INVESTIGATING MARKERS OF CARDIOVASCULAR DISEASE RISK

IN FEMALE HOSPITAL EMPLOYEES

by

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Abstract

Shift work is an increasingly popular work pattern and there is evidence that it is associated with an increased risk of cardiovascular disease (CVD). The causal pathways linking shift work and CVD risk are unclear, however, there is evidence that endothelial dysfunction and high reactivity to mental stress may play a role in disease progression. No studies in women have investigated the impact of a history of shift on 1) endothelial function assessed using two distinct tests (RH-FMD and HGEX-FMD) and 2) cardiovascular and cortisol reactivity to an acute stressor. The purpose of this thesis was to compare 1) RH-FMD and HGEX-FMD and 2) cardiovascular and cortisol stress reactivity in a group of female shift workers (SW) and non-shift workers (NSW). 20 healthy SW (41.1 ± 11.4 years) and 19 NSW (41.6 ± 11.4 years) participated. Each participant preformed two RH-FMD and two HGEX-FMD tests. Brachial artery diameter and blood velocity were assessed using Echo and Doppler ultrasound, respectively. For stress reactivity, serum cortisol, heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured before, during, and after a speech and mental arithmetic stress task. All values are means ± SD. There was no difference between SW and NSW in terms of RH-FMD (SW: 7.34 ± 2.78%, NSW: 7.86 ± 3.47, p=0.610) or HGEX-FMD (SW: 5.46 ± 3.24%, NSW: 4.14 ± 2.10, p=0.15). HR, SBP, and DBP stress reactivity were not significantly different between groups (HR: ΔSW: 22.35 ± 15.68 beats·min⁻¹, ΔNSW: 20.42 ± 10.53 beats·min⁻¹, p=0.66; SBP: ΔSW: 20.93 ± 10.72 mmHg, ΔNSW: 19.57 ± 11.92 mmHg, p=0.72; DBP: ΔSW: 14.27 ± 6.58 mmHg, ΔNSW: 11.20 ± 6.28 mmHg, p=0.16), however the magnitude of difference in DBP reactivity between groups (3.07 mmHg) had a moderate effect size (d = 0.5). Cortisol stress reactivity was significantly higher in SW than NSW (8.53 ± 6.04 µg/dL vs. 1.41 ± 2.92 µg/dL). These preliminary data suggest that shift work experience did not have a negative impact on endothelial function, but did result in elevated HPA-axis reactivity and modest increases in cardiovascular reactivity to acute mental stress.
Co-Authorship

This thesis presents the work of Ira Carson in collaboration with Dr. Kyra Pyke, Morgan Batson, and Dr. Joan Tranmer. Design of the complete study was a collaborative effort between Ira Carson, Dr. Kyra Pyke, Dr. Joan Tranmer, and Morgan Batson. In the findings described in manuscript 1 (Chapter 3) and manuscript 2 (Chapter 4) Ira Carson was responsible for developing the research question, reviewing the background literature, designing the study protocol, conducting the data collection and analysis (Morgan Batson assisted with all data collection, and in the analysis of the RH-FMD data reported in Chapter 4), interpreting the results, and drafting all thesis chapters. All aspects of this were done with important consultation with Dr. Kyra Pyke. Dr. Kyra Pyke was the Co-principal investigator on the research grant funding this study and is the co-author of the manuscript in Chapter 3 titled “A preliminary study of cardiovascular and cortisol reactivity in female shift and non-shift workers”, and the manuscript in Chapter 4 titled “Brachial artery flow-mediated dilation of female shift and non-shift workers in response to reactive hyperemia and handgrip induced increases in shear..."
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List of Abbreviations

AUC – area under curve
BH₄ – tetrahydrobiopterin
BP – blood pressure
CHD – coronary heart disease
CVD – cardiovascular disease
DBP – diastolic blood pressure
DDNN – day, day, night, night
eNOS – nitric oxide synthase
FMD – flow-mediated dilation
HGEX – handgrip exercise
HGEX-FMD – handgrip exercise mediated flow-mediated dilation
HPA axis – hypothalamic-pituitary-adrenal axis
HR – heart rate
IMT – intima-media thickness
MVC – maximum voluntary contraction
NO – nitric oxide
NSW – non-shift worker
RH – reactive hyperemia
RH-FMD – reactive hyperemia mediated flow-mediated dilation
ROS – reactive oxygen species
SBP – systolic blood pressure
SW – shift worker
Chapter 1

Introduction

Cardiovascular disease (CVD) is a major cause of death worldwide (31). In Canada alone CVD accounts for approximately 25-30% of annual deaths (54) and results in more than 20 billion dollars in lost wages, decreased productivity, and hospital costs (48). This makes understanding the etiology of the disease and developing early detection and prevention programs important. Lifestyle factors play a large role in the development of CVD (58), and given the significant time spent at work, the working environment itself warrants study as a possible contributor.

There is growing evidence that shift work patterns in particular are associated with increased CVD risk (44, 49, 55). Shift work can be defined as working rotating morning, afternoon, and evening shifts, or working constant shifts at irregular hours (i.e. permanent nights shifts) (10). In Canada approximately one quarter of the labor force works shift work, with women comprising 37% of full-time and 70% of part-time workers (55). Specifically, there is some evidence that shift workers (SW) show more early signs of atherosclerosis (38), a precursor to CVD, and are at a significantly higher risk of coronary heart disease (CHD) than non-shift workers (NSW) (25, 27). Large scale cohort studies have also identified shift work as an independent risk factor for the progression of hypertension(52).While it is becoming clear that shift work is related to CVD risk, the causal pathways are still unclear.

Emerging evidence points to the vascular endothelium as a physiological link between shiftwork and CVD. The endothelium is a single layer of cells that lines all arteries, and is essential for control of vascular tone (14) and regulating arterial functions such as
anticoagulation, leucocyte adhesion, and smooth muscle proliferation (16). Disruption of proper endothelial function is therefore thought to contribute importantly to the development of atherosclerotic plaques, eventually leading to CVD (11). Several studies have indicated that acute exposure to shift work has a deleterious impact on endothelial function. In a variety of shift working samples these studies have identified that relative to a day rotation or day-off baseline, within SW endothelial function is impaired immediately post night shift (3, 26, 47). However, the chronic effect of shift work (i.e. comparing SW (after recovery from a night shift or rotation) vs. NSW) on endothelial function has been minimally investigated. Existing reports suggest a shift work-associated chronic impairment. However, these studies are small, use non-standard techniques (45, 56), or have only been done in men (53).

Endothelial function can be assessed non-invasively by taking advantage of how the endothelium reacts to a blood flow-associated shear stress stimulus (the friction of flowing blood on the arterial wall). When exposed to an increase in shear stress, endothelial cells release vasodilators in a response known as flow-mediated dilation (FMD) (35, 43). Low magnitudes of FMD indicate poor endothelial function (7). The established way to measure FMD in humans is through reactive hyperemia (RH): transient increases in shear stress achieved via the release of a temporary limb occlusion (7, 36). RH-mediated FMD (RH-FMD) is an independent predictor of cardiovascular events in both healthy (42) and at-risk populations (17).

In recent years an alternative method to increase shear stress for FMD assessment, dynamic handgrip exercise (HGEX), has been garnering greater attention (39). HGEX mediated FMD (HGEX-FMD) uses an exercise stimulus to create a sustained, intensity-dependent increase in brachial artery shear stress (40, 57). The difference in the shear stress stimulus profile created with RH vs. HGEX-FMD is important because the nature of the FMD response (i.e. its magnitude
and mechanisms) appears to be dependent on the shear stress profile (33, 41). Indeed, acute stress (46), diabetes (4), and a history of smoking (12) have been shown to impact RH and HGEX-FMD differently, indicating that these two tests may provide distinct information regarding endothelial function. A provocative preliminary report showed that HGEX, but not RH-FMD, was impaired in young healthy smokers. In a sample of older individuals with a longer smoking history, both RH-FMD and exercise mediated FMD have been shown to be impaired (15). This suggests that in some cases HGEX-FMD could provide a more sensitive test of emerging endothelial dysfunction, which would make it a useful tool in young and asymptomatic groups. Furthermore, FMD that occurs during daily activity is a result of exercise-induced shear stress. Since this may have functional relevance in terms of perfusion (20, 51), it is important that the factors influencing exercise-mediated FMD are well characterized. Understanding how a history of shift work impacts both RH and HGEX-FMD may provide a more comprehensive picture of endothelial function than performing either test alone. Currently it is unknown how a history of shift work affects HGEX-FMD.

Another potential pathway linking shift work and CVD risk involves psychological and physiological responses to stress. Stress can be defined as any disruption of homeostasis by internal or external factors that requires constant autonomic, hormonal, or behavioral adjustment (32). The major physiological stress response systems are the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the important stress hormone cortisol. Cardiovascular and cortisol reactivity (the magnitude heart rate (HR), blood pressure (BP), and cortisol elevation) to acute psychologically stressful tasks (termed mental stress tasks) have been shown to be predictive of future cardiovascular risk (8, 19). A meta-analysis of 36 prospective studies found that heightened cardiovascular reactivity to acute mental stress was associated with future hypertension and increased carotid intima-media thickness (IMT) (a
measure of atherosclerotic changes (8)). Additionally, cortisol responses to stress are higher in hypertension-prone men and women (1), and a prospective study in 479 healthy men and women reported a significant association between heightened cortisol reactivity and future hypertension (19). While this cannot be determined by observations of association, cardiovascular and cortisol reactivity may also play a causal role in the development of CVD via several pathways (8, 19). For example, frequent exposure to daily stress may result in a clinically relevant elevation of resting blood pressure in hyper-responsive individuals (8, 18), and heightened cortisol responses to stress could contribute to the development of atherosclerosis via a negative impact on endothelial function (6).

The effect of shift work on stress reactivity has been minimally investigated with only one study examining cardiovascular reactivity and none investigating cortisol reactivity. Systolic blood pressure (SBP) reactivity was reported to be non-significantly higher in SW compared to NSW (24). While primary experiments are lacking, there is evidence that shift work affects the physiological stress response systems. Female nurses on permanent night and rotating shift schedules are seen to have higher sympathetic activation compared to day workers (9, 21). Furthermore, levels of urinary norepinephrine, a neurotransmitter of the sympathetic nervous system, have been reported to be elevated in female hospital SW reporting high job strain (13). In terms of the HPA-axis, altered diurnal cortisol secretion (28, 34, 56) and elevated chronic cortisol levels (30) have been observed in shift working populations.

In conjunction with impacting physiological stress systems, shift work is also known to increase self-reported chronic stress (5, 23). Previous studies have characterized chronic stress using measures of job strain, work-life balance, and recovery time from work-induced fatigue (37). In general SW report higher job strain (5), substantial interruptions in social (2) and family
life (23), and an increased time needed to recover from work-induced fatigue (22). There is also evidence of gender differences in the experience of chronic stress (50). Chronic stress in SW is relevant to acute stress reactivity because it can amplify cardiovascular responses to acute mental stress (29).

Collectively, this evidence suggests that shift work may influence cardiovascular and cortisol responses to acute mental stress. It is important to know if shift work increases stress reactivity because it would provide additional insight regarding the association between the function of the stress response systems and shift working patterns. It may also direct attention to a new mechanistic pathway leading from shift work to CVD risk.

In summary, while existing evidence suggests that CVD risk is elevated in SW, the mechanisms are still unclear. There is preliminary evidence to suggest that both endothelial dysfunction and dysregulation of stress response systems may be involved, but these factors require further investigation. Women comprise a large proportion of the shift working population and a study devoted to this group is important as gender differences may influence the pathways between shift work and CVD risk. With this as background, the aim of the following study was to investigate how shift working history impacts RH-FMD, HGEX-FMD, and cardiovascular and cortisol stress reactivity in women.

**Specific Objectives**

The objectives of this thesis were two-fold.

1. To characterize and compare cardiovascular and cortisol stress reactivity in healthy female SW and NSW (Chapter 3)
2. To compare brachial artery endothelial function in healthy female SW and NSW using HGEX- and RH-FMD (Chapter 4).
Specific Hypotheses

1. Female SW will have greater cardiovascular and cortisol stress reactivity compared to NSW.

2. Female SW will exhibit evidence of endothelial dysfunction as indicated by a lower HGEX-FMD compared to NSW. RH-FMD will not differ between SW and NSW.

The findings of this study are presented in two manuscripts (Chapters 3 and 4). These data were collected as part of a larger protocol that included additional characterization of vascular health. In this larger protocol RH and HGEX-FMD was tested first and stress reactivity was tested second. Sample size was initially powered for RH-FMD, the primary outcome variable for the larger overall study. In order to detect moderate differences in FMD between SW and NSW based on the variability of precious acute shift work studies (3, 26), a sample size of 128 participants (64 participants per group) was needed. However, due to the practicality of recruiting participants and the exploratory nature of this pilot study, the sample size was reduced to 39 participants (20 SW and 19 NSW).
References


Chapter 2

Literature Review

This review will focus on 1) shift work and CVD risk, 2) the endothelium and its role in the development of CVD, 3) current methods of assessing endothelial function, 4) endothelial function in SW, 5) the importance of cardiovascular and cortisol stress reactivity in the prediction and progression of CVD, and 6) evidence for modulation of cardiovascular and cortisol stress reactivity in SW.

2.1 Shift work and CVD risk

Shift work can be defined as work at changing times of day (i.e. morning, afternoon and night shifts), or work at constant but unusual hours of the day (i.e. permanent nights shifts) (21). In Canada, approximately 28% (4.1 million people) of the workforce is employed as SW. This trend is reflected in other developed nations with approximately 15% of workers in the United States (102) and 20% of workers in Europe (93) employed in shift working positions. Occupations with the highest percentage of SW include police officers, nurses and doctors, and primary industry workers (114). Patterns of shift work cause circadian stress (interruption of the sleep-wakefulness rhythm) (81), that may act through physiological, behavioral, and psychosocial pathways to result in an increased risk of CVD (55, 59, 77, 81, 82, 106) (Figure 1).
Within the past three decades there have been many large-scale prospective studies exploring the association between shift work and CVD risk. In 1988 Kawachi and colleagues (1995) (55) began a prospective study following 121 700 female nurses aged 42-67. Of the initial cohort, 79 109 worked rotating night shifts at least 3 nights per month. At the start of the trial all subjects were free of diagnosed CVD and stroke. After 4 years of follow up, the age adjusted relative risk of CVD (non-fatal myocardial infarction and fatal coronary heart disease (CHD)) was 1.38 in women who had done shift work compared to nurses who had never done shift work. Within SW, those who had done < 6 years of shift work had a relative risk of 1.21, while those who had done > 6 years had a relative risk of 1.51. This increased risk still existed after controlling for smoking and other CVD risk factors. Other cohort studies involving both men and women have established connections between shift work and increased risk of fatal CVD (106), hypertension (77), coronary heart disease (59), and early signs of atherosclerosis (82). Currently, the mechanistic pathways linking shift work to CVD are not well understood (81). However, evidence suggests that the vascular endothelium and physiological stress responses may play a role.

