The Effects of a Hydration Intervention on Cardiac Function, Autonomic Activity, and Cerebral Oxygenation during Phlebotomy

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the faculty of
the College of Arts and Sciences of Ohio University

In partial fulfillment
of the requirements for the degree
Master of Science

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This thesis titled
The Effects of a Hydration Intervention on Cardiac Function, Autonomic Activity, and Cerebral Oxygenation during Phlebotomy

by
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has been approved for
the Department of Psychology
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Abstract

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The Effects of a Hydration Intervention on Cardiac Function, Autonomic Activity, and Cerebral Oxygenation during Phlebotomy

Director of Thesis: Stephen M. Patterson

Many medical procedures rely on donated blood and unfortunately shortages do occur. Retention of donors is hindered in part by vasovagal reactions in response to blood donation. Research indicates donors’ symptoms can be reduced by consuming water before donating, but no study has investigated the physiology underlying the efficacy of water consumption in a blood donation context. Therefore, the present study investigated the effects of a water intervention on cardiovascular, autonomic, and cerebral function during phlebotomy. Participants randomized to either a predonation hydration intervention (PHI) \((n = 14)\) or a control group \((n = 15)\) completed a baseline period, a blood draw, and a recovery period. Plasma osmolality was significantly lower at the end of the blood draw in the PHI than in the control group, indicating that the PHI was more hydrated. Across the blood draw and recovery periods, participants in the PHI had lower blood pressure, pulse pressure, and baroreflex sensitivity than participants in the control group. Although prior research in non-blood donation contexts indicates water consumption in healthy, young individuals produces either no change or a slight increase in blood pressure, the current study found that relative to a control group, a PHI reduces blood pressure during phlebotomy. The reduction in blood pressure may be due to reductions in pulse pressure and baroreflex sensitivity.
Acknowledgements

First and foremost, I would like to thank my mentor, Dr. Stephen Patterson, for his expertise, advice, and support. I sincerely appreciate his help with the current study as well as my other research and academic pursuits in graduate school thus far. Additionally, I would like to thank three fellow graduate students for their help in collecting and analyzing this study’s data, Matthew Wilkinson, Katrina Hamilton, and Cari Hollenbeck. Their dedication, talent, humor, and intelligence were integral to finishing this study. I would also like to thank my dissertation committee members, Drs. Christopher France and Peggy Zoccola for their advice, feedback, and support that were critical to fine-tuning and completing this study. Furthermore, I would like to thank Dr. Thad Wilson for generously allowing me to use his lab space and physiology equipment to conduct my study and for his expert advice and guidance that contributed to the study design. I am also thankful for Nurse Lauren Mente’s help with performing the study protocol and in particular drawing blood from all of the participants. Lastly, I would like to thank my wife, Dr. Farhana Hamid-Scanlin and my parents, Jerry and Robin Scanlin, for their support and love throughout this entire process.
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Introduction

Currently, no viable synthetic blood supply for transfusions exists. Therefore, blood donors are vital to maintaining an adequate blood supply for the 5 million annual patients who receive transfusions for procedures related to traumatic injuries, cancers, sickle-cell anemia, and other conditions (The American Red Cross, 2014). Despite the ever present demand for blood, temporary regional and seasonal shortages in its supply occur, in part due to the limited number of blood donors. In fact, only 38% of the U.S. population is eligible to donate blood and worse yet, less than 10% of Americans do so in a given year. This is problematic since half of the national blood supply is transfused to Americans over 65 years old and this population is expected to nearly double by 2030 (Centers for Disease Control and Prevention [CDC], 2013). In order to reduce the shortages in the blood supply that occur presently and are only expected to worsen in the future, research is needed to help the American Red Cross and other blood collection agencies increase recruitment of blood donors.

To address this need, researchers have investigated methods to improve the blood donation experience and in particular on how to attenuate vasovagal reactions (e.g. dizziness, nausea, and fainting), which are experienced by as much as 16% of blood donors and pose a hindrance to retention of donors (Eder, Hillyer, Dy, Notari, & Benjamin, 2008a; Eder et al., 2008b; France, Rader, & Carlson, 2005; Newman, 2002; Newman, Pichette, Pichette, & Dzaka, 2003). More specifically, higher subjective ratings of adverse vasovagal sensations in response to blood donation are negatively associated with blood donors’ intention to donate again in the future (Ditto & France, 2006a; Ditto & France, 2006b; Hanson & France, 2009; Olatunji, Etzel, & Ciesielski,
2010) and with their return behavior (Ditto & France, 2006b; France, France, Roussos, & Ditto, 2004; France et al., 2005). Although vasovagal reactions can be uncomfortable and at times dangerous for blood donors, they are not unexpected and are believed to be an evolutionarily beneficial response to hemorrhaging (Alboni, Alboni, & Bertorelle, 2008). However, during blood donation, where a non-life-threatening amount of blood is being intentionally withdrawn, vasovagal reactions are maladaptive and adverse.

In light of this problem, researchers have proposed interventions aimed at attenuating or preventing the vasovagal response among blood donors. The two most prominently studied are an applied muscle tension (AMT) intervention and a predonation hydration intervention (PHI). Previous research indicates that both AMT (repeatedly contracting and relaxing muscles of the abdomen, buttocks, and legs every five seconds) and a PHI (self-administration of a specific volume of water before donating blood) produce significant reductions in donors’ vasovagal symptoms as reported by the donors themselves, nurses, and phlebotomists (Ando et al., 2009; Ditto, Byrne, & Holly, 2009; Ditto & France, 2006a; Ditto, France, Albert, & Byrne, 2007; Ditto, Wilkins, France, Lavoie, & Adler, 2003; France et al., 2010; Hanson & France, 2004; Holly, Torbit, & Ditto, 2012; Newman et al., 2007). However, while researchers have established that each of these interventions are efficacious at reducing vasovagal symptoms in blood donors, less is known about the underpinning physiological mechanisms of these interventions’ vasovagal symptom attenuating effects.

Research on the physiological effects of these interventions is warranted because they are targeted at reducing vasovagal reactions, which during blood donation are due to a collection of physiological responses to hypovolemia (Claydon & Hainsworth, 2003;
Silvani et al., 1990; Triedman, Cohen, & Saul, 1993), acute orthostatic challenge (Fu, Witkowski, Okazaki, & Levine, 2005; Goswami et al., 2009; Grasser, Goswami, Rössler, Vrecko, & Hinghofer-Szalkay, 2009), and/or anxiety (Ditto & France, 2006b; Holly, Balegh, & Ditto, 2011; Labus, France, & Taylor, 2000; Viar, Etzel, Ciesielski, & Olatunji, 2010). Vasovagal reactions are physiologically characterized by an initial increase in sympathetic nervous system (SNS) stimulation followed by a reduction in SNS and an increase in parasympathetic nervous system (PNS) stimulation (Alboni et al., 2008; Hainsworth, 2004). The end result can include: vasodilation (widening of the vasculature’s diameter), bradycardia (a heart rate [HR] below 60 beats per minute), a reduction in cardiac output (CO, the amount of blood the heart’s left ventricle ejects in a minute) and impairments in cardiac baroreflex sensitivity (BRS), which is a measure of how well the heart adjusts to acute changes in blood pressure (BP) in order to maintain BP homeostasis (Alboni et al., 2008; Hainsworth, 2004; Zöllei et al., 2004). Collectively, these responses can cause reductions in BP and consequently decreases in cerebral perfusion as indicated by reductions in cerebral oxygenation (O₂, oxygenated hemoglobin minus deoxygenated hemoglobin within the brain) and impaired cerebral autoregulation (AI, a correlation coefficient between cerebral BP and cerebral blood flow that assesses how well cerebral blood flow is maintained despite cerebral BP changes) during the vasovagal response (Alboni et al., 2008; Claydon & Hainsworth, 2003; Claydon, Schroeder, Norclicfè, Jordan, & Hainsworth, 2006; Hainsworth, 2004; Madsen et al., 1998; Paulson, Strandgaard, & Edvinsson, 1990). Ultimately, the reductions in cerebral perfusion lead to presyncopal vasovagal reactions, and if severe enough, can cause vasovagal syncope (Alboni et al., 2008; Hainsworth, 2004; Wieling et al., 2009).
Researchers have started to study how blood donation interventions affect the pathophysiology of the vasovagal response in blood donors. For example, initial research has found AMT elicits hemodynamic changes that may protect against the vasovagal response during blood donation. More specifically, during a blood donation procedure, participants who performed AMT relative to those in a control condition experienced a pattern of increased SNS stimulation and reduced PNS activity as indicated by a greater heart rate (HR) and diastolic BP (DBP), lower pre-ejection period (PEP; a measure of the time between left ventricular contraction and the aorta valve opening with lower values indicating more SNS activity), a reduction in high frequency heart rate variability (HF HRV, a measure of variability in heart rate indicative of the heart’s PNS activity), and a smaller reduction in CO (Ditto et al., 2009). Additionally, a study by Kowalsky et al. (2011) found that after controlling for participants’ body mass index (BMI) and number of prior blood donations, participants in an AMT condition had an attenuated reduction in \( O_2 \) relative to participants in a control condition. Therefore, AMT’s attenuation of vasovagal symptoms during blood donation is likely due to central cardiovascular changes (i.e. increases in HR and CO) that in turn increase BP and cerebral perfusion.

Although a limited number of studies have investigated the physiological effects of an AMT intervention for blood donors, the present study is the first to investigate the physiological responses (i.e. autonomic, cardiovascular, and cerebral perfusion) to a PHI in a blood donation context. Given the absence of similar studies, the current study’s hypotheses were informed by the findings of multiple studies performed in non-blood donation contexts that indicate water consumption elicits several physiological changes that may prove protective against the pathophysiology of the vasovagal response and
consequently help explain a PHI’s vasovagal-symptom-attenuating effects (Ando et al., 2009; France et al., 2010; Hanson & France, 2004; Newman et al., 2007). Interestingly, water is not a pharmacologically inert substance. In fact, acute increases in hydration are associated with acute decreases in plasma osmolality (POSM; measure of electrolyte-water balance used to assess hydration status) (Endo et al., 2001; Freund, Claybaugh, Hashiro, & Dice, 1988; Joannides, Moore, de la Gueronniere, & Thuillez, 1999; Williams, Seckl, & Lightman, 1989), which if dramatic enough, can stimulate the SNS via osmolality receptors in the hepatic system and result in a pressor response (i.e. acute increase in BP) (May & Jordan, 2011; McHugh et al., 2010).

