Hydration status influences the measurement of arterial stiffness

Aaron R. Caldwell, Matthew A. Tucker, Jenna Burchfield, Nicole E. Moyen, Alf Z. Satterfield, Ashley Six, Brendon P. McDermott, Sean W. Mulvenon and Matthew S. Ganio

Human Performance Laboratory, College of Education and Health Professionals, University of Arkansas, Fayetteville, AR, USA

Summary

Consensus guidelines have attempted to standardize the measurement and interpretation of pulse wave velocity (PWV); however, guidelines have not addressed whether hydration status affects PWV. Moreover, multiple studies have utilized heat stress to reduce arterial stiffness which may lead to dehydration. This study utilized two experiments to investigate the effects of dehydration on PWV at rest and during passive heat stress. In experiment 1, subjects (n = 19) completed two trials, one in which they arrived euhydrated and one dehydrated (1·2[1·0]% body mass loss). In experiment 2, subjects (n = 11) began two trials euhydrated and in one trial did not receive water during heat stress, thus becoming dehydrated (1·6 [0·6]% body mass loss); the other trial subjects remained euhydrated. Using Doppler ultrasound, carotid-to-femoral (central) and carotid-to-radial (peripheral) PWVs were measured. PWV was obtained at a normothermic baseline, and at a 0·5°C and 1°C elevation in rectal temperature (via passive heating). In experiment 1, baseline central PWV was significantly higher when euhydrated compared to dehydrated (628[95] versus 572[91] cm s^{-1}, respectively; $P<0·05$), but peripheral PWV was unaffected (861[117] versus 825[149] cm s^{-1}; $P>0·05$). However, starting euhydrated and becoming dehydrated during heating in experiment 2 did not affect PWV measures ($P>0·05$), and independent of hydration status peripheral PWV was reduced when rectal temperature was elevated 0·5°C (−74[45] cm s^{-1}; $P<0·05$) and 1·0°C (−70[48] cm s^{-1}; $P<0·05$). Overall, these data suggest that hydration status affects measurements of central PWV in normothermic, resting conditions. Therefore, future guidelines should suggest that investigators ensure adequate hydration status prior to measures of PWV.

Correspondence

Matthew S. Ganio, Human Performance Laboratory, University of Arkansas, 155 Stadium Dr. HPER 321, Fayetteville, AR 72701, USA
E-mail: msganio@uark.edu

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Introduction

Arterial stiffness is an independent predictor of cardiovascular mortality that, in general, refers to mechanical properties of the arterial wall, making it elastic or compliant under the strain of blood flow. The gold standard for measuring arterial stiffness is pulse wave velocity (PWV) which assesses the velocity of a pulse wave travelling in a segment of an artery (Townsend *et al.*, 2015). In the last 20 years, the use of PWV in research has, according to experts, 'literally exploded' (Townsend *et al.*, 2015), likely due to the ease of measurement and the value of information obtained from the measurement. Therefore, multiple consensus guidelines have been issued in an attempt to standardize the measurement and thereby ensure high-quality research (Laurent *et al.*, 2006; Van Bortel *et al.*, 2012; Townsend *et al.*, 2015).While these guidelines have been useful in standardizing the measurement of PWV, none of the current guidelines have detailed how, or even if, hydration status affects the measurements of arterial stiffness.

Decreases in hydration status (i.e. 'hypohydration' or 'dehydration', hereby referred to the latter throughout for consistency) is reflected by changes in blood volume, and there is some evidence that changes in blood volume can acutely affect arteries. Chronic kidney disease patients who undergo dialysis experience large changes in blood volume (Katzarski, 1996) which leads to acute reductions in arterial stiffness (Covic *et al.*, 2000; Di Iorio *et al.*, 2010). It is unknown if changes also will occur when blood volume changes during periods of dehydration in healthy individuals.

There is some mechanistic rationale as to why dehydration may affect arterial stiffness. In healthy young adults, dehydration reduces endothelial function (Arnaoutis *et al.*, 2016), and reductions in endothelial function are strongly correlated

with increased PWV (McEniery *et al.*, 2006). However, it is possible that hydration status affects measures of arterial stiffness through multiple mechanisms. For example, the mode of dehydration, whether through exercise in the heat (Arnaoutis *et al.*, 2016) or reductions in total body water by dialysis (Covic *et al.*, 2000; Di Iorio *et al.*, 2010), should be considered.

The importance of examining dehydration in conjunction with heat stress and PWV is highlighted by the increased research in this area (Brunt *et al.*, 2016; Kaldur *et al.*, 2016; Moyen *et al.*, 2016b). With an acute exposure to passive heating, some studies have shown improvements in PWV (Ganio *et al.*, 2011), but not all (Kaldur *et al.*, 2016; Moyen *et al.*, 2016b). Heat stress leads to dehydration (i.e. a reduction in blood volume) if not fully compensated for with fluid intake. There is little evidence that prior studies investigating PWV and heat stress controlled hydration status prior to or during heat stress (Ganio *et al.*, 2011; Kaldur *et al.*, 2016; Moyen *et al.*, 2016b). Given the variability of sweating between individuals and lack of control for hydration status, it is possible that varying levels of dehydration partially explain conflicting findings of studies examining the effect of heat stress on arterial stiffness.