**Figure 1.** Model for pathways from shift work to CVD. Adapted from Puttonen et. al (2010) (81).
2.2 The Endothelium and Atherogenesis

The endothelium is a single layer of cells that lines all arteries. It plays a key role in vasoregulation and vasoprotection by synthesizing and releasing a variety of modulating factors in response to physical and chemical stimuli (104). The endothelium influences vascular tone through the secretion of vasodilatory substances including nitric oxide (NO) (47, 86). NO is produced by nitric oxide synthase (eNOS), an enzyme constitutively expressed within endothelial cells. Activated via transduction of the shear stress (friction) of red blood cells moving against endothelial cells, eNOS facilitates the conversion of L-arginine to NO and L-citrulline (26, 78, 89). Diffusion of NO into the surrounding smooth muscle leads to increased cyclic guanosine monophosphate (cGMP) production and decreased intracellular calcium, resulting in relaxation of the vascular smooth muscle. This phenomenon whereby increased blood flow associated shear stress leads to endothelium dependent dilation is called flow-mediated dilation (FMD).

Most often, endothelial dysfunction is detected as the impairment of FMD, or more rarely, impairment of endothelial dependent dilation to an infused chemical stimulus; both of which may result from a decrease in NO bioavailability. Endothelial dysfunction occurs naturally with aging (16), but can be accelerated by many factors including obesity (45), smoking (17), insulin resistance (94), renal failure (6) and psychosocial stress (72). These factors may contribute to endothelial dysfunction through several diverse mechanisms, many of which involve oxidative stress (reviewed by Griendling and FitzGerald, 2003). When NO reacts with reactive oxygen species (ROS) it becomes converted to peroxynitrite (8), thereby losing its vasoprotective and vasodilatory function.

Interruption of NO bioavailability and endothelial dysfunction is considered an initial and perpetuating event in the development of atherosclerosis (28, 85). Atherosclerosis is the
thickening of artery walls caused by the formation of hard plaques consisting of cells, lipids, and connective tissue (28). These plaques are a product of an inflammatory response within the artery (66). Retention of low-density lipoprotein (LDL) within the subendothelium, a space immediately deep to the endothelium, is the first step in plaque formation. Once trapped in the arterial wall, LDL is oxidized by ROS (10), the source of which includes uncoupled NO synthase, xanthine oxidase, NADH, and NADPH oxidase (15). Oxidized LDL molecules damage endothelial cells, which subsequently express and secrete cell adhesion molecules and signaling chemokines. Chemokines localize monocytes while cell adhesion molecules facilitate their extravasation into the subendothelium (85). Once in the subendothelium monocytes differentiate into macrophages that phagocytose oxidized LDL. These cholesterol filled macrophages are termed foam cells, and accumulate to form fatty streaks – the earliest recognizable lesion in atherogenesis (7). Fatty streaks progress to fibrous plaques through a cascade of further events including proliferation of vascular smooth muscle, and these lesions can be further complicated by platelet aggregation (68). In addition to controlling vascular tone, endothelial derived NO confers vasoprotection by limiting the following: monocyte adhesion to the endothelium (37), excess proliferation of smooth muscle (24), and platelet aggregation (39).

2.3 Assessment of Endothelial Function

The non-invasive assessment of endothelial function by brachial artery FMD was first introduced in 1992 by Celermajer and colleagues (18). Lower FMD magnitudes indicate impeded function and greater magnitudes suggest normal function (18, 108). A variety of methods can be used to increase brachial artery shear stress and induce FMD. These include reactive hyperemia (RH), dynamic handgrip exercise (HGEX), and distal skin warming. Currently, the most commonly used method in FMD assessment is RH (84).
2.3.1 Reactive hyperemia-mediated FMD

Measuring endothelial function using reactive hyperemia-mediated FMD (RH-FMD) involves the occlusion and release of blood flow to a limb in order to create a transient upstream conduit artery shear stress stimulus (18). Occlusion is achieved by applying a pressure cuff on the forearm or lower leg in order to test the FMD of the respective conduit vessels; the brachial or radial, and femoral or popliteal artery. The brachial artery is the most common location for RH-FMD assessment (84) at least in part because the endothelial function observed there has been shown to correlate with the endothelial function seen in coronary arteries (5, 98). For example, Takase and colleagues (1998) observed a strong correlation (r=0.78) between FMD of the brachial and coronary arteries. This is important because it indicates that the FMD of the brachial artery can be used as a surrogate index of coronary artery FMD, a region where endothelial dysfunction may contribute to ischemia or myocardial infarction. Further clinical studies have demonstrated RH-FMD to be an independent predictor of cardiovascular events in both healthy individuals (88, 90, 91), and those with established cardiovascular disease (38, 53, 73, 80).

2.3.2 Handgrip exercise-mediated FMD

Handgrip exercise-mediated FMD (HGEX-FMD) assessment uses forearm contractions to increase brachial artery blood flow in a stepwise manner, resulting in a controlled and sustained shear stress stimulus. Shoemaker and colleagues first described HGEX-FMD in 1997 (92). Exercise causes increases in shear stress through rapid vasodilatation of the resistance vessels that supply the exercising muscle, resulting in increases in blood flow in the upstream conduit arteries (25). Since these increases in blood flow are exercise intensity-dependent, experimenters can precisely target and maintain the magnitude of the desired shear stress stimulus by manipulating the exercise intensity (31, 58).
HGEX-FMD differs from RH-FMD due to the shear stress stimulus profile (sustained and controlled vs. transient and uncontrolled). This is important since the shear stress transduction and dilatory mechanisms involved in the FMD response are dependent on the shear stress profile. This concept is supported by in vitro evidence (33) and the following observations in humans. A report by Szigyarto and colleagues (2013) (97) found that acute mental stress impaired RH-FMD but not HGEX-FMD in a group of young healthy males. In addition, a study of type 1 diabetic patients revealed impaired FMD though distal hand heating, but not RH-FMD (9). Similarly, Grzelak and colleagues (2010) (41) examined a type 1 diabetic population and found a greater magnitude of endothelial dysfunction between diabetic patients and controls when using HGEX-FMD compared to RH-FMD. Collectively, these data suggest that RH and HGEX-FMD provide different information regarding endothelial function, and that the distinct transduction pathways that RH and HGEX-FMD engage have differing vulnerabilities to impairment by the same vascular insult.

Compared to RH-FMD, HGEX-FMD may be a more sensitive test for detecting early endothelial dysfunction in some populations. A study by Findlay and colleagues (2013) (31) compared RH and HGEX-FMD in a group of young healthy male smokers and non-smoking controls. They found that HGEX-FMD, but not RH-FMD, was impaired in the smoking group. Since long-term smoking exposure is known to cause endothelial dysfunction when measured with RH-FMD (17), these results suggest that HGEX-FMD may be more sensitive in detecting early smoking-induced endothelial impairment. Furthermore, HGEX-FMD may have greater functional relevance than RH-FMD in terms of perfusion (46, 105). This is because in daily activity FMD is caused by exercise-induced shear stress, not the release of a limb-occlusion.
As with many types of tests, there are common criticisms of HGEX-FMD. HGEX results in patterns of repeating reverse and forward flow due to repeated contraction and relaxation of the exercising muscle (83). An oscillatory shear stress pattern has been shown to negatively influence endothelial function both in vitro (36) and ex vitro (118) studies. Therefore, it was a concern that the HGEX-FMD response may not be an appropriate index of endothelial function since the endothelium may be negatively influenced by the fluctuating shear stress. Pyke and colleagues (2008) examined FMD responses to a stable shear stimulus (forearm heating) and fluctuating shear stimuli caused by forearm heating, rhythmic cuff inflation, and HGEX. In all cases FMD was determined by the mean shear stimulus, and independent of the shear stimulus pattern. Additionally, King and colleagues (2013) found that varying patterns of HGEX contraction and relaxation, which produce different shear stress patterns, had no impact on FMD response when the same mean shear stress stimulus was achieved. As a whole, these data indicate that the fluctuating shear stress pattern created with HGEX is not an obstacle for FMD assessment.

A further concern regarding the application of HGEX-FMD involves the possibility of conducted vasodilation in the brachial artery. Conducted vasodilation is the process where exercise-induced vasodilation of small arterioles near the site of muscle activation results in cell to cell communication and vasodilation of upstream arteries (111). If conducted vasodilation occurred in the brachial artery during HGEX, then the observed brachial artery dilation would not be exclusively reflective of FMD. In their 2008 study, Pyke and colleagues induced HGEX without permitting an increase in brachial artery shear stress in an effort to isolate any potential conducted vasodilation in the brachial artery (83). This was achieved by compressing the artery such that blood velocity (and therefore shear stress) remained at baseline levels during HGEX. The results showed that brachial arterial diameter did not increase during forearm exercise without increases in shear stress, therefore providing evidence that HGEX does not result in conducted vasodilation in the brachial artery.
In summary, HGEX has been validated as a viable method of increasing shear stress for the purposes of brachial artery FMD assessment. HGEX-FMD may provide distinct information compared to RH-FMD about endothelial function, and could have differing vulnerability to impairment by a given vascular insult than RH-FMD (9, 41). Additionally, HGEX-FMD may be a more sensitive test of early endothelial dysfunction (31), and have use for testing how various factors (e.g. shift work, mental stress, and smoking) affect this mechanism of perfusion during daily activity (46, 105).

2.4 Shift Work and Endothelial Dysfunction

Prior to the study reported in chapter 4 of this thesis, HGEX-FMD had never been investigated in relation to shift work. Working a single night shift or several consecutive shift shifts has been shown to acutely impair endothelial function as assessed by RH-FMD. Kim and colleagues (2011) (57) tested the RH-FMD of 22 healthy female nurses (mean age = 30 years) at baseline (defined as a regular workday with no previous or subsequent night shift) and immediately following 3 consecutive night shifts. They observed decreases in post-shift FMD that were independently related to a longer history of shift work (baseline FMD 13.33±3.53%, post-shift FMD 7.62±2.38%, p<0.001). Other studies on the acute effects of shift work have found similar results in physicians immediately following a 24-hour shift (4), and in young healthy cardiology trainees immediately following a night shift (83).

To date, few experiments have examined the chronic impact of shift working history on endothelial function. Suessenbacher and colleagues (2011) compared the peripheral endothelial function of 48 male industrial SW and 47 NSW (mean age =43) using a technique known as peripheral arterial tonometry (PAT). All workers had a minimum of 5 years of experience and were matched for risk factors such as smoking, BMI, and family history of CHD. SW were found to have significantly reduced endothelial function compared to the non-shift working group.
A study by Wong and colleagues (2012) (117) also compared endothelial function by PAT in a small group of male and female paramedics who worked rotating shifts (n=14) and day-shifts (n=7). Similar to Sussenbacher’s study, rotating SW were reported to have impaired endothelial function. A major limitation of these studies is that PAT uses measurements of finger arterial pulse-wave amplitude and this is an index of the magnitude of RH following occlusion(96). The RH magnitude is determined by the degree of forearm resistance vessel dilation during occlusion and this may not be wholly endothelial dependent (27). While there is evidence that PAT and RH-FMD are correlated (r=0.55) (60), the RH magnitude may not necessarily reflect the endothelial function in the brachial artery. Only one study has examined how shift working history affects RH-FMD. Wehrens and colleagues (2012) (110) compared RH-FMD in small group of male SW and NSW and found a non-significantly lower trend for FMD in SW. To date no studies have examined how shift working history affects RH-FMD in women, and it is unknown if there are gender differences regarding the impact of shift on endothelial function.

In summary, several previous studies have shown that single or consecutive night shifts acutely impair endothelial function within SW when measured with RH-FMD (4, 57, 99). Studies using PAT have been used to suggest that shift working history impairs endothelial function, however, PAT may be limited because it does not directly assess endothelial dependent dilation (27, 96). Only one small study to date has examined how a history of shift work affects RH-FMD in men (110). While this study reported a trend for decreased RH-FMD in male SW, the impact of shift work on RH-FMD in women is unknown. Furthermore, no studies to date have examined the chronic impact of shift work on HGEX-FMD. This is important because HGEX-FMD may have different vulnerability to shift work than RH-FMD (31), be an indicator of early endothelial dysfunction (31), and provide insight as to how shift work affects perfusion during daily living (46).
2.5 Stress Reactivity

2.5.1 Cardiovascular stress reactivity as a predictor of cardiovascular risk

Cardiovascular stress reactivity refers to the magnitude of change in heart rate and blood pressure during an aversive, challenging, or engaging laboratory stressor (100). Cardiovascular reactivity to stress has been shown to be a predictor of future cardiovascular risk. A study by Matthews and colleagues (2004) (69) found SBP and DBP stress reactivity to be significant predictors of future hypertension 13 years after testing in a population of 4100 men and women. Other studies have shown similar findings with regard to the association between cardiovascular stress reactivity and prediction of future blood pressure after 6.5 (70) and 10 years (74). Carroll and colleagues (2001), examined the blood pressure response to stress in 795 middle aged men and followed them for 10 years. Blood pressure reactivity was associated with future hypertension (defined by taking antihypertension medication or blood pressures >160 and >90 mmHg) even after correcting for confounders such as baseline blood pressure and age. Hypertension risk was reported to increase approximately 2-7% per mmHg increase in blood pressure reactivity to mental stress. Another study by Matthews and colleagues (2006) (71) examined how blood pressure responses to a video game mental stress task in 2800 healthy young woman predicted future coronary artery calcifications (a marker of subclinical atherosclerosis). At 13 year follow-up, each 10 mmHg change in systolic blood pressure reactivity was associated with a 24% increased odds of having significant calcifications.

Most convincingly, a recent meta-analysis of 36 prospective studies by Chida and Steptoe (2010) (19) examined HR, SBP, and DBP stress reactivity in relation to future hypertension, left ventricular mass, subclinical atherosclerosis, and clinical cardiac events. Overall, the meta-analysis revealed that greater cardiovascular reactivity to stress was associated with a poorer future cardiovascular status. Elevated SBP and DBP stress reactivity were most
consistent in predicting incident hypertension and increased carotid intima-media thickness (IMT). IMT is a measurement of the thickness of the tunica intima and media, the two innermost arterial walls, and is associated with the development of atherosclerosis (13).

