THE EFFECT OF PASSIVE HEAT STRESS ON ARTERIAL WAVE REFLECTION, PULSE WAVE VELOCITY, AND CEREBRAL BLOOD FLOW

by

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ABSTRACT

It is well known that the prevalence of cardiovascular events is increased in extreme weather conditions. It has previously been shown that cerebral blood flow (CBF) decreases with heat stress, while the effect of heat stress on augmentation index and pulse wave velocity (PWV) has not been clearly investigated. The main purpose of this study was to determine the effects of passive heat stress on CBF, PWV, and wave reflection. We measured the correlation between baseline measurements of Augmentation Index (AIx), PWV, wave reflection transit time (Tr) and changes in CBF. In addition, the correlation between changes in AIx, PWV, Tr and changes in CBF were also explored. We gathered data from sixteen apparently healthy young adults. Changes in variables from baseline to passive heat stress were evaluated with a paired t-test. While mean CBF did not significantly decline, it was found to trend downwards from baseline to passive heat stress (15 cm/s to 14.9 cm/s, p=0.06). AIx was found to significantly decrease from a baseline of -1% to -12% during passive heat stress (p<0.05). PWV and Tr did not significantly change from baseline. There were no significant correlations found between variables. The present study is the first to demonstrate a decrease in AIx during passive heat stress. In conclusion, these data suggest that while CBF and AIx both decline under passive heat stress, it is not likely that a correlation between CBF and AIx leads to cardiovascular events in the heat.
Chapter 1
INTRODUCTION

Heat is an environmental change that affects the body’s maintenance of homeostasis. The response to these challenges involves many body systems, including the cardiovascular system. When thermal stress is combined with other unusual demands, such as exercise, the challenges for the cardiovascular system are even greater. Clinical and epidemiological data also suggest a strong connection between thermal stress and cardiovascular events. The risk of injury in the heat is often overlooked. Heat waves have killed more people in the United States than all of the other natural disasters combined (Klinenberg, 2002). More than 400 Americans die from heat-related illnesses in a typical year (Klinenberg, 2002). Of particular importance, was the large number of ‘excess’ deaths during the 1995 Chicago heat wave of individuals with a prior ‘heart condition’ (Semenza et al., 1996). Heat stress has been associated with an increase in orthostatic hypotension (OH) (and thus, orthostatic intolerance) events. Orthostatic hypotension is defined by the American Autonomic Society (AAS) and the American Academy of Neurology (AAN) as a systolic blood pressure decrease of at least 20 mm Hg or a diastolic blood pressure decrease of at least 10 mm Hg within three minutes of standing up. It is important to note that orthostatic hypotension is a physical symptom, not a disease (Bradley & Davis, 2003). Orthostatic intolerance (OI) is a syndrome defined more generally as the
development of symptoms during upright standing that are relieved by recumbence (Stuart, 2011). Thus, it can be deduced that OH is a more specific physical characteristic of OI. OI has been shown to be increased during heat stress compared with normothermic conditions (Wilson, et al., 2006). In a study conducted on the prevalence and severity of clinical events studied during a heat wave versus “normal” summer temperatures, the prevalence of OI-related events was significantly higher during the heat wave, suggesting the association between OI and heat (Pathak et al., 2005). OI has also been identified through evidence as a risk factor for ischemic stroke (Eigenbrodt et al., 2000). Experimental investigation of cardiovascular responses to passive heat stress is vital in understanding the mechanisms behind heat-related injuries and deaths.

Cardiovascular Effects of Heat Stress

In this review I will focus on heat stress due to increased body surface temperature. This kind of heat stress is often referred to as passive heat stress. Other kinds of heat stress include heating due to waste heat produced by muscles during exercise, and heating due to an upward resetting of the body’s temperature set point, as occurs in fever. These forms of heat stress are quite different than passive heating, since they are the result of physiological changes that begin inside the body.

The most well known physiological responses to heat involve an elevation in skin blood flow and sweating to protect against heat-related injury (Crandall and Gonzalez, 2010). The skin blood flow increase is achieved through large increases in
vascular conductance associated with cutaneous vasodilation. To prevent large decreases in arterial blood pressure, cardiac output increases, along with decreases in vascular conductance of non-cutaneous beds. This elevation in cardiac output is mainly due to an increase in heart rate as stroke volume has been shown to not change or only very slightly increase in young healthy heat-stressed subjects (Minson et al, 1998). A large increase in cardiac output will generate more endothelial shear stress, which is a major contributor to production of nitric oxide, a known vasodilator and modulator of vascular tone. (Kellogg et al, 1998).