Two different paradigms have been commonly used to assess the physiological effects of water consumption in healthy, young to middle-aged participants. The first is a prolonged supine or seated rest period and the second is an orthostatic tolerance (OT) test, which measures the time elapsed until induction of vasovagal reactions after progressive tilting of the body and sometimes also combined with lower-body negative pressure. Several of these studies have demonstrated that water consumption increases plasma norepinephrine (NE) levels (Scott, Greenwood, Gilbey, Stoker, & Mary, 2001), muscle sympathetic nerve activity (MSNA) (Callegaro et al., 2007; Scott et al., 2001), vasoconstriction (Brown, Barberini, Dulloo, & Montani, 2005; Callegaro et al., 2007; Claydon et al., 2006; Girona, Grasser, Dulloo, & Montani, 2014; Lu et al., 2003; Nozawa, Yana, Kaeriyama, Mizuta, & Ono, 2009; Schroeder et al., 2002; Scott et al., 2001), pulse pressure (PP, the mathematical difference between systolic and diastolic BP commonly used as a non-invasive index of stroke volume) (Olatunji, Aaron, Michael, & Oyeyipo, 2011), BP (Callegaro et al., 2007; Claydon et al., 2006; Girona et al., 2014; Hai
et al., 2013; Nozawa, et al., 2009; Schroeder et al., 2002), and OT (Claydon et al., 2006; Lu et al., 2003; Olatunji et al., 2011; Schroeder et al., 2002). However, it is important to note that several similar studies have found no change in SNS activity (Joannides et al. 1999; Jordan et al., 2000; Lu et al., 2003), vasoconstriction (Joannides et al., 1999; Schroeder et al., 2002), and BP (Brown et al., 2005; Joannides et al., 1999; Jordan et al., 2000; Lu et al., 2003; Routledge, Chowdhary, Coote, & Townend, 2002; Scott et al., 2001). These mixed findings may be due to methodological differences between studies with regards to sample sizes, the volume or temperature of water participants consumed, physiological monitoring equipment used, and the time duration between consumption of water and the start and end times of physiological measures. Additionally, unlike the elderly or patients with autonomic disorders, water consumption in healthy, young adults increases BRS, which can attenuate or mask the pressor response to water consumption (Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; May & Jordan, 2011; Schroeder et al., 2002). Overall, studies on the physiological effects of water loading are limited in number and their findings are mixed. However, several of these studies suggest that water consumption prior to blood donation may produce a pressor response or at the very least help young, healthy blood donors maintain BP stability during the acute anxiety, hypovolemic, and/or orthostatic challenges that threaten to lower BP and cause vasovagal reactions during blood donation.

As previously discussed, during vasovagal reactions a cascade of cardiovascular changes can lead to reductions in BP and subsequent reductions in cerebral perfusion. Some of the most commonly used measures of cerebral perfusion include $O_2$ and AI (Panerai, 2009; Tobias, 2006). To the author’s knowledge, no studies have investigated
the effect of water loading on $O_2$ in non-blood donation or blood donation contexts. Moreover, no study of blood donation has investigated AI in response to water consumption, but some studies in non-blood donation contexts have found that participants’ AI decreases in a water loading condition relative to a control condition (Claydon et al., 2006; Schroeder et al., 2002). This is notable because prior findings indicate that a lower AI improves time tolerated during OT testing (Claydon & Hainsworth, 2003). As such, water loading before blood donation may reduce donors’ vasovagal symptoms via increased vasoconstriction and BP and as a result improve cerebral perfusion.

Although peripheral cardiovascular and cerebral perfusion responses to water consumption may be protective against vasovagal reactions, central cardiovascular responses to water consumption do not appear to be protective. Several studies investigating the physiological effects of water have demonstrated mixed effects in regards to HF HRV activity, HR, stroke volume (SV, volume of blood ejected by the left ventricle each time it contracts), and CO. Although the majority of studies have found water consumption increases HF HRV (Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Routledge et al., 2002; Schroeder et al., 2002), one study found no change in HF HRV (Schroeder et al., 2002). Similarly, some studies have found water loading decreases HR (Ando et al., 2009; Brown et al., 2005; Callegaro et al., 2007; Claydon et al., 2006; Girona et al., 2014; Lu et al., 2003; Routledge et al., 2002 Schroeder et al., 2002), but others have found no effect (Claydon et al., 2006; Joannides et al., 1999; Jordan et al., 2000; Nozawa et al., 2009; Schroeder et al., 2002; Scott et al., 2001). In regards to SV, previous research has found that it either increases (Brown et al., 2005;
Claydon et al., 2006; Girona et al., 2014; Schroeder et al., 2002) or remains unchanged after water loading (Girona et al., 2014; Lu et al., 2003; Schroeder et al., 2002). Furthermore, the majority of studies have found water consumption does not affect CO (Brown et al., 2005; Claydon et al., 2006; Joannides et al., 1999; Girona et al., 2014; Lu et al., 2003; Schroeder et al., 2002), but one study found that 90 minutes after participants consumed body-temperature water, CO increased (Girona et al., 2014). Collectively, these mixed results suggest that even if CO’s components, HR and SV, are altered by water loading, they are changed such that they counteract each other leaving CO unaltered by water consumption. Therefore, the previously reported effects of water loading such as increases in OT (Claydon et al., 2006; Lu et al., 2003; Schroeder et al., 2002), BP (Callegaro et al., 2007; Claydon et al., 2006; Freund et al., 1988; Girona et al., 2014; Hai et al., 2013; Nozawa et al., 2009; Schroeder et al., 2002), and cerebral perfusion (Claydon et al., 2006; Schroeder et al., 2002) in non-blood donation contexts and the reduction of vasovagal symptoms in blood donation contexts (Ando et al., 2009; France et al., 2010; Hanson & France, 2004; Newman et al., 2007) are unlikely due to central cardiovascular responses and more likely due to increases in peripheral SNS activity and vasoconstriction.

Lastly, changes in blood plasma volume could potentially influence vasovagal reactions since BP increases when plasma volume increases (Powers & Howley, 2009). However, the majority of research in non-blood donation contexts indicates that blood plasma volume does not acutely change in response to water consumption at rest (Callegaro et al., 2007; Geelen et al., 1984; Jordan et al., 2000; Williams et al., 1989) or during OT testing (Lu et al., 2003). In contrast, a study by Endo et al. (2001) found a
biphasic change in plasma volume, as measured by hematocrit (Hct, volume percentage of red blood cells in blood), such that Hct increased 5 minutes after water consumption and decreased 25 minutes later. However, participants in the Endo et al. (2001) study consumed 1 L of water in 2 minutes, which is more water and in less time than what other studies’ participants received. Furthermore, in a blood donation context, water consumption prior to donation produces negligible increases in plasma volume (Saito, Shimazu, Miyamoto, Maemura, & Satake, 2013). Moreover, even in the absence of water loading, homeostatic mechanisms quickly adapt to reductions in intravascular plasma volume during blood donation procedures and replenish most of it from the interstitial spaces within minutes of the procedure (Janetzko, Klüter, Kirchner, & Klotz, 2001; Menke, Stöcker, & Sibrowski, 2004; Saito et al., 2013). Therefore, the vasovagal-attenuating effects of a PHI during blood donation are unlikely due to changes in plasma volume.

As discussed above, although several studies indicate a PHI can reduce blood donors’ vasovagal reactions, no studies have investigated the physiological effects of water consumption in a blood donation context. However, several studies performed in non-blood donation contexts suggest water consumption may be physiologically protective against vasovagal reactions. More specifically, these studies found water loading can increase SNS activity, vasoconstriction, PP, BP, cerebral perfusion, and orthostatic tolerance. All of these changes could help reduce vasovagal reactions during blood donation. In contrast, the central cardiovascular responses to water loading characterized by increases in HF HRV, decreases or no change in HR, and no effect on CO do not appear to be protective against the pathophysiology of the vasovagal response.
Overall, previous research indicates that water loading before blood donation reduces donors’ vasovagal symptoms and non-blood donation studies demonstrate that water loading exerts multiple physiological effects, some of which may protect against vasovagal reactions during blood donation (see Table 1). Taken together these separate but related lines of research suggest a PHI may produce physiological changes that protect against the pathophysiology of the vasovagal response. Based on these previous findings, the goal of the present study was to examine the physiological responses (autonomic, cardiovascular, and cerebral perfusion) before, during, and after a simulated blood donation procedure among participants randomly assigned to either a PHI or a control group.
Table 1  
*Summary of Prior Studies' Findings on the Physiological Responses to Water Consumption*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Resting</th>
<th>OT</th>
<th>Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSNA</td>
<td>↑</td>
<td>N/A</td>
<td>Callegaro et al., 2007; Scott et al., 2001</td>
</tr>
<tr>
<td>NE</td>
<td>No Δ , ↑</td>
<td>No Δ</td>
<td>Jordan et al., 2000; Lu et al., 2003; Scott et al., 2001</td>
</tr>
<tr>
<td>E</td>
<td>N/A</td>
<td>No Δ</td>
<td>Lu et al., 2003</td>
</tr>
<tr>
<td>HF HRV</td>
<td>No Δ , ↑</td>
<td>↑</td>
<td>Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Routledge et al., 2002; Schroeder et al., 2002</td>
</tr>
<tr>
<td>HR</td>
<td>No Δ , ↓</td>
<td>No Δ, ↓</td>
<td>Ando et al., 2009; Brown et al., 2005; Callegaro et al., 2007; Claydon et al., 2006; Girona et al., 2014; Joannides et al., 1999; Jordan et al., 2000; Lu et al., 2003; Nozawa et al, 2009; Routledge et al., 2002 Schroeder et al., 2002; Scott et al., 2001;</td>
</tr>
<tr>
<td>PP</td>
<td>↑</td>
<td></td>
<td>Olatunji et al., 2011</td>
</tr>
<tr>
<td>Variable</td>
<td>Resting</td>
<td>OT</td>
<td>Studies</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>----</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>SV</td>
<td>No Δ, ↑</td>
<td>No Δ, ↑</td>
<td>Brown et al., 2005; Claydon et al., 2006; Girona et al., 2014; Lu et al., 2003; Schroeder et al., 2002</td>
</tr>
<tr>
<td>CO</td>
<td>No Δ, ↑</td>
<td>No Δ</td>
<td>Brown et al., 2005; Claydon et al., 2006; Girona et al., 2014; Joannides et al., 1999; Lu et al., 2003; Schroeder et al., 2002</td>
</tr>
<tr>
<td>TPR</td>
<td>No Δ, ↑, ↓</td>
<td>No Δ, ↑</td>
<td>Brown et al., 2005; Callegaro et al., 2007; Claydon et al., 2006; Girona et al., 2014; Joannides et al., 1999; Lu et al., 2003; Nozawa et al., 2009; Schroeder et al., 2002; Scott et al., 2001</td>
</tr>
<tr>
<td>BP</td>
<td>No Δ, ↑, ↓</td>
<td>No Δ, ↑</td>
<td>Brown et al., 2005; Callegaro et al., 2007; Claydon et al., 2006; Girona et al., 2014; Hai et al., 2013; Joannides et al., 1999; Jordan et al., 2000; Lu et al., 2003; Nozawa et al, 2009; Olatunji, et al., 2011; Routledge et al., 2002; Schroeder et al., 2002; Scott et al., 2001</td>
</tr>
<tr>
<td>Variable</td>
<td>Resting</td>
<td>OT</td>
<td>Studies</td>
</tr>
<tr>
<td>----------</td>
<td>---------</td>
<td>----</td>
<td>---------</td>
</tr>
<tr>
<td>BRS</td>
<td>No Δ , ↑</td>
<td>No Δ , ↑</td>
<td>Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Schroeder et al., 2002</td>
</tr>
<tr>
<td>AI</td>
<td>N/A</td>
<td>↓</td>
<td>Claydon et al., 2006; Schroeder et al., 2002</td>
</tr>
<tr>
<td>Hct</td>
<td>No Δ , ↑, ↓</td>
<td>No Δ</td>
<td>Callegaro et al., 2007; Endo et al., 2001; Geelen et al., 1984; Jordan et al., 2000; Lu et al., 2003; Williams et al., 1989</td>
</tr>
<tr>
<td>POSM</td>
<td>↓</td>
<td>N/A</td>
<td>Endo et al., 2004; Joannides et al., 1999; Williams et al., 1989</td>
</tr>
</tbody>
</table>

Note: AI = Autoregulation index, BP = Blood pressure, BRS = Baroreflex sensitivity, CO = Cardiac output, E = Epinephrine, Hct = Hematocrit, HF HRV = High frequency heart rate variability, HR = Heart rate, MSNA = Muscle sympathetic nerve activity, N/A = Not applicable or not available, NE= norepinephrine, OT = Orthostatic tolerance, POSM = Plasma osmolality, PP = Pulse pressure, SV = Stroke volume, TPR = Total peripheral resistance, ↓ = Decrease/lower, ↑ = Increase/greater, No Δ = No change.
Methods