2.5.2 Cortisol stress reactivity as a predictor of cardiovascular risk

Cortisol, one of the primary stress hormones regulated by the hypothalamic-pituitary-adrenal (HPA) axis, can increase 10 fold in response to severe stress (87) and can remain elevated for up to an hour following an acute stressor (76). Similar to cardiovascular stress reactivity, cortisol stress reactivity refers to the magnitude of change in cortisol during an acute laboratory stress task, and there is some evidence to suggest that it may predict future cardiovascular risk (43). Compared to cardiovascular reactivity, however, cortisol reactivity has received significantly less attention.

There is both indirect and direct evidence to support the status of cortisol stress reactivity as a predictor of cardiovascular risk. Excessive secretion of cortisol is known to cause increases in blood pressure. This has been observed in studies involving the infusion of cortisol in normotensive adults (22), and in individuals with Cushing’s syndrome: a condition characterized by over-secretion of cortisol. Furthermore, men and women at high risk of hypertension (high resting SBP) have been observed to have heightened cortisol responses to laboratory public speaking tasks (1). A study by Alevizaki and colleagues (2007) (3) examined the relationship between cortisol levels in CVD patients prior to undergoing a stressful procedure (coronary angiograph), and the extent of arterial blockage revealed by the angiograph. Pre-angiography cortisol levels were significantly correlated with the number of vessels with severe blockage (an indicator of CVD severity) as well as femoral artery IMT. It should be noted that this study did not measure true reactivity because there was no assessment of unstressed baseline cortisol levels.
Two prospective studies by Hamer and colleagues (2009, 2012) (42, 43) provide the strongest evidence for a relationship between cortisol stress reactivity and future cardiovascular health. In their 2009 study, cortisol reactivity to a 5-minute Stroop Colour-Word task and 5 minute mirror tracing task was measured in 514 healthy older men and women (mean age 62.9). In the Stroop task the name of one colour is presented in the font of another colour (e.g. blue written in red) and the participant is asked to identify the font colour (95). The mirror tracing task involves tracing the outline of a figure while only looking at its reverse image in a mirror. The 40% of the study population that had a notable cortisol response to stress also demonstrated a higher risk of significant coronary artery calcification. In their 2012 study Hamer and colleagues again examined reactivity to a 5-minute Stroop and 5-minute mirror tracing task in 479 initially healthy older men and women. At three years follow-up there was a significant association between cortisol stress reactivity and hypertension after adjusting for confounding variables such as age, sex, resting cortisol BP, smoking, and BMI. The odds of developing hypertension increased 59% per standard deviation change in cortisol stress reactivity.

2.5.3 Cardiovascular reactivity as a mechanism for cardiovascular disease development

All of the studies examining the relationship between cardiovascular stress reactivity and CVD risk are observational, therefore it is not possible to determine the existence or direction of cause and effect. In this sense, underlying factors that contribute to future hypertension and atherosclerotic risk may cause heightened stress reactivity, as opposed to the opposite scenario (19). For example, although the observed association between reactivity and poor cardiovascular status persisted with correction for traditional risk factors like smoking and obesity, it is possible that other underlying factors that predispose to cardiovascular impairment also influence stress reactivity. However, it is possible that heightened reactivity may make a mechanistic contribution to the development of cardiovascular risk. Elevated cardiovascular reactivity may lead to future CVD due to repeat episodes of acute elevated BP. In support of this deleterious impact of
repeated experiences of high reactivity, a report by Light and colleagues (1999) (61) demonstrated that high reactivity was predictive of increases in tonic BP over a 10 year period, but only when associated with high reported levels of daily stress.

2.5.4 Cortisol reactivity as a mechanism for cardiovascular disease development

As stated above for cardiovascular stress reactivity, conclusions regarding cause and effect for associations between cortisol stress reactivity and poor cardiovascular outcomes cannot be made. However, some evidence suggests that mechanistic connections may exist. Increased cortisol stress reactivity may lead to cardiovascular disease due to the consequences of frequent exposure to excessive circulating cortisol concentration. Cortisol affects areas of the brain that are involved in the control of BP, such as the hypothalamus and limbic system (115). It can also directly affect BP regulation through the action of glucocorticoid receptors in the heart, vascular smooth muscle of resistance vessels, and the kidneys (56). Acute elevation in blood cortisol via infusion results in an increase in BP (22), and excessive levels of cortisol may lead to hypertension. Hypertension is observed in individuals with Cushing’s syndrome (a condition characterized by over secretion of cortisol) (113), and blood pressure normalizes in these patients when lowered cortisol levels are restored using medication (29). Furthermore, urinary cortisol excretion is higher in hypertensive individuals compared to normotensive individuals (63), and plasma cortisol is reported to be elevated in young people with high BP (109).

Cortisol induced hypertension does not appear to act via increasing sympathetic nervous activity (65), but may be mediated in part through the nitric oxide (NO) system, which helps regulate blood pressure through vasodilation (112). Cortisol may inhibit NO bioavailability in a number of ways. First, Cortisol has been shown to directly block NO synthesis by down-regulating expression of NO synthase (eNOS) within the vascular endothelium (107), and reducing available plasma nitrate (14), a precursor to NO. Second, Cortisol may also enhance
reactive oxygen species (ROS) production (49). ROS react directly with NO rendering it non-functional, and also oxidize tetrahydrobiopterin (BH₄), a necessary cofactor for eNOS (44). In the absence of BH₄, eNOS uses O₂ as a substrate instead of L-arginine, and produces further ROS instead of NO (32).

2.6 Evidence for Modulation of Cardiovascular and Cortisol Reactivity in SW

While evidence suggests that cardiovascular and cortisol reactivity to acute stress is associated with CVD development, no studies involving shift working populations have examined cortisol reactivity, and only one has examined cardiovascular reactivity to acute stress. Kario and colleagues (2002) (54) compared BP reactivity in two groups of female nurses (33 night workers and 54 day workers) and found SBP reactivity to be non-significantly higher in night workers. There is also evidence that elevated chronic stress, a condition reported in some groups of SW (11, 50, 81), is associated with heightened cardiovascular and cortisol stress responses. A study by Low and colleagues (2009) (64) examined 159 adolescents over the course of 3 years and found that those experiencing chronic negative stressors (assessed using questionnaires) had amplified hemodynamic responses to an acute mental stress task. Furthermore, these individuals showed increased carotid IMT, placing them at greater risk for future atherosclerosis (13). A study by Wirtz and colleagues (2013) (116) investigated how chronic stress in the form of occupational role stress (high tension caused by inconsistent expectations at work) impacted cortisol stress reactivity in 43 healthy men aged 22-65. Higher occupational role stress was associated with greater cortisol reactivity, suggesting again that chronic stress amplifies HPA-axis responses to acute stress. The following sections will review evidence regarding the effect of shift work on the physiological stress systems, and chronic psychosocial stress.
2.6.1 Sympathetic nervous activity and shift work

In general, autonomic activity follows a diurnal pattern with sympathetic activity being higher during the day and parasympathetic activity being highest at night (12). Participating in shift work can alter this 24 hour pattern. Furlan and colleagues (2000) (35) examined autonomic activity via heart rate variability in 22 healthy male SW for 24 hours during each of 3 different shifts: 6 AM to 2 PM, 2 PM to 10 PM, and 10 PM to 6 AM. In all workers the 24 hour cycle of autonomic activity adapted to the work and sleeping times dictated by the different shifts. Sympathetic activity was highest during work and parasympathetic activity was greatest during sleep regardless of time of day. Individuals who work rotating day and night shifts therefore experience frequent pattern changes in their autonomic cycles.

In addition to changing autonomic cycles, there is evidence that shift work can specifically increase sympathetic nervous activity. A study by Chung and colleagues (2009) (20) assessed autonomic function by heart rate variability in 10 permanent night-shift nurses and 10 permanent day-shift nurses. Night-shift nurses were found to have significantly elevated sympathetic activation during sleep compared to day-shift nurses. Similarly elevated sympathetic activity has been found in hospital nurses working on a rotating 3-shift-system (morning, afternoon, and evening) compared to public health nurses working only day shifts (48), and in paramedics and dispatchers working rotating day and night shifts compared to their colleagues working only day shifts (117). There are also reports that levels of urinary norepinephrine, a neurotransmitter of the sympathetic nervous system, is elevated in female hospital SW reporting high job strain (34). Collectively, this evidence suggests that shiftwork alters autonomic rhythms and contributes to elevations in sympathetic nervous activity.
2.6.2 HPA-axis activity and shift work

The HPA axis is a signaling pathway consisting of the hypothalamus, anterior pituitary gland, and adrenal cortex, and is a main component of the stress response system (101). Under non-stressful conditions the HPA axis secretes cortisol in a diurnal pattern; concentrations are highest in the morning and lowest at night (79). This diurnal pattern is disturbed by changes in lighting, eating schedules, and activity (101), all of which are characteristics of shift work. Indeed there is evidence that diurnal patterns of secreted cortisol are altered in shift working populations. A study by Lindholm and colleagues (2012) (62) reported elevated waking cortisol in media SW compared to day workers. Increased cortisol secretion has also been observed during the day in shift-working paramedics (117), and in nurses immediately following a night shift (75). Conversely, some studies have found that cortisol secretion is decreased (30) or unchanged (23) in shift working populations. These inconsistencies may be due to factors such as time of sampling (i.e. on a day off vs. during a work shift) and the population of SW being sampled (23).

In addition to diurnal changes, there are reports that shift work impacts chronic cortisol concentrations. A study by Manenschijn and colleagues (2011) (67) examined hair cortisol, a method of characterizing long-term cortisol levels (103), in 33 male SW and 89 male day workers. After controlling for age, BMI, and frequency of hair washing, younger SW were shown to have significantly higher hair cortisol compared to age matched day workers. This would indicate that shift work at a young age is associated with elevated chronic cortisol levels.

2.6.3 Chronic psychosocial stress and shift work

There are several different pathways by which shift work may cause psychosocial stress. These include greater job strain, decreased work-life balance, and poor recovery from work-induced fatigue (81). Job strain can be defined as a measure of high job demand and low control over one’s job (52). Shift work has been associated with lower job control compared to non-shift
work. In a study of 5940 Danish employees, Bøggild and colleagues (2001) (11) found conflicts at work and low decision-making responsibility (indicators of low job control) to be higher in SW compared to NSW. In addition to job strain, irregular working hours have been seen to decrease work-life balance. Not only does shift work interrupt one’s social life to a greater extent than non-shift work (2), it has also been associated with higher rates of interference between work and family (51). As part of the Maastrich cohort study (50), the working patterns, hours and schedules of 12905 Danish employees were examined using self-administered questionnaires. The study revealed that SW often felt they needed more post-work recovery time compared to NSW, indicating a lessened ability to recover from work-induced fatigue. As a whole, these data suggest that shift work contributes to increases chronic psychosocial stress through elevated job strain, decreased work-life balance, and a lessened ability to recover from work-induced fatigue.

In summary, there is evidence that shift work affects the physiological stress response systems: the autonomic nervous system (20, 35, 117) and hypothalamic-pituitary-adrenal (HPA) axis (62, 75, 117). Shift work is also reported to increase chronic mental stress (11, 51), which may heighten acute stress responses (64, 116). Collectively, although it has been minimally studied this supports the hypothesis that shift work may impact cardiovascular and cortisol reactivity to acute mental stress.

2.7 Summary and Conclusions

Shift work is associated with an increased risk of CVD. While direct mechanisms are unclear, endothelial dysfunction and heightened cardiovascular and cortisol stress reactivity may play a role. Endothelial function is an integral part of proper vascular function, and when impaired, can potentiate atherogenesis. Endothelial function can be measured non-invasively by RH-FMD and HGEX-FMD. While RH-FMD is a useful tool for predicting future CVD events in healthy and at-risk populations, HGEX-FMD is a newer technique that may provide distinct
information regarding endothelial function as well as information regarding the way in which different vascular insults affect perfusion (46, 105) during daily activities. Acute shift work reportedly impairs RH-FMD, however the chronic effect of a history of shift work on RH-FMD is unclear, particularly in women. Furthermore, no studies have examined how a history of shift work affects HGEX-FMD. Cardiovascular and cortisol stress reactivity are associated with increased cardiovascular risk. Only one study has examined cardiovascular reactivity in a small group of SW and none have examined cortisol reactivity a shift working population. Evidence that shift work effects the physiological stress response systems and chronic psychological stress levels suggests that shift work may alter acute cardiovascular and cortisol responses to stress.
2.8 References


24. **Cornwell T, Arnold E.** Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP [Online]. ... *Physiol. ...*. http://ajpcell.physiology.org/content/267/5/C1405.short [22 Jun. 2013].


33. Frangos JA, Huang TY, Clark CB. Steady Shear and Step Changes in Shear Stimulate Endothelium via Independent Mechanisms — Superposition of Transient and Sustained Nitric Oxide Production flow through the release of factors such as the vasoactive compounds prostacyclin (PGI 2) and of. 665: 660–665, 1996.


Moseley JV, Linden W. Predicting blood pressure and heart rate change with cardiovascular reactivity and recovery: results from 3-year and 10-year follow up. *Psychosom. Med.* 68: 833–43, [date unknown].


86. Robert F. Discovery of Endothelium-Derived Relaxing Factor and Its Importance in the Identification of Nitric Oxide. .


Chapter 3

A preliminary study of cardiovascular and cortisol stress reactivity in female shift and non-shift workers

3.1 Introduction

Shift work is an increasingly common work pattern world-wide with approximately 15-25\% of the work force in North America and Europe employed in shift working positions (40, 47, 51). Shift work can be defined as working rotating day and evening shifts or working constant but non-traditional hours (i.e. permanent night shifts) (28), and there is evidence that it is associated with an increased risk of CVD (49). However, the causal pathways involved in the shift work-CVD connection remain incompletely understood.

