largely due to LF power at baseline rather than as function of post consumption changes due to drinking (i.e., no dose*time interaction). When all dose groups were collapsed into a water group, there was a main effect for dose but no main effect for time or significant dose*time interaction (Table 1, Figure 1B).

Water consumption had a profound dose-dependent effect on HF power with a significant dose*time interaction (Table 3; Figure 1C). Simple main effects revealed significant differences between baseline and post-water consumption for the 400mL and 600mL conditions (Table 3). When all dose groups were collapsed into a water group, there was a significant water*time interaction (Table 3, Figure 1D). Simple main effects revealed a significant difference between baseline and post water consumption. The alternative model – that HF was significantly different than baseline – was 35.8 times more likely than the null model, which is considered very strong evidence for the alternative model relative to the null model.

2.2.2. Interactions between osmopressor effect of water dose and RTT effects

Water consumption had a significant effect on LF for the main effect of dose, while the main effect of time and the time*dose interaction were on the border of statistical significance (Table 3; Figure 1E). Simple main effects revealed significant differences between baseline and post-water consumption for the 200mL and 600mL conditions (Table 4). Water consumption had a significant effect on HF for the main effect of dose, but there was no main effect of time or a dose*time interaction (Table 4; Figure 1F).

3. Experiment 2

In Experiment 2 participants ate a standardized meal then consumed a measured dose of water in order to address the final aim, to establish a dose-dependent effect of water consumption with food on HRV, and on SNS outflow assessed via PEP. Food and water consumption have independent and complex effects on autonomic outflow, and while it was expected that the combined effects would differ from those of water consumption alone, no directional hypotheses were posed as it was unknown how dose-dependent water consumption would modify the expected food-related changes in SNS outflow, and how water consumption and food would interact to modify HRV.

3.1. Method

3.1.1. Participants

18 healthy males aged 18 – 21yrs (mean=18.8, SD=1.1) participated in the study in exchange for undergraduate course credit. Screening and participation requirements were as before. Participants provided informed consent, and the study was approved by the Sydney University HREC.

3.1.2. Design and procedure

Using a between-within design, participants were assigned a random experimental schedule, which differed only in the amount of water ingested as before (dose, 3 levels between subjects, 200ml, 400ml, or 600ml; six participants per condition) with a standardized mixed meal¹ (572 calories; 39% carbohydrates,

¹ A commercially-available meal replacement bar and an oat bran bar.

38% fat, 23% protein by calories). All sessions commenced between 11:30pm and 1:30pm, and were controlled as for Experiment 1. In addition, fasting status was confirmed by assessment of fasting blood glucose (all participants < 5.0mmol/L) via fingerprick (Accutrend Plus, Roche Diagnostics, USA).

ECG and ICG were recorded for an initial 5 minutes (baseline), after which participants received their food/water combination and were asked to eat/drink as fast as was comfortable. Immediately subsequent to the end of the meal, a further 45 minutes of ECG and ICG were taken, which was epoched into 9 non-overlapping 5-minute intervals for within-subjects analysis. The laboratory temperature was constant at 24°C, and subjects remained seated in an armchair throughout.

3.1.3. Data acquisition and analysis

Thoracic impedance (Z) and ECG were recorded simultaneously by the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) via six Ag/AgCl electrodes (de Geus & Van Doornen 1996; de Geus et al. 1995). Electrodes on the cervical spinal process and lower back were utilized to send HF current (350 μ A; 50kHz) through the torso, and two electrodes just below the sternal notch and xiphoid process measured impedance. These sites were always at least 2" lower and higher respectively from the relevant rear electrodes. ECG electrodes were attached to the right collarbone (-ve), in the intercostal space at the left later margin of the rib cage approximately equal to the height of the xiphoid process, (+ve), and approximately between the 8th to 10th ribs on the right-hand side (ground). Electrode sites were cleaned with abrasive gel and isopropyl alcohol.