Participants

Fifty college students were recruited for this study via flyers and emails at Ohio University. Eligible participants had to: have donated blood before, but not within 56 days of study participation for whole blood donors and not within 112 days for double red blood cell donors, weigh $\geq 110$ lbs., have no diagnosed chronic diseases, no history of syncope, not be pregnant, have a normal body temperature and a non-anemic Hct reading (i.e. above 40% for men or 37% for women), and not have exercised strenuously in the past 12 hours. A total of 21 participants were excluded from the final analyses (see Figure 1), leaving a final sample of 29 participants, 14 in the PHI and 15 in the control group. The groups did not differ in descriptive statistics and baseline physiological function, with the exception of pulse pressure, which was significantly higher in the PHI than in the control group at baseline (see Table 2).
Figure 1. Flow chart of participant recruitment.
Table 2

Baseline Descriptive Statistics and Physiological Activity by Group

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PHI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>21.4 ± 1.68</td>
<td>21.36 ± 1.39</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>23.67 ± 2.94</td>
<td>25.64 ± 3.46</td>
</tr>
<tr>
<td>Blood Draw Fear</td>
<td>0.20 ± 0.41</td>
<td>0.21 ± 0.43</td>
</tr>
<tr>
<td>Prior Whole Blood Donations (n)</td>
<td>3.73 ± 3.31</td>
<td>4.42 ± 2.82</td>
</tr>
<tr>
<td>Prior Plasma Donations (n)</td>
<td>0.33 ± 1.29</td>
<td>0.79 ± 2.67</td>
</tr>
<tr>
<td>Prior Double Red Cell Donations (n)</td>
<td>0.07 ± 0.26</td>
<td>0.07 ± 0.27</td>
</tr>
<tr>
<td>Cigarette Smokers (n)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>53.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Body Temperature (ºF)</td>
<td>98 ± 0.44</td>
<td>97.68 ± 0.57</td>
</tr>
<tr>
<td>Total Body Water by Weight (%)</td>
<td>50.4 ± 5.33</td>
<td>48.34 ± 4.40</td>
</tr>
<tr>
<td>Plasma Osmolality (mmol/Kg)</td>
<td>280.20 ± 4.32</td>
<td>279.38 ± 5.18</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.07 ± 3.07</td>
<td>41.96 ± 3.26</td>
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<tr>
<td>Blood Draw Duration (minutes)</td>
<td>9.33 ± 4.65</td>
<td>9.89 ± 3.76</td>
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<tr>
<td>Systolic Blood Pressure (mmHg)</td>
<td>129.25 ± 9.56</td>
<td>135.92 ± 14.29</td>
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<tr>
<td>Diastolic Blood Pressure (mmHg)</td>
<td>67.48 ± 7.15</td>
<td>64.61 ± 7.91</td>
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<tr>
<td>Mean Arterial Blood Pressure (mmHg)</td>
<td>85.31 ± 7.36</td>
<td>83.72 ± 10.02</td>
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<tr>
<td>Pulse Pressure (mmHg) *</td>
<td>61.77 ± 5.48</td>
<td>71.32 ± 9.25</td>
</tr>
<tr>
<td>Total Peripheral Resistance(dyn•s/cm⁵)</td>
<td>1568.22 ± 449.27</td>
<td>1531.76 ± 383.68</td>
</tr>
<tr>
<td>Heart Rate (beats per minute)</td>
<td>66.19 ± 9.69</td>
<td>62.75 ± 9.45</td>
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<tr>
<td>Stroke Volume (mL/beat)</td>
<td>83.14 ± 18.82</td>
<td>92.45 ± 21.11</td>
</tr>
<tr>
<td>Cardiac Output (L/minute)</td>
<td>5.52 ± 1.48</td>
<td>5.77 ± 1.32</td>
</tr>
<tr>
<td>Fast Fourier Transformed High Frequency HRV (ms²/Hz)</td>
<td>4340.80 ± 6703.08</td>
<td>6605.60 ± 8525.30</td>
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<tr>
<td>Baroreflex Sensitivity (ms/mmHg)</td>
<td>22.81 ± 12.52</td>
<td>32.56 ± 23.24</td>
</tr>
<tr>
<td>Oxygenation at Voxel 8 (µM)</td>
<td>0.86 ± 2.19</td>
<td>1.16 ± 3.17</td>
</tr>
</tbody>
</table>

Note: All values are means ± standard deviations. * = significant group difference, $p < 0.01$. PHI = Predonation Hydration Intervention. HRV = Heart rate variability.
Procedure

Consent, eligibility, and baseline measures. After providing consent, participants had their height, weight, temperature and Hct assessed by a registered nurse and then completed several demographic and eligibility criteria questions. Eligibility criteria for this study was similar to standards used by the American Red Cross with several modifications such that donors had to also: have no chronic diseases, have donated blood in the past, and not have exercised strenuously for 12 hours prior to participating. If eligible after these measures, participants were randomized to one of two groups, PHI or control, stratified by gender. Next, to control for potential confounding, participants were asked to void their bladder of urine because bladder distension increases SNS activity and BP (Fagius & Karhuvaara, 1989). Upon returning to the laboratory, participants were asked to lay supine on a blood draw table for 10 minutes and during this time researchers equipped the participants with physiological monitoring equipment. After 10 minutes, a bioelectrical impedance analysis (BIA) monitor was used to assess total body water by weight (TBW/Kg), as a measure of baseline hydration. Then participants sat up for 5 minutes. Immediately after reaching a seated position, recording of all continuous, physiological variables (i.e. HR, SV, CO, mean arterial pressure [MAP], total peripheral resistance [TPR, resistance to blood flow in the systemic vasculature], and O₂) started and continued for the remainder of the study. During this 5 minute period, participants in the PHI consumed 500 mL of room-temperature, bottled water and participants in the control group were asked to remain still. After 5 minutes, participants returned to a supine position for a 20 minute water absorption period. One minute into the water absorption period, participants’ brachial BPs were attained via an
automated BP cuff (SunTech Medical, Inc., Morrisville, NC) for calibration of continuous BP measures obtained via the Finometer PRO. At the end of the 20 minute supine period, a second TBW/Kg value was obtained and participants read a brief statement on the study’s purpose tailored to their assigned group (PHI or control) (see Appendix A).

**Blood draw period.** After baseline assessments were obtained, a registered nurse sanitized the participant’s left arm and then inserted an 18 gauge intravenous catheter into a vein at the antecubital fossa. A total of 450 mL of blood was collected into a 500 mL anticoagulant blood pack (Fenwal, Lake Zurich, IL) via a 20” tubing-set attached to the catheter. Furthermore, a standard weight gauge was used to continuously track the volume of blood collected into the blood pack during the blood draw. At 0 (i.e. start of blood loss), 150, 300, and 450 mL of total blood loss, three separate 4 mL blood samples were collected into vacutainers (Becton, Dickinson & Co., NJ). Therefore, a total of 498 mL of blood was collected (i.e. 48 mL into vacutainers and 450 mL into a blood pack). Immediately after each fourth of the blood draw (i.e. 0, 150, 300, and 450 mL of blood loss), Hct analyses were conducted with blood samples removed from a vacutainer via a micropipette. All vacutainers were kept on ice until the end of the blood draw, at which point they were centrifuged and the plasma was pipetted into Eppendorf tubes and frozen at -80°C for later analyses of POSM and catecholamines.

**Recovery period.** Upon completion of the blood draw, the catheter was removed and the registered nurse elevated and applied pressure to participants’ left arms for the first 2 minutes of the 6 minute supine recovery period. Following the first 2 minutes of the supine recovery period, a third TBW/Kg measure was obtained. After the 6 minute supine
recovery period, in order to simulate typical post-donation activities, participants sat upright for 1 minute, then stood and walked in place for 1 minute, and then sat upright for 10 minutes. At the end of the recovery periods, the physiologic monitoring equipment was disconnected, participants completed the 11-item blood donation reaction inventory (BDRI; assesses self-reported presyncopal symptoms), a debriefing was conducted, and participants received financial compensation for their time. Table 3 outlines when each physiological measure was assessed during the study.
Table 3

*Timeline of Study Measures*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Intake</th>
<th>Baseline</th>
<th>Blood Draw</th>
<th>Recovery Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 Mins Supine</td>
<td>5 Mins Seated</td>
<td>20 Mins Supine</td>
<td>0 mL</td>
</tr>
<tr>
<td>Cardiovascular Reactivity&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cerebral Oxygenation</td>
<td></td>
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<tr>
<td>Heart Rate Variability</td>
<td></td>
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<tr>
<td>Plasma Osmolarity</td>
<td></td>
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<tr>
<td>Hematocrit</td>
<td></td>
<td></td>
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<tr>
<td>Catecholamines</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total Body Water by Weight</td>
<td></td>
<td></td>
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</tbody>
</table>

Note:  
<sup>a</sup> Although cardiovascular reactivity and cerebral oxygenation were measured during the two minute pressure period, they were not analyzed during that time. In contrast, HRV analyses included the last minute of the two minute pressure period and the four minutes of the recovery period.  
<sup>b</sup> Cardiovascular reactivity = mean arterial pressure, systolic blood pressure, diastolic blood pressure, pulse pressure, stroke volume, heart rate, cardiac output, and total peripheral resistance.
Equipment

**Finometer PRO** (Finapres Medical Systems, Amsterdam, Netherlands). The Finometer PRO is a noninvasive device that measures beat-to-beat cardiovascular function (Finapres Medical Systems, 2012). A finger BP cuff combined with a volume clamp measured finger arterial pressure which was then filtered at specific frequencies in order to reconstruct the brachial artery’s MAP. The brachial MAP values calculated by the Finometer PRO were calibrated by obtaining a separate, non-continuous measure of brachial BP via an automated auscultatory BP device. Additionally, the Finometer PRO used the Modelflow algorithm and imputed data on participants’ gender, age, height, and weight to calculate SV, CO, and TPR. After data collection, AcqKnowledge 3.9 was used to derive systolic (SBP) and diastolic (DBP) blood pressure from the MAP waveform. The Finometer Pro has been shown to have high concurrent validity with the thermodilution technique, the gold standard of measuring CO (De Wilde, Schreuder, Van Den Berg, and Jansen, 2007). Furthermore, a study by Leonetti et al. (2004) has previously shown this device to be sensitive to changes in cardiovascular reactivity during phlebotomy.

**Cardiotachometer 1000** (CWE Inc., Ardmore, PA). Heart rate and HF HRV were calculated from continuous electrocardiograms measured by the Cardiotachometer 1000 using a modified limb lead II electrode placement.

**The functional near infrared spectroscopy (fNIR) model 1,000** (BIOPAC systems, Inc., Goleta, CA). The fNIR is a non-invasive, portable measure of O₂ in the dorsolateral pre-frontal cortex (Ayaz et al., 2011). It consists of a forehead sensor
attached to a control unit and a computer operating the Cognitive Optical Brain Imaging Studio (COBI) software (BIOPAC systems, Inc., 2014), which collects, saves, and analyzes raw data. The forehead sensor has four light emitting diodes (LEDs) and 10 photodetectors that together create 16 voxels (i.e. measurement locations). Only data from voxels eight and 10 were analyzed as they are located on the center of the forehead, providing the best adhesion of the fNIR’s sensor. The LEDs emit light wavelengths in the 730-850 nm range, which are primarily absorbed by hemoglobin (Hb) in the brain’s blood supply (Ayaz et al., 2011; Ferrari, Mottola, & Quaresima, 2004). The COBI software entered light absorbency data, as measured by the photodetectors, into a modified beer-lambert law to continuously calculate changes relative to baseline in oxygenated (HbO₂) and deoxygenated hemoglobin (Hb) (BIOPAC Systems, Inc., 2014; Ferrari et al., 2004). The baseline data for HbO₂ and Hb were collected over a 10 second period at the end of the 10 minute supine baseline period. Changes relative to baseline in O₂ were calculated by subtracting Hb values from HbO₂ values.