Cardiovascular and cortisol reactivity to acute mental stress (characterized as the magnitude of the increase in HR, BP, and cortisol during a stressor) have been shown to be predictive of future cardiovascular risk, and may also play a mechanistic role in the development of CVD (7, 19). The effect of shift working history on stress reactivity has been minimally studied (25), however, shift work is known to impact the physiological stress response systems: the sympathetic nervous system and HPA axis. For example, shift work had been associated with reductions in HR variability (8, 22) and increases in urinary norepinephrine under high job strain (13), indicating an elevation of sympathetic nervous activity. In terms of HPA axis activity, SW show altered diurnal patterns of cortisol secretion (36, 45) and elevated hair cortisol (a measure of chronic cortisol levels) compared to day workers (34). In addition to physiological stress-related effects, shift work has been observed to increase levels of self-reported chronic stress (characterized by job strain and work-life balance) (3, 24) and prolonged exposure to chronic stress has been shown to amplify cardiovascular responses to acute stressors (33). Collectively,
this evidence suggests that shift work may influence cardiovascular and cortisol reactivity to acute mental stress.

With this as background, the objective of the present study was to compare the cardiovascular and cortisol stress reactivity of healthy female SW and NSW in response to a laboratory mental stress task. We hypothesized that SW would have greater cardiovascular and cortisol reactivity to mental stress. If high reactivity contributes mechanistically to CVD progression this could help to further elucidate the causal pathways linking shift work experience to CVD.

3.2 Methods

Participants Thirty-nine healthy, non-smoking female hospital employees aged 23–65 years from Kingston General Hospital were recruited for this study. Here we report the data from thirty-eight subjects for whom we had stress reactivity data. Health status was confirmed via a medical screening questionnaire. Exclusion criteria were assessed via self-report and included cardiovascular or metabolic disease, use of alpha or beta-blocking mediations, and morbid obesity (BMI>40). The study contained two experimental groups: SW and NSW. Shift work was defined as working rotating day and night shifts ranging between 8 and 12 hours, and NSW was defined as working 8 hour day shifts. The SW group consisted of 19 women in current shift work positions, with a minimum of 6 years of shift work experience. The NSW group consisted of 19 women in current non-shift, workday positions with a minimum of 6 years of experience. All NSW had never participated in shift work with the exception of three women who had done shift work 10 or more years ago. The Health Sciences Research Ethics board at Queen’s University approved the study procedures, and all of the participants completed a consent form approved by the same board.
3.2.1 Participant monitoring

At the beginning of the visit baseline BP was assessed using an automatic BP monitor (BPM-100, BpTru, Coquitlam, Canada). HR was monitored continuously throughout the experiment with a 3-lead electrocardiogram. Throughout the experiment BP was measured continuously with finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). ECG and Finometer signals were recorded and stored with the program LabChart (AD Instruments, Colorado Springs, USA) for future analysis.

3.2.2 Experimental procedure

This experiment was part of a larger study evaluating vascular function and structure in these participants. All testing was done at the same time of day between 0800 and 1000h with the exception of one participant (NSW) who began testing at 1615h. Testing was done in a quiet room with a maintained temperature of 21°C. Participants were instructed to fast for 12 h prior to visiting the lab (43), and to avoid caffeine, alcohol, and vigorous exercise for 12 h before testing. All SW participants were tested at least 12 hours following the most recent night shift. At the beginning of the visit a 22-gauge catheter (BD Nexivia, BD Medical Supplies, Mississauga, Canada) was inserted in the right antecubital vein and a blood draw was performed for baseline serum cortisol determination following a 40 min acclimatization period. Immediately prior to the stress task participants lay supine for 10 min of rest. Participants then received instructions for a stress task, had 10 min of preparation time, completed the task, and recovered for 15 min. Participants were asked to rate their level of stress on a scale of 0-10 pre and post-stress task. Three additional blood draws were performed following the stress task (Figure 2).
**Figure 2.** Protocol Timeline. MAT = mental arithmetic task. Arrows represent time points when blood samples were taken. Blood sample 1 was taken 50 min before baseline rest and blood sample 4 was taken 10 min after the final rest period. X represents time points when participants were asked to rate their stress on a scale from 0-10.
3.2.3 Blood lipids and glucose

Prior to initial rest and instrumentation a small sample of blood was used for analysis of blood lipids and fasting glucose using a Cholestech LDX System (Alere Inc., Ottawa, Canada). If a venous catheter could not be successfully inserted blood samples were taken by finger-prick. LDL values were not obtained in 5 SW and 6 NSW as the values fell outside of the range of the device.

3.2.4 Mental Stress Task

The mental stress task protocol was based on the Trier Social Stress Test (TSST) (26) and consisted of instructions, preparation time, a speech task and a mental arithmetic task (MAT) all administered by an unfamiliar researcher. In the speech task, the participant was asked to defend herself against a false accusation of shoplifting (39). Participants were given 10 min to prepare their speech followed by 5 min of speaking time. The researcher provided a standard prompt if the participant stopped speaking at any point. Immediately following the speech task participants were given instructions for the 5 min MAT. The participant was asked to serially subtract 17 from a 4-digit prime number. At planned intervals during the MAT participants were told that their answers were incorrect even when correct. Participants were informed that their speech and MAT responses were being video recorded for facial expression analysis.

3.2.5 Serum cortisol sampling

Venus blood samples were obtained from the right antecubital vein using a 22-gauge catheter (BD Nexivia, BD Medical Supplies, Mississauga, Canada) and collected into silicone coated serum tubes (BD Vacutainer, BD Medical Supplies, Mississauga, Canada). Samples were kept at room temperature for at least 30 min following collection in order for clotting to occur as per BD Medical Supplies instructions. After clotting, samples were spun for 10 min at 21°C, at a rate of 2680 r min⁻¹ and 1200 relative centrifugal force (RCF) (IEC-Centra MP4R; International
Equipment Company, Mass, USA). Once spun, the samples were aliquoted and stored at –80.0°C until analysis. It was not possible to collect full sets of serum cortisol samples in 8 SW and 12 NSW due to technical difficulties when inserting the venous catheter or keeping the catheter line patent during the entire data collection.

3.2.6 Derogatis Stress Profile and effort-reward imbalance ratio

Life and work stress was assessed using the Derogatis Stress Profile (DSP) and effort-reward imbalance (ERI) ratio, respectively. The DSP uses questions about environmental factors, personality mediators, and emotional responses to generate a total stress score expressed as a T-score. A T-score of 50 is defined as the mean for the general population and higher T-scores indicate greater stress (20). The ERI ratio questionnaire uses questions about effort, reward, and over commitment at work. An ERI ratio > 1 indicates a poor work situation where effort exceeds reward, and a ratio <1 indicates an optimal work situation where reward exceed or equals effort (29). Surveys were given to participants following data collection to be completed on the participant’s own time. Surveys were returned within approximately one week of data collection with the exception of one NSW did not complete the DSP. The DSP and ERI questionnaires have been shown to be reliable and valid measures of life stress (11) and work stress, respectively (30).

3.3 Data Analysis

Stress reactivity: BP, HR and Subjective Ratings

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR were analyzed offline in 3-s average time bins. Reactivity to the stress tasks was characterized as the change in SBP, DBP, and HR from the mean of the last 3 min of baseline to the mean of the first 3 min of the speech task and MAT (21, 37) (ΔSBP, ΔDBP, and ΔHR). Due to poor finometer signals, SBP and DBP reactivity was not collected in one NSW. Subjective stress reactivity was characterized as the change in subjective rating from baseline to immediately post-stress task (Δstress rating).
**Serum cortisol analysis and cortisol reactivity**

Serum samples were thawed on the day of the assay. Serum cortisol was determined in duplicate, according to the manufacturer’s instructions, using an enzyme immunoassay (ELISA) kit (cat. no. 11-CORHU-E01, ALPCO Diagnostics, Salem, United States). Cortisol reactivity to the stress task was characterized as the change in cortisol from pre-task baseline to the highest post-stress sample (42). Cortisol stress reactivity was only calculated in participants who had full sets of blood samples (1 pre-stress sample and 3 post-stress samples (Figure 2)).

### 3.3.1 Statistical analysis

Independent sample t-tests were used to compare participant characteristics. Similar to other reports (21, 37), independent t-tests were used to compare stress reactivity via the following parameters: Δstress ratings, ΔSBP, ΔDBP, ΔHR, and Δcortisol between SW and NSW. The level of significance was set at p < 0.05. Effect size for differences between groups in SBP, DBP, HR, subjective stress, and cortisol reactivity were examined with Cohen’s d using the following equation:

\[
Cohen's\ d = \frac{mean\ 1 - mean\ 2}{pooled\ standard\ deviation}
\]

All statistics were calculated using SigmaPlot 11.0, and all data were expressed as means ± SD.

### 3.4 Results

#### 3.4.1 Participant characteristics

Mean age, waist circumference, BMI, blood lipids, glucose, resting SBP and DBP pressure, heart HR, and serum cortisol are shown in Table 1. None of these variables were significantly different between groups (p>0.05). Non-physiological participant characteristics are shown in Table 2. Life stress as measured by the Derogatis Stress Profile (DSP) was not significantly different between groups (p=0.684), however, job stress, measured by the effort-reward imbalance (ERI) ratio, was significantly higher in SW (p=0.016
Table 1. Physiological Participant Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>NSW</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.5 ± 11.3</td>
<td>41.6 ± 11.4</td>
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</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>91.7 ± 8.5</td>
<td>89.4 ± 12.3</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 3.9</td>
<td>25.4 ± 4.7</td>
<td>0.728</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>188.4 ± 36.2</td>
<td>181.2 ± 29.9</td>
<td>0.513</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>81.8 ± 40.0</td>
<td>82.6 ± 43.0</td>
<td>0.951</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>61.9 ± 15.6</td>
<td>62.8 ± 10.3</td>
<td>0.836</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>114.0 ± 36.0</td>
<td>103.3 ± 31.0</td>
<td>0.429</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>85.1 ± 11.8</td>
<td>87.7 ± 11.0</td>
<td>0.488</td>
</tr>
<tr>
<td>Resting SBP (mmHg)</td>
<td>112.9 ± 7.9</td>
<td>116.6 ± 12.6</td>
<td>0.282</td>
</tr>
<tr>
<td>Resting DBP (mmHg)</td>
<td>76.3 ± 5.8</td>
<td>78.3 ± 7.3</td>
<td>0.354</td>
</tr>
<tr>
<td>Heart Rate (beats·min⁻¹)</td>
<td>62.2 ± 8.9</td>
<td>64.1 ± 7.3</td>
<td>0.473</td>
</tr>
<tr>
<td>Serum Cortisol (µg/dL)</td>
<td>9.8 ± 3.2</td>
<td>8.6 ± 3.6</td>
<td>0.490</td>
</tr>
</tbody>
</table>

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are means ± SD.

a SW: n=15, NSW: n=13
b SW: n=11, NSW: n=7
Table 2. Non-physiological Participant Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>NSW</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Years SW experience</strong></td>
<td>16.9 ± 9.5</td>
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<td>n/a</td>
</tr>
<tr>
<td><strong>Baseline Subjective Stress Rating (out of 10)</strong></td>
<td>2.0 ± 1.7</td>
<td>1.9 ± 1.6</td>
<td>0.923</td>
</tr>
<tr>
<td><strong>Life &amp; Job Stress Descriptors</strong>(^a)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DSP T-score</td>
<td>110.1 ± 21.9</td>
<td>106.5 ± 31.4</td>
<td>0.687</td>
</tr>
<tr>
<td>ERI Ratio</td>
<td>1.7 ± 1.2</td>
<td>0.9 ± 0.4</td>
<td>0.013</td>
</tr>
</tbody>
</table>

DSP = Derogatis Stress Profile. ERI = effort-reward imbalance. A copy of the DSP and ERI questionnaire is in Appendix B. Higher DSP T-scores indicate greater stress (20). An ERI ratio > 1 indicates that effort exceeds reward, and a ratio <1 indicates that reward exceed or equals effort (29). Values are means ± SD.

\(^a\) SW: n =19, NSW: n=18
3.4.2 ΔSubjective stress rating, Δcardiovascular, and Δcortisol reactivity.

There were no significant differences between groups in baseline subjective stress ratings, HR, SBP, DBP, or serum cortisol (Table 1, Table 2). The stress task caused an increase in subjective stress ratings that was not significantly different between groups (Figure 3A). SW and NSW also did not differ in HR, SBP, or DBP stress reactivity (Figure 3B-D). Although it did not reach significance, the magnitude of difference in DBP stress reactivity between groups (3.07 mmHg) was a moderate effect size ($d = 0.5$). SW had significantly higher cortisol stress reactivity than NSW (Figure 3E).
Figure 3. A) Subjective stress ratings, effect size: $d=0.4$ B) Heart rate stress reactivity, effect size: $d=0.1$. C) Systolic blood pressure reactivity, effect size: $d=0.1$. D) Diastolic blood pressure reactivity, effect size: $d=0.5$. E) Serum cortisol reactivity, effect size: $d=1.5$. Bars represent group means and error bars represent standard deviation. All values are means ± SD. * represents significance from SW.
3.5 Discussion

This study was designed to compare cardiovascular and cortisol reactivity to a laboratory mental stress task in healthy female SW and NSW. A key novel finding was that SW had higher cortisol stress reactivity. There were no significant group differences in HR, SBP, or DBP stress reactivity, however, the impact of group on DBP stress reactivity (greater in SW) had a moderate effect size. These results extend previous evidence indicating that shift work effects chronic/diurnal HPA-axis function, and demonstrate that shift work may result in heightened HPA-axis reactivity to an acute stressor. Although underpowered to detect a significant difference in the present small sample, the results also suggest that cardiovascular reactivity, particularly DBP, might be elevated in SW.

3.5.1 Cardiovascular reactivity

This study used a mental arithmetic and public speaking task in order to cause a cardiovascular stress response. On average, we observed an increase in HR of approximately 17 bpm, and a rise in SBP and DBP of approximately 20 and 12 mmHg, respectively. These magnitudes of change are in agreement with previous studies that have examined HR (21, 25), SBP, (5, 25) and DBP (5) stress reactivity in men and women using mental arithmetic, public speaking, anger recall, cognitive, and video game stress tasks.

To date, only one other study has examined cardiovascular stress reactivity in a shift-working population (25). Similar to the findings of Kario et al., (2002) who examined stress reactivity in 54 female night-shift nurses and 33 day-shift nurses, the current study did not reveal a significant difference between SW and NSW with regard to HR or SBP reactivity. Both studies used similar tasks to elicit cardiovascular responses: a mental arithmetic task, and one form of a coping task (public speaking or anger recall). In addition, baseline participant characteristics
between studies were also similar with regard to age, BMI, and resting BP, which is important since all three factors can affect cardiovascular stress reactivity (41, 46).