3.1.4. PEP

The thoracic impedance signal was amplified and rectified to give thoracic impedance (Z), of which the maximum negative deflection of the signal dZ/dt was used to calculate the B-point (Lozano et al. 2007), as many records contained no well-defined B-notch or upstroke (Sherwood et al. 1990). The R-

wave to B-point interval was taken as an analogue of pre-ejection period (PEP). dZ/Dt position for each epoch was identified by first averaging ICG complexes as per VU-DAMS design. Two coders (JH; DA), blind to participant and water dose level, then located the maximal positive deflection in each averaged ICG complex for each participant. When ICG complexes had multiple peaks, dZ/Dt was scored at the first peak. For complexes with no clear peak, dZ/Dt was located at the area midpoint. All discrepancies over 2ms between double codes were rechecked without reference to the size or direction of discrepancy. All records were analysed within a single analysis session

3.1.5. HF-HRV

RR intervals were automatically detected by VU-DAMS software (v 3.4, Vrije Universiteit, Amsterdam) and inspected for accuracy. LF and HF HRV were calculated by interpolating the RR-series and resampling at 4hz, splitting the signal into 1024 point series (50% overlap), convoluting with a smoothness prior matrix (Tarvainen et al. 2002) to achieve stationarity, then performing an additional quadrature convolution and discrete Fourier transform on the available periods via Welch's method. Power spectral density was calculated between standard frequency bands (as for Experiment 1).

3.1.6. Statistical analysis

All measures were adjusted for baseline (i.e., Time Point-Baseline), with baseline measures presented in table 5. Time and dose-dependent effects were measured using two-way repeated-between measures ANOVA [time (9) * water dose (3)]. Generalized eta squared (η_G^2) was calculated for all comparisons. PEP, IBI, and LF comparisons were significant on Mauchly's Test of Sphericity, and were Greenhouse-Geisser corrected. Corrected p values are reported in these cases. HF power did not violate assumptions of Sphericity, and was uncorrected. The level of statistical significance was set at $p = 0.05$. All statistical processing was performed in the JASP statistical package. JASP files are available at <https://osf.io/uqztv/>.

3.2. Results

As shown in Figure 2A, baseline corrected PEP decreased over the course of the 45-minute recording period (Table 6). Bayesian repeated measures ANOVA revealed that the main effect of time model was preferred to the null model by a Bayes factor of 4.85×10^{12} , which is considered extreme evidence for the alternative hypothesis (Lee and Wagenmakers, 2013). The trajectory of the baseline PEP decrease did not vary between dose groups and there was no overall baseline corrected PEP difference between dose groups (Table 6). There were no main effects for time or dose, or time*dose interactions for baseline corrected IBI, LF, or HF (Table 6; Figures 2B-D).

4. Discussion

4.1. Dose dependency effects of water consumption

The central findings of the present study confirm previous observations that water consumption can form a significant confound to HRV measurement. These changes are sufficient to affect the establishment of an experimental baseline, and show a straightforward dose-dependency for HF as the effects increase in strength for 200mL, 400mL, 600mL. The maximal dose used here was broadly equivalent to the large doses used in other studies, and replicates the magnitude (Brown et al. 2005; Routledge et al. 2002) and timescale (Jordan et al. 2000; Newman et al. 2007) of effects outlined previously.

While data is log transformed here to meet the criteria of normality, the full magnitude of the effect in ms^2/Hz is profound (for HF after 600mL, 1018 to 2354; an increase of 128%) – the raw spectral power approximately doubles. This is well in excess of most experimental effects observed with HRV as a dependent variable, an increase which is approximately the same magnitude as the decrease from a significant postural change (specifically, the transition from 30° to supine; Porta et al. 2007). It requires no extrapolation to see the potential scope of the problem – unlike undiagnosed medical conditions, unrevealed medication or stimulant use etc. where individual participants are occasionally affected, every participant may be in some way affected by alterations of HRV after a normal activity of daily living.