**Enzyme-linked immuno-sorbent assays (ELISA)** (Labor Diagnostika Nord, Nordhorn, Germany). The ELISAs were performed in accordance with the manufacturer’s protocol for the quantification of epinephrine (E) and NE in the participants’ blood plasma samples, which were collected in Ethylenediaminetetraacetic acid (EDTA) vacutainers at 0, 150, 300, and 450 mL of blood loss. All samples were run in duplicate. This assay has a standard range of 0/1- 200 ng/ml for E and 0/5 – 1,000 ng/ml for NE and a sensitivity of 10 pg/ml for E and 50 pg/ml for NE. Also it has been shown to be precise with intra-assay coefficient of variation percentages (CV) ranging from 9.8 to 16.1% for NE and 6.9 to 15.8% for E and inter-assay CV % ranging from 8.5% to 15.0% for NE and 13.2 to 18.2 %
for E. The average intra-assay CV % for the present study was 26.8% and 16.9% for NE and E, respectively. Additionally, the ELISA kit has strong concurrent validity with high-performance liquid chromatography, another common method for plasma NE ($r = 0.96$) and E ($r = 0.99$) quantification.

**The MultiScan 5000 bioelectrical impedance analysis (BIA) monitor** (Bodystat, Isle of Man, UK). The BIA monitor sends an electrical signal through the body to calculate impedance (Z), which is the opposition to electric current. The monitor uses Z and imputed data on participants’ height and weight to determine participants’ total (TBW), extracellular (EBW) and intracellular (ICW) body water (Brodie, Moscrip, & Hutcheon, 1998).

**HemataSTAT microhematocrit centrifuge** (Separation Technology, Inc., Sanford, FL). The HemataSTAT assessed Hct in five separate blood samples collected from participants. A capillary blood sample was collected at intake via a finger stick lancet and venous blood samples were collected into EDTA vacutainers at 0, 150, 300, and 450 mL of blood loss during the blood draw period. Each sample was run in duplicate, averaged and used to calculate percent plasma volume change values relative to baseline.

**Vapro 5520 vapor pressure osmometer** (Wescor, Inc., Logan, UT). Using plasma samples separated from blood collected in heparin vacutainers at 0 and 450 mL of blood loss during the blood draw, POSM was measured via the Vapro 5520 in triplicate and averaged.
Questionnaires

**Health, demographics, and blood donation history survey.** This experimenter-designed questionnaire assessed eligibility criteria (e.g. age, blood donation history, weight, etc.) and other variables (e.g. cigarette smoking, gender, blood draw fear, etc.) that are associated with cardiovascular reactivity and vasovagal reactions during blood donation.

**Blood donation reaction inventory (BDRI).** The BDRI is an 11-item measure of sensations experienced during or directly after blood donation. For example, one item reads, “indicate the degree to which you experienced dizziness at the blood donation clinic today by circling a number between 0 ("not at all") and 5 ("to an extreme degree").” The BDRI is commonly used to assess efficacy of blood donation interventions at reducing vasovagal symptoms and for predicting future donor return behavior. Prior research indicates the BDRI has good internal consistency ($\alpha = 0.93$), high concurrent validity with phlebotomist ratings of donor reactions ($r = 0.46, p < 0.001$), and good predictive validity with donor return behavior (OR = 0.96, $p < 0.001$) (France, Ditto, France, & Himawan, 2008; France et al., 2004; Meade et al., 1996). The current study also found the 11-item and 4-item BDRI scales had good internal consistency, $\alpha = 0.90$ and $\alpha = 0.87$, respectively.

Data Acquisition

All continuous, physiological data except $O_2$ were recorded using the AcqKnowledge 3.9 software (BIOPAC systems, Inc., Goleta, CA). The MAP waveform was used to derive SBP and DBP. When signal losses occurred in the SBP and DBP waveforms, they were replaced with values obtained from the previous cycle. Data for
each of these variables was visually examined for artifacts (e.g. loss of signal, impossible values). When an artifact value was identified, it was replaced with the mean value of the data 30 seconds before and after it. Using these derived BP values, pulse pressure was calculated by subtracting DBP from SBP. Baroreflex sensitivity was calculated using the spontaneous sequence method on the interbeat interval and SBP data in the AcqKnowledge 4.4 software. Furthermore, HR data was transferred to the Kubios 2.1 software (University of Eastern Finland) to perform a spectral analysis of HRV with a low correction filter. Variations of the HF HRV analyses were run to include different transformations (fast-Fourier and autoregressive) and different units (normalized and actual units). Cerebral oxygenation was recorded in the COBI software.

Data Reduction

Baseline sample characteristics. In order to describe the group samples and identify potential confounds, baseline group differences on eligibility, health, demographics, and physiological data were assessed via independent samples $t$-tests for all continuous data and chi-square analyses for all categorical data.

Cardiovascular reactivity. The continuous data for HR, SV, CO, MAP, SBP, DBP, and TPR\(^1\) were reduced to means for the following epochs: the first 8 minutes of the 20 minute baseline; four separate 10 second epochs during the blood draw (0, 150, 300, and 450 mL of blood loss); and four separate 1 minute epochs during the 6 minute supine recovery period (minutes 3, 4, 5, and 6). The first 2 minutes of the supine

\(^1\) Although the study included hypothesized changes in PEP, it was not possible to collect data on this variable. The Finometer PRO can only measure a limited amount of variables during any given protocol and as such it was eliminated so that other variables could be recorded.
recovery period were discarded from analyses because of the R.N.’s interactions with each participant (e.g. pressure applied to participants’ IV site and communications with the participant). Change scores were calculated for each of these variables such that mean baseline values (first 8 eight minutes of the 20 minute supine baseline) were subtracted from each of these variables’ mean values at the blood draw and recovery time points.

In accordance with recommendations made by the Task Force of the European Society of Cardiology (1996), HF HRV was calculated over multiple 5 minute epochs within the EKG waveform data. More specifically, there was one epoch for the first 5 minutes of the 20 minute supine baseline, one epoch during the last 5 minutes of the blood draw, and one epoch during the first 5 minutes of the supine recovery period. Change scores relative to the baseline epoch were calculated for HF HRV during these blood draw and recovery periods.

Cerebral oxygenation. The COBI software recorded continuous changes over time in HbO₂ and HB relative to 10 seconds of baseline data collected at the end of the 10 minute supine, baseline period. Oxygenation data was calculated as HbO₂ minus Hb and the resulting data was reduced to means for the following 10 second epochs during the blood draw (0, 150, 300, and 450 mL of blood loss); and 1 minute epochs during the supine recovery period (minutes 3, 4, 5, and 6).

Plasma volume. Hematocrit was measured in duplicate and averaged at baseline (i.e. intake) and at 0, 150, 300, and 450 mL of blood loss. These Hct mean values were used to calculate percent change scores relative to baseline for plasma volume in accordance with methods used in prior research on hydration and plasma volume changes
(Jordan et al., 2000). The equation used was \((Hct_T - Hct_B) / (Hct_B) \times 100\) where \(Hct_T\) represented the Hct value at a time point (e.g. 0, 150, 300, or 450 mL of blood loss) and \(Hct_B\) represented the Hct value at baseline.

**Sympathetic activity.** Both E and NE were measured in duplicate and averaged for each time point. Furthermore, since catecholamines were only measured during the blood draw time points, the change scores of mean values were calculated relative to the start of the blood draw for 150, 300, and 450 mL of blood loss.

**Presyncopal symptoms.** Means were calculated for scores on the 4-item and 11-item BDRI scales.

**Plasma osmolality.** Plasma osmolality was measured in triplicate and averaged for plasma samples collected at 0 and 450 mL of blood loss during the blood draw.

**Total body water by weight.** At three separate time points during the study, TBW/Kg was calculated by dividing TBW in liters by weight in Kg and reported as a percentage. Change scores were calculated by subtracting participants’ TBW/Kg for the first baseline (i.e. before the PHI consumed water) from their TBW/Kg for the second baseline (i.e. after the PHI consumed water), and from their TBW/Kg for the recovery period.

**Pulse pressure.** Pulse pressure (PP) was calculated by subtracting mean DBP from mean SBP at the following epochs: the first 8 minutes of the 20 minute baseline; four separate 10 second epochs during the blood draw (0, 150, 300, and 450 mL of blood loss); and four separate 1 minute epochs during the 6 minute supine recovery period (minutes 3, 4, 5, and 6). Afterwards, change scores were calculated such that baseline
mean PP values were subtracted from mean PP values at each of the blood draw and recovery time points.

**Baroreflex sensitivity.** The spontaneous sequence method was used to assess arterial baroreflex sensitivity in the time domain (Bertinieri et al., 1985; Bertinieri et al., 1988; Hughson, Quintin, Annat, Yamamoto, & Gharib, 1993). Importantly, this measure only assesses heart rate changes elicited by PNS activity in response to BP changes (Iida et al., 1999; La Rovere, Pinna, & Raczak, 2008; Parati, Di Rienzo, & Mancia, 2000). As such, this measure cannot be used to make inferences about other variables typically altered by the baroreflex such as vasoconstriction (Dutoit et al., 2010). This method has strong concurrent validity with the phenylephrine technique, the gold standard technique for measuring BRS, and is less invasive (Parlow, Viale, Annat, Hughson, & Quintin, 1995). In the current study, ascending BRS sequences were characterized by three or more consecutive cycles of SBP increasing by 1 mmHg co-occurring with successive increases in the interbeat interval (time duration between heart beats) of 5 ms or more per cycle. Similarly, descending BRS sequences were characterized by three or more consecutive cycles of SBP decreasing by 1 mmHg co-occurring with successive decreases in the interbeat interval of 5 ms or more per cycle. Furthermore, the interbeat interval and SBP values within each identified sequence had to correlate by .80 or more to be included in the BRS analyses. Regression coefficients (i.e. the slope between ms and mmHg values) were calculated for all valid ascending and descending sequences identified. These coefficients are also known as BRS values. Then ascending and descending sequences’ regression coefficients were averaged separately and together (i.e. combined BRS) during three time periods: minutes 2 through 9 of the 20 minute supine
baseline, across the entire blood draw period, and across the entire 6 minutes of the supine recovery period. Furthermore, a minimum of two sequences were required for an average BRS value to be calculated for ascending, descending, or combined BRS. Change scores for ascending, descending, and combined BRS values were each calculated by subtracting mean baseline BRS values from mean BRS values at the blood draw and recovery time points.

Area under the curve. In addition to change score values, area under the curve (AUC) values were calculated for all previously discussed physiological variables in accordance with methods outlined by Pruessner et al. (2003). Both AUC with respect to ground (AUCG) and with respect to increase (AUCI) were calculated. Although similar conceptually, these methods are calculated slightly different; AUCG assesses the AUC relative to zero and AUCI assesses the AUC relative to a baseline value for the variable of interest (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

Data Analyses

Group differences in the mean values on all baseline descriptive and physiological variables were assessed via independent samples t-tests (see Table 2). Chi-square tests were conducted for categorical baseline descriptive variables. Additionally, change scores were calculated for all continuous, physiological variables except POSM. The calculated change scores (or means in the case of POSM) were entered as the dependent variables in a series of repeated measures analyses of variance (ANOVA) testing Time and Group main effects and their interaction. Greenhouse-Geisser corrections were made when violations of the sphericity assumption occurred in the omnibus tests. Additionally, Bonferroni-adjusted post-hoc pairwise comparisons (i.e. t-tests) were made for significant
Time main effects. Tests of simple main effects followed significant omnibus tests for interactions. All effect sizes for ANOVAs are reported as partial eta squared values and \( p < 0.05 \) were considered significant.