Although we did not find a significant group difference in cardiovascular reactivity in the current study, the magnitude of difference in DBP reactivity between SW and NSW (~3 mmHg) had a moderate effect size \((d=0.5)\). Changes of 1 mmHg in SBP and DBP reactivity have been suggested to result in a 3-7% increase in risk of future hypertension (5). This suggests that the higher DBP reactivity in SW could potentially indicate a 9-21% increase in risk. Considering the variation of the DBP response in both groups, approximately 70 SW and NSW (140 participants total) would be required to achieve a power of 0.8 to detect the observed group difference in DBP reactivity as significant (alpha 0.05). Unlike DBP, the difference in SBP between groups had a small effect size and was far from reaching significance. Given that stress reactivity increases with age (46), any impact of SW on reactivity may be more apparent in an older sample. It is unclear why shift work might impact DBP reactivity exclusively. SBP and DBP are often reported as independent predictors of cardiovascular risk in previous models (7), making it possible that DBP reactivity could be elevated in SW even though SBP remains similar between groups.

We hypothesized that SW would have heightened cardiovascular stress reactivity for several reasons. First there is evidence that shift work increases sympathetic nervous activity (22, 52). Since the sympathetic nervous system is responsible for increasing HR and BP, elevated sympathetic nervous activity may result in higher HR and BP responses to stress. Furthermore, previous research (and the present study) report SW to have high chronic work stress measured by effort/reward imbalance (ERI), and there is evidence that chronic stress may be associated with elevated reactivity (6, 33). In addition, studies have found that employees who report high
ERI have increased sympathetic nervous activity in the form of heightened HR and BP during and immediately following work (48).

3.5.2 The importance of cardiovascular reactivity

Although there is strong evidence that elevated cardiovascular reactivity is associated with poor future cardiovascular health status (7), it is difficult to determine the existence or the direction of causality. However, several causal pathways have been suggested and seem likely (7). It is possible that hyper-reactive individuals who encounter frequent daily stress experience vascular changes in response to the bursts of high BP that eventually lead to an increase in their resting BP (6). This is supported by evidence showing that high cardiovascular reactivity only predicts future BP in individuals with high daily stress (31). Under these circumstances, SW would be especially vulnerable to high reactivity-induced damage as they report frequent daily stress in the form of high job stress (3, 23). Furthermore, recurring bouts of acute reactivity-induced hypertension may eventually result in cardiovascular insults similar to those caused by chronic hypertension. Nevertheless, it is also possible that heightened stress reactivity not is a causal mechanism, but rather a symptom of one of many underlying conditions that are contributing to CVD (7).

3.5.3 Cortisol stress reactivity

This is the first study to compare cortisol stress reactivity in SW and NSW. Baseline serum cortisol levels were found to be 9.36 ± 4.37 µg/dL and 9.07 ± 3.15 µg/dL in SW and NSW, respectively. These values fall within the normal range (4-27 µg/dL) for morning serum cortisol in women (17). There was wide variability in the cortisol response to the mental stress task. Approximately 22% of participants had no cortisol response to stress (defined by a net decrease in serum cortisol from baseline), while individuals who had responses showed increases in cortisol ranging from 15 - 190% from baseline. Previous studies have also observed large
variability in cortisol reactivity including no change (19, 42), increases of 40-50% (18), and increases of up to 250% (27). Most commonly, cortisol concentrations for stress reactivity have been assessed using salivary cortisol (2, 18, 19, 27). While this study used serum cortisol, salivary and serum cortisol concentrations are known to accurately reflect each other (16).

Cortisol stress reactivity was found to be significantly higher in SW compared to NSW, and this difference had a large effect size ($d=1.5$). While no previous studies have compared cortisol reactivity to acute stress in a SW vs. NSW sample, there is evidence that shift work affects HPA axis activity. Reports have shown changes in diurnal cortisol secretion patterns with SW experiencing increased (32, 36, 52) and decreased (12) waking cortisol concentrations compared to day workers. Hair cortisol, a technique used to assess long-term cortisol levels, has also been shown to be elevated in SW (34). Similarly, exposure to chronic work stress, a characteristic of shift work that was observed in the current study, is correlated with increased waking cortisol (38). Future research is required to determine the existence and nature of association between these diurnal and chronic cortisol levels and acute reactivity in SW.

### 3.5.4 The importance of cortisol reactivity

Compared to cardiovascular stress reactivity there has been significantly less research regarding the ability of cortisol reactivity to predict future risk. Nevertheless, two prospective studies have shown associations between heightened cortisol reactivity in healthy men and women, and increased risk of hypertension (19) and significant coronary artery calcification (18). Furthermore, hypertension-prone men and women (high resting systolic BP) have demonstrated elevated cortisol responses to public speaking tasks (2). While, as described with respect to cardiovascular reactivity, issues regarding direction of causality exist, there is evidence suggesting that cortisol reactivity could be involved in mechanisms leading to CVD. For example, infusion of cortisol in normotensive individuals has been reported to cause increases in
BP (9), and the very high cortisol concentrations see in Cushing’s syndrome patients are thought to be a likely cause of hypertension (50). There is also evidence that high cortisol has a negative impact on endothelial function. For example, the FMD reductions observed in individuals with Cushing’s syndrome (1) and following cortisol-elevating bouts of acute mental stress (4) are ameliorated by reducing cortisol concentration. A reduction in endothelial function may result in attenuated vasoprotection and potentiate atherosclerosis progression, because the endothelium is essential for controlling vascular tone (14), anticoagulation, and smooth muscle proliferation (15).

Given the detrimental effects of excess cortisol, exposure to daily mental stress-associated high cortisol concentrations ‘bursts’ over an extended period of time may lead to hypertension and atherosclerosis in high-reacting individuals.

3.5.5 Limitations

One major limitation of this study was the small sample size overall, and in particular in the cortisol stress reactivity analysis. Due to technical challenges and personal preferences of some participants, it was not possible to insert a catheter and obtain blood samples for all participants. While this small sample size indicates that the results should be regarded as preliminary, a significant difference with a large effect size was detected between groups. In terms of cortisol measurement, the act of inserting a needle has been seen to cause rapid increases in serum cortisol (35). Therefore, it is possible that baseline cortisol levels were elevated and did not reflect true resting status. However, since there was one hour between initial catheter insertion and the baseline blood draw, it is likely that serum cortisol concentrations had returned to a near resting state. This study only included women working a single shift work pattern making the results less generalizable to male SW or different shift patterns. There was also a wide age-range within the sample, which is important because stress reactivity increases with age (46). However, our sample size did not allow an age comparison. Finally, physical activity levels were not assessed in the study population. This is a limitation because physical fitness (10) and activity
have been seen to reduce cardiovascular stress reactivity. Therefore, a greater level of physical activity in the SW might have offset a negative impact of cardiovascular reactivity.

3.5.6 Conclusions

In this study, an acute mental stress task resulted in significantly higher cortisol stress reactivity in a group of shift-working vs. non-shift working female hospital employees. While there was no significant difference in cardiovascular reactivity between SW and NSW, the magnitude of group difference for DBP reactivity had a moderate effect size. These preliminary data suggest that SW have increased HPA-axis reactivity to acute stress, and may also have modestly increased cardiovascular stress reactivity. This is important because high HPA-axis and cardiovascular reactivity are associated with future cardiovascular risk, and may be a potential pathway that leads from shift work to CVD. Future research is required to confirm if heightened cortisol and cardiovascular reactivity exist in larger samples that are homogeneous in age, contain men, and include different shift work patterns.
3.6 References


27. **Kirschbaum C, Prussner JC, Stone AA.** Persistent High Cortisol Responses to Repeated Psychological Stress in a Subpopulation of Healthy Men In humans and subhuman primates, repeated ex- result in rapid habituation of cortisol responses. showed that monkeys subjected to 72-hour shock avoidan. 474: 468–474, 1995.


Chapter 4

Brachial artery flow-mediated dilation of female shift and non-shift workers in response to reactive hyperemia and handgrip exercise induced increases in shear stress

4.1 Introduction

There is evidence that shift work, defined as working a rotation of day and evening shifts, or consistently working shifts that have irregular hours (i.e. permanent night shifts) (19), is associated with an increased risk of cardiovascular disease (CVD) (15, 20, 37). However the causal pathways underlying the increased risk are still unclear. Proper vascular endothelial cell function is essential for the overall health of the vascular system (21) and a shift work associated dysfunction of these cells may play a mechanistic role in the connection between shift work and CVD. Endothelial function has been shown to be impaired in SW immediately after an acute bout of shift work (1, 34), and immediately after working sequential night shifts (17). However, the chronic impact of shift work on endothelial function is less clear. Only a small number of studies, done in men (38), or with non-standard techniques (32, 39) have compared endothelial function in SW (measured well after a shift rotation to minimize acute effects) vs. NSW controls.

The established technique for non-invasive assessment of endothelial function involves increasing blood flow-associated shear stress in a conduit artery to cause a flow-mediated dilation (FMD). Low magnitudes of FMD indicate poor endothelial function (4). Different methods of FMD testing vary in the procedure used to increase shear stress in the artery. The most common form of FMD testing uses the release of a temporary forearm occlusion (reactive hyperemia (RH)) to create a transient shear stress stimulus in the brachial artery (4, 25). Brachial artery RH
mediated FMD (RH-FMD) is an independent predictor of cardiovascular events in both healthy (31) and at-risk populations (11).

Handgrip exercise (HGEX) can be used to create a sustained, intensity-dependent increase in brachial artery shear stress, stimulating an HGEX mediated FMD response (HGEX-FMD) (29, 40). The difference in the shear stress stimulus profile created with RH vs. HGEX-FMD is important because the nature of the FMD response (e.g. its magnitude and mechanisms) appears to be dependent on the shear stress profile (23, 30). Moreover, acute mental stress (33), diabetes (2), and smoking (7) have been shown to impact RH-FMD and HGEX-FMD differently, indicating that these two tests may provide distinct information regarding endothelial function. In particular, there is evidence that HGEX-FMD may sometimes provide a more sensitive test of endothelial dysfunction. HGEX-FMD, but not RH-FMD has been shown to be impaired in young healthy smokers (7), and group differences between other at-risk vs. healthy control groups have been found to be larger with HGEX- vs RH-FMD (12). Furthermore, HGEX-FMD may have greater functional relevance than RH-FMD in terms of perfusion because FMD during daily activity is caused by exercise-induced shear stress and not release of a limb-occlusion. The impact of shift working history on RH-FMD has been minimally studied (38) and no studies to date have investigated how shift working history impacts HGEX-FMD.

With this as background the objective of the present study was to compare the effects of chronic shift work on RH and HGEX-FMD in healthy female hospital employees. We hypothesized that the SW would have impaired HGEX-FMD, but not RH-FMD compared to NSW.
4.2 Methods

4.2.1 Participants

Thirty-nine healthy, non-smoking female hospital employees aged 23–65 years from Kingston General Hospital were recruited for this study. Here we report the data from thirty-eight subjects for whom we had full RH- and HGEX-FMD data. This sample is different from the sample in Chapter 3 in that it includes one more SW who had full FMD data but no stress reactivity data, and one less NSW who had stress reactivity data but incomplete FMD data. Health status was confirmed via a medical screening questionnaire. Exclusion criteria were assessed via self-reported and included cardiovascular or metabolic disease, use of alpha or beta-blocking medications, and morbid obesity (BMI>35). The study contained two experimental groups: SW and NSW. Shift work was defined as working 12 hour rotating day and night shifts, and NSW was defined as working 8 hour day shifts. The SW group consisted of 20 women in current shift work positions, with a minimum of 6 years of shift work experience. The NSW group consisted of 19 women in current non-shift workday positions with a minimum of 6 years of experience. Those in the NSW group had never participated in shift work with the exception of three women who had done shift work 10 or more years ago. The Health Sciences Research Ethics board at Queen’s University approved the study procedures, and all of the participants completed a consent form approved by the same board.

4.2.2 Experimental procedure

All testing was done at the same time of day between 0800 and 1000h with the exception of one participant (NSW) who began testing at 1615h. Testing was done in a quiet room with a maintained temperature of 21°C. Participants were instructed to fast for 8 h prior to visiting the lab (35), and to avoid caffeine, alcohol, and vigorous exercise for 12 h before testing. All SW participants were tested at least 12 hours following their most recent night shift. Menstrual phase
during the experimental visit was not controlled. 7 of the participants were in the follicular phase, 8 were in the early luteal phase, 4 were in the late luteal phase, 7 were menstruating, 2 were aphasic, and 10 were post-menopausal. At the beginning of the visit a 22-gauge catheter (BD Nexivia, BD Medical Supplies, Mississauga, Canada) was inserted in the right antecubital vein. After an initial blood draw, subjects lay supine for 20 min of rest and instrumentation. Blood pressure was measured on the right arm while ultrasound images were obtained from the left arm. Participants completed two RH-FMD and two HGEX-FMD trials, all separated by 10 min of rest (Figure 4).
**Figure 4.** Protocol Timeline. RH-FMD: Reactive hyperemia flow-mediated dilation, HGEX-FMD: Handgrip exercise flow-mediated dilation. The arrow represents the time point when the blood sample was taken.
4.2.3 Blood lipids and glucose

Prior to initial rest and instrumentation a small sample of blood was used for analysis of blood lipids and fasting glucose using a Cholestech LDX System (Alere Inc., Ottawa, Canada). If a venous catheter could not be successfully inserted blood samples were taken by finger-prick. LDL values were not obtained in 5 SW and 6 NSW as the values fell outside of the range of the device.

4.2.4 Blood pressure and heart rate monitoring

Heart rate (HR) was monitored continuously throughout the experiment with a 3-lead electrocardiogram. Blood pressure was measured continuously with finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). All signals were recorded on LabChart (AD Instruments, Colorado Springs, USA) for future analysis.

4.2.5 Brachial artery blood velocity and diameter measurements

Brachial artery blood velocity was obtained using Doppler ultrasound operating at 4 MHz (Vivid i2, GE Medical Systems, Mississauga, Canada). The Doppler shift frequency spectrum was analyzed via a Multigon 500P TCD spectral analyzer where mean velocity was determined as a weighted mean of the spectrum of Doppler shift frequencies. The corresponding voltage output was sampled continuously (Powerlab, AD Instruments, Colorado Springs, USA) and stored (LabChart, AD Instruments, Colorado Springs, USA) for analysis at a later time. Brachial artery diameter was obtained using ultrasound imaging technology operating at 12-MHz in B-mode (Vivid i2, GE Medical Systems, Mississauga, Canada). Ultrasound parameters were set to optimize longitudinal B-mode images of the lumen/arterial wall interface. All data were collected an insonation angle of 68° for reasons previously described (28). Image recording from the Vivid i was achieved with a VGA to USB frame grabber (Epiphan Systems, Ottawa, Canada) and
images were recorded as .avi files on an independent computer using commercially available software (Camtasia Studio, TechSmith, Michigan, USA).