4.2. Water-task interactions

Of greater concern, water appears to amplify the previously noted effects of serial attention on LF HRV. That is, water consumption affects the measurement of a task effect separately to its effect on baseline HRV. As LF HRV fell from pre-task to task when this measurement was adjusted for baseline, it is consequently reasonable to expect this decrease is largely driven by a change in task response.

Considering that water may augment performance in visual attention tasks, this potentially reveals an interesting tripartite relationship between visual attention performance, autonomic outflow and water consumption. In the literature, LF power decreases appear to be driven by “time on task” variables (Mulder & Mulder 1981), presumably more a function of continuous visual attention than cognitive load. The assumption in much of the literature relating hydration to task performance is that water is correcting sub-optimal hydration or dehydration, without an immediate concern for the mechanism involved (Edmonds & Burford 2009; Edmonds et al. 2013). However, the potential positive effects of drinking water (i.e., elevated hydration from a normal baseline) are not necessarily mediated by the same mechanism as the removal of potential negative effects due to dehydration; in many studies on hydration, participants are exercised or water restricted (from 1% to 4% of bodyweight; see Secher & Ritz 2012 for review). It is an open question as to whether changes in autonomic outflow are directly responsible for improvements in visual attention previously noted consequent to drinking (Rogers et al. 2001; Suhr et al. 2004).

Our task did not record the latency of responding, so the present work cannot determine if water consumption improved attention. However, it is likely that a ceiling effect would be observed, as the vigilance task used was extremely simple and was performed only once at full wakefulness in each session –typically, similar tasks are performed at multiple points over time to record lapses in attention. However, no participants reported difficulties at any point and it is unlikely that many, if any, lapses of attention occurred.

4.3. Water-Food interactions

Finally, concurrent consumption of a mixed meal appears to antagonize the osmopressor-mediated relationship between water and HRV entirely. While we did not entertain a directional

hypothesis that this would be the case, the result is both clear and straightforward to explain - during eating and subsequent digestion, water no longer represents a hypoosmotic stimulus. The converse was not true, as water did not appear to antagonize the normal autonomic postprandial response (e.g., Lu et al. 1999). While this might suggest that a concurrent meal may remove potential artifacts from HRV changes subsequent to drinking, this also implies that water does not interfere with the profound changes in ANS subsequent to feeding – PEP and IBI changes were profound and consistent in all participants.

This data addresses only the situation of concurrent food + water consumption – perhaps the effects may be combined if not delivered in sequence. For instance, if the original response to water consumption is observed subsequent to changes in hepatic portal vein osmolality (which is well established in 30 minutes; Lechner et al. 2011) is followed by food consumption and digestion, the effects may well not be straightforwardly antagonistic. Shannon et al. (2002) attempted this in patients with postprandial hypotension, and observed a significant improvement in the response to a meal when it was preceded by water consumption.

4.4. Water as a confound in HRV research

Our observations show that water consumption is a straightforward confound to HRV research, and should be strictly controlled wherever possible. If drug administration in tablet or capsule form is assessed for HRV-based changes, a standardized minimal amount of water should be provided. This is of increased importance in experimental models where autonomic changes are provoked by exercise, as dehydration is expected and fluids are often provided, although previous research perhaps suggests exercise is a sufficiently powerful autonomic stimulus that hypo-osmotic and osmotic fluids behave similarly during recovery (Vanderlei et al. 2015). As the pressor response seems to decay around 90 minutes (Jordan et al. 1999), adequate pre-hydration is most likely the best solution. While 200mL of water did not provoke a significant change in either LF or HF HRV, this is not a definitive statement of dose threshold –this study is statistically underpowered to observe a single small-magnitude effect.