Similar to the above described ANOVAs, independent samples \( t \)-tests were conducted to assess group differences on mean AUC values for each physiological variable. Furthermore, the AUC values were used to test mediation with several different methods outlined by Baron and Kenny (1986) and Preacher and Hayes (2004). In regards to the Baron and Kenny (1986) method, three regression analyses were conducted for each physiological variable. The first regression tested the effects of group (PHI, control) on BDRI scores. The second regression tested the effects of group (PHI, control) on the value of the physiological variable. The third regression was a hierarchical analysis to predict BDRI scores with the respective physiological variable in the first block and the groups (PHI, control) in the second block. Complete mediation was considered present if the effect of group status on BDRI scores decreased to zero after controlling for a physiological mediator and partial mediation was present if the effect was reduced, but not to zero. Additionally, indirect effects were statistically tested with the Sobel and bootstrapping methods (Preacher & Hayes, 2004). The Sobel method assessed whether the direct effect of group status on the BDRI scores was significantly different from the indirect effect of the group status on the BDRI scores after controlling for the mediator. The bootstrapping method is similar to the Sobel method, but does not rely on any normality assumptions and as such was better suited for studies, such as the current one, with small sample sizes which may violate the normality assumption.
Results

Baseline Sample Characteristics

There were no significant differences between the PHI and control groups on any variables related to demographics, health, and blood donation experience. In regards to baseline physiological function there was only one group difference. Pulse pressure was significantly lower in the control group ($M = 61.77, SD = 5.48$) than in the PHI ($M = 71.32, SD = 9.25$) at baseline, $t(27) = 3.41, p < .01, d = 1.26$. Table 2 provides baseline descriptive and physiological function statistics.

Cardiovascular Reactivity

Group differences in cardiovascular function were assessed via a series of 8 (Time: 0, 150, 300, and 450 mL of blood loss; and minutes 3, 4, 5, and 6 of the supine recovery period) x 2 (Group: PHI, control) repeated measures ANOVAs for change scores relative to baseline for MAP, SBP, DBP, SV, HR, CO, and TPR. Results revealed significant Time main effects for: MAP, $F(7, 189) = 3.41, p < .05, \eta_p^2 = .112$; SBP, $F(7, 189) = 3.11, p < .05, \eta_p^2 = .103$; SV, $F(7,189) = 9.91, p < .0001, \eta_p^2 = .268$; HR, $F(7,189) = 7.35, p < .0001, \eta_p^2 = .214$; CO, $F(7, 189) = 9.08, p < .0001, \eta_p^2 = .252$; and a marginal Time main effect for TPR, $F(7, 189) = 2.84, p = .066, \eta_p^2 = .095$), with a trend towards increased TPR across the blood draw and recovery periods as compared to the baseline period.

Bonferroni-adjusted post-hoc tests revealed significant differences between time-points for SV, HR, and CO, all $ps < .01$. More specifically, across groups and relative to the baseline, SV was greater at the start of the blood draw than at minute 6 of the supine recovery; greater at 150 mL of blood loss than at 450 mL of blood loss or at minutes 3
through 6 of the supine recovery; and greater at 300 mL of blood loss than at minutes 3, 5, and 6 of the supine recovery. Furthermore, as compared to baseline, HR was greater at the start of the blood draw than at 150 mL and 300 mL of blood loss; and greater at 450 mL of blood loss than at 150 mL of blood loss. Additionally, relative to baseline, CO was greater at the start of the blood draw than at 300 and 450 mL of blood loss and minutes 3 through 6 of the supine recovery; and greater at 300 mL of blood loss than at minute 6 of the supine recovery. Bonferroni adjusted post-hoc tests found no significant differences between time points for MAP and SBP. There were significant Group main effects for MAP, \( F(1, 27) = 7.28, p < .02, \eta_p^2 = .212 \), SBP, \( F(1, 27) = 6.93, p < .02, \eta_p^2 = .204 \), and DBP, \( F(1, 27) = 6.27, p < .02, \eta_p^2 = .189 \), such that across all time points and relative to baseline participants in the control group had higher MAP, SBP, and DBP than participants in the PHI group (see Figures 2, 3, & 4, respectively). No other significant effects were found.
Figure 2. Mean arterial blood pressure (MAP) change scores (± SE) for the predonation hydration intervention (PHI) and control groups during the blood draw and recovery periods.
Figure 3. Systolic blood pressure (SBP) change scores (± SE) for the predonation hydration intervention (PHI) and control groups during the blood draw and recovery periods.
Figure 4. Diastolic blood pressure (DBP) change scores (± SE) for the predonation hydration intervention (PHI) and control groups during the blood draw and recovery periods.

A 2 (Time: last 5 minutes of the blood draw and first 5 minutes of the 6 minute supine recovery) x 2 (Group: PHI, control) repeated measures ANOVA of change scores of HF HRV relative to baseline found no significant Time or Group main effects and no Group x Time interactions. The results were unaffected by transformation method (i.e. either a fast Fourier transformation [FFT] or an autoregressive [AR] analysis) or by units (i.e. either normalized or actual units). That said, there was a marginal Time main effect for HF HRV in normalized units using both the FFT method, $F(1, 27) = 4.02, p = .055,$
\[ \eta_p^2 = .129 \] and the AR method, \( F(1, 27) = 3.05, p = .092, \eta_p^2 = .101 \), with a trend towards a reduction in HF HRV across the blood draw and recovery periods as compared to the baseline period.

**Cerebral Oxygenation**

Complete \( O_2 \) data was only available for seven participants in the control group and seven participants in the PHI due to hardware malfunctions with the fNIR model 1,000. Group differences in \( O_2 \) over time were assessed by conducting a series of 8 (Time: start of blood draw, 150, 300, and 450 mL of blood loss; and minutes 3, 4, 5, and 6 of the supine recovery period) x 2 (Group: PHI, control) repeated measures ANOVAs for \( O_2 \) change scores relative to baseline for voxel eight and 10. No significant Time or Group main effects were found for voxel eight. However, for voxel eight there was a marginal Time x Group interaction, \( F(7, 84) = 2.66, p = .083, \eta_p^2 = .181 \), such that there was a trend towards lower \( O_2 \) during the recovery period for the PHI as compared to the control group. In regards to voxel 10, no significant main effects or interactions were found even though \( O_2 \) values obtained from voxel 8 and 10 were significantly correlated with each other, \( r(12) = .95, p < .001 \).

**Plasma Volume**

It is important to note, that although there were 29 participants with complete cardiovascular reactivity data, one participant had missing data for Hct because of equipment failure. A 4 (Time: 0, 150 mL, 300 mL and 450 mL of blood loss) x 2 (Group: PHI, control) repeated measures ANOVA for percent change in plasma volume relative to baseline (i.e. intake) was conducted to determine group differences in plasma volume changes over time. The results revealed a significant Time main effect, \( F(3, 78) \)
Bonferroni-adjusted post-hoc tests indicated that relative to baseline, plasma volume was lower at 450 mL of blood loss than it was at the start of the blood draw, \( p < .01 \). No other significant effects were found.

**Sympathetic Activity**

Group differences in E and NE during the blood draw were assessed by conducting two separate 3 (Time: 150, 300, and 450 mL of blood loss) x 2 (Group: PHI, control) repeated measure ANOVAs for change scores relative to the start of blood loss for E and NE.

**Epinephrine.** One participant was removed from the analysis because of an outlier value for E at time four that was not physiologically feasible and was more than three standard deviations from the mean. Therefore, the analysis of E included 28 participants. The results indicated there was a Time main effect, \( F(2, 52) = 21.18, p < .0001, \eta^2_p = .449 \). Bonferroni-adjusted post-hoc tests indicated that across groups the change relative to the start of the blood draw for E was higher at 450 mL of blood loss than it was at either 150 or 300 mL of blood loss, all \( p < .05 \). There was also a Group main effect, \( F(1, 26) = 5.99, p < .05, \eta^2_p = .187 \), such that across all time points the PHI had higher E than the control group (see Figure 5). No Group x Time interaction was found.

**Norepinephrine.** In regards to NE, one participant was removed from the analysis because of equipment error with the plasma sample for time one. Therefore, the final analysis consisted of 28 participants. The results revealed a significant Time main effect, \( F(2, 52) = 3.94, p < .05, \eta^2_p = .132 \). Bonferroni-adjusted post-hoc tests demonstrated that across groups and relative to baseline, NE decreased from 150 mL to
300 mL, $ps < .05$. There also was a marginal interaction for Time x Group, $F(2, 52) = 2.50, p = .092, \eta_p^2 = .088$, with a trend of a greater decrease in NE over time in the PHI than in the control group. There was no significant main effect for Group.

![Graph showing Epinephrine (E) change scores (± SE) for the predonation hydration intervention (PHI) and control groups during the blood draw periods.](image)

**Figure 5.** Epinephrine (E) change scores (± SE) for the predonation hydration intervention (PHI) and control groups during the blood draw periods.

**Presyncopal Symptoms**

There was no difference between the PHI ($M = 10.64, SD = 10.20$) and control group ($M = 6.07, SD = 6.05$) on the 11 item BDRI, $t(27) = .160, p > .05$. Similarly there was no difference between the PHI ($M = 5.86, SD = 4.94$) and control group ($M = 3.60, SD = 3.42$) on the 4 item BDRI, $t(27) = .162, p > .5$. 
Exploratory Analyses

**Plasma osmolality.** Plasma osmolality did not differ between the PHI ($M = 279.38$, $SD = 5.18$) and control group ($M = 280.20$, $SD = 4.32$) at the start of the blood draw, $t(27) = .464$, $p > .05$, so no covariation was needed for the following ANOVA. A 2 (Time: 0 and 450 mL of blood loss) x 2 (Group: PHI, control) repeated measures ANOVA of mean POSM values revealed a significant Time main effect, $F(1, 27) = 17.77$, $p < .001$, $\eta_p^2 = .397$, such that across all participants mean POSM decreased from 0 to 450 mL of blood loss. Additionally, as can be seen in Figure 6, there was a significant Time x Group interaction $F(1, 27) = 6.32$, $p < .02$, $\eta_p^2 = .190$. Simple main effect follow up tests for the significant interaction between Group and Time revealed that the PHI and control group did not differ in POSM at the start of the blood draw but at the end of the blood draw the PHI had significantly lower POSM than the control group, $p < .05$. Additionally, simple main effect follow up tests revealed that POSM did not differ from start to end of the blood draw in the control group but in the PHI group POSM was significantly lower at the end of the blood draw relative to the start of the blood draw, $p < .0001$. No other significant effects were found.
Figure 6. Mean (± SE) plasma osmolality (POSM) for the predonation hydration intervention (PHI) and control groups at 0 and 450 mL of blood loss, * = $p < .05$.

**Total body water by weight.** In order to assess group differences in TBW/Kg, a 2 (Time: second baseline period and the recovery period) x 2 (Group: PHI, control) repeated measures ANOVA for change in TBW/Kg relative to the first supine baseline period (i.e. prior to water consumption) was conducted. Results revealed a significant Time main effect, $F(1, 25) = 76.14, p < .0001, \eta_p^2 = .753$, such that across groups and relative to the supine baseline period, TBW/Kg decreased from the second supine baseline period (i.e. 20 minutes after water consumption) to the recovery period after the blood draw ($p < .05$) (see Figure 7). No other significant effects were found.
Figure 7. Total body water by weight (TBW/Kg) change scores (± SE) for the predonation hydration intervention (PHI) and control groups before and after the blood draw.