4.2.6 Blood viscosity

Blood viscosity was measured in order to calculate shear stress during HGEX-FMD and RH-FMD. Blood was drawn from the antecubital vein during rest and promptly tested using a viscometer at a shear rate 225s\(^{-1}\) (DV-II+ Pro, Brookfield Engineering Laboratories, Middleborough, USA). In cases where a blood sample was not obtainable (1 SW, 2 NSW) and blood viscosity could not be measured, the mean viscosity of all other participants in the same group was used in shear stress calculations.

4.2.7 FMD tests

The variability of FMD reported by our group is similar to other reports in terms of intra- and inter-observer analysis (27, 33).

**RH-FMD**

Prior to each RH-FMD test, subjects lay supine for 10 min of rest. During rest, the ultrasound probe was placed over the left brachial artery and positioned to achieve an optimal image and blood velocity signal. The real time blood velocity was displayed on a computer screen as a 3 s moving average. In each RH-FMD trial, brachial artery blood velocity and diameter were recorded for 1 min of baseline, the last minute of a 5 min cuff inflation to 250mmHg, and for 3 min after cuff release (Figure 4). The cuff was placed just below the antecubital fossa, and distal to the site of brachial artery ultrasound measurements. The results from both RH-FMD trials were averaged to form one response in each subject with the exception of 11 SW and 16 NSW who only had one RH-FMD response due to inadequate ultrasound scans.
**HGEX-FMD**

Prior to each HGEX trial subjects rested supine for at least 10 minutes. During this time a handgrip device was placed in the left hand and a brachial artery image and blood velocity signal were acquired on the left arm. Blood velocity and diameter were recorded for 1 min of baseline, followed by 6 min of handgrip exercise, and 3 min of recovery (Figure 4). The aim of exercise was to reach and maintain a target brachial artery shear stress of 12 dynes/cm². The blood velocity required to achieve the target shear stress was calculated for each subject as:

\[
\text{required blood velocity} = \frac{(12 \times \text{BA diameter})}{(4 \times \text{Blood Viscosity})}
\]

For this calculation, brachial artery diameter was measured using caliper measurements from the ultrasound image.

Real time blood velocity was displayed on a computer screen as a 6 s moving average. Subjects initially exercised at 30 percent of their maximum voluntary contraction (MVC), which was determined at the start of the experimental procedure by squeezing the handgrip device with maximal force. To reach the target blood velocity, the subjects were coached through small increases and decreases in exercise intensity. The subject received visual feedback on their exercise intensity via a force output line displayed on a computer screen. Contractions for handgrip exercise were performed in time with a 1 s contraction/5 s relaxation duty cycle metronome. The results from both HGEX-FMD trials were averaged to form one response in each subject with the exception of 9 SW and 6 NSW who only had one HGEX-FMD response due to inadequate ultrasound scans.

**4.2.8 Data Analysis**

**Brachial artery blood velocity**

Blood velocity was analyzed offline in 3 s average time bins, using the data-acquisition software program LabChart (AD Instruments, Colorado Springs, USA).
**Brachial artery diameter**

Vessel diameter was analyzed using automated edge-detection software (Encoder FMD and Bloodflow v3.0.3, Reed Electronics, Perth, Australia) as previously described (7). Following software analysis, the diameter data was compiled in 3 s time bins. Missing data due to erroneous wall tracking were interpolated. The experimenter was blinded to shift working group (SW or NSW) when performing the artery diameter analysis.

**FMD**

RH-FMD is reported as the percent change in diameter from baseline (before cuff occlusion) to the peak 3 s average diameter time bin following cuff release. In 4 SW and 3 NSW the diameter during the last minute of cuff occlusion was used as baseline diameter due to superior image quality. HGEX-FMD is reported as the percent change in diameter from baseline to the average diameter recorded in the last minute of exercise. If image quality during the last minute of exercise did not meet analysis standards (n=2 NSW), the first 15 seconds of post-exercise recovery was used to calculate exercise diameter. Diameter during the post-exercise recovery period has been shown to be an adequate surrogate for the diameter in the last minute of handgrip exercise (18).

**Shear Stress**

Shear stress in the brachial artery was calculated using the equation:

\[ 4 \times \text{blood viscosity} \times \text{blood velocity/brachial artery diameter} \]

For RH-FMD, the peak shear stress stimulus and the area under the curve (AUC) of the shear stress stimulus from cuff release until the time of peak diameter until peak diameter are reported. For HGEX-FMD, the shear stress stimulus was calculated as a mean for every minute during the HGEX trial, and is reported for baseline and the average of the last 5 min of exercise (the steady state period). In 9 HGEX trials (6 SW, 3 NSW) diameter was only analyzed during the first and
last minute of exercise due to image quality. In these trials, the average diameter during the last minute of exercise was used to calculate shear stress for the last 5 min of exercise.

**MAP and HR**

MAP and HR were analyzed offline in 3 s average time bins. MAP and HR during the FMD tests are reported as 1 min average during baseline, and during the last minute of hyperemia (RH-FMD), and all 6 min of exercise (HGEX-FMD).

**4.2.9 Statistical analysis**

The primary outcome variables in this experiment were RH-FMD and HGEX-FMD. An independent t-test was used to compare RH-FMD and HGEX-FMD between groups. The level of significance was set at $p < 0.05$. A mixed model analysis of variance (ANOVA) (factors: between subjects - group (SW vs NSW) and within subjects –time (baseline and last minute of hyperemia or exercise) was used to examine changes in HR and MAP during HGEX and RH. Statistics were calculated using SigmaPlot 11.0 (Systat Software Inc., Chicago, USA) and IBM SPSS, Version 20 (SPSS Inc., Chicago, USA). All data were expressed as means ± SD.

**4.3 Results**

**4.3.1 Baseline characteristics**

Baseline characteristics for all subjects are displayed in Table 3. There were no significant differences between groups with the exception of blood viscosity ($p = 0.014$), shear stress ($p = 0.008$), and blood velocity ($p = 0.025$), which were higher in SW.
### Table 3. Baseline characteristics of shift workers and non-shift workers.

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>NSW</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42 ± 11</td>
<td>41 ± 3</td>
<td>0.885</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>91.6 ± 8.3</td>
<td>90.5 ± 11.6</td>
<td>0.734</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 3.5</td>
<td>25.7 ± 4.6</td>
<td>0.971</td>
</tr>
<tr>
<td>HGEX Brachial Artery Diameter (mm)</td>
<td>0.28 ± 0.20</td>
<td>0.29 ± 0.03</td>
<td>0.852</td>
</tr>
<tr>
<td>RH Brachial Artery Diameter (cm)</td>
<td>0.29 ± 0.03</td>
<td>0.28 ± 0.03</td>
<td>0.231</td>
</tr>
<tr>
<td>Blood Viscosity (centipois)</td>
<td>4.2 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>0.014*</td>
</tr>
<tr>
<td>HGEX Shear Stress (dynes⋅cm²)</td>
<td>4.1 ± 1.6</td>
<td>2.9 ± 0.8</td>
<td>0.008*</td>
</tr>
<tr>
<td>Blood Velocity (cm⋅sec²)</td>
<td>7.1 ± 2.7</td>
<td>5.4 ± 1.3</td>
<td>0.025*</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>189.4 ± 36.2</td>
<td>180.2 ± 30.5</td>
<td>0.406</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>79.9 ± 40.2</td>
<td>84.4 ± 43.5</td>
<td>0.737</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>62.4 ± 15.4</td>
<td>63.3 ± 10.4</td>
<td>0.830</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>114.8 ± 34.9</td>
<td>103.3 ± 32.9</td>
<td>0.380</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dL)</td>
<td>85.1 ± 11.8</td>
<td>88.1 ± 11.2</td>
<td>0.428</td>
</tr>
<tr>
<td>Shift Work Experience (years)</td>
<td>16.87 ± 9.50</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

SW, shift worker; NSW, Non-shift worker; BMI, body mass index; HGEX, handgrip exercise; RH, reactive hyperemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are means ± SD. * indicates significant difference (p<0.05).

---

a SW: n=15, NSW: n=13
4.3.2 HR and MAP

HR and MAP data during the RH and HGEX protocols are reported in Table 4.

**HGEX**

For HR there was a main effect of time such that HR increased from baseline to exercise (p<0.001). There was also a main effect of group such that HR was slightly higher in NSW (p<0.034) (time x group interaction p=0.672). For MAP there was a main effect of time such that MAP increased from baseline to exercise (p<0.001). There was no main effect of group (p=0.509) or time x group interaction (p=0.288).

**RH**

For HR there was a main effect of time such that HR increased from baseline to the last minute of hyperemia (p<0.040). There was no main effect of group (p=0.112) or time x group interaction (p=0.072). For MAP there was a main effect of time such that MAP increased from baseline to the last minute of hyperemia (p<0.001). There was no main effect of group (p=0.564) or time x group interaction (p=0.054).
Table 4. HR and MAP in shift workers and non-shift workers during the handgrip exercise and reactive hyperemia protocols.

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>NSW</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Handgrip Exercise</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>59.2 ± 7.3</td>
<td>64.3 ± 8.1</td>
<td>Main effect of time, <em>p &lt; 0.001</em></td>
</tr>
<tr>
<td>Last min of ex</td>
<td>63.1 ± 7.0</td>
<td>68.7 ± 8.7</td>
<td>Group: <em>p = 0.034</em></td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>107.2 ± 9.6</td>
<td>103.8 ± 13.3</td>
<td>Time x group, <em>p = 0.672</em></td>
</tr>
<tr>
<td>last min of ex</td>
<td>110.4 ± 10.4</td>
<td>108.5 ± 14.5</td>
<td>Main effect of time, <em>p &lt; 0.001</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Group, <em>p = 0.509</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time x group, <em>p = 0.288</em></td>
</tr>
<tr>
<td><strong>Reactive Hyperemia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>59.6 ± 7.9</td>
<td>63.3 ± 8.5</td>
<td>Main effect of time, <em>p = 0.040</em></td>
</tr>
<tr>
<td>Last min of hyperemia</td>
<td>60.1 ± 8.2</td>
<td>65.1 ± 8.81</td>
<td>Group: <em>p = 0.112</em></td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>106.6 ± 11.1</td>
<td>105.0 ± 17.1</td>
<td>Time x group, <em>p = 0.072</em></td>
</tr>
<tr>
<td>Last min of hyperemia</td>
<td>110.2 ± 12.0</td>
<td>106.3 ± 16.7</td>
<td>Main effect of time, <em>p &lt; 0.001</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Group: <em>p = 0.564</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time x group, <em>p = 0.054</em></td>
</tr>
</tbody>
</table>

The factor time has two levels: baseline and last minute of handgrip exercise or reactive hyperemia. HR, heart rate; MAP, mean arterial pressure; SW, shift workers; NSW, non-shift workers. Values are means ± SD.
4.3.3 Shear stress

Baseline shear stress was significantly higher in SW (p=0.008) (Table 3). During HGEX, mean shear stress during the last 5 minutes of exercise was significantly greater in SW compared to NSW (Figure 5 A, B). The group difference in shear stress was due to experimenter error in shear stress targeting (for a description of targeting refer to the HGEX-FMD section in methods (page 69)). A subset of participants was formed, excluding participants with the largest targeting error. In this subset there was no group difference in the shear stress stimulus (Figure 5C, D). For the RH protocol there was no significant group difference in shear stress AUC from baseline until the time of peak diameter measurement (Figure 6).

4.3.4 FMD

There was no significant difference in %HGEX-FMD or %RH-FMD between groups (Figure 7A, B). There was also no significant group difference in %HGEX-FMD in the subset matched for the HGEX shear stress stimulus (Figure 7C).
Figure 5. A) Shear stress expressed as one minute averages during baseline (1 min), handgrip exercise (HGEX) (6 min) and recovery (3 min). B) Mean shear stress during last 5 min of HGEX.* denotes significant difference from NSW (p<0.05). C) Shear stress expressed as one minute averages as described for panel A during HGEX-FMD when the 3 SW and NSW with the largest shear targeting errors were removed D) Average shear stress during last 5 min of exercise in the same subset as panel C. SW, shift worker; NSW, non-shift worker. Values are means ± SD.
**Figure 6:** Area under curve (AUC) of the shear stress stimulus during the RH protocol from baseline to the time of peak diameter measurement. SW, shift worker; NSW, non-shift worker. Values are means ± SD.
Figure 7. %FMD in SW and NSW. A) %RH-FMD B) %HGEX FMD. C) %HGEX FMD with matched shear stresses between groups. SW, shift worker; NSW, non-shift worker. Values are means ± SD
4.4 Discussion

This study was designed to compare brachial artery endothelial function in female SW and NSW using RH- and HGEX-FMD. The primary finding was that contrary to our hypothesis SW did not have an impaired FMD response relative to NSW regardless of the shear stress stimulus profile used in testing. These findings suggest that a history of rotating shift work may not lead to impaired endothelial function in women.

4.4.1 The impact of shift work on endothelial function

RH-FMD

In the present study no difference in %RH-FMD between SW and NSW was observed. Only one previous study comparing a small group of male SW and NSW has investigated how a history of shift work affects RH-FMD (38). Similar to the present study, this previous work did not identify a significant difference between groups, however, there was a trend for lower %RH-FMD in SW (p=0.08). It is possible that the present study did not detect a similar trend due to sex differences in the impact of shift work on endothelial function, in that it may have a more deleterious impact in men (the protective impact of estrogen on endothelial function is discussed below). Two other reports have compared endothelial function in SW and NSW with fingertip peripheral arterial tonometry (PAT). These studies observed impairment in middle aged male (32, 39) and female (39) rotating SW compared to NSW. PAT uses measurements in the finger to provide an indication of resistance vessel vasodilation during an occlusion induced RH (32). While PAT can be used as an index of endothelial function, resistance vessel dilation during occlusion may not be wholly endothelial dependent, and is distinct from conduit artery function. These results are therefore difficult to compare to RH-FMD. Furthermore, the SW in both of these studies adhered to different shift patterns than the SW in the present study, and these other work patterns may have a more deleterious impact on endothelial function.