However, if fluids are definitely required and the total fasting of experimental subjects is impractical, the osmopressor effect might potentially be avoided via the provision of isotonic or hypertonic fluids. As far as the authors can ascertain, this has not been attempted – while the differences between hypotonic and iso/hypertonic fluids were classically investigated as intragastric or intravenous infusions, there is no comparison of HRV so far comparing hypotonic to isotonic fluid drinking. Boschmann & Thielecke (2007) recorded an increased in energy expenditure subsequent to drinking water vs. drinking physiological saline but no change in HR or SBP for either. Of course, sodium consumption has its own relationship to cardiovascular regulation (e.g. Weinberger 1996) but these effects on baseline HRV are not presently quantified.

Controlling water consumption may sometimes be impractical or undesirable. For instance, it may be detrimental to performance (especially in a physical task), or impractical for recruitment at some time periods, or difficult to maintain throughout a study of several hours duration. It should be recognized that fasting, too, has its own coordinated effects on autonomic outflow. Pivik & Dykman (2004), for instance, describe substantial increases in morning LF and HF HRV during morning fasting which are not observed in groups fed a standard meal subsequent to a previous overnight fast.

Irrespective of the above, the most prudent option would seem to be that which provides the most consistency for experimental results – there is often no ‘ideal’ platform for the investigation of an experimental effect in free-living human participants, but rather simply one which has the fewest drawbacks. As the absence of a response is invariably more predictable than the presence of one, this would dictate a tentative hierarchy for research; that, in increasing order of preference, participants should receive a) a controlled and predictable amount of water, b) a fluid which represents less of an experimental confound, or most preferably c) no fluids from a period 90-120 minutes previous to experiment commencement through to experiment end.

4.5. Limitations

There are some limitations with the present data. Firstly, Experiment 1 is statistically equivocal with regards to smaller amounts of water (200mL or less). Due to the nature of the analysis conducted, it is impossible to state a sufficient lower limit below which a response does not occur – the significance of any difference would change markedly with the sample size involved. In addition, with regards to the effect of water on task-mediated HRV change, the present work only establishes a unidirectional conclusion. That is, measured spectral power is affected irrespective of any effect on performance, as latency of responding was not recorded. As powerful HRV changes are driven by increases in central sympathetic and vagal outflow, how these affect the measured response to other tasks is unresolved.

Likewise, while altered HRV response to task is specifically confined to the LF band. This has no bearing on other HRV-mediated task effects, such as the classic HF reduction to potentially stressful tasks with a related attentional component (Delaney & Brodie 2000; Porges & Raskin 1969; Suess et al. 1994). As these tasks are typically defined by vagal rather than baroreflex-mediated changes, the present result cannot be generalised.

Finally, we cannot assess the integrated effects of gastric distention and bladder filling consequent to food/water consumption. Gentilcore et al. (2008) showed that a distinct haemodynamic response can be observed with intragastric/intraduodenal infusion of as little as 300mL of fluid; it is unclear how this might interact with the measurement of HRV accompanied with the osmopressor effect subsequent to drinking. Likewise, bladder filling may be involved in the response subsequent to drinking, and contribute a potential change in HRV. When under stretch, mechanoreceptors in the bladder walls activate afferent parasympathetic signals to the nucleus of the solitary tract, which increases global parasympathetic outflow – this is the reason studies taking various visceral or autonomic measurements typically request that participants either arrive with an empty bladder or empty their bladder before the experiment begins.

4.6. Conclusion

In conclusion, the consumption of water before or during cardiac psychophysiological investigation should receive immediate attention as a source of error, especially within small sample experiments. It is unclear at present precisely how much existing research is affected by the osmopressor response. We feel it is unfair to single out existing study protocols likely affected, considering how commonly uncontrolled food and water consumption appear to be (Heathers 2014). It seems prudent, however, to consider the evidence here in the ongoing assessment of previous work which explicitly states water was provided to participants *ad libitum* or where water consumption was uncontrolled. Likewise, water consumption before or during experimentation should be minimal in order to measure stable baseline values of HRV indices, and to prevent change over time during experimentation. Alternatively, in participant samples where water consumption cannot be controlled, time and amount of water consumption might be included as a covariate. Future research may address the question of using water to provoke changes in HRV, which may in turn amplify or interact with similar ANS-mediated task effects.