**Pulse pressure.** An 8 (Time: 0, 150, 300, and 450 mL of blood loss; and minutes 3, 4, 5, and 6 of the supine recovery period) x 2 (Group: PHI, control) repeated measures ANOVA of PP change scores relative to baseline revealed a significant Time main effect, $F(7, 189) = 3.28, p < .01, \eta_p^2 = .107$. Bonferroni-adjusted post-hoc tests revealed that across groups and relative to the baseline, PP was greater at 150 mL of blood loss than at minute 6 of the supine recovery period, $p < .01$ (see Figure 8). Additionally, there was a significant Group main effect, $F(1, 27) = 4.53, p < .05, \eta_p^2 = .144$, such that the PHI had...
lower PP than the control group throughout the blood draw and recovery periods. No significant interaction was found.

Baroreflex sensitivity.

*Combined baroreflex sensitivity.* A 2 (Time: blood draw, and recovery) x 2 (Group: PHI, control) repeated measures ANOVA of change relative to baseline for combined BRS values was conducted to determine group differences in combined BRS over time. There was no significant Time main effect but there was a significant Group
main effect, $F(1, 27) = 6.66, p < .02, \eta^2_p = .198$. Additionally, as can be seen in Figure 9, there was a significant Time x Group interaction $F(1, 27) = 6.03, p < .05, \eta^2_p = .182$.

Simple main effect follow up tests for the significant interaction between Group and Time revealed that during the blood draw period the PHI had significantly lower combined BRS than the control group, $p < .01$. Additionally, simple main effect follow up tests revealed that among the PHI, combined BRS did not differ between blood draw and recovery periods, but among the control group combined BRS was significantly lower during the recovery period than during the blood draw period, $p < .01$. 
Figure 9. Combined baroreflex sensitivity (BRS) change scores (± SE) for the predonation hydration intervention (PHI) and control groups during the blood draw and recovery periods, * = p < .05.

Ascending baroreflex sensitivity. It is important to note, that although there were 29 participants with complete cardiovascular reactivity and combined BRS data, only 24 participants (15 control, and 9 PHI) had complete data for the analysis of BRS values for ascending sequences; five participants were excluded because they had less than two ascending BRS sequences occur during at least one of the three time points. A 2 (Time: blood draw, and recovery) x 2 (Group: PHI, control) repeated measures ANOVA of change relative to baseline for ascending BRS values was conducted to determine group
differences in ascending BRS over time. Results revealed a significant Time main effect, \( F(1, 22) = 4.92, p < .05, \eta^2_p = .183 \). Post-hoc tests revealed significantly lower ascending BRS relative to baseline during the recovery period than during the blood draw period, \( p < .05 \). Additionally, there was a marginal Group main effect, \( F(1, 22) = 3.955, p = .059, \eta^2_p = .152 \), with a trend towards a greater reduction in ascending BRS across time relative to baseline in the PHI as compared to control group. No other significant effects were found.

**Descending baroreflex analysis.** The analysis of descending BRS sequences included 28 participants; one participant in the control group was excluded because less than two descending sequences occurred during the baseline period. A 2 (Time: blood draw, and recovery) x 2 (Group: PHI, control) repeated measures ANOVA of change relative to baseline for descending BRS values was conducted to determine group differences in descending BRS over time. No significant time or group main effects were found but there was a marginally significant interaction between Time and Group, \( F(1,26) = 3.28, p = .082, \eta^2_p = .112 \), with a trend towards a greater reduction in descending BRS values during the blood draw period in the PHI as compared to the control group. Also there was a trend towards a decrease in descending BRS values from the blood draw period to the recovery period in the control group whereas descending BRS values tended to stay the same across the blood draw and recovery periods in the PHI.

**Area under the curve.** Regardless of the type of mediation test, AUC method, or BDRI scale (11-item or 4-item) used, the results revealed that the relationship between group and BDRI scores was not mediated by any of the physiological variables.
Furthermore, there were no significant group differences on any of the cardiovascular reactivity or oxygenation variables’ AUCG values. However, there were significant group differences for AUCI values on MAP, SBP, combined BRS, and descending BRS and a marginally significant group difference on AUCI values for DBP, PP, and ascending BRS. More specifically, MAP AUCI was significantly lower in the PHI ($M = 1679.15, SD = 7725.97$) than in the control group ($M = 9384.41, SD = 8667.29$), $t(26) = 2.47, p < .05, d = 0.94$. Systolic BP was significantly lower in the PHI ($M = 1160.03, SD = 14942.30$) than in the control group ($M = 14443.8331, SD = 13114.72$), $t(26) = 2.51, p < .05, d = 0.94$. Combined BRS was significantly lower in the PHI ($M = -6375.97, SD = 5332.86$) than in the control group ($M = 174.43, SD = 5335.17$), $t(26) = 3.24, p < .01, d = 1.23$. Descending BRS was significantly lower in the PHI ($M = -5906.92, SD = 5742.44$) than in the control group ($M = -1756.54, SD = 3792.44$), $t(25) = 2.23, p < .05, d = 0.85$. DBP was marginally lower in the PHI ($M = 3024.17, SD = 7049.80$) than in the control group ($M = 8640.29, SD = 7421.39$), $t(26) = 2.04, p = .051, d = 0.78$. Pulse pressure was marginally lower in the PHI ($M = -1863.02, SD = 10053.26$) than in the control group ($M = 5804.06, SD = 10098.35$), $t(26) = 2.01, p = .055, d = 0.76$. Ascending BRS was marginally lower in the PHI ($M = -4689.23, SD = 4738.76$) than in the control group ($M = 1641.40, SD = 8074.48$), $t(21) = 2.03, p = .056, d = 0.96$. Descending BRS was marginally lower in the PHI ($M = -5425.52, SD = 5803.74$) than in the control group ($M = -1756.54, SD = 3792.44$), $t(26) = 1.980, p = .058, d = 0.75$. Regardless of transformation methods or units used for HRV, there were no group differences on AUCG or AUCI values for HRV.
In addition to the cardiovascular and oxygenation variables discussed above, the other physiological measures’ AUC values were analyzed. There were no group differences on AUCG or AUC1 values for TBW/Kg. There were no group differences in POSM AUCG values but there was a significant group difference in POSM AUC1 values with lower values in the PHI ($M = -1099.82, SD = 963.94$) than in the control group ($M = -295.88, SD = 724.16$), $t(24) = 2.40, p < .05, d = 0.94$. There were no significant group differences in AUCG and AUC1 values for plasma volume change. There were also no significant group differences on AUCG and AUC1 values for NE. In contrast, the AUC1 values for E was significantly higher in the PHI ($M = 3240.08, SD = 7581.00$) than in the control group ($M = -3457.23, SD = 6937.15$), $t(27) = -2.48, p < .05, d = 0.92$. However, the groups did not differ on AUCG values for E.
Discussion

The current study was the first to assess cardiovascular, cerebral, and autonomic reactivity to water consumption in a blood donation context. Based on previous studies performed in non-blood donation contexts, it was expected that BP would be higher in a PHI than in a control group during phlebotomy. In contrast, the current study found BP was lower in the PHI than in the control group possibly due to reductions in PP and impairments in BRS. However, regardless of group assignment, participants experienced decreases in SV, PP, HF HRV, and CO, an initial decrease followed by an increase in E and HR, and no change in BP. As such the current study’s results generally support and extend prior studies’ findings on the physiological effects of mild hypovolemia (Cooke, Ryan, & Convertino, 2004; Fortrat, Nasr, Duvareille, & Gharib, 1998; Haberthür, Seshächinger, Seeberger, & Gysi, 2003; Zollei et al., 2004).

Results from the current study indicated water loading participants prior to a simulated blood donation procedure elicits several physiological changes. First, it should be noted that across groups, the simulated blood donation procedure resulted in mild hypovolemia as indicated by the significant reduction in PV at 450 mL of blood loss relative to the start of blood draw and by the significant reduction in TBW/Kg during the recovery period as compared to the baseline period. Second, participants in the PHI experienced a significantly greater decrease in POSM over the blood draw period than participants in the control group, indicating they were more hydrated during the blood draw period. Third, relative to the control group, participants in the PHI exhibited significantly lower MAP, SBP, DBP, and PP throughout the blood draw and recovery periods, which may have been due to the significantly greater impairment in combined
BRS in the PHI as compared to the control group during the blood draw period. Furthermore, during the supine recovery period, there was a trend towards a greater reduction in O₂ relative to baseline in the PHI as compared to the control group. Additionally, across all blood draw time points and relative to control, the PHI had higher E levels. Moreover, there was a trend of a greater reduction in NE levels during the second half of the blood draw in the PHI relative to the control group. Together, these results suggest that a PHI may not protect against the pathophysiological changes characteristic of a vasovagal response during blood donation. Next, the study’s main findings, limitations, and strengths will be discussed.

Although, data indicated participants were slightly hypovolemic, regardless of group status, there was no change over time in MAP, SBP, and DBP. Previous findings on blood pressure responses to phlebotomy are mixed, with some studies also finding no change over time in BP (Haberthür et al., 2003; Rea et al., 1991), others finding increases in BP (Fortrat et al., 1998; Zöllei et al., 2004) and one study finding a decrease in BP (Leonetti et al., 2004). These mixed findings may be due to differences in the amount of time needed to complete the blood draw, which across studies ranged from 5 to 14 minutes, or due to differences in the amount of blood withdrawn from participants, which across studies ranged from 350 to 480 mL (Fortrat et al., 1998; Haberthür et al., 2003; Leonetti et al., 2004; Rea et al., 1991; Zöllei et al., 2004). Since the current study’s participants had 498 mL of blood withdrawn in an average of 9.60 ± 4.18 minutes, this study’s findings may differ from previous findings due to differences in the phlebotomy procedure. Relatedly, previous studies have found hypovolemia induced by mild lower body negative pressure (LBNP) equal to or less than -20 mmHg (similar to 400 to 500

Although the previously reported effects of mild hemorrhage and mild LBNP on BP are mixed, both manipulations of hypovolemia have been shown to independently reduce central venous pressure (CVP; a measure of how much blood is returning to the heart) (Duranteau et al., 1995; Hirsch et al., 1989; Johnson et al., 2014; Mark et al., 1978; Mark et al., 1982; Norsk et al., 1986; Rae et al., 1991; van Hoeyweghen et al., 2001; Zoller et al., 1972). Reductions in CVP during hypovolemia can in turn reduce SV (Cooke et al., 2004; Klabunde, 2011). The present study did not measure CVP. However, SV was measured and found to decrease during the blood draw and recovery periods, regardless of group status. This finding is consistent with previous research demonstrating that mild hypovolemia induced by blood loss (Leonetti et al., 2004) or by mild LBNP decreases SV in humans (Brown et al., 2003; Convertino, Cooke, & Holcomb, 2006; Convertino et al., 2013; Franke et al., 2003; Fu et al., 2009; Hanson et al., 1998; Hinojosa-Laborde, Rickards, Ryan, & Convertino, 2011; Johnson et al., 2014; Rickards, Ryan, Cooke, & Convertino, 2011; van Hoeyweghen et al., 2001).

Furthermore, the current study also found that regardless of group, PP was lower at
minute six of the recovery period than at 150 mL of blood loss during the blood draw period. Pulse pressure is considered an index of SV because of the high correlation between the two measures (Cecconi & Rhodes, 2011; Convertino et al., 2006; Dufour et al., 2011; Johnson et al., 2014). Prior studies have found mixed results with regards to the effects of mild hypovolemia on PP. Some studies have found mild LBNP decreases PP (Convertino et al., 2006; Duranteau et al., 1995; Zoller et al. 1972) and others have found no effect (Mark et al., 1978; Rea et al., 1991). Additionally, some prior studies have found mild hemorrhage reduces PP (Leonetti et al., 2004) whereas others have no change (Fortrat et al., 1998; Rea et al., 1991). Therefore, although prior studies’ findings are mixed, the current study found that mild hemorrhage reduces SV and PP, which is consistent with hypovolemia resulting from blood loss.