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The hospital employees in this study worked a rotating shift pattern comprised of two 12-hour days, two 12-hour nights, and five days off (DDNN). This work pattern could have contributed to the lack of difference in %RH-FMD between groups. Unlike schedules consisting of permanent night shifts, or alternating weeks of permanent day and night shifts, the DDNN pattern may result in lower levels of circadian disruption (physiological, behavioral, and psychosocial consequences related to interruption of the sleep-wakefulness rhythm(26)).

Circadian disruption can cause behavioral changes such as poor nutrition and physical inactivity leading to increased adiposity (26). These changes may in turn lead to endothelial dysfunction through pathways including dyslipidemia (36) and hyperglycemia (16). In support of limited circadian disruption, the SW and NSW groups in the present study had similar waist circumference, BMI, lipids, and fasting glucose.

Baseline characteristics such as age (3), BMI (13), high cholesterol (24), and blood glucose (16) could influence FMD independently of shift work as mentioned above. If age, BMI, cholesterol, and blood glucose had been higher in the NSW that could have had a deleterious impact on RH-FMD in this group, making it more difficult to detect a negative impact of shift work history. However, these factors were well matched in our current sample and were therefore unlikely to contribute to the lack of group differences. Conversely, blood viscosity, baseline blood velocity, and baseline shear stress were significantly higher in SW. A number of factors can cause changes in blood viscosity, with hydration and red blood cell concentration having the greatest impact (8). No previous studies, however, have examined blood viscosity in SW and it is unclear why it was higher in this group. The greater 3 s binned blood velocity in the SW was likely influenced by the faster baseline heart rate. The higher baseline shear stress observed in SW can be explained by the combined effects of a higher blood viscosity and blood velocity. Any physiological significance of these modestly elevated baseline variables is unclear. While a
higher shear stress stimulus in the SW could have masked an RH-FMD impairment, despite the higher blood viscosity the shear stress stimulus for RH-FMD was not different between groups.

It is possible that the relatively young age of the SW (41.6 years) in this study contributed to the lack of difference in %RH-FMD between groups. Approximately 73% of SW were premenopausal and therefore had normal levels of estrogen secretion. There is evidence that estrogen has vasoprotective effects (22) and premenopausal status may mitigate any vascular consequences that could result from shift work. The work of Celermajer et al., (1994) suggests that an age related decline in FMD begins in women after age 50 (older than in men). When only older participants were compared (>50 years; n=6 in each group), the group difference in RH-FMD widened to 2.2% (lower in SW). This would suggest that youth might play a protective role in the SW, however not all women over 50 yrs. were post-menopausal and more research is required to elucidate the interaction between shift work, age, menopausal status and endothelial function.

**HGEX-FMD**

This was the first study to investigate how a history of shift work affects HGEX-FMD and, similar to RH-FMD, the results revealed no difference in %HGEX-FMD between groups. Compared to previous HGEX studies HR and MAP increases during exercise were typical (14, 29), and similar between groups. Shear stress during the HGEX protocol was significantly higher in the SW compared to NSW (12.6 vs. 11.4 dynes·cm²). This difference was caused by experimenter error in over-targeting shear stress in SW and under-targeting in NSW. In order to determine whether differences in shear stress were masking potential differences in %HGEX-FMD, a subset of subjects with matched shear stress was analyzed. Within the subset, the targeting was more accurate (SW: 12.3 dynes·cm2, NSW 11.9 dynes·cm2) resulting in no group
differences in shear stress stimulus between groups. The non-significantly higher %HGEX-FMD in SW in the subset confirmed that shift work had no detrimental effect on endothelial function.

It may be advantageous to test both RH- and HGEX-FMD because the different shear stress profiles may cause FMD through different transduction pathways (9). While there are conflicting reports regarding how these transduction pathways differ (24, 40), there is evidence that the RH- and HGEX-FMD may be vulnerable to different vascular insults. For example, HGEX- but not RH-FMD has been shown to be impaired in young healthy smokers (7) while the opposite has been observed following acute mental stress in healthy young men (7). Additionally, healthy control vs. type 1 diabetic group differences in FMD stimulated by sustained limb heating-induced increases in shear stress (similar to exercise) (2) and HGEX (12) have been shown to be larger than group differences in RH-FMD. We had originally hypothesized that only HGEX-FMD would be impaired in SW in light of these studies indicating earlier or greater impairment vs. RH-FMD (7, 12). While no group difference in HGEX-FMD was observed, results may be different in other SW samples consisting of men, older women, or individuals working different shift patterns. The assessment of endothelial function using two shear stress profiles that may utilize distinct transduction pathways strengthens the evidence that endothelial function was not impaired in the current population of female SW.

4.5 Limitations

This study only included women working a single shift work pattern, which makes the results less generalizable to male SW or different shift patterns. Furthermore, the shift working group was not homogenous in terms of job position as it included emergency room nurses, cardiology nurses, personal care assistants, medical laboratory technicians, and sonographers. Due to the different working environment and demands of each position, the effects of shift work may have varied between individuals. We counted both part-time and full-time work towards
years of experience. As a result, some shift workers may not have had enough exposure to shift work in order to experience vascular changes. We used the number of days since last menstrual period as opposed to hormone levels or body temperature to determine menstrual phase. This may have led to error when estimating menstrual phase at testing. Furthermore, menstrual phase at time of testing was not controlled and this may have increased variability in FMD (22). However, our records indicate that the same number of SW and NSW were tested during the high estrogen phases, therefore menstrual cycle phase was unlikely to have prevented detection of a shift work associated impairment in FMD. Physical activity level was not measured in this study. Activity can influence FMD, and a greater level of physical activity in the SW might have offset a negative impact of shift work (5). Finally, this study is preliminary and limited by its small sample size. We were underpowered to detect a moderate shift work effect size; in order to detect a HGEX-FMD difference of 1% as significant with a power of 0.8 and alpha of 0.5, we would have needed approximately 70 SW and NSW (140 participants total). If this study had been adequately powered we might have detected a higher %HGEX-FMD compared to NSW. While this would be contrary to our initial hypothesis, it is possible that factors such as greater physical activity at work (6) could contribute to enhancing %FMD in SW (5).

4.6 Conclusion

This study was the first to investigate the chronic impact of a history of shift work on both RH and HGEX-FMD and the first to examine RH-FMD in shift working women. There was no significant difference in RH or HGEX-FMD between SW and NSW. This suggests that rotating shift work done in a DDNN pattern may not have a chronic effect on endothelial function in women. Future research is required to establish how other patterns of shift work impact RH and HGEX-FMD in women, the impact of menopausal status and, whether similar FMD results exist in larger and distinct shift working populations.
4.7 References


9. Frangos JA, Huang TY, Clark CB. Steady Shear and Step Changes in Shear Stimulate Endothelium via Independent Mechanisms — Superposition of Transient and Sustained Nitric Oxide Production flow through the release of factors such as the vasoactive compounds prostacyclin (PGI2) and of. 665: 660–665, 1996.


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Chapter 5

General Discussion

This thesis examined two possible pathways that may contribute to increased CVD risk in female SW. Manuscript one (Chapter 3) investigated cardiovascular and cortisol reactivity to acute stress. Heightened reactivity has been associated with increased future risk of hypertension (7, 11) and atherosclerotic changes (7), but has been minimally studied in SW. We found that cortisol stress reactivity was significantly higher in SW vs. NSW, and that the difference in DBP reactivity between SW and NSW had a moderate effect size. These preliminary data suggest that shift work exposure results in increased HPA-axis reactivity to stress and may also modestly increase cardiovascular stress reactivity. While the traditional risk cardiovascular risk factors reported in Table 3 suggest that this sample was currently at low cardiovascular risk, they may be subject to future cardiovascular damage via stress reactivity pathways.

Manuscript two (Chapter 4) examined brachial artery endothelial function using an RH and HGEX-induced shear stress stimulus. While a night shift has been shown to acutely impair RH-FMD within groups of SW (1, 13, 18), the chronic effect of shift working history on endothelial function has only been minimally investigated using RH-FMD (21) and other non-standard techniques (17, 22). Additionally, no previous studies have investigated the effect of shift working history on HGEX-FMD. We found no difference in endothelial function when assessed by RH or HGEX-FMD. These preliminary data suggest that shift work experience did not have a deleterious impact on endothelial function. The current unimpaired cardiovascular state in SW suggested by the FMD data is in agreement with the low risk status determined by the traditional cardiovascular risk factors reported in Table 3.
5.1 Integrating Endothelial Function and Stress Reactivity Results

Interestingly, SW showed no difference in %FMD but had higher cortisol stress reactivity. There is evidence that elevated cortisol causes impairment in FMD possibly through inhibition of NO synthesis (2, 3). This difference in reactivity but not FMD may be explained by the fact that stress reactivity is a predictor of future cardiovascular risk while FMD assessed current vascular function. In previous stress reactivity studies, negative cardiovascular effects were observed anywhere from 3 to 13 years after initial testing (11, 14, 16). Although the vascular function of these SW was similar to NSW, it may be impaired in the future particularly after menopause. It is also possible that FMD and stress reactivity are influenced by different aspects of the shift working environment. One example is circadian interruption. Since FMD seems to be impaired in SW following a 24 hour shift (1, 18), or consecutive night shifts (13), it is possible that interruptions in circadian rhythms caused by these work patterns contributed to the observed endothelial dysfunction. The SW in this study followed a schedule that contained 5 rest days following the DDNN work pattern, and this may have enabled recovery from any significant circadian disruption, preventing the development of chronic endothelial dysfunction. However, shift work associated factors other than circadian disruption, such as chronic stress, may influence stress reactivity. While chronic stress has also been shown to impact FMD (15) the level of chronic stress in the reported sample may have been below a threshold required to influence endothelial function, but above a threshold required to influence stress reactivity.

5.2 Strengths and Limitations

5.2.1 Strengths

In previous studies examining stress reactivity, stress responses have been characterized using single measurements of BP and HR at various time points throughout the stress task (5, 16). This is a limitation because it may result in underestimation of the stress response. The current
study continuously monitored HR and BP throughout the entire stress task, allowing for full characterization and a greater likelihood of capturing the true cardiovascular response to stress. Additionally, previous RH-FMD studies in SW only measured the FMD response, and did not measure the shear stress stimulus (1, 12, 18, 21). This poses a problem since different magnitudes of shear stress stimuli could cause differences in %FMD between individuals and groups. This study measured shear stress AUC, which has been shown to be the best characterization for the RH stimulus (4). In studies that do report the stimulus, blood viscosity is not usually measured, and as a result shear rate (an estimate of shear stress without viscosity) is used instead of shear stress. If blood viscosity happens to differ between subjects, a similar shear rate will actually represent a distinct shear stress. Since blood viscosity was higher in SW, we would have underestimated the shear stress stimulus in this group if we had used shear rate instead of shear stress. Finally, this study examined endothelial function using both RH- and HGEX-FMD, which is advantageous because the different shear stress profiles may cause FMD through different transduction pathways (10). Overall, testing endothelial function with two possibly distinct transduction pathways strengthened the evidence that shift work did not have a negative impact on endothelial function in the tested sample.

5.2.2 Limitations

This study was limited by a sample that was heterogeneous in terms of job position and age. The SW group included emergency room nurses, cardiology nurses, personal care assistants, medical laboratory technicians, and sonographers. Due to the different working environment and demands of each position, the impact of shift work on FMD and stress reactivity may have varied between individuals. There was also high variation in terms of age with participants ranging from 23 years to 61 years old. Since FMD decreases (6), and stress reactivity increases (20) with age, it is important to explore the impact of shift work within different age groups (young vs old). However, our sample size was too small to allow for a meaningful exploration of these
comparisons. Additionally, the small sample size of this study resulted in a lack of statistical power to detect moderate differences when comparing FMD and cardiovascular reactivity between SW and NSW. Sample size was particularly small for cortisol stress reactivity (11 SW, 7 NSW) due to missing blood samples. However, we were still able to detect a significant group difference in cortisol reactivity ($d=1.5$) between SW and NSW.

The shear stress stimulus during HGEX was higher in SW than NSW, which could have masked potential differences in %FMD. This mismatch in shear stimulus was caused by experimenter error in shear stress targeting during exercise (NSW group below target, SW group above target). Further analysis of a subset of subjects who had more accurate targeting and group matched shear stress still revealed no group difference in %HGEX-FMD. Indeed, although not significantly different, SW exhibited higher HGEX-FMD than NSW when shear stress was matched. In addition, it was necessary to exclude a large number of RH-FMD and HGEX-FMD images during data analysis because of poor image quality caused by the challenges of performing vascular ultrasound in a sample of middle-aged women. This resulted in only having one FMD response of each type for several participants (RH: 11 SW and 16 NSW; HGEX: 9 SW and 6 NSW), which may have increased the variability of the FMD results.

A comparison of the medications being taken by SW and NSW is not currently available. This is important because antihypertensive and cholesterol lowering medications could make it appear that baseline characteristics such as blood pressure and blood lipids were matched between groups, while differences in pharmaceutical control could be significant. Additionally, antihypertensive medications could have influenced BP stress reactivity. Finally, physical activity was not measured in the sample of SW and NSW included in this thesis. This is a limitation because fitness and activity can increase FMD (8) and decrease cardiovascular stress reactivity (9,
Therefore, a greater level of physical activity in the SW might have offset a negative impact of shift work on FMD or cardiovascular stress reactivity.

5.3 Technical Obstacles

As mentioned previously it was difficult to obtain clear ultrasound images in this female study population, particularly in middle-aged and older individuals. Challenges in capturing defined arterial walls may have stemmed from the small arterial diameter of these participants. We also faced difficulties in successfully inserting the venous catheter, and keeping the catheter line patent in order to get a complete panel of blood samples over the 3.5 hour duration of the visit. Some participants were not comfortable with having a catheter, others had non-compliant veins, and in some cases the catheter was compromised because of excessive arm movement throughout the study. There were also challenges in scheduling participants for this study, especially in terms of working around the busy schedules of the SW.

5.4 MSc Experience

Although challenging at times, completing this MSc has given me many valuable experiences. I was able to mentor a group of undergraduate students who were helping on the project. This included teaching them how to do data analysis, use lab equipment, and help with key aspects of data collection. I also had the unique opportunity to collaborate with Nursing MSc student Morgan Batson, who brought a different scientific background and viewpoint to the study. Throughout this project I have learned many new technical skills including ultrasound imaging, biochemical analysis of cortisol samples using ELISA kits, and measurement of blood viscosity. I have also gained valuable study design and project management experience in the form of determining an experimental protocol, scheduling participants, organizing and scheduling volunteers to assist during data collections, and communicating with biotech companies when researching and ordering new equipment.
5.5 Conclusions and perspectives

No difference was found in RH- or HGEX-FMD between SW and NSW, indicating that shift work experience did not have a negative impact on endothelial function. As such, endothelial dysfunction may not be a pathway leading from shift work to CVD in nurses who work a DDNN pattern. In terms of stress reactivity, SW had significantly higher cortisol stress reactivity, and the difference in DBP reactivity between SW and NSW had a moderate effect size. This would suggest that SW have increased HPA-axis reactivity to acute stress, and may also have modestly increased cardiovascular stress reactivity. Elevated HPA-axis and cardiovascular reactivity are associated with future cardiovascular risk, and this may be a potential mechanistic pathway that leads from shift work to CVD. Future research should include testing a population, examining both a younger and older (post-menopausal) sample, investigating the effects of different shift patterns (permanent night shifts or other rotating patterns), and looking at other indicators of life-stress and stress reactivity. If similar patterns of elevated stress reactivity are found in larger studies, there may be the need to develop interventions targeted at increasing coping strategies or changing work structure or content to reduce stress in shift working environments.
5.6 References


10. Frangos JA, Huang TY, Clark CB. Steady Shear and Step Changes in Shear Stimulate Endothelium via Independent Mechanisms — Superposition of Transient and Sustained Nitric Oxide Production flow through the release of factors such as the vasoactive compounds prostacyclin ( PGI 2 ) 2 and of. 665: 660–665, 1996.


Appendix A

Consent Form

School of Kinesiology and Health Studies
Queen's University

Dr. Kyra E. Pyke and Dr. Joan Tranmer Principle Investigators

Study performed in Room 400D, School of Kinesiology and Health studies Building 28 Division St.

CONSENT FORM
FOR RESEARCH PROJECT ENTITLED:

Stress reactivity and vascular health in women who perform shift work

Please read this form carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and any associated risks. Your signature on this form also means that you want to take part in this study.
Purpose of the Study:

You are being invited to participate in a research study directed by Dr. Kyra Pyke and Dr. Joan Tranmer to evaluate the impact of shift work on vascular function and responses to acute mental stress in women. Dr. Pyke or an associated investigator will read through this consent form with you on your first visit to the laboratory and will describe the procedures in detail and answer any questions you may have. This study has been reviewed for ethical compliance by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

The purpose of the study is to examine the impact of a history of shift work on vascular function and responses to acute mental stress.

Benefits For You:

There are no direct benefits to you by participating in this study.

Description of Experiment and Risks:

What will happen? During this study, you will take part in the specific experimental procedures outlined below.

HEART RATE MEASUREMENTS:

Heart rate is continuously monitored by an electrocardiogram (EKG) through 6 spot electrodes on the skin surface. The electrodes are placed
on the chest and abdomen and they can detect the electrical activity that makes your heart beat.

RISKS: **This procedure is entirely safe.** In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.

**BLOOD PRESSURE MEASUREMENTS:**

A small cuff is fit around your finger. This cuff inflates to pressures that match the blood pressure in your finger, so you feel the cuff pulsing with your heart beat. It shines infrared light through your finger to measure changes in the size of your finger with each heart beat. A larger cuff will be periodically inflated around your upper arm to calibrate the finger cuff.

RISKS: **This technique is non-invasive and poses no risk.**

**LIMB BLOOD FLOW AND BLOOD VESSEL DIAMETER MEASUREMENTS:** The blood flowing through your brachial (above the elbow) artery can be detected, and your artery size measured using Doppler and imaging ultrasound. A probe will be placed on the skin over your artery and adjustments in its position will be controlled by hand by the investigator. High frequency sound (ultrasound) will penetrate your skin. The returning sound provides information on blood vessel size and blood flow.

RISKS: **This technique is non-invasive and poses no risk.**
**FOREARM OCCLUSION:** A blood pressure cuff will be secured just above or below your elbow on your left arm. This cuff will be inflated to 300mmHg for 5 min to limit blood flow into your forearm. You may feel a strong pressure and some mild tingling with cuff inflation but it should not be uncomfortable. If there is pain, immediately notify the investigator and the cuff will be deflated and repositioned. Upon cuff release there will be a large rush of blood into your forearm. This may feel warm and you may experience mild tingling but no discomfort.

**RISKS:** *This technique is non-invasive and poses no risk.*

**HANDGRIP EXERCISE:** You will be asked to perform handgrip squeezing exercise. You will be asked to perform maximal contractions (duration ~2s) and up to 10 min of rhythmic contractions at an intensity that is 10-60% of your maximal force.

**RISKS** *You may experience muscle soreness in the muscles of your forearm for 24-72 hours after performing the handgrip exercise, much as you would if you had been lifting weights.*

**ARTERIAL THICKNESS AND PRESSURE MEASUREMENTS:** An ultrasound probe will be placed on the front of your neck over your left carotid artery to take a picture of the vessel. A small pencil like pressure transducer will be placed over your pulse on the right side of your neck and on your femoral artery pulse in your upper thigh. This will allow us to measure the change in pressure in your artery
over the cardiac cycle. These measurements will be performed periodically while you rest.

**RISKS:** This technique is non-invasive and poses no risk.

**PRESSURE IN YOUR FOOT:** A small infrared (light) sensor will be taped to the top of your foot to take continuous measurements of your pulse. This will be used to measure how quickly the pressure wave created by your beating heart travels through your arteries. This tells us about the stiffness of your arteries.

**RISKS:** This technique is non-invasive and poses no risk.

**MENTAL CHALLENGES:** During your visit you will be periodically presented with mentally challenging tasks. A description of each task is provided below. Upon your arrival in the laboratory, and after each task you will be asked to rate your level of stress on a scale of 0-10.

*Response to a scenario:* You will presented with a scenario and asked to prepare (10 min) and deliver a short verbal response (5 min). Your verbal response will be video recorded for subsequent analysis by graduate students involved in the project.

*Mental Arithmetic:* You will be asked to subtract a constant from numbers that are provided to you by an administrator and to verbally report your answer. You will be encouraged to do this as quickly as possible.
RISKS: These tasks may cause your heart rate and blood pressure to increase as they would in moderate intensity exercise. This does not pose any greater risk than exercise.

BLOOD SAMPLING: In order to quantify the thickness of your blood (blood viscosity), and other factors that may relate to vascular function we will draw a venous blood sample. This will be done via sampling with a needle from vein at your hand or elbow. In order to make the vein easy to identify, a non-latex tourniquet will be briefly applied to your upper arm. A needle will be inserted into the full vein the same way that it is done if you donate blood or have blood taken for medical tests.

RISKS: There may be some mild soreness and mild bruising at the site of the needle insertion. In rare cases more significant soreness and significant bruising can occur. Puncturing a blood vessel increases the risk of clot formation, but this is very rare with venous blood sampling. If you have symptoms outside of what is described here, please contact Dr. Pyke or Morgan Baston.

SALIVARY CORTISOL MEASUREMENTS

Cortisol is a hormone released in response to stress. When you arrive at the laboratory and at specific time points during the experiment, we will take samples of your saliva. This will be done by having you chew on a swab that absorbs your saliva.

RISKS: This procedure is non invasive and poses no risk.
**Derogatis Stress Profile (DSP)** – This is a self-report questionnaire that is used to quantify feelings about health and stress related factors.

RISKS: **This poses no risk.**

**Effort Reward Balance Index** – This is a self-report questionnaire that is used to quantify feelings about working conditions.

RISKS: **This poses no risk.**

**Health and Work Environment Questionnaire** – This is a self-report questionnaire that is used to quantify factors related to medical history, current health status and your work environment.

RISKS: **This poses no risk.**

How long will it take?

When you arrive at the laboratory you will be shown all of the equipment that we will use and asked to complete the questionnaires. This will take approximately 30-40min.
At the beginning of the physiological testing a researcher will measure your height, weight and waist circumference. A venous blood draw will then be performed. While lying down and resting, you will be instrumented for heart rate, blood pressure and blood flow (ultrasound) measurements. After a 30 min rest period ultrasound will be used to take measurements on your brachial artery in your upper arm. You will then be asked to perform the mentally challenging task and ultrasound measurements will be repeated. The whole visit will take approximately 2-2.5h.

Talking and Movements:

Talking or moving during the times that we are taking measurements will cause variations in the measurements we are making. If you have any discomfort, please let us know immediately and we can temporarily break from data collection. However, if everything is comfortable, please maintain a very quiet posture. Even very slight movements interfere with our experiments.

Special Instructions:

Participants are asked to not exercise for a full day prior to the study, to not drink alcohol or caffeine from 6pm onward the night prior to the study, and to consume no food and/or beverages other than water from 8pm onward the night prior to the study. You should empty your bladder immediately prior to starting the test. When the study is finished, we will have you sit in the laboratory for a short time to allow you to readjust to the upright posture. Juice will also be
provided. These precautions should be enough to prevent any sensations of dizziness. Please be aware that sensations of dizziness are not normal and you should let us know if you experience any discomfort before you leave the laboratory.

Attached Medical Screening Forms:

This questionnaire asks some simple questions about your health. This information is used to guide us with your entry into the study. Current health problems indicated on this form which are related to cardiovascular diseases exclude you from the study.

Safety Precautions:

Safety precautions for the study will include the following:

- Before entering the study you will be screened using a medical screening form. You will not be able to enter the study if anything is found which indicates that it is dangerous for you to participate.

- We will continuously monitor your heart rate and blood pressure, and you will be laying on your back. These precautions allow us to quickly identify if you are experiencing an unusual response and simply stopping the experimental manipulation will allow you to quickly recover.
Blood sampling will be performed by trained personnel using sterile equipment.

Confidentiality:

All information obtained during the course of the study is strictly confidential and will not be released in a form traceable to you, except to you and your personal physician. Your data and any personal health information reported on the health questionnaire, will be kept in locked files which are available only to the investigators and research assistants who will perform statistical analysis of the data. There is a possibility that your data file, including identifying information, may be inspected by officials from the Health Protection Branch in Canada in the course of carrying out regular government functions. The study results will be used as anonymous data for scientific publications and presentations, or for the education of students in the School of Kinesiology and Health Studies at Queen’s University.

Study Compensation

You will receive $25 to offset any travel costs incurred as a part of participation.

Freedom to Withdraw from the Study
Your participation in this study is voluntary. You may refuse to participate or you may discontinue participation at any time during the duration of the study without penalty and without affecting your future medical care. By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

**Subject Statement and Signature Section**

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I will receive a copy of this consent form for my information.

If at any time I have further questions, problems or adverse events, I will contact:

Kyra E. Pyke, Ph.D.
pykek@queensu.ca
(Principal Investigator)
Room 301C School of Kinesiology and Health Studies Building
28 Division st. Queen’s University, Kingston, ON, K7L 3N6
Tel: (613) 533-6000, ext, 79631

Morgan Baston
(Graduate Student Investigator)
School of Nursing and Community Health and Epidemiology
5bm28@queensu.ca

Ira Carson
(Graduate Student Investigator)
School of Kinesiology and Health Studies
8ic2@queensu.ca
(613) 533-6000 x 79377

Jean Cote Ph.D.
Department head
Room 225, Physical Education Centre
Queen’s University, Kingston, ON, K7L 3N6
Tel: (613) 533-6601

If I have any questions concerning research subject’s rights, I will contact:

Dr. Albert F. Clark, Chair of the Queen’s University Health Sciences and
Affiliated Teaching Hospitals Research Ethics Board
Office of Research Services
Fleming Hall, Jemmett Wing 301
Queen’s University, Kingston, ON, K7L 3N6
By signing this consent form, I am indicating that I agree to participate in this study.

______________________  _______________________
Subject Signature        Person obtaining consent Signature

______________________  _______________________
Subject Name (please print)  Person obtaining consent Name (please print)

______________________  _______________________
Date (day/month/year)      Date (day/month/year)
Appendix B
Derogatis Stress Profile and Effort-Reward Imbalance questionnaires

All questionnaires are used with permission for research led by Dr. Joan Tranmer

Effort-Reward Imbalance

Job Status
We kindly ask you to answer the following questions and statements about your current working conditions. By doing so you contribute to a better scientific understanding of associations between modern working life and health.

1. What is your current occupation/job title? ________________________________

2. What is your position at work?
   a. Manager
   b. Supervisor
   c. Employee, non-manual
   d. Employee, manual
   e. Self-employed

3. How many years have you worked in your current position? _______________

4. Apart from your main employment, do you have any other jobs?
   □ Yes
   □ No

5. In total, how many hours a week do you spend working for pay? _________ hours
6. Are you scheduled on shift work?

☐ Yes
☐ No

If you **AGREE** for the following questions please also indicate how much you are generally distressed by this situation

*(1 = I am not at all distressed; 2 = I am somewhat distressed; 3 = I am distressed; 4 = I am very distressed).*

7. I have constant time pressure due to a heavy work load.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed

8. I have many interruptions and disturbances in my job.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed

9. I have a lot of responsibility in my job.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed
10. I am often pressured to work overtime.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed

11. My job is physically demanding.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed

12. Over the past years, my job has become more and more demanding,

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed

If you DISAGREE for the following questions please also indicate how much you are generally distressed by this situation
(1 = I am not at all distressed; 2 = I am somewhat distressed; 3 = I am distressed; 4 = I am very distressed).

13. I receive the respect I deserve from my superiors.

Agree _______  Disagree _______

1 _______  2 _______  3 _______  4 _______
Not at all distressed  Somewhat distressed  Distressed  Very distressed

14. I receive the respect I deserve from my colleagues.

Agree _______  Disagree _______

1 _______  2 _______  3 _______  4 _______
Not at all distressed  Somewhat distressed  Distressed  Very distressed

15. I experience adequate support in difficult situations.

Agree _______  Disagree _______

1 _______  2 _______  3 _______  4 _______
Not at all distressed  Somewhat distressed  Distressed  Very distressed

If you AGREE for the following questions please also indicate how much you are generally distressed by this situation.
(1 = I am not at all distressed; 2 = I am somewhat distressed; 3 = I am distressed; 4 = I am very distressed).

16. I am treated unfairly at work.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed

17. I have experienced or I expect to experience an undesirable change in my work situation.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed

18. My job promotion prospects are poor.

Agree _______ Disagree _______

1 _______ 2 _______ 3 _______ 4 _______
Not at all distressed Somewhat distressed Distressed Very distressed
19. My job security is poor

Agree ________  Disagree ________

1 ________ 2 ________ 3 ________ 4 ________
Not at all distressed  Somewhat distressed  Distressed  