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Figure captions

Figure 1

Dose-dependent (A) and overall (B) effects of water dose on LF power between baseline and post-consumption, and dose-dependent (C) and overall (D) effects of water dose on HF power between baseline and post-consumption. Overall water effects represent the average of the three water conditions (200mL, 400mL, 600mL). Dose-dependent effects of water dose on LF power (E) and HF power (F) between post-consumption and during the reaction time task. Note: Error bars represent standard errors, which are corrected to remove between-subject variability.

Figure 2

Dose-dependent effects of water consumption on the pre-ejection period (A), interbeat interval (B), LF Power (C), and HF Power (D). All measures were subtracted from baseline.

Table 1. Descriptive statistics for the effects of water consumption on cardiovascular variables

Dose	Baseline		Post		Task	
	Mean	SD	Mean	SD	Mean	SD
LF power						
0mL	6.86	1.04	6.91	0.82	6.83	0.62
200mL	7.26	0.91	7.45	0.92	6.84	0.71
400mL	7.01	1.11	7.51	0.79	7.16	0.61
600mL	7.26	0.76	7.70	0.59	7.05	0.85
Average water	7.18	0.74	7.56	0.69	-	-
HF power						
0mL	6.30	1.13	6.17	0.90	6.36	1.11
200mL	6.45	1.05	6.64	1.35	6.68	1.44
400mL	6.59	1.17	6.91	1.06	7.04	1.01
600mL	6.50	1.00	7.34	0.87	7.16	1.03
Average water	6.51	0.97	6.97	0.97	-	-

Table 2. Effect of dose and time on LF power

	F-test	<i>p</i>	η_G^2	BF₁₀
All doses				
Main effect (time)	F(1,9) = 2.65	0.138	0.03	1.49
Main effect (dose)	F(3,27) = 3.79	0.022	0.07	2.77
Interaction effect (time*dose)	F(3,27) = 0.85	0.476	0.02	0.22
Collapsed dose groups				
Main effect (time)	F(1,9) = 1.52	0.249	0.02	0.56
Main effect (dose)	F(1,9) = 11.16	0.009	0.09	14.43
Interaction effect (time*water)	F(1,9) = 2.9	0.12	0.01	0.62

Table 3. Effect of dose and time on HF power

	F-test	<i>p</i>	η_G^2	BF_{10}
All doses				
Main effect (time)	F(1,9) = 11.83	0.007	0.02	1.35
Main effect (dose)	F(3,27) = 2.57	0.075	0.06	5.66
Interaction effect (time*dose)	F(3,27) = 7.12	0.001	0.03	1.05
Simple time main effect (0mL)	F(1,9) = 0.66	0.438	0.004	0.48
Simple time main effect (200mL)	F(1,9) = 1.29	0.285	0.01	0.61
Simple time main effect (400mL)	F(1,9) = 14.26	0.004	0.02	7.21
Simple time main effect (600mL)	F(1,9) = 18.23	0.002	0.18	15.07
Collapsed dose groups				
Main effect (time)	F(1,9) = 2.3	0.164	0.01	0.42
Main effect (water)	F(1,9) = 5.24	0.048	0.07	13.29
Interaction effect (time*water)	F(1,9) = 17.79	0.002	0.02	1.53
Simple time main effect (0mL)	F(1,9) = 0.66	0.438	0.004	0.49
Simple time main effect (Collapsed water)	F(1,9) = 29.54	< 0.001	0.06	35.75