Heat Stress, Pulse Wave Analysis, and Pulse Wave Velocity

An increase in vascular conductance is equivalent to a reduction in total peripheral resistance (TPR). Alterations in peripheral vascular resistance can affect the timing and magnitude of reflected pressure waves. Arterial wave reflection occurs when a pulse pressure wave reaches a location where the arterial properties change, most often at points where blood vessels divide or branch, although it can also occur at an atherosclerotic lesion, a stent, or other abnormality. Changes in large artery stiffness or peripheral resistance can alter wave reflection, because these changes alter the properties of different size arteries to different extents. A decrease in peripheral resistance decreases wave reflections, while an increase in peripheral resistance enhances wave reflections (Westerhof et al., 1972). This same group later demonstrated more specifically, that augmentation index (AIx) is most sensitive to changes in arterial stiffness (Westerhof et al., 2012). The physiological significance of
wave reflections arises mainly from the ability of reflected waves to alter the systolic load and diastolic perfusion of the heart, which is dependent on the reflected wave’s magnitude and timing. When optimally timed, the reflected pressure wave returns to the central aorta during diastole and enhances diastolic perfusion in the coronary circulation (Mitchell et al., 2004) without increasing the workload on the heart, because central aortic pressure during systole is not enhanced. This “late-returning” pressure wave is therefore advantageous. When the reflected wave returns to the central aorta during systole, it increases ventricular afterload, thus increasing workload and reducing stroke volume, without increasing diastolic coronary perfusion. This is detrimental to the heart, and may contribute to cardiovascular events. When researching the effects of heat stress on radial pulse pressure and heart rate, Huang et al. found that after hand immersion in 45°C water for a two-minute period, heat stress significantly reduced augmentation index (Huang et al. 2011). Previous research has shown that passive heat stress causes reductions in systemic vascular resistance (Ganio et al., 2011), suggesting that this will result in an alteration of wave reflection. The effects of arterial dilation on wave reflection have been examined in various studies. Kelly et al. (1989) found that beta-blockers, dilevalol and atenolol, which have vasodilatory effects, decreased pulse wave velocity (PWV) and decreased wave reflection. They and many others use augmentation index (AIx), discussed below, as a quantitative measure of wave reflection. Boutouyrie et al. (2010) found that treatment of hypertension with a calcium channel blocker plus an angiotensin receptor blocker (CCC+ARB) was more effective at reducing central blood pressure than treatment.
with a calcium channel blocker plus a beta-blocker (CCC+BB), even though both treatments produced similar brachial arterial pressures. This study, like the earlier Conduit Artery Function Evaluation (CAFE) study (William B, Lacy PS. et al, Circulation 2006), which compared CCB+ACEI to BB+diuretic, showed that wave reflection is clinically important. The Boutouyrie (2010) study also showed that the two different treatments had similar effects on PWV, but different effects on wave reflection, whether wave reflection was measured as AIX or as HR-corrected AIX. Those treated with BB had a 10 bpm fall in HR while those treated with CCB had a 0.5 bpm fall in HR. This large decrease in HR in the BB group meant that a returning wave would more likely arrive in systole in the BB group than in the CCB group, and this is likely the explanation for why AIX was higher in the BB group than in the CCB group, despite similar PWVs. In addition, an association between alterations of the reflection pressure wave and orthostatic hypotension has been made. Tabara et al. (2005) observed a significant association between subjects diagnosed with orthostatic systolic hypotension (a systolic blood pressure decrease of ≥ 20 mmHg upon standing) and a higher basal carotid AIX, suggesting the relevance of the reflection pressure wave in orthostatic intolerance.

As noted earlier, a normal cardiovascular alteration due to heat stress is an increase in cardiac output, predominantly through an increase in heart rate (Crandall and Gonzalez, 2010). An increase in heart rate could alter the timing of reflected wave arrival at the heart, relative to the phase of the cardiac cycle. Previous research has demonstrated an inverse linear relationship between augmentation index and heart rate
(Wilkinson et al., 2000). This data found that within-individuals for every 10 beats per minute (bpm) increase in heart rate, augmentation index fell by about 4%. This is likely due to alterations in the timing of the reflected pressure wave, and possible changes in the absolute duration of systole (Wilkinson et al., 2000). An alteration in heart rate and its effect on AIx could also contribute to orthostatic intolerance during heat.

Pulse wave velocity (PWV) is typically identified as a measurement of arterial stiffness by describing the velocity of the pressure pulse along the arterial tree (Cecelja and Chowienczyk, 2012). PWV has been described using the Bramwell-Hill equation (Arterial compliance = 1/PWV^2 x blood density), where PWV is proportional to the inverse of the square root of compliance. Arterial stiffness, as measured by carotid-femoral PWV, is an important predictor of cardiovascular mortality and morbidity in hypertensive patients, type 2 diabetes, end-stage renal disease, and elderly (Laurent et al. 2001). It was originally thought that PWV decreases as arterial compliance increases (Bramwell & Hill, 1922) yet it has been found that central PWV and peripheral PWV did not significantly change in healthy adults with an increase in core temperature up to 1.5°C, although the extent of the change was significantly correlated with normothermic central PWV; the largest decreases in arterial stiffness with heating occurred in individuals with the highest baseline stiffness (Ganio et al., 2011). Ganio et al. also noted that a relatively minor increase in blood temperature with passive heating is sufficient to decrease blood viscosity to the point where physiologically significant increases in arterial compliance occurs in the absence of changes in PWV.
In addition, loss of hypotonic sweat may increase blood viscosity, which would counter the effects of increases in blood temperature on blood viscosity, causing arterial compliance to remain the same (Ganio et al., 2011).

Heat Stress and Cerebral Blood Flow

The mechanisms responsible for reducing orthostatic tolerance during heat stress are unclear. Factors that have been studied include cerebral perfusion pressure, cerebral blood flow, and tissue oxygenation. Wilson et al. (2006) demonstrated an increase in orthostatic intolerance with decreased cerebral blood flow with and without heat stress. It is likely that heat-stress induced decreases in cerebral perfusion contribute to increases in orthostatic intolerance. Given this hypothesis, Wilson et al. demonstrated that when subjects were heat stressed the reduction in cerebral vascular conductance and perfusion were significantly greater than when subjects were normothermic (Wilson et al. 2006). The effects of whole-body heating on cerebral vascular regulation remain uncertain. In general, previous research has shown that mild and moderate passive heat stresses lead to a decrease in cerebral blood velocity. In a study on the relationship between dynamic cerebral autoregulation and hyperthermia, subjects were exposed to heat stress by pumping 50°C water through a water perfused suit for a duration of ~45-60 minutes (sufficient enough to increase core temperature ~1°C). Cerebral blood flow at the middle cerebral artery significantly decreased, while heart rate and forearm vascular conductance increased (Low et al, 2009). Mild heating itself has been reported to reduce cerebral blood flow velocity
(Wilson et al, 2002). Whole body heat stress has been found to significantly reduce cerebral blood velocity and increase cerebral vascular resistance. In addition, combined heat and orthostatic stress (simulated using lower body negative pressure) causes even further increases in cerebral vascular resistance and decreases in cerebral blood velocity than with orthostatic stress alone (Wilson et al, 2006). Lastly, peripheral thermo-applications of hot packs decreased cerebral blood flow velocity by 6.9% (Doering et al, 1996).