Overall, the increased use of PWV in both clinical and research settings confirms the need for standardization of testing parameters, such as hydration status. Thus, the overall purpose of this research was to assess whether dehydration affects the measurement of PWV in healthy, young adults. The primary objective of experiment 1 was to examine the influence of hydration status on PWV at a normothermic baseline. In the second experiment (i.e. experiment 2), individuals began well hydrated, and we examined whether progressive dehydration during passive heat stress affected PWV. Overall, we hypothesized that dehydration would alter peripheral and central PWV at normothermic baseline and during passive heat stress.

Methods

Ethical approval

Written informed consent was obtained from all subjects before participating in this study. Study procedures and the informed consent were approved by the Institutional Review Board at the University of Arkansas and were in accordance with current guidelines of the *Declaration of Helsinki*.

Study design

To determine the effects of dehydration on measures of PWV at rest and during heat stress, two separate experimental studies were completed at the Human Performance Laboratory within the University of Arkansas as part of larger studies involving male and female participants. Some gender differences exist between the experiments which led to large differences in baseline central PWV (mean difference 146 [40]cm s^{-1}; $P<0{\cdot}01$) and peripheral PWV (mean difference 141[49]cm s^{-1}; $P<0{\cdot}01$). However, gender differences were negligible with passive heating between experiments ($P = 0{\cdot}53$ and $0{\cdot}46$, central and peripheral PWV, respectively).

Within each experiment, subjects were tested in a randomized order separated by a minimum of 48 h. In experiment 1, subjects arrived either in a euhydrated state and maintained euhydration by ingesting warm water (37°C) throughout the trial at a rate to minimize body mass loss (Trial 'E-E'), or arrived in a dehydrated state and did not receive water throughout heating (Trial 'D-D'). Subjects were passively heated to 1°C elevation in rectal temperature. In experiment 2, subjects were euhydrated at the beginning of two trials and were then passively heated to 1°C elevation in rectal temperature. However, in one trial, subjects received warm water (37°C) throughout the trial to remain euhydrated (Trial 'E-E'), and in the other trial, they did not receive fluid, thus becoming progressively dehydrated during passive heating (Trial 'E-D'). In both experiments, measures of PWV were obtained at a normothermic baseline and at a 0·5°C, and 1°C elevation in rectal temperature.

In experiment 1, subjects were provided with a digital scale (BalanceFrom High Accuracy Bathroom Scale, BalanceFrom LLC, China) to take home for 1 week. Subjects, in a euhydrated state, recorded their morning body masses before consuming breakfast and without clothing for five consecutive days. The average of the five body masses were used to establish a baseline body mass for the two subsequent experimental trials (Cheuvront & Kenefick, 2014). Prior to the start of the E-E and E-D experimental trials, subjects were encouraged to consume an additional 1000 ml of water the night before testing and 500 ml, 2–3 h prior to arrival at the laboratory. In the D-D trial, subjects only consumed ~250 ml of water (a water bottle was provided to all subjects) over the 24 h prior to the start of the trial and avoided foods with high moisture content (e.g. soups and fruits). Subjects recorded food consumed for 24 h prior to their first trial and replicated this diet for the 24 h preceding the subsequent trial. Subjects refrained from alcohol and exercise for 24 h, food for 4 h and caffeine for 12 h before participating in any trial. Pretest compliance was verified with a 24-hour history questionnaire upon arrival each day.

Subjects

Table 1 provides an overview of subject characteristics. Subjects were excluded if they were smokers, taking medications, hypertensive (resting systolic blood pressure > 139 mmHg), or reported any cardiovascular, metabolic or neurological diseases. Furthermore, at a familiarization visit, subjects had body composition determined via dual-energy X-ray absorptiometry (DXA). Female subjects were only tested during the early follicular phase of the menstrual cycle (days 1–9) to

Table 1 Subject characteristics, mean (*SD*).

	Experiment 1	Experiment 2
Sex (males / females)	0 / 19	11 / 0
Age (y)	23·0 (3·8)	24·4 (2·7)
Height (cm)	162·9 (5·8)	179·0 (4·9)
Body mass (kg)	72·7 (17·4)	76·7 (9·1)
Body mass index (kg/m^2)	27·3 (6·1)	24·0 (2·4)
Adiposity (% Body Fat)	36·5 (12·3)	18·1 (7·3)

control for the thermoregulatory responses that are affected by menstrual cycle (Stephenson & Kolka, 1985; Moyen *et al.*, 2016a).

Experimental protocol

In both experiments, upon arrival to the laboratory, subjects voided their bladder, providing a small urine sample, and obtained a nude body mass. To classify hydration status, a urine specific gravity (U_{SG}) of ≤1·020 was designated as euhydrated, and a urine specific gravity >1·020 was dehydrated (Sawka *et al.*, 2007; Armstrong *et al.*, 2010). If subjects reported to the E-E or E-D trial with a U_{SG} > 1·020, they were given ~500 ml of water to drink and did not start the trial until they produced a urine sample with a U_{SG}≤1·020.