Additionally, participants in the present study, regardless of group status, demonstrated an initial reduction from baseline in HR after 150 mL of blood loss, which recovered by the end of blood draw and remained stable during the recovery period. This finding is inconsistent with other studies that find either no change or a slight increase in HR in response to mild hypovolemia induced by hemorrhage (Fortrat et al., 1998; Haberthür et al., 2003; Leonetti et al., 2004; Zöllei et al., 2004) or mild LBNP (Brown et al., 2003; Convertino et al., 2013; Cooke, et al., 2008; Duranteau et al., 1995; Franke et al., 2003; Fu et al., 2009; Hachiya et al., 2012; Hanson et al., 1998; Johnson et al., 2014; Mark et al., 1978; Norsk et al., 1986; Rea et al., 1991; Rickards et al., 2011; van Hoeyweghen et al., 2001; Zoller et al., 1972). However, regardless of group status, in the present study participants’ HF HRV was marginally lower during the recovery period than during the blood draw period. This potential withdrawal of vagal activity during the
recovery period may explain the return to baseline in HR values during the recovery period after the initial drop in HR during the blood draw period. Similar studies have found either a reduction or no change in HF HRV in response to mild hemorrhage (Fortrat et al., 1998; Haberthür et al., 2003; Zollei et al., 2004) or mild LBNP (Cooke & Convertino, 2005; Cooke et al., 2008). Furthermore, the present study found a reduction in CO during the blood draw and recovery periods, regardless of group, which is consistent with the above discussed SV and HR findings from the current study and with previous studies that have found mild blood loss (Leonetti et al., 2004) and mild LBNP both reduce CO (Ahn et al., 1989; Baily, Leuenberger, Leaman, Silber, & Sinoway, 1991; Brown et al., 2003; Franke et al., 2003; Fu et al., 2009; Duranteau et al., 1995; Hachiya et al., 2012).

In the present study, although SV, HR, and CO decreased in response to the blood draw procedure, BP did not change over time most likely because of a compensatory, albeit marginally significant, increase in TPR. Similar studies have found that hypovolemia induced by phlebotomy (Leonetti et al., 2004) or by mild LBNP increases vasoconstriction (Duranteau et al., 1995; Franke et al., 2003; Fu et al., 2009; Mark et al., 1982; Zoller et al., 1972). Such increases may be due to increased SNS activity since the systemic vasculature is predominately innervated by the SNS and not by the PNS (Klabunde, 2011). For example, prior studies have found that hypovolemia induced by mild hemorrhage or by mild LBNP increases sympathetic nerve activity (Convertino et al., 2006; Rea et al., 1991), NE (Duranteau et al., 1995; Fortrat et al., 1998; Haberthür et al., 2003; van Hoeyweghen et al., 2001; Norsk et al., 1993), and E (Fortrat et al., 1998).
In the present study, although E initially decreased from baseline, afterwards it steadily increased across the blood draw period. In contrast, during the blood draw period, NE decreased from baseline and never recovered to or exceeded baseline values. There are a couple of possible explanations about why the current study’s NE findings are inconsistent with prior research. First, only a limited number of studies have investigated NE reactivity to mild hypovolemia and as such it is difficult to fully elucidate the expected response of NE. Secondly, the current study’s intra-assay coefficient of variation for the NE ELISA was almost double the manufacturer’s recommended limit indicating that measurement error likely influenced the results. Again, there was a marginally significant trend towards increased TPR in the present study. Arteries and veins contain alpha-1 and alpha-2 adrenoceptors, which cause the vasculature’s smooth muscle to constrict after binding NE and E, and beta-2-adrenoceptors, which primarily bind E to cause vasodilation in some organs such as the lungs and pancreas (Klabunde, 2011). Although E preferentially binds beta-2 adrenoceptors at low concentrations, at higher concentrations the affinity of alpha-1 and alpha-2 adrenoceptors for E increases and leads to vasoconstriction. Therefore, the marginal increase in TPR across the blood draw period may have been due to vasoconstriction induced by the increasing E concentration during the blood draw. Similarly, beta-1 receptors in the heart increase HR after binding locally released NE and circulating NE and E. In the present study, during the blood draw, both HR and E decreased from the start of the blood draw to 150 mL of blood loss and then steadily increased until the end of the blood draw. This suggests that the effects of blood loss on HR were in part driven by the chronotropic effects of E.
Although some studies of healthy human participants in non-blood donation contexts have found water consumption reduces HR (Ando et al., 2009; Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Lu et al., 2003; Routledge et al., 2002; Schroeder et al., 2002) and increases HF HRV (Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Routledge et al., 2002; Schroeder et al., 2002), SV (Brown et al., 2005; Girona et al., 2014; Schroeder et al., 2002), CO (Girona et al., 2014), vasoconstriction (Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Lu et al., 2003; Nozawa et al., 2009; Schroeder et al., 2002; Scott et al., 2001), PP (Olatunji et al., 2001), and BP (Callegaro et al., 2007; Girona et al., 2014; Hai et al., 2013; Nozawa et al., 2009; Schroeder et al., 2002), the current study found no group differences in HR, HF HRV, SV, CO, or TPR and found that MAP, SBP, DBP, and PP were lower in the PHI than in the control group throughout the simulated blood donation procedure. Therefore, although BP in the PHI as compared to the control group was significantly reduced across the blood draw and recovery periods, there were no group differences in the common cardiovascular determinants of BP such as SV, HR, CO, and TPR. Since at least one of these variables would have to change for there to be a significant group difference in BP, it is possible the study was underpowered to find group differences in some of these variables. However, across the blood draw and recovery periods, relative to the control group the PHI had a lower PP, which is considered a surrogate measure for SV. Although, there was no significant group difference in the actual measure of SV, the group difference in PP suggests the PHI may have had lower SV than the control group, which in turn would explain why SBP, DBP, and MAP were lower in the PHI than in the control group throughout the blood draw and recovery periods. Interestingly, a lower PP
in the PHI as compared to the control group was unexpected since prior studies in non-
blood donation contexts have found acute water loading increases PP (Olatunji et al.,
2011) and SV (Brown et al., 2005; Claydon et al., 2006; Girona et al., 2014; Schroeder et
al., 2002). It is difficult to further explain this unexpected finding of a lower PP in the
PHI than in the control group since variables such as HR and TPR, two of the
determinants of SV, did not differ between the groups. Nonetheless, the present study
observed a lower PP, an index of SV, in the PHI than in the control group, which may
explain the lower BP observed in the PHI relative to the control group throughout the
blood draw and recovery periods.

Typically reductions in BP and/or PP such as those that occurred in the PHI, can
activate the arterial baroreflex, which consists of mechanoreceptors in the carotid sinus
and aortic arch that are sensitive to changes in BP and PP (Klabunde, 2011). When BP
and/or PP decrease from physiological set points, the baroreflex increases SNS activity,
HR, SV, CO, and TPR and consequently BP. This homeostatic mechanism produces
opposite hemodynamic effects when BP increases with or without an increase PP.
However, as will be discussed next, the PHI exhibited impairments in BRS which may
have contributed to the lower BP maintained throughout the blood draw and recovery
periods as compared to the control group. More specifically, similar to a prior study by
Zöllei et al. (2004), the present study found that regardless of group status, there was a
significant reduction in ascending BRS during the recovery period as compared to the
blood draw period. Additionally, the PHI had marginally lower ascending BRS
throughout the blood draw and recovery periods relative to the control group. This
indicated that in response to the mild hypovolemia, there was impairment in participants’
ability to decrease HR in response to increases in BP, and possibly more so in the PHI group. In contrast to the Zöllei et al. (2004) study, which found no change in descending BRS in response to phlebotomy, the current study found a marginally significant trend towards a greater reduction relative to baseline in descending BRS in the PHI as compared to the control group during the blood draw period. This suggests that participants in the PHI were less capable than the control group of increasing HR in response to decreases in BP during the blood draw period. In contrast to a similar phlebotomy study by Fortrat et al. (1998), which found no effect of phlebotomy on combined BRS, the current study found that relative to baseline, the combined BRS (analysis of both ascending and descending sequences) was significantly lower in the PHI than in the control group during the blood draw period. This indicated that during the blood draw, participants in the PHI were less capable of responding to BP changes in either direction with appropriate, compensatory HR changes. These findings are inconsistent with previous research from non-blood donation contexts that indicate water consumption increases BRS (Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Schroeder et al., 2002). However, the cause of the impairment in BRS is not evident and since this is the first study of the effects of water loading on BRS during mild hemorrhage in humans, more research is needed. Therefore, the lower BP in the PHI as compared to the control group during the blood draw and recovery periods was likely due to reduced PP and impaired BRS.

To the author’s knowledge, no study has assessed the effects of water loading on catecholamine reactivity in response to mild hypovolemia induced by phlebotomy. Studies of the effects of water loading on catecholamine reactivity in young, healthy
human participants in non-blood donation contexts are limited in number and have had mixed findings. More specifically, some studies have found water consumption increases NE (Scott et al., 2001) and others have found no change in NE or E (Jordan et al., 2000; Lu et al., 2003). In contrast, the current study found that participants in the PHI had significantly higher concentrations of E and marginally lower NE than participants in the control group during the blood draw procedure. Although participants in the PHI had a higher concentration of E than participants in the control group, they had lower BP and PP, which was unexpected because increases in E can cause increased BP and increased PP (Klabunde, 2011). However, although the concentration of E was higher in the PHI than the control group, it may not have been sufficiently high enough to increase BP and PP for participants in the PHI. For example, it is possible that cardiovagal activity was higher in the PHI than in the control group which would mitigate the pressor effects of the higher concentration of E in the PHI as compared to the control group. This notion is partially supported by the marginally lower descending BRS value in the PHI as compared to the control group during the blood draw period, which suggests the PHI had a reduced capability to withdraw PNS activity in order to increase HR when BP was lowered. However, unlike previous studies which have found water loading increases HF HRV (Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Routledge et al., 2002; Schroeder et al., 2002), the current study found no difference between the PHI and control group in terms of HF HRV. Despite the limited support for higher cardiovagal activity in the PHI relative to the control group, it is possible that increased cardiovagal activity offset the pressor effects of E.
Contrary to expectations, in the present study water loading did not increase BP during mild hypovolemia, which may explain the trend towards lower O₂ in the PHI as compared to the control group. Prior research indicates that reductions in O₂ occur in response to blood donation (Menke et al., 2004; Torella, Cowley, Thorniley, & McCollum, 2002). Although no study has previously investigated the effects of water loading on O₂ during blood donation, research in non-blood donation contexts indicates that water loading improves cerebral autoregulation (Claydon et al., 2006; Shroeder et al., 2002), which could potentially attenuate reductions in O₂ during blood donation. However, in the present study there was a trend towards lower O₂ in the PHI as compared to the control group throughout the recovery period. It is likely that the lower BP observed in the PHI as compared to the control group throughout the blood draw and recovery periods reduced the O₂ of participants in the PHI. This suggests that autoregulation of cerebral perfusion was impaired in the PHI as compared to the control group in response to mild hypovolemia. However, direct measures of autoregulation were not assessed in the current study.