Table 4. Effect of dose and time on LF and HF power

		F-test	<i>p</i>	η_G^2	BF_{10}
LF					
	Main effect (time)	F(1,9) = 4.88	0.055	0.08	1.49
	Main effect (dose)	F(3,27) = 3.03	0.047	0.07	16.82
	Interaction effect (time*dose)	F(3,27) = 2.68	0.067	0.03	0.35
	Simple time main effect (0mL)	F(1,9) = 0.29	0.606	0.003	0.43
	Simple time main effect (200mL)	F(1,9) = 5.29	0.047	0.13	2.37
	Simple time main effect (400mL)	F(1,9) = 1.68	0.227	0.06	0.76
	Simple time main effect (600mL)	F(1,9) = 7.1	0.026	0.18	3.6
HF					
	Main effect (time)	F(1,9) = 0.08	0.79	0.0004	0.23
	Main effect (dose)	F(3,27) = 5.58	0.004	0.11	1330.09
	Interaction effect (time*water)	F(3,27) = 2.16	0.116	0.005	0.17

Table 5. Impact of water and meal on cardiovascular variables

		F-test	p	η_G^2	BF₁₀
PEP					
	Main effect (time)	F(1.7, 23.72) = 14.12	< 0.001	0.21	> 100000
	Main effect (dose)	F(2,14) = 0.91	0.424	0.09	0.55
	Interaction effect (time*dose)	F(3.39,23.72) = 0.63	0.624	0.02	0.06
IBI					
	Main effect (time)	F(2.99,35.83) = 2.19	0.11	0.06	3.16
	Main effect (dose)	F(2,12) = 0.76	0.49	0.08	0.58
	Interaction effect (time*dose)	F(5.97,35.83) = 0.56	0.76	0.03	0.07
LF					
	Main effect (time)	F(4.36,52.29) = 1.18	0.331	0.05	0.2
	Main effect (dose)	F(1,12) = 2.89	0.095	0.2	1.48
	Interaction effect (time*dose)	F(8.71,52.29) = 0.57	0.809	0.04	0.07
HF					
	Main effect (time)	F(8,96) = 1.8	0.086	0.06	0.11
	Main effect (dose)	F(2,12) = 1.88	0.195	0.16	0.87
	Interaction effect (time*dose)	F(16,96) = 1.57	0.159	0.1	0.51

Table 6. Descriptive statistics for cardiovascular variables at baseline for experiment 2

Dose	PEP		IBI		LF power		HF power	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
200mL	68.12	5.2	932	75.37	8.03	0.53	7.5	0.63
400mL	63.9	5.42	938	186.4	7.92	0.26	7.27	0.88
600mL	68.18	10.7	779	97.16	7.43	1.11	6.78	1.26

Supplementary material

Guidelines for reporting articles on psychiatry and heart rate variability (GRAPH) checklist items

Topic	Item number	Checklist item	Reported on page number
Selection of participants			
Clinical group recruitment and selection	1a	Psychiatric group recruitment details and illness assessment methods.	N/A
Control group recruitment and selection	1b	Control group recruitment details and methods to rule out psychiatric illness.	N/A
Inclusion criteria	1c	Description of inclusion criteria (e.g., absence of physical health conditions).	8,9
Disease characteristics	1d	Description of disease duration, severity, psychiatric comorbidities, and medication status.	9
Demographics	1e	Details on age, gender distribution, physical activity level, alcohol intake, and nicotine intake.	8,9
IBI collection			
Hardware / software details	2a	Brand, electrode configuration (if applicable)	10
IBI collection details	2b	Raw sampling rate, length of data collection, time of day, filtering, participant posture, and instructions.	10,11
IBI analysis and cleaning			
IBI calculation	3a	IBI calculation and resampling methods.	10
IBI artifact identification	3b	IBI artifact identification method (e.g., algorithm, manual inspection).	10
IBI data loss	3c	Reasons for loss (e.g., persistent ectopy, equipment failure)	10
IBI cleaning	3d	Artifact cleaning methods and the percentage of beats were corrected.	10
HRV calculation			
Method of analysis used	4a	Metrics used and the software/script used for HRV calculation, log transformation (if applicable)	10,11
Frequency bands used	4b	Specification of bands and how they were interpreted.	10,11