By quantifying the effects of passive heat stress on cerebral blood flow, pulse wave reflection and PWV, this will provide information that will allow us to better understand other underlying factors that play a role in cardiovascular events in hot environments. This research is novel in that the correlation between cerebral blood flow, arterial wave reflection, and wave propagation has not been looked at under this type of variable. Therefore research on this topic will provide greater insight into this phenomenon.
Specific Aims and Hypotheses

It is well known that the prevalence of cardiovascular events is increased in extreme weather conditions, and that heat stress reduces orthostatic intolerance. It is also known that passive heat stress alters wave reflection, and there is at least one study that suggests a relationship between orthostatic intolerance and high baseline AIx (Tabara et al., 2005). The main purpose of this study was to determine the effects of passive heat stress on cerebral blood flow (CBF), pulse wave velocity, and wave reflection. We measured the correlation between changes in CBF and baseline measurements of wave reflection and wave propagation as well as changes in CBF and changes in wave reflection and changes in wave propagation. Since this study was somewhat exploratory, several measures of wave reflection and propagation were assessed, including AIx, AIx corrected to a heart rate of 75 bpm, PWV, and reflection time. Significant correlations, where found, suggest future lines of investigation, including studies in other patient groups (e.g. elderly). The relationship between wave reflection and cerebral blood flow has never been looked at during heat stress. This study provides novel information about physiological mechanisms related to heat-related cerebrovascular events. Measurement of the correlation between CBF and measures of wave reflection under different temperatures is a useful step toward a better understanding of the relationship between heating and brain blood flow. The specific aims of this study were as follows:
**Aim 1:** To determine whether or not passive heat stress will cause changes in cerebral blood flow.

**Hypothesis 1:** During passive heat stress, cerebral blood flow will be significantly less than that in a control condition.

**Aim 2:** To determine whether passive heat stress causes changes in wave reflection (AIx, AIxHR=75) or wave propagation (PWV, reflection time) and to describe the magnitude of potential changes in apparently healthy adults.

**Hypothesis 2:** During passive heat stress, augmentation index will be significantly less and reflection time will be significantly greater than at baseline. PWV will not significantly change from baseline.

**Aim 3:** To determine whether there is a relationship between baseline measures of wave reflection (AIx, AIxHR=75) or wave propagation (PWV, reflection time) and changes in cerebral blood flow during passive heat stress.

**Hypothesis 3:** Changes in cerebral blood flow during passive heat stress will be correlated with baseline measures of wave reflection and wave propagation.

**Aim 4:** To determine whether there is a relationship between changes in wave reflection and wave propagation and changes in cerebral blood flow during passive heat stress.
**Hypothesis 4:** Changes in cerebral blood flow during passive heat stress will be correlated with changes in wave reflection and wave propagation.
Chapter 2

METHODS

Participants

Sixteen (n=16, 6 male, 10 female) apparently healthy young adult (between 18-35 years of age) subjects were recruited for the study. Limiting the upper range of ages to 35 ensured ruling out subjects that may have begun the process of arterial stiffness due to aging (more specifically, aortic stiffness) that has been shown to occur after age 50 (Mitchell et al., 2004). Exclusion criteria included: any current or past history of cardiovascular disease, smokers, those currently taking medications that affect cardiovascular function, obesity (BMI>30), hypertension (blood pressure >140/90), or any self-reported cardiovascular or metabolic diseases. Informed consent was obtained. The experimental procedures have been approved by the Institutional Review Board (IRB) for Human Subjects Research at the University of Delaware. A copy of the human subjects consent form can be found in Appendix A.

Instrumentation and Preparation

Three limb leads were used to monitor the electrocardiogram (ECG). Brachial artery blood pressure was measured at the right arm (GE Dash 2000). Blood pressure was also measured, and cardiac output was estimated, with a finger tip pressure waveform sensor (Finometer). The Finometer height correction sensor was wrapped
around the subject’s left forearm, and finger blood pressure cuff was placed around the middle phalanx on the same arm. An additional blood pressure cuff was placed on the subject’s left upper arm to calibrate the Finometer.

Skin temperature was measured with thermocouples placed on the subject at six different locations (upper back, lower back, chest, abdomen, thigh, and calf) using hypoallergenic clear tape (Kendall Curity™, Mansfield, MA). An oral thermometer (OT-1, Physitemp Instruments Inc., Clifton, NJ) was used to measure core temperature.

The subject was then dressed in a water-perfused suit (Figure 2.1) that covered the entire body except for head, hands, and feet (Med-Eng Systems Inc., Pembroke, ON, Canada). The suit was attached to tubes that allow inflow and outflow of water from water baths with digital temperature controls (Thermo Fisher Scientific Neslab RTE 7). Backup thermometers were placed in each water bath to confirm the correct temperature.

Trans-cranial Doppler ultrasound (Figure 2.2) was used to measure cerebral blood velocity at the right middle cerebral artery (MCA). A 2 MHz probe was held in place at the subject’s MCA using plastic headgear that can be adjusted. Carotid blood velocity was measured using ultrasonography (Logiq GE ultrasound) and a 12 L-RS probe.

Pulse wave analysis (PWA) and pulse wave velocity (PWV) were obtained by the use of a tonometer (model SPC-350, Millar Instruments Inc.). The tonometer was positioned at the carotid artery (Figure 2.3) using a magnetic clamp (Irwin Quick-
Grip). For calculation of PWV the tonometer was positioned to take measurements at the femoral artery as well. Measurements from the tonometer put into SphygmoCor 2000 software. Three additional ECG limb leads were used for monitoring heart rate and rhythm into the SphymoCor 2000 software for PWV measurements.

**Heating**

Thermoneutral (34-36°C) water was pumped through the suit for 20 minutes before a baseline set of measurement begins. After the first set of measurements was recorded, hot (46-48°C) water was pumped through the suit for a duration sufficient enough to increase core temperature ~1°C. Digital oral temperature and skin temperatures were recorded manually from thermocouples during each set of measurements. Mean skin temperature from thermocouple measurements was computed using a formula previously described (Taylor et al., 1989).

Formula 1. Mean Skin Temperature

\[ T_{sk} = 0.22 \text{ chest} + 0.21 \text{ upper back} + 0.19 \text{ lower back} + 0.14 \text{ abdomen} + 0.14 \text{ thigh} + 0.11 \text{ calf} \]

**Cerebral Blood Flow Velocity**

Cerebral blood velocity was measured using a trans-cranial Doppler ultrasound (Multigon Industries, Yonkers, NY) at the middle cerebral artery (MCA). Maximum and mean cerebral blood velocity from the trans-cranial Doppler ultrasound were
transferred into PowerLab 16/35 (Model PL3516, ADInstruments, Colorado Springs, Colorado) and then subsequently into LabChart Pro software. A 30-second clip was analyzed from each temperature measurement period to determine cerebral blood velocity maximum and mean.