While some of the findings from this study were unexpected based on previous research, the group differences observed in this randomized, experimental design were likely due to differences in hydration. Plasma osmolality was measured as a manipulation check because prior research has shown that acute increases in hydration are associated with acute decreases in POSM (Geelen et al., 1984; Williams et al., 1989). In the current study, no change in POSM occurred from start to finish of the blood draw in the control group. This is consistent with previous research that has found no effect on POSM by hypovolemia induced by mild hemorrhage (Fortrat et al., 1998) or mild LBNP
(Norsk et al., 1986). However, as expected POSM decreased in the PHI from start to finish of the blood draw. At the end of the blood draw, which took on average 9.6 minutes, there was a 4% reduction in POSM among the PHI relative to the control group, which is substantial since even a one percent increase from a person’s basal mean can initiate the sensation of thirst (Armstrong, 2005). Therefore, relative to the control group, POSM in the PHI decreased on average 29.6 minutes after water consumption, which is slightly longer than reported in previous research. A study by Endo et al. (2001) found that after participants consumed 1 L of water, POSM started to decrease after 10 minutes and the greatest reduction occurred after 40 minutes.

Interestingly, although the POSM results indicated that by the end of the blood draw the participants in the PHI were more hydrated than those in the control group, TBW/Kg, another measure of hydration, did not mimic these results. Results for TBW/Kg did not differ between the groups at baseline prior to when the PHI consumed the water, indicating both groups started the study at a similar hydration level. However, TBW/Kg did not differ between groups relative to baseline at minute 19 during the supine period prior to the blood draw (i.e. 19 minutes after the participants in the PHI consumed water) or at minute 2 of the 6 minute recovery period. This absence of a group difference in TBW/Kg may be due to prediction error with the BIA monitor, as previous research indicates it is not able to measure changes in TBW of less than 1 L (Armstrong, 2005). In contrast, group differences in POSM may have been detected because measures of POSM have high reliability and are sensitive to changes as small as a 1% loss in body weight during exercise-induced dehydration protocols (Armstrong, 2005; Popowski et al., 2001).
Limitations

There were several methodological limitations to this study, some of which may help explain the unexpected findings in this study. First, it is possible that the study was underpowered for some of the analyses. For example, there were no group differences in HR, SV, CO, or TPR despite the group differences in MAP, SBP, and DBP. In order for blood pressure to differ between the two groups, there would have to be a group difference on one of these cardiovascular determinants of BP. Interestingly, PP, a surrogate measure of SV, did significantly differ between the two groups. Therefore, it is possible there was insufficient power to assess group differences on the SV data collected by the Finometer PRO. The study was also underpowered to find differences in BDRI scores and likely also underpowered for mediational analyses.

Secondly, the study did not measure MSNA, which would have allowed for BRS analyses of the sympathetic efferent arm of the baroreflex. The present study only included BRS measures of the cardiovagal efferent arm of the baroreflex. When the baroreflex is activated by changes in BP and/or PP, compensatory changes occur in HR and vasoconstriction to maintain BP homeostasis. However, prior research indicates that the responses in the cardiovagal and sympathetic efferent arms are not correlated (Dutoit et al., 2010). Therefore, the current study’s BRS analyses only explain the cardiovagal component of the baroreflex. As such, inferences could not be made about the sympathetic component. Given that prior research indicates that water consumption results in a pressor response because of increased MSNA, NE, and TPR, measuring the sympathetic efferent arm of the baroreflex would have been particularly informative of about the hemodynamic differences between the PHI and control group. However, this
measure was excluded from the current study because of its invasive nature and because
of the time, money, and expertise needed to perform the measurement procedure.

Additionally, the present study did not measure several other endocrine measures
that may have influenced the BP results. However, prior research indicates that mild
hypovolemia does not affect BP-regulating hormones such as: vasopressin (Duranteau et
al., 1995; Fortrat et al., 1998; Goldsmith, Francis, Cowley, & Cohn, 1982; Norsk et al.,
1986; Norsk et al., 1993) atrial natriuretic peptide (Duranteau et al., 1995; Fortrat et al.,
1998), endothelin (Fortrat et al., 1998), renin (Brown, Davies, Lever, Robertson, &
Verniory, 1966; Duranteau et al., 1995; Fortrat et al., 1998; Mark et al., 1978; Norsk et
al., 1993), and aldosterone (Duranteau et al., 1995). Although the literature of the
endocrine effects of acute water consumption in non-blood donation settings in healthy
adults is limited, prior research indicates that it does not affect vasopressin (Jordan 2000),
atrial natriuretic peptide (Freund et al., 1988; Yamasaki, Nishiuchi, Kojima, Saito, &
Saito, 1988), renin (Freund et al., 1988; Jordan 2000) and aldosterone (Kimura et al.,
1986). As such, the current study’s findings were unlikely due to any of these endocrine
measures.

Furthermore, it is possible that the differences between the current study’s
physiological findings and those of previous studies could be due to differences in
characteristics of the water provided to participants such as temperature, tonicity, or
volume. The current study used 500 mL of bottled (hypotonic), room-temperature water
because the majority of the literature on the physiological effects of water on healthy,
young to middle-aged individuals has used room-temperature water that that is hypotonic
(mineral, tap, or distilled water) (Callegaro et al., 2007, Claydon et al., 2006, Endo et al.,
2001, Geelen et al. 1984; Girona et al., 2014; Lu et al., 2003; Routledge et al., 2002; Schroeder et al., 2002; Scott et al., 2001). However, a study by Girona et al. (2014) found that consuming cold water or body-temperature water produced a pressor response, but consuming room-temperature water caused a decrease in BP. The Girona et al. (2014) study and the current study are the only studies to find room-temperature water decreases BP. However, these limited findings indicate water temperature can affect cardiovascular function. Therefore, future research on a PHI for blood donors should consider using cold or body-temperature water instead of room-temperature water.

Tonicity of the water can also alter physiological functioning. For example, a study by Brown et al. (2005) with a randomized cross-over design investigated the cardiovascular effects of consuming the same volume of distilled water to that of an isotonic saline solution. Although consuming distilled water increased participants’ SV and TPR, it decreased their HR which likely mitigated any changes in BP. In contrast, the saline condition produced a small but significant increase in participants’ DBP due to increases in SV without any other compensatory cardiovascular changes. The Brown et al. (2005) study demonstrated that tonicity can alter the hemodynamic effects of water. More specifically, isotonic solutions may be more likely to produce a pressor effect than a hypotonic solution. However, other research by McHugh et al. (2010) found that regardless of volume, consumption of a saline solution did not alter BP, whereas a hypotonic water solution increased BP. Given the limited and mixed research findings on the effects of water tonicity on hemodynamics, it is unclear whether the current study’s findings would have been different with an isotonic solution instead of the hypotonic solution.
Studies on the physiological effects of water consumption varied with regards to the volume of water that participants consumed. However, the most frequently used volume was 500 mL (Callegaro et al., 2007; Claydon et al., 2006; Girona et al., 2014; Routledge et al., 2002; Schroeder et al., 2002; Scott et al., 2001). As such, the current study had the PHI consume this volume of water. Although it is possible that other volumes may produce different physiological effects, larger volumes may produce uncomfortable stomach distention and lower volumes may be physiologically inert.

It should also be mentioned that the current study performed the blood draw 20 minutes after the PHI consumed water because many of the physiological effects of water occur between 20 to 40 minutes after water consumption (Brown et al., 2005; Callegaro et al., 2007; Girona et al., 2014; Nozawa et al., 2009; Routledge et al., 2002; Schroeder et al., 2002; Scott et al., 2001). Together the blood draw and recovery periods lasted 15 to 20 minutes. Therefore, it is likely the study measured the peak of the physiological responses to water consumption. Still, it is possible that performing the blood draw slightly earlier or later with regards to water consumption may alter its hemodynamic effects. More research is needed to determine if there is a better time to perform phlebotomy after a PHI. Another limitation of the current study was that there was no baseline period prior to water consumption in the PHI. Instead, the baseline period started right after the PHI consumed water. That said, as mentioned above, most physiological changes in response to water consumption take about 20 minutes. Therefore, the current study used the first 8 minutes of the 20 minute baseline as the baseline for most statistical analyses. So it is unlikely that the baseline period used in the current study’s analyses was problematic. In fact, there were no group differences on all
of the physiological variables at baseline except for pulse pressure. It is possible the lower PP in the PHI at baseline relative to the control group was due to water consumption. However, it is unclear why PP would decrease in response to water consumption.

Lastly, the study was limited in part due to equipment problems. For example, duplicate measures of Hct did not agree within 2% for the venous blood samples collected during the blood draw periods. Therefore, the current study’s plasma volume results should be interpreted with caution since the manufacture of the HemataSTAT states that the difference between duplicate measures of Hct should not exceed 2%. However, if the current study had excluded participants that had duplicate values differing by more than two percentage points, only 11 participants (2 controls, 9 PHI) would have had complete Hct data. Therefore, for the purposes of this study, all duplicate values were included even if they differed by more than two percentage points. The low reliability of the duplicate Hct measures for venous blood samples may be because these samples were collected in vacutainers with EDTA, an anticoagulant. Especially, since the duplicate capillary blood samples collected via finger stick at intake agreed within 2%. Additionally, the fNIRs monitor used to assess O₂ had an irreparable hardware failure after only half of the participants had been recruited. As such, O₂ analyses were likely underpowered. Furthermore, the ELISA analyses for NE had an intra-assay CV well above the manufacturer recommended amount. Therefore, the NE results may not be valid.

Despite the limitations, this study had many strengths. First, and foremost, no other study, to the author’s knowledge has assessed the physiological effects of a PHI for
phlebotomy. Secondly, internal validity was high because it was a randomized, gender stratified experiment of a homogenous sample of participants. Additionally, it included a multitude of physiological measures, many of which were recorded on a continuous basis, to provide a detailed investigation of the effects of a PHI for phlebotomy. Although the results were at times unexpected, these findings provide a starting point for further research. Future research should replicate this study as well as modify the characteristics of the PHI. It is possible that the physiological effects of water consumption will differ in response to changes in its volume or tonicity as well as the time duration between consumption and the phlebotomy procedure.

**Conclusion**

Similar to prior studies on phlebotomy, the current study found that it induces hypovolemia, which subsequently elicits several physiological responses. Regardless of group assignment, in response to hypovolemia participants in the current study experienced decreases in SV, PP, HF HRV, and CO, an initial decrease followed by an increase in E and HR, and no change in BP. These findings generally support those of prior studies on the physiological responses to hypovolemia. However, the main findings of the present study were unexpected since prior studies performed in non-blood donation contexts have found that acute water loading can exert multiple effects that may prove protective against vasovagal reactions such as increases in: SNS activity, vasoconstriction, PP, BP, cerebral perfusion, and OT. In contrast to these previous findings, the current study found that during and after a simulated blood donation procedure, participants in the PHI exhibited significantly lower MAP, SBP, and DBP and marginally lower \( \text{O}_2 \) as compared to the control group. The lower BP in the PHI as
compared to the control group may be due to the lower PP and BRS also exhibited by the PHI relative to the control group. Although the current study’s findings were unexpected, it was the first study to investigate the physiological effects of water loading before phlebotomy. As such, more research is needed to confirm and extend the present findings.
References


sensitivity within healthy, young humans. *Hypertension, 56*(6), 1118-1123. doi: 10.1161/hypertensionaha.110.158329


Appendix A: Blood Draw Instructions

Participants in the PHI received the following:

“During a blood draw some people may feel slightly lightheaded or dizzy, which is very similar to the lightheadedness experienced if one gets up too quickly. The same thing is occurring physically in both situations. There is a small, short-term reduction in oxygen levels available to the brain. In this study, we are testing the potential benefits of drinking water shortly before donating blood to offset these small reductions in brain oxygen levels that typically accompany blood draw. Accordingly, we had you consume water prior to beginning the blood draw today.”

Participants in the control group received the following:

“During a blood draw some people may feel slightly lightheaded or dizzy, which is very similar to the lightheadedness experienced if one gets up too quickly. The same thing is occurring physically in both situations. There is a small, short-term reduction in oxygen levels available to the brain. In this study, we are interested in measuring the physiological changes that accompany a blood draw.”