Subjects were then dressed in a water-perfused, tube-lined suit that covers the entire body, except the head, face, hands and feet (Allen-Vanguard Technologies, Ottawa, Canada). This suit permits the control of skin and core temperature by changing the temperature of the water perfusing the suit. Subjects then laid supine for ~30 min while warm water, 34°C (i.e. normothermic skin temperature), was perfused through the suit. After this period, a blood sample was obtained and passive heating began by perfusing 49°C water through the suit. Measures (described below) were obtained just prior to heating (baseline) and at 0·5°C and 1·0°C rectal temperature increase. After the last measure, another 10 ml blood sample was obtained and cold water was immediately perfused through the suit to cool subjects. Once subjects were cooled and all instrumentation removed, another nude body mass was obtained and subjects provided another urine sample.

Experimental measures

Core body temperature was measured via a rectal thermometer (Physitemp Inc., Clifton, NJ) inserted 15 cm past the anal sphincter. Skin temperature thermocouples were taped to the skin on the right side of the body. Mean skin temperature was calculated as previously described (Ramanathan, 1964) by weighting skin temperature from the right anterior thigh, chest, lateral calf, and triceps. Blood pressure was measured with an automated sphygmomanometer blood pressure cuff in duplicate at each time point (Tango+; SunTech Medical, Inc., Morrisville, NC). Mean arterial pressure (MAP) was calculated as one-third systolic plus two-thirds diastolic. Subjects were also fitted with a heart rate monitor (Polar Electro Inc., Lake Success, NY). Heart rate (HR), rectal and skin temperature were sampled continuously via a data acquisition system (LabChart 7, ADInstruments, Colorado Springs, CO) and averaged at each time point over the period in which PWV measurements took place. Pre- and postblood samples were analysed for serum osmolality (S_{osm}). These, along with urine osmolality (U_{osm}), were analyzed using freezing point depression osmometry (model 3250, Advanced Instruments, Norwood, MA).

PWV is the preferred method to evaluate arterial compliance given that PWV is proportional to the inverse of the square root of compliance; thus, PWV decreases as arterial compliance increases (Laurent *et al.*, 2006). Specifically, PWV was measured with Doppler ultrasound (GE GoldSeal LOGIQ eBT08) and calculated as the distance between measurements sites divided by the time delay between the two waveforms (Boccara *et al.*, 2006). A three-lead ECG was utilized to calculate the time delay from the R-wave to the foot of the pulse wave. The time delay was averaged from a minimum of 10 cardiac cycles wherein the standard deviation between the 10 measurements was less than 5 cm s^{-1}. Central PWV was calculated from the carotid and femoral arteries, while peripheral PWV was calculated from carotid and radial arteries using the postanalysis foot-to-foot method (Boccara *et al.*, 2006). Analysis of the ultrasound images was performed by a trained individual who was unaware of the experimental visit (i.e. whether the participant was dehydrated or euhydrated). All PWV measures were performed on the left side of the body with consistent probe location being assured by marking the skin at each trial. Carotid and femoral measurement order was randomized between subjects but consistent within-subject for each trial. The distance between arterial measurement sites for central PWV was calculated as the combined distance from suprasternal notch site to the umbilicus and from the umbilicus to femoral site minus the distance from carotid to the suprasternal notch. The distance between arterial sites for peripheral PWV was calculated as the distance between the suprasternal notch and the radial site minus the distance from the carotid to the suprasternal notch. Distances between sites were calculated for each individual trial by measuring from the proximal side of the ultrasound probe with a commercially available retractable cloth tape measure.

Statistical analysis

Data were analysed using SAS 9·2 (SAS Institute, Cary, NC). Paired samples *t*-tests were used to identify baseline differences in body mass and hydration measures between trials in each experiment. To answer the research question of experiment 1 (i.e. the influence of baseline hydration status on normothermic PWV), repeated-measures ANOVA, with an ANCOVA for differences in MAP, was used to determine differences between the D-D and E-E trial at baseline (i.e. before heat stress). To examine the effect of heat stress and hydration status, a separate two-way repeated-measures

ANOVA, with MAP as a time-varying covariate (Winer *et al.*, 1991), was used for both experiments to examine changes in PWV as rectal temperature increased (Δrectal). Pairwise comparisons with a Bonferroni correction were conducted if a significant main effect or interaction was identified, again with PWV adjusted for MAP. For all other experimental measures, a two-way repeated-measures ANOVA was utilized with pairwise comparisons being made with a Bonferroni correction. All data are reported as mean (SD). Significance was set at $P<0{\cdot}05$.

Results

Experiment 1 (E-E & D-D)

In experiment 1, subjects reported, at the start of the trial, either euhydrated or dehydrated when compared to baseline euhydrated body mass for the E-E and D-D trials, respectively (Table 2). Accordingly, in the D-D trial, subjects had greater ($P<0{\cdot}05$) baseline U_{SG}, U_{osm} and S_{osm} versus the E-E trial (Table 2). In E-E, there were no pre- to postchanges in urinary markers of hydration. In the D-D trial, S_{osm} increased during heating in comparison with the E-E trial ($P<0{\cdot}05$; Table 2). Also, as intended, subjects had a greater reduction in body mass in the D-D trial ($P<0{\cdot}01$; Table 2).

Mean skin temperature increased similarly between trials during heating (35·24 [0·49] to 37·79 [0·49] to 37·89 [0·77]°C at baseline, 0·5 and 1·0°C Δ rectal, respectively; $P<0{\cdot}05$). HR also was not different between trials ($P>0{\cdot}05$) and increased from baseline to 1·0°C Δ rectal ($P<0{\cdot}05$; Table 3). There were no significant differences in MAP between trials or throughout heating ($P>0{\cdot}05$; Table 3).

Normothermic, baseline dehydration resulted in a lower central ($P = 0{\cdot}03$; Fig. 1a), but not peripheral ($P = 0{\cdot}20$; Fig. 1b) PWV versus baseline euhydration when adjusted for MAP as a time-varying covariate. Baseline MAP, but not HR, was slightly lower when dehydrated compared to euhydrated ($P = 0{\cdot}04$; Table 3). Furthermore, a follow-up simple regression analysis demonstrated that the change in MAP explained less than 1% of the variation in central PWV between trials ($r = 0{\cdot}01$; $P>0{\cdot}05$). Therefore, the minor difference in MAP did not influence the observed differences in central PWV.

The omnibus F-test indicated changes in central PWV from baseline to a 1·0°C increase in rectal temperature were dependent on condition (i.e. interaction; $P = 0{\cdot}04$). However, pairwise comparisons revealed no significant differences between trials ($P>0{\cdot}05$). There was not a main effect of trial (611 [69] versus 578 [75] cm s^{-1}, respectively; $P = 0{\cdot}14$). Further, independent of trial, there were no significant changes in central PWV during heating, (600 [74] versus 586 [57] versus 598 [62] cm s^{-1}, for baseline, 0·5°C and 1·0°C Δ rectal respectively; $P = 0{\cdot}42$).

During heat stress, peripheral PWV did not differ between trials ($P = 0{\cdot}58$), but independent of trial peripheral PWV decreased with passive heating ($P<0{\cdot}01$). Specifically, there was a reduction in peripheral PWV from baseline to 0·5°C and 1·0°C Δ rectal temperature (843 [68] versus 737 [73] and 758 [75] cm s^{-1}, respectively; $P<0{\cdot}01$). There were no differences in peripheral PWV between 0·5°C and 1·0°C Δ rectal ($P>0{\cdot}05$).

Experiment 2 (E-E & E-D)

As designed, at the start of the trials, there were no differences ($P>0{\cdot}05$) in any measure of hydration status (Table 4). At the end of the E-D trial, subjects had greater U_{SG}, U_{osm} and S_{osm} ($P<0{\cdot}05$; Table 4) compared to E-E. In the E-D trial, subjects lost a greater amount of body mass versus E-E ($P<0{\cdot}01$; Table 4).

Similar to experiment 1, mean skin temperature did not differ between trials ($P>0{\cdot}05$), but increased significantly during

Table 2 Mean (SD) hydration status for experiment 1.

	E-E	D-D
Body Mass (kg)		
Baseline euhydrated body mass	72·6 (16·7)	72·6 (16·7)
Pre	72·5 (16·7)	71·8 (16·6)
Post	72·2 (16·9)	71·0 (16·5)
Change (Post/Pre)	−0·5 (0·6) %	−1·1 (0·5)%[a]
Change (Prebaseline)	−0·1 (1·1) %	−1·2 (1·0)%[a]
Urine specific gravity		
Pre	1·007 (0·005)	1·025 (0·004)[a]
Post	1·011 (0·006)	1·028 (0·004)[a]
Change (Post/Pre)	0·004 (0·005)	0·003 (0·003)
Urine osmolality (mmol/kg)		
Pre	299·6 (197·6)	987·4 (209·3)[a]
Post	394·6 (94·9)	1071·0 (110·2)[a]
Change (Post/Pre)	94·9 (209·9)	83·5 (87·9)
Serum osmolality (mmol kg^{-1})		
Pre	284·1 (2·3)	288·3 (3·2)[a]
Post	283·6 (3·7)	293·3 (3·9)[a]
Change (Post/Pre)	−0·5 (3·5)	5·0 (2·7)[a]

Euhydrated body mass is the average of 5 consecutive morning, fasted, euhydrated nude body weights.
[a]indicates significant difference from E-E trial at the corresponding time point ($P<0{\cdot}05$). Subjects began euhydrated and remained euhydrated (E-E) or began euhydrated and became dehydrated (E-D) during heating.

Table 3 Mean (SD) hemodynamic responses for experiment 1.

	Baseline	0·5°C	1·0°C
Mean arterial pressure (mmHg)			
E-E	80·3 (7·1)	80·1 (7·0)	78·2 (6·6)
D-D	74·7 (6·6)[a]	77·3 (10·1)	78·0 (9·6)
Heart rate (bpm)			
E-E	67 (9)	89 (12)[b]	99 (13)[b]
D-D	69 (10)	85 (11)[b]	102 (10)[b]

Subjects began euhydrated and remained euhydrated (E-E) or began dehydrated and continued dehydrated (D-D) during heating.
[a]Indicates significant difference, paired t-test, from E-E trial at the corresponding time point ($P<0{\cdot}05$).
[b]Indicates significant difference from baseline ($P<0{\cdot}05$).

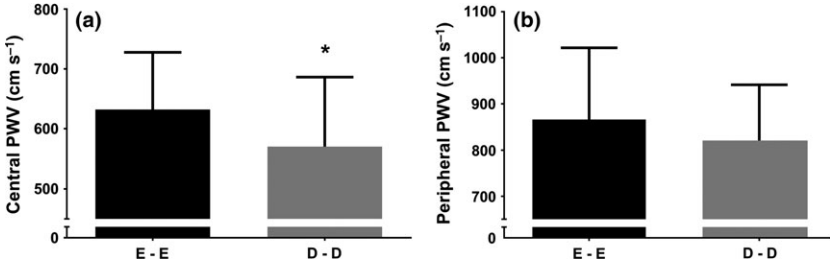

Figure 1 Mean (±SD) (a) central and (b) peripheral pulse wave velocity (PWV) in experiment 1 at baseline (rested; no heat stress) between trials E-E (arriving euhydrated) and D-D (arriving dehydrated). * Indicates a significant difference P<0·05 between experimental trials.

Table 4 Mean (SD) hydration status for experiment 2.

	E-E	E-D
Body mass (kg)		
Pre	73·8 (9·1)	73·8 (8·5)
Post	73·7 (9·0)	72·6 (0·6)
Change (Post/Pre)	−0·1 (0·7)%	−1·6 (0·6)%[a]
Urine specific gravity		
Pre	1·009 (0·007)	1·011 (0·006)
Post	1·005 (0·002)	1·012 (0·007)[a]
Change (Post/Pre)	−0·004 (0·007)	0·001 (0·010)
Urine osmolality (mmol kg^{-1})		
Pre	309·7 (190·7)	424·9 (209·3)
Post	200·0 (59·0)	465·9 (209·3)[a]
Change (Post/Pre)	−109·8 (195·2)	41·4 (359·5)
Serum osmolality (mmol kg^{-1})		
Pre	290·4 (3·1)	289·5 (4·2)
Post	284·4 (3·8)	290·3 (2·6)[a]
Change (Post/Pre)	−5·9 (4·3)	0·8 (3·2)[a]

Subjects began euhydrated and remained euhydrated (E-E) or began dehydrated and continued to dehydrate (D-D) during heating.
[a]indicates significant difference from E-E trial at the corresponding time point (P<0·05).

Table 5 Mean (SD) hemodynamic responses for experiment 2.

	Baseline	0·5°C	1·0°C
Mean Arterial Pressure (mmHg)			
E-E	85·3 (5·3)	80·9 (10·0)	80·3 (9·9)
E-D	82·9 (6·1)	82·7 (7·7)	82·9 (12·8)
Heart Rate (bpm)			
E-E	58 (15)	82 (16)[a]	92 (19)[a]
E-D	60 (12)	83 (18)[a]	95 (19)[a]

[a]Indicates significant difference from baseline (P<0·05). Subjects began euhydrated and remained euhydrated (E-E) or began euhydrated and dehydrated (E-D) throughout passive heating.

passive heating (grand mean = 33·10 [3·00] to 37·70 [3·50] to 38·00 [3·70]°C, baseline to 0·5 to 1·0°C rectal temperature increase, respectively; P<0·05). Overall, there were no differences in HR between trials, but HR did increase over time with heating (Table 5; P<0·05). There were no changes in MAP during heating or between trials (Table 5; P>0·05).

Central PWV did not change during heating between trials (P = 0·15), and there were no overall differences between trials (P = 0·78; Fig. 2a). Peripheral PWV also did not differ between conditions during passive heating (P = 0·55; Fig. 2b). However, independent of condition, peripheral PWV decreased from baseline (710 [62] cm s^{-1}) to 0·5°C (637 [57] cm s^{-1}) and 1·0°C (640 [49] cm s^{-1}; P<0·01). There were no differences in peripheral PWV between 0·5°C and 1·0°C Δ rectal (P>0·05).

Discussion

Although evidence suggests that fluid balance can affect measures of arterial stiffness, to our knowledge no one has systematically modified hydration status prior to measures of arterial stiffness in a repeated-measures fashion. Likewise, heat stress may (Mitchel *et al.*, 1994; Ganio *et al.*, 2011; Kaldur *et al.*, 2016) or may not (Moyen *et al.*, 2016b) alter measures of arterial stiffness, and it is possible that differences in hydration status before and/or during heat stress confounded previous data. Considering this work, we utilized two separate experiments to test whether hydration status affected normothermic central and peripheral PWV (experiment 1), and whether hydration status during passive heating affected PWV (experiment 2). Our main findings are that normothermic, central PWV, but not peripheral PWV, was significantly reduced when subjects were dehydrated (Fig. 1). However, hydration status, both prior to and during heating, did not alter either peripheral or central PWV responses to passive heating (Fig. 2).

Our data support the notion that overall body fluid balance can influence the measurement of arterial stiffness. In patients with chronic kidney disease, arterial stiffness is reduced in direct proportion to the blood volume loss that occurs during dialysis (Covic *et al.*, 2000; Gusbeth-Tatomir & Covic, 2007; Di Iorio *et al.*, 2010). In our study, we systematically manipulated hydration status via fluid restriction, which led to approximately a 1·2% reduction in body mass. Urinary and blood markers of hydration status confirmed that the subjects were mildly dehydrated in comparison with the E-E trial (Table 2). Despite minor changes in body mass, baseline central PWV was reduced by ~10% when subjects arrived dehydrated (Fig. 1). Because fluid restriction leads to hypovolemic hyperosmotic dehydration (Cheuvront & Kenefick, 2014), we are unable to determine whether changes in central PWV were an osmotic or volume-driven response. Future research should attempt to isolate different types of dehydration to observe if

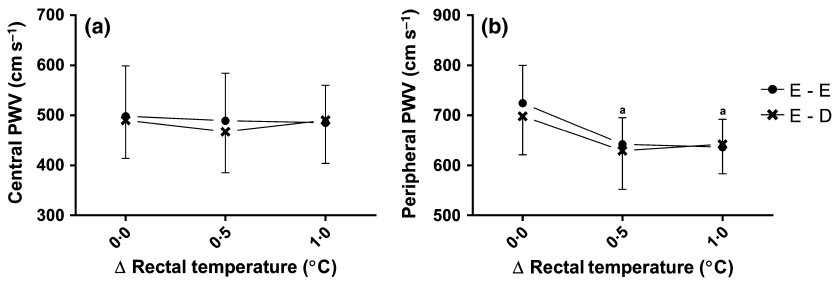

Figure 2 Mean (±SD) (a) central and (b) peripheral pulse wave velocity (PWV) in experiment 2 between trials E-E, where subject arrive and stay euhydrated, and E-D where subjects arrive euhydrated but do not receive water during passive heating. [a]Indicates a significant difference $P<0{\cdot}05$ from normothermic baseline, independent of trial.

volume and osmotic stresses have independent or combined effects on the measurement of PWV.

However, previous research (Covic *et al.*, 2000; Di Iorio *et al.*, 2010) indicates that the effect of dehydration on PWV is likely a volume-driven response. The reduction in blood volume during dehydration may in turn lead to lower shear stress in the artery, which is known to influence arterial stiffness (Glagov *et al.*, 1993). Also, blood pressure can influence the measurement of arterial stiffness (Stewart *et al.*, 2003). Hypovolaemia can acutely reduce blood pressure (Coble *et al.*, 2015) and thereby reduce arterial stiffness. Multiple analyses have concluded that dialysis causes acute hypovolaemia which drives a reduction in arterial stiffness and blood pressure (Covic *et al.*, 2000; Di Iorio *et al.*, 2010). However, while both blood pressure and central PWV are reduced following dialysis, these changes appear to be independent of one another (Agarwal & Light, 2008). In accordance with the current guidelines (Townsend *et al.*, 2015), we collected and reported MAP throughout both experiments. In our analyses, we adjusted for MAP as a time-varying covariate within a repeated-measures design in a fashion previously described by Winer *et al.* (1991). Despite this adjustment, central PWV was significantly reduced when individuals were dehydrated (D-D trial) at baseline. Therefore, while it is prudent to collect MAP while measuring PWV, adjusting for differences in MAP does not eliminate the effect of dehydration on PWV. Therefore, the reduction in central PWV in experiment 1 is a result of dehydration that is not affected by fluctuations in MAP.

Reduced arterial stiffness is typically perceived as beneficial because it indicates a more compliant artery, and a chronically lower PWV is associated with lower risk of a cardiac event (Townsend *et al.*, 2015). Despite our findings that acute dehydration reduces central stiffness, it is not advisable to purposefully reduce fluid intake given the medical maladies associated with chronic dehydration (Chan *et al.*, 2002; Strippoli *et al.*, 2011). Further, recent research would suggest that acute dehydration can negatively affect endothelial function (Arnaoutis *et al.*, 2016). Clearly, more research is required to fully understand the effects of hydration on arterial health and how the vasculature reacts to acute and chronic dehydration. Regardless of these seemingly contradictory findings, and more salient to the purpose of this study, we show that acute dehydration affects measures of central PWV; thus, hydration status needs to be controlled for prior to PWV measurements.

This study also examined the effect of acute dehydration during passive heating on PWV. In experiment 1, subjects arrived 1·2% dehydrated (with a further 1·1% reduction during heating), and in experiment 2, subjects had a 1·6% reduction in body mass during passive heating. Measures of PWV during heating in both experiments were not influenced by hydration status (Fig. 2). It is important to point out that the baseline, normothermic differences in central PWV disappeared when PWV was measured during heating. This suggests that, from a methodological standpoint, if measures of PWV during passive heating are of interest, mild dehydration is not of concern.

Independent of hydration status, we observed significant reductions in peripheral PWV during passive heating. Our findings indicate that differences in hydration status during heating do not explain why some research shows changes in PWV with passive heating (Ganio *et al.*, 2011) and some does not (Moyen *et al.*, 2016b). There may be other reasons why we observed reductions in peripheral PWV with heat stress. These changes were likely observed in the peripheral PWV, unlike the central PWV, considering the muscular (peripheral) arteries have a greater capacity to dilate. During heat stress, vasodilation occurs in the periphery to increase blood flow to the skin in attempt to maximize heat loss. Overall, heat stress acts as an arterial vasodilator, much like nitrates (Pauca *et al.*, 2005), which reduces stiffness of the peripheral, but not central, arteries. Interestingly, this effect occurs independent of changes in brachial blood pressure (Pauca *et al.*, 2005).

Also, many have proposed that changes in peripheral or central arterial stiffness during heat stress may be dependent upon normothermic arterial stiffness (Ganio *et al.*, 2011; Kaldur *et al.*, 2016; Moyen *et al.*, 2016b). At a young age, the central, elastic, arteries are very compliant already, while the peripheral, muscular arteries are relatively stiff. Therefore, there may be a greater capacity for a reduction in stiffness in the peripheral arteries (i.e. floor effect). Regardless of the mechanism, further studies should examine the effect of heat stress on arterial stiffness, especially given that long-term sauna use (i.e. increase in core temperature) reduces incidences of sudden cardiac death, fatal coronary heart disease and fatal cardiovascular disease (Laukkanen *et al.*, 2015).

Limitations

In this study, we utilized a standard fluid restriction protocol that is effective in inducing dehydration (Tucker *et al.*, 2016). However, we did not directly measure changes in total body water or blood volume in this study so we are unable to definitively determine the magnitude of blood volume or total body water reduction that occurred. Further, subjects in this study only experienced mild dehydration, as defined by body mass loss (Cheuvront & Kenefick, 2014), during passive heating. Nonetheless, the amount of heat stress that subjects underwent is comparable to most studies involving passive heating (Ganio *et al.*, 2011; Kaldur *et al.*, 2016; Moyen *et al.*, 2016b).

Also, it should be acknowledged that the primary finding of changes in normothermic central PWV (Fig. 1) occurred in the experiment that only included females. Although some studies have reported differences between PWV in males and females (Ahimastos *et al.*, 2003; Niboshi *et al.*, 2006), there is no evidence to suggest that the acute response to a perturbation (such as dehydration) would differ between males and females. This is supported by the fact that in both experiments (i.e. both males and females), we observed an identical response with regard to peripheral PWV. Nonetheless, until further research is conducted, our findings regarding hydration status affecting baseline, normothermic central PWV can only be applied to young females.

Conclusions

In young healthy adults, hydration status affects the measurement of central PWV. Our fluid restriction protocol induced only mild dehydration (i.e. <2% loss in body mass) and given that this mild dehydration commonly occurs in day-to-day living (Cheuvront & Kenefick, 2014), it is important for clinicians to evaluate hydration status prior to PWV measurements. However, mild changes in hydration status that occur during heat stress do not confound PWV measures. Future research utilizing measures of arterial stiffness should use simple measures of hydration status (e.g. urine specific gravity via a refractometer) and attempt to standardize hydration status between visits and individuals.

Further, our results indicate that passive heat stress can acutely reduce peripheral, but not central, PWV. This effect is likely the result of dilation occurring in the periphery to increase skin blood flow. Overall, it appears that passive heat stress tends to have positive effects on the vasculature with clinical trials indicating improvements in both macrovascular and microvascular functions (Brunt *et al.*, 2016).

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Conflict of Interest

The authors declare no conflict of interest.

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References

Agarwal R, Light RP. Arterial stiffness and interdialytic weight gain influence ambulatory blood pressure patterns in hemodialysis patients. *Am J Physiol Renal Physiol* (2008); **294**: F303–F308.

Ahimastos AA, Formosa M, Dart AM, *et al.* Gender differences in large artery stiffness pre- and post puberty. *J Clin Endocrinol Metab* (2003); **88**: 5375–5380.

Armstrong LE, Pumerantz AC, Fiala KA, *et al.* Human hydration indices: acute and longitudinal reference values. *Int J Sport Nutr Exerc Metab* (2010); **20**: 145–153.

Arnaoutis G, Kavouras SA, Stratakis N, *et al.* The effect of hypohydration on endothelial function in young healthy adults. *Eur J Nutr* (2016); **56**: 1211–1217.

Boccara F, Simon T, Lacombe K, *et al.* Influence of pravastatin on carotid artery structure and function in dyslipidemic HIV-infected patients receiving antiretroviral therapy. *AIDS* (2006); **20**: 2395–2398.

Brunt VE, Eymann TM, Francisco MA, *et al.* Passive heat therapy improves cutaneous microvascular function in sedentary humans via improved nitric oxide-dependent dilation. *J Appl Physiol* (1985) (2016); **121**: 716–723. jap 00424 02016.

Chan J, Knutsen SF, Blix GG, *et al.* Water, other fluids, and fatal coronary heart disease: the Adventist Health Study. *Am J Epidemiol* (2002); **155**: 827–833.

Cheuvront SN, Kenefick RW. Dehydration: physiology, assessment, and performance effects. *Compr Physiol* (2014); **4**: 257–285.

Coble JP, Grobe JL, Johnson AK, *et al.* Mechanisms of brain renin angiotensin system-induced drinking and blood pressure: importance of the subfornical organ. *Am J Physiol Regul Integr Comp Physiol* (2015); **308**: R238–R249.

Covic A, Goldsmith DJ, Panaghiu L, *et al.* Analysis of the effect of hemodialysis on peripheral and central arterial pressure waveforms. *Kidney Int* (2000); **57**: 2634–2643.

Di Iorio B, Nazzaro P, Cucciniello E, *et al.* Influence of haemodialysis on variability of pulse wave velocity in chronic haemodialysis patients. *Nephrol Dial Transplant* (2010); **25**: 1579–1583.

Ganio MS, Brothers RM, Shibata S, *et al.* Effect of passive heat stress on arterial stiffness. *Exp Physiol* (2011); **96**: 919–926.

Glagov S, Zarins CK, Masawa N, *et al.* Mechanical functional role of non-atherosclerotic intimal thickening. *Front Med Biol Eng* (1993); **5**: 37–43.

Gusbeth-Tatomir P, Covic A. Causes and consequences of increased arterial stiffness in chronic kidney disease patients. *Kidney Blood Press Res* (2007); **30**: 97–107.

Kaldur T, Unt E, Ööpik V, *et al.* The acute effects of passive heat exposure on arterial stiffness, oxidative stress, and inflammation. *Medicina* (2016); **52**: 211–216.

Katzarski KS. Monitoring of blood volume during haemodialysis treatment of acute renal and multiple organ failures. *Nephrol Dial Transplant* (1996); **11**(Suppl 8): 20–23.

Laukkanen T, Khan H, Zaccardi F, *et al.* Association between sauna bathing and fatal cardiovascular and all-cause mortality events. *JAMA Intern Med* (2015); **175**: 542–548.

Laurent S, Cockcroft J, Van Bortel L, *et al.* , European Network for Non-invasive Investigation of Large A. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* (2006); **27**: 2588–2605.

McEniery CM, Wallace S, Mackenzie IS, *et al.* Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension* (2006); **48**: 602–608.

Mitchel JF, Fram DB, Aretz TA, *et al.* Effect of low-grade conductive heating on vascular compliance during *in vitro* balloon angioplasty. *Am Heart J* (1994); **128**: 21–27.

Moyen N, Burchfield J, Butts C, *et al.* Effects of obesity and mild hypohydration on local sweating and cutaneous vascular responses during passive heat stress in females. *Appl Physiol Nutr Metab* (2016a); **41**: 879–887. In Press.

Moyen NE, Ganio MS, Burchfield JM, *et al.* Effect of passive heat stress on arterial stiffness in smokers versus non-smokers. *Int J Biometeorol* (2016b); **60**: 499–506.

Niboshi A, Hamaoka K, Sakata K, *et al.* Characteristics of brachial-ankle pulse wave velocity in Japanese children. *Eur J Pediatr* (2006); **165**: 625–629.

Pauca AL, Kon ND, O'Rourke MF. Benefit of glyceryl trinitrate on arterial stiffness is directly due to effects on peripheral arteries. *Heart* (2005); **91**: 1428–1432.

Ramanathan NL. A new weighting system for mean surface temperature of the human body. *J Appl Physiol* (1964); **19**: 531–533.

Sawka MN, Burke LM, Eichner ER, *et al.* American college of sports medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc* (2007); **39**: 377–390.

Stephenson LA, Kolka MA. Menstrual cycle phase and time of day alter reference signal controlling arm blood flow and sweating. *Am J Physiol* (1985); **249**: R186–R191.

Stewart AD, Millasseau SC, Kearney MT, *et al.* Effects of inhibition of basal nitric oxide synthesis on carotid-femoral pulse wave velocity and augmentation index in humans. *Hypertension* (2003); **42**: 915–918.

Strippoli GF, Craig JC, Rochtchina E, *et al.* Fluid and nutrient intake and risk of chronic kidney disease. *Nephrology* (2011); **16**: 326–334.

Townsend RR, Wilkinson IB, Schiffrin EL, *et al.* , American Heart Association Council on H. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: a Scientific Statement From the American Heart Association. *Hypertension* (2015); **66**: 698–722.

Tucker MA, Gonzalez MA, Adams JD, *et al.* Reliability of 24-h void frequency as an index of hydration status when euhydrated and hypohydrated. *Eur J Clin Nutr* (2016); **70**: 908–911.

Van Bortel LM, Laurent S, Boutouyrie P, *et al.* , European Society of Hypertension Working Group on Vascular S, Function, European Network for Noninvasive Investigation of Large A. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens* (2012); **30**: 445–448.

Winer BJ, Brown DR, Michels KM. *Statistical Principles in Experimental Design* (1991). McGraw-Hill, NewYork.