*Carotid Blood Velocity and Flow*

Carotid blood flow was computed from mean carotid blood velocity times vessel cross sectional area. Velocity was measured using a 10 MHz linear phased array ultrasound transducer (Logiq e, GE Healthcare, Waukesha, WI). The average of five waveform cycles was used to determine peak systolic (PS), end diastolic (ED), maximum and mean carotid blood flow velocity during each temperature period. Diameter was measured from the ultrasound image of the artery, acquired with the hardware measured previously.

*Pulse Wave Analysis*

To measure pulse waves for calculation of pulse wave analysis, a tonometer (Millar Instruments, Houston, Texas) was placed over the carotid artery while the subject was in the supine position. The tonometer was connected to the SphymoCor Px System (AtCor Medical, Sydney, Australia) and the carotid pressure wave was recorded. A central pressure wave was calculated non-invasively from the arterial pressure wave via a transfer function. The augmentation index (AIx) was described as the approximate amplitude of the central systolic arterial wave reflection. AIx is
calculated as \((Ps - Pi)/(Ps - Pd)\), where \(Ps\) = peak systolic pressure, \(Pi\) = the inflection point occurring in systole either before or after peak systolic pressure, and \(Pd\) = diastolic pressure. Raw Aortic AIx was determined \((\text{Aortic Pressure}/\text{Pulse Pressure})\) as well as Aortic AIx adjusted to a heart rate of 75.

**Pulse Wave Velocity**

Pulse wave velocity (PWV) was calculated using the SphygmoCor System with the patient in the supine position. The distances from the heart (estimated from the jugular notch) to the carotid and femoral measurement sites were measured with a cloth tape measure. The difference in distance was recorded. The upstroke of the carotid and femoral pulse waves relative to the R wave of the ECG was measured to calculate PWV. The difference in the rise times was computed. The distance difference is divided by the time difference to obtain the estimate of large artery pulse wave velocity.

**Arterial Pressure, Heart Rate, and Cardiac Output.**

Arterial blood pressure was measured on a beat-to-beat basis at the middle finger of the left hand using servocontrolled finger photoplethysmography positioned at the level of the heart (Finometer; Finapres Medical Systems). The finger pressure was calibrated to the brachial artery pressure according to the manufacturer’s recommended calibration procedures. In addition, brachial artery pressures were used to verify absolute Finometer blood pressure measurements via an automated
oscillometric sphygmomanometer (Dinamap Dash 2000; GE Medical Systems Waukesha, WI). Heart rate was monitored using a three-lead electrocardiogram (Dinamap Dash 2000; GE Medical Systems). Cardiac output was estimated using by the Finometer using Modelflow® Technology. An approximate voltage of blood pressure and cardiac output was transferred into PowerLab software and subsequently converted into mmHg or L/min, respectively.

**Statistical Analysis**

Data was evaluated using Microsoft Excel. A paired t-test was performed between baseline and heating for each variable. For correlations, Pearson’s r correlation was calculated and linear regression analysis was performed. Statistical significance is set at p<0.05 for all analyses.

The carotid blood velocity data for subject P014 during heating, 185.6 cm/s, appeared to be an outlier when viewed on a graph with the other carotid velocity data. The other subjects had a mean velocity of 50.9 cm/s and a standard deviation of 7.9 cm/s (n=14). Therefore the Z score of the data from P014, using the mean and standard deviation from the rest of the subjects, was Z=12.56. The probability of getting a Z score that extreme or more extreme, i.e. Pr(|z|>=12.36), is Pr=4.3E-35. In other words, it would never happen. Therefore, we deemed this point an outlier and eliminated it from consideration. As a result, the carotid blood velocity results included data from 15 subjects.
Figure 2.1: Water-perfused suit

Figure 2.2: Doppler ultrasound probe
Figure 2.3: Carotid tonometry
Chapter 3

RESULTS

Temperature and Hemodynamic Responses

Temperature and hemodynamic responses to whole-body heating are shown in Table 1. Core temperature increased by 0.9 +/- 0.25 degrees Celsius (mean +/- standard deviation) from baseline to the heating measurement period (P<0.05, Fig.3.1). Similarly, mean skin temperature increased from 35.2°C at baseline to 37.8°C after heat stress (Fig.3.2), heart rate increased from 63.4 bpm to 89.6 bpm (Fig.3.3), and cardiac output (Fig.3.4) increased from 4.9 L/min to 6.6 L/min (P<0.05). Mean arterial blood pressure did not change over the same temperature increase (P=0.25, Fig.3.5).

Wave Reflection and Propagation

Aortic augmentation Index (AIx) decreased from a baseline measurement of -1% to a heat stressed measurement of -12% (P<0.05, Fig.3.6), while Aortic AIx (HR = 75bpm) did not change from baseline to the heating period (P=0.69, Fig.3.7). Likewise, aortic wave reflection time did not change between these core body temperatures (P=0.27, Fig.3.8). In addition, pulse wave velocity did not change with an increase in core body temperature (P=0.95, Fig.3.9).
**Cerebral Blood Flow Velocity**

Mean cerebral blood flow velocity, measured by Doppler ultrasound in the right middle cerebral artery, was 15.0 cm/s at baseline and 14.9 cm/s during heating. This change was not quite significant, although a trend was evident (p=0.06, Fig. 3.10). Similarly, maximal cerebral blood flow velocity did not significantly decrease but did show a downwards trend from 32.1 cm/s to 28.9 cm/s (P=0.08, Fig.3.11).

**Carotid Blood Flow Velocity**

Interestingly, carotid blood velocity measured by Doppler ultrasound at the right common carotid artery showed a significant increase from 27.8 cm/s at baseline to 36.2 cm/s after the heating period (P<0.05, Fig.3.12). In the 6 subjects we were able to get a measurement from, diameter of the carotid vessel significantly decreased from baseline to heating (P<0.05). After computing for cross-sectional area and multiplying velocity by this measurement, carotid blood flow still significantly increased from 26.2 at baseline to 36.2 during heating (P<0.05).

**Correlations**

There were no significant correlations between baseline Aortic AIx and ∆CBFV (r = 0.22, P = 0.43, Fig.3.13), baseline Aortic AIx(HR=75) and ∆CBFV (r = 0.24, P = 0.39, Fig.3.14), baseline aortic reflection time and ∆CBFV (r = 0.12, P = 0.67, Fig.3.15), and baseline PWV and ∆CBFV (r = 0.23, P = 0.41, Fig.3.16). In
addition, there was no correlation between the ∆Aortic AIx and ∆CBFV (r = -0.33, P = 0.23, Fig.3.17), the ∆Aortic AIx(HR=75) and ∆CBFV (r = -0.016, P = 0.55, Fig. 3.18), the ∆Aortic reflection time and ∆CBFV (r = 0.22, p = 0.42, Fig.3.19), and ∆PWV and ∆CBFV (r = -0.07, P = 0.79, Fig.3.20).

Table 3.1  Mean subject data for each variable ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Heating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>63 ± 11</td>
<td>90 ± 10*</td>
</tr>
<tr>
<td>Cardiac Output (L/min)</td>
<td>4.9 ± 1.2</td>
<td>6.6 ± 1.8*</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td>78 ± 5.8</td>
<td>77 ± 8.4</td>
</tr>
<tr>
<td>Mean Skin Temperature (°C)</td>
<td>35.2 ± 0.5</td>
<td>37.8 ± 0.6*</td>
</tr>
<tr>
<td>Core Temperature (°C)</td>
<td>36.5 ± 0.3</td>
<td>37.4 ± 0.3*</td>
</tr>
<tr>
<td>Pulse Wave Velocity (m/s)</td>
<td>5.5 ± 0.6</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Aortic AIx (%)</td>
<td>-1 ± 0.2</td>
<td>-12 ± 0.1*</td>
</tr>
<tr>
<td>Aortic AIx @ HR=75(%)</td>
<td>-8 ± 0.1</td>
<td>-6 ± 0.1</td>
</tr>
<tr>
<td>Aortic Tr (sec)</td>
<td>176.0 ± 34.2</td>
<td>161.9 ± 33.4</td>
</tr>
<tr>
<td>Max CBFV (cm/s)</td>
<td>32.1 ± 11.6</td>
<td>28.9 ± 8.7</td>
</tr>
<tr>
<td>Mean CBFV (cm/s)</td>
<td>15.5 ± 5.5</td>
<td>14.1 ± 5.4</td>
</tr>
<tr>
<td>Carotid blood velocity (cm/s)</td>
<td>27.8 ± 4.5</td>
<td>36.2 ± 5.9*</td>
</tr>
<tr>
<td>Carotid blood flow (cm³/s)</td>
<td>26.2 ± 4.3</td>
<td>36.2 ± 8.7*</td>
</tr>
</tbody>
</table>

*Denotes significance from baseline at P<0.05.
Figure 3.1: Core temperature.

Figure 3.2: Mean skin temperature.
Figure 3.3: Heart rate.

Figure 3.4: Cardiac output.
Figure 3.5: Mean Arterial Pressure.

Figure 3.6: Aortic AIx.
Figure 3.7: Aortic AIx (HR=75).

Figure 3.8: Aortic reflection time.
Figure 3.9: Pulse wave velocity (PWV).

Figure 3.10: Mean CBFV.
Figure 3.11: Maximal CBFV.

Figure 3.12: Carotid blood velocity.
Figure 3.13: Carotid blood flow.

Figure 3.14: Correlation between baseline AIx and ΔCBFV.
Figure 3.15: Correlation between baseline Aortic AIx (HR = 75) and ΔCBFV.

Figure 3.16: Correlation between baseline Aortic Reflection Time and ΔCBFV.
Figure 3.17: Correlation between baseline PWV and ΔCBFV.

Figure 3.18: Correlation between ΔAIx and ΔCBFV.
Figure 3.19: Correlation between \( \Delta \text{Aortic AIx} \) and \( \Delta \text{CBFV} \).

Figure 3.20: Correlation between \( \Delta \text{Aortic Reflection Time} \) and \( \Delta \text{CBFV} \).
Figure 3.21  Correlation between $\Delta$PWV and $\Delta$CBFV ($p=0.79$).
Mean skin temperature, heart rate, and cardiac output all increased as hypothesized. An increase in heart rate and subsequently cardiac output is expected based upon previous research done demonstrating classic cardiovascular responses to increases in skin temperature. This resulted in increases in core temperature, heart rate and cardiac output (Rowell et al., 1969). With little to no increase in stroke volume, an increase in cardiac output is contributed to nearly entirely by an increase in heart rate (Minson et al., 1998). Mean arterial pressure data also fell in line with responses to heat observed by previous investigators. An elevation in skin blood flow and sweating to prevent heat-related injury is achieved through large increases in vascular conductance associated with cutaneous vasodilation. Thus, mean arterial pressure is maintained by large increases in cardiac output, and changes in mean arterial pressure from baseline normothermic measurements to heating measurements are minimal.

Pulse wave velocity (PWV) did not change from baseline after passive heat stress. This is consistent with previous findings by Ganio et al. (2011) who used a very similar intervention (whole-body heat stress by diffusing 49°C water through water-perfused suit) and found no significant alterations in PWV with core temperature...
increases of 0.5°C, 1.0°C, and 1.5°C. However, they found that individuals with increased normothermic baseline arterial stiffness had a greater decrease in stiffness (larger changes in PWV) versus those with lower baseline stiffness. Given findings that direct heating of isolated arteries increases arterial compliance (Mitchel et al. 1994) and that increases in shear stress (which we can surmise have occurred with a significant increase in cardiac output) are capable of modulating arterial elasticity (Kinlay et al. 2001), it seems intuitive that whole body passive heat stress would lead to a reduction in PWV. Given their negative findings, they hypothesized a contrary explanation that the reserve to increase vascular compliance in an already compliant bed is diminished, which results in greater increases in compliance to heat stress in stiffer vessels (Ganio et al., 2011). This is a relevant point when taking into consideration our subject population. In a group of apparently healthy young adults, it is not surprising that mean PWV at baseline normothermia is 5.5 +/- 0.6 m/s. Average pulse wave velocity in a population < 30 years of age with normal blood pressure (≥120/80 and <130/85 mmHg) was found to be 6.6 m/s (Reference Values for Arterial Stiffness’ Collaboration, 2010). With our subject population’s better than average PWV, there is not a great reserve for increasing compliance and decreasing this velocity.

Consequently, we found that aortic reflection transit time (Aortic Tr) is also not altered under passive heat stress. These findings are compatible with an unchanged PWV. Aortic reflection time is dependent on distance or velocity. Thus, Aortic Tr
would only change if the site of reflection or speed of wave propagation were changing. Therefore, the current finding of an insignificant change in Aortic Tr is consistent with a lack of change in PWV. Moreover, concurrent with our findings, a recent study which looked at the opposite intervention of cold exposure found a lack of change in aortic transit time as well (Sanchez-Gonzalez et al. 2013).

Our results demonstrated a significant decrease in raw aortic augmentation index (AIx) from normothermic measurements to a heat stressed state. Accordingly, previous findings that used localized heat stress (hand immersion in 45°C water for a two minute period) significantly reduced augmentation index (Huang et al., 2011). Furthermore, our current findings are consistent when compared to those found by Edwards et al., who quantified the effects of both facial cooling and whole-body cooling on augmentation index and systolic blood pressure (Edwards et al., 2008, 2006). They found that with facial cooling as well as whole-body cold exposure, AIx was significantly increased when compared with that at baseline temperature. Thus, it would make sense that whole-body passive heat stress would have a reverse effect. While these results are mainly attributed to the resultant vasoconstriction associated with cold exposure, it doesn’t appear that the resultant decrease in AIx from heat exposure is due to the same physiological principles. It was originally hypothesized that because heat stress can lead to large changes in vascular conductance, AIx would likely be reduced. Decreases in large artery stiffness or peripheral resistance can decrease wave reflection (Westerhof et al., 2012). Due to the fact that we saw no
changes in pulse wave velocity under heat stress (the most widely used measurement of arterial stiffness), it is unlikely that changes in AIx were due to alterations in arterial stiffness. More probable is a decrease in AIx due to alterations in heart rate, affecting the timing of reflected wave’s arrival at the aorta. Previous research has demonstrated an inverse linear relationship between AIx and heart rate (Wilkinson et al., 2000). This study found that for every 10 beats per minute increase in heart rate, AIx fell by about 4%. This is likely due to modifications in the absolute duration of systole during each heart beat. As noted earlier, the physiological significance of wave reflections arises from their magnitude as well as timing. A reflected pressure wave that returns to the central aorta during systole increases ventricular afterload, having detrimental effects on the heart. An increase in heart rate will decrease the duration of systole, making it less likely that a reflected pressure wave will arrive at the heart during systole.

The resultant decrease in AIx with an increase in heart rate is instrumental in understanding the opposing results of Aortic AIx adjusted to demonstrate AIx at a heart rate of 75 beats per minute. Thus, since we are contributing a significant decline in raw Aortic AIx to the stimulating effect of heat stress on heart rate, it is clear that it would not have the same effect on a measurement adjusted for heart rate. Without the contributing factor of an increase in heart rate, absolute duration of systole at this measurement remains the same, and there is little to no effect of heat stress on Aortic AIx at a heart rate of 75 bpm.
Cerebral blood flow velocity (CBFV) did not significantly decrease from baseline normothermic temperature to heating, but did demonstrate a trending decline. This trend is consistent with other research findings on cerebral blood flow under passive heat stress circumstances (Wilson et al. 2002, 2006, Low et al. 2009, Brothers et al. 2009, Fan et al. 2008). Heat stress has been found to reduce blood velocity at the middle cerebral artery (MCA) by up to 30% in humans after a passive increase in core temperature of 1.0-1.5°C (Crandall, Brothers, Zhang, 2011). Assuming that the diameter of the MCA does not change throughout heat stress, reductions in velocity are proportional to flow. While we did not see as large a decrease in cerebral blood flow, both maximal CBFV and mean CBFV declined by ~10%. Whole-body heat stress decreases cerebral perfusion partially by reductions in arterial carbon dioxide tension (Wilson et al. 2006, Brothers et al. 2009). The mechanisms underlying reductions in cerebral perfusion independent of reduced arterial carbon dioxide tension remain unknown. One proposed mechanism is increased cerebral sympathetic nerve activity. It has been shown that sympathetic nerve activity to muscle and skin increases during passive heat stress (Niimi et al. 1997, Cui et al. 2002). The role of sympathetic control of the cerebral vasculature remains controversial; although animal studies have shown that cerebral arteries are richly innervated with sympathetic nerve fibers (Nelson & Rennels, 1970). Furthermore, recent evidence has been shown in human studies that support cerebral sympathetic nerve activity. Unilateral trigeminal ganglion stimulation led to a reduction in cerebral perfusion (Visocchi et al. 1996), while a stellate ganglionic blockade led to an increase in cerebral perfusion (Ide et al. 1996).
Thus, it is feasible that increases in cerebral sympathetic nerve activity due to heat-stress may contribute to reductions in cerebral blood flow.

Interestingly, carotid blood velocity demonstrated a significant increase from normothermic baseline temperature to heat stress. It has been shown in vivo and ex vivo that the carotid artery vasoconstricts under hyperthermic conditions (Mustafa et al. 2004, 2007). Given these findings, a measurement of carotid blood velocity is not proportional to flow and cross-sectional area of the vessel needs to be taken into consideration. We were able to calculate diameter of the carotid in 6 subjects which significantly decreased from baseline. After calculating and dividing velocity by cross-sectional area, carotid blood flow was still found to significantly increase. Given that this measurement was taken at the right common carotid artery, which branches into the internal carotid artery and external carotid artery, it is not solely an indication of cerebral blood flow such as the MCA. The internal carotid supplies oxygenated blood to the brain and eyes, while the external carotid supplies the throat, neck glands, tongue, face, mouth, ear, scalp and the dura mater. Consequently, it is plausible to speculate that the significant increase at this artery is due to an increase of blood flow to other locations than the cerebrum such as the skin and scalp to allow for dissipation of heat through sweating.

There were no significant correlations found between baseline values of AIx, Aortic AIx, Aortic Tr, PWV and ΔCBFV. In addition, no significant correlations were found between changes in AIx, Aortic AIx, Aortic Tr, PWV and ΔCBFV. While the
correlation between AIx and cerebral blood flow has not been previously been looked at under a heat stressed condition, a correlation has been found examining the influence of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, on arterial stiffness and cerebral blood flow (Kielsten et al., 2006). Administration of ADMA increased arterial stiffness (as measured by AIx) and significantly decreased cerebral perfusion. Aside from this study, there is limited research done to examine the correlation between AIx and cerebral blood flow, especially under heat stress. Taking into consideration that Aortic AIx, Aortic Tr, and PWV were all found to be insignificantly changed under heat stress, a significant correlation between these variables and the ΔCBFV would not be expected.

Limitations

In our current study, middle cerebral artery velocity was used as an index of cerebral blood flow. It is recognized that this velocity is only representative of flow if the diameter of the vessel remains unchanged. This assumption has been validated in humans by studying the effects of changes in MAP and variations in CO₂ on this vessel diameter. These investigators found that the diameter of this vessel was either not changed or only minimally affected by these changes (Giller et al. 1993). Furthermore, it has been shown that MCA velocity reliably correlates with changes in cerebral blood flow, and any problems with reliability are likely due to wide between-patient variations in absolute blood flow velocity at rest (Bishop et al. 1986). Thus, it is assumed that MCA velocity is reflective of cerebral blood flow.
Another possible limitation that we found as investigators is the possibility that the position of the Doppler probe was altered from the normothermic measurement to the heat stressed measurement. There may have been movement of the probe in between measurements which may have altered the strength of the signal, not indicating the true $\Delta$CBFV from normothermia to heat stress.

As opposed to cerebral blood velocity, carotid blood velocity is not a reliable index of flow due to its likelihood of changing diameter during hyperthermia. For this reason, we planned to calculate cross-sectional area of the vessel from its diameter in order to accurately calculate flow. Due to technical difficulties with our ultrasound machine, we were unable to recover images of the carotid vessel in our first nine subjects in order to measure diameter and calculate cross-sectional area. Thus, we only have accurate carotid blood flow measurements from six of our subjects.

Admittedly, tonometry measurements taken at the carotid artery are not validated and not always ideal for applanation tonometry because it is surrounded by loose tissue and is affected by breathing. In a comparison study performed on radial and carotid tonometry, it was found that beat-to-beat variability was lower for the radial than the carotid site, and additionally operator index was $\sim$58% higher at the radial site (Adji et al. 2006). This suggests that radial tonometry is a superior measurement of augmentation index to carotid tonometry. On the contrary, there have been a handful of investigations done that demonstrate the reliability of tonometry at the carotid artery, suggesting that carotid tonometry-derived AIx is an applicable
measurement of central augmentation index and should be widely used in cardiovascular studies (Chen et al., 1996). In the context of the current study, carotid artery tonometry was used due to the correlations being researched and its relative proximity to the cerebrum (and thus middle cerebral artery).

Lastly, a limitation of the interpretation of the results of this study in comparison to other studies is a lack of consistency in the magnitude of heat stress. Some studies have reported increases in core temperature as low as 0.5°C while others have reported increases from 1.0°C-1.8°C (Ganio et al. 2011, Brothers et al. 2009, Low et al. 2009, Nelson et al. 2011, Doering et al. 1996).

In conclusion, passive whole-body heat stress to increase core temperature ~0.9°C decreases cerebral blood flow and significantly reduces AIx. Additionally, current data showed that passive heating did not affect central arterial stiffness (as measured by pulse wave velocity). Lastly, passive heating led to a significant increase in carotid blood velocity. These present findings support and are consistent with prior observations. Future research should include studies done in elderly as opposed to a young healthy population. Hellon and Lind (1956) showed that an older population (ages 44-57) have a lower tolerance to heat, as demonstrated by a delayed onset of sweating (when compared to a middle-aged group ages 18-23) during heat exposure and an overall higher skin and core temperatures (Hellen and Lind, 1956). Our subjects likely had a greater tolerance to heat and ability to dissipate heat than had we tested an older population.
REFERENCES


Appendix A

INFORMED CONSENT FORM

Research Study: Cardiovascular and Cerebrovascular Responses during Whole Body Heating

Investigators: Kimberly Ashton, Gregory Gillispie, William Rose, Ph.D., David Edwards, Ph.D.

1. INTRODUCTION

You are invited to take part in a research project to gain information about blood pressure and flow during heat exposure. Your participation is voluntary. You may withdraw your participation in this study at anytime without penalty.

2. PURPOSE

The purpose of this research is to understand how changes in body temperature affect blood pressure and flow patterns in the body. This is important because the risks of some cardiovascular problems including heart attacks and strokes are greater during the summer months. A better understanding of these physiological mechanisms is important in reduction of these risks. We will non-invasively record your heart rate, blood pressure, blood flow, and brain blood flow during skin surface heating (45-48ºC; 113-118ºF). Temperature will also be measured under your tongue and on your
skin of your abdomen, legs, chest, and back. You have been asked to participate as one of approximately 30 subjects in this study because you are a young, healthy person.

This procedure has been used previously by various investigators with similar protocols. Temperatures as high as 50°C have been used in the water perfusion suit. Cardiovascular changes have also been studied using more extreme conditions including environmental chambers. Our main focus is to study the cardiovascular changes due to environmental stress with a slight change in core body temperature (~0.8-1.0°C). Numerous past studies have increased core temperature using passive heat stress up to 1.5-2.0°C, thus we feel confident in the safety of your health with an increase in ~0.8-1.0°C. Healthy, young adults have considerable tolerance to temperature changes. Should core temperature increase greater than 1.5°C, we will terminate the study.

3. PROCEDURES

Your participation will involve one testing session that will last approximately 2 hours. You will wear a body suit lined with watertight tubes through which water will be pumped to cause a neutral or warm temperature. You will not get wet, except for possible sweating, since the tubes are watertight. This makes the heating effect considerably less intense than actually being immersed in water of an equivalent temperature. You should not consume alcoholic or caffeinated beverages for 12 hours prior to the testing. You should bring a pair of shorts and a T-shirt to be used during the study.
You will be one of ~30 healthy young volunteers that will be recruited. In order to participate in this study, you must be sedentary to recreationally active (do not exercise more than 4 times per week), have blood pressure in the normal range, non-smoker, non-obese, free from cardiovascular disease, and not receiving medication that could influence the results of this study. You will also fill out a questionnaire to make sure you fit the criteria. We will measure height and weight to assess your BMI. If you are female, you will need to submit a urine sample, for pregnancy testing, and will be excluded and referred to doctor if tested positive. Once qualified for the study, you will first sit quietly for 3 minutes and then your blood pressure will be recorded at the upper left arm to make sure you do not have high (above 140/90 mmHg) or too low (below 100/60 mmHg) blood pressure.

If you pass the screening, you will put on the tube-lined suit and lie on a padded table for the experiment. Three ECG electrodes will be placed on your skin: one on each side of your chest and one on your lower left waist. The ECG electrodes are about the size of a quarter with adhesive on the skin side and a wire on the other side. They will be used to monitor your heart rate. The tube-lined suit will cover the entire body except the head, hands, and feet. Zippers along the forearm will allow for measurements on the arms. Temperature of the water will be adjusted by water baths with digital temperature controls and backup thermometers will be placed in each water bath to confirm the correct temperature. You will wear plastic headgear, which will hold a probe against the skin of your right temple. This probe will monitor brain blood flow. A similar probe will be positioned on the right side of the neck to
measure blood flow in the carotid artery. A device that looks like a small plastic sleeve will next be placed on your left finger to measure blood pressure. A tonometer, which is a device about the size of a pencil, will be positioned at your neck, and groin (upper thigh) in succession to measure your pulse. Small thermometers will be applied to your abdomen, leg, chest, and back to measure your skin temperature, and an oral thermometer will be used to monitor internal body temperature. We will zip up the suit and you will lie quietly, while thermoneutral water (34-35°C; 93-95°F) pumps through the suit for baseline measurements for 20 minutes.

Then warm water (45-48°C; 113-118°F; feels like a hot tub) will pump through the suit after which we will take another set of measurements. The warm water will continue to be pumped through the suit until you have reached a sufficient increase in core temperature (this typically takes about 45-60 minutes) at which point we will take the final set of measurements. The water will not touch your skin, since the tubes are watertight, but you may perspire during exposure to the warm water. It is important to note that if your internal body temperature changes by greater than 1.0°C degree Celsius or more at any time the water temperature will be adjusted to minimize further body temperature changes. If your internal temperature changes by more than 1.5°C from baseline, we will terminate the study. If, at any time, you would like to stop the procedure, just let us know you are not comfortable participating anymore, and the tube-lined suit and measuring devices will be immediately removed.

4. CONDITIONS OF SUBJECT PARTICIPATION
Your results for the tests are confidential and will be assigned a number that will be used on spreadsheets, notes, and data analysis documents. You will be allowed to receive your blood pressure and heart rate data following the completion of testing. Your name and subject number will only appear on a single master document, which will be locked in a filing cabinet in the principal investigator’s office. Other records will be stored in locked filing cabinet and on password-protected computers. The duration of data retention is indefinite. Your name will be omitted in any publications related to this research.

In the event of physical injury during laboratory testing procedures, you will receive first aid, at that time. If you require additional medical treatment, you will be referred to the closest emergency room and will be responsible for the cost. You may withdraw from the study at any time without penalty.

5. RISKS AND BENEFITS

The risks associated with this study include mild discomfort with the pressure and flow probes. Side effects of heating may be sweating, increased heart rate, discomfort, nausea, and fatigue. Since you are young and healthy, your body should adjust to the heat, just as it does when you are outside during the summer. As a result of your participation, you may have a greater knowledge about your blood pressure and heart rate at rest, and during heat exposure. In addition, your participation in the
study may provide new, useful information about changes in blood pressure and flow during heating.

6. FINANCIAL CONSIDERATIONS

You will not be compensated financially for participating in this research study.

7. CONTACTS

If you have questions about the research study, you may call: Kimberly Ashton, (609-915-9669) or Dr. William Rose (302-831-1064) Assistant Professor, Department of Kinesiology and Applied Physiology. If you have questions regarding the rights of individual who agree to participate in this research, you may call (302-831-2137), the chair of the University of Delaware Institutional Review Board.

8. SUBJECT'S ASSURANCES

I have read the above informed consent document. The nature, demands, risks and benefits of the project have been explained to me. I knowingly assume the risks involved, and understand that I may withdraw my consent and discontinue my participation in this study at any time without penalty or loss of benefit to myself. A copy of this consent form has been given to me.
9. CONSENT SIGNATURES

Participant’s Signature: ______________________________

Date:____________

Participant’s Name (printed): ______________________________

I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. I have provided the subject with a copy of this informed consent document.

Signature of the Investigator: ______________________________ Date:________
Appendix B

UD CLASSIFIEDS ADVERTISEMENT

Research Participation Opportunity:

The Physiology Lab is performing a study about the effects of heating on the cardiovascular system. Healthy young, non-smoking individuals between the ages of 18-35 years are invited to participate. The study will take approximately 2.5 hours. Days and times are flexible. By participating in this study, you will receive a free blood pressure screening. Participants will have a greater knowledge about their blood pressure and heart rate at rest, and during heat exposure. In addition, by participating in the study, you may provide new, useful information about changes in blood flow to the brain and vascular function during heating.

In order to participate, you must:

1. Be between the ages of 18-35 years
2. Have no heart, lung, or brain disorder/disease
3. Have a healthy BMI (less than 30)
4. Be sedentary to recreationally active
5. Not smoke
6. Not receive any medications that could affect the results of the study

Please include your availability for the next 2 weeks in your response. Please wear or bring sweat shorts and a T-shirt for the study.

For further information, please contact Kimberly Ashton: kashton@udel.edu
Appendix C

IRB APPROVAL LETTER

[Image of IRB Approval Letter]

Thank you for your submission of Amendment/Modification materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

- 1 -

Generated on IRBNet
If you have any questions, please contact Jody-Lynn Berg at (302) 831-1119 or jberg@udel.edu. Please include your study title and reference number in all correspondence with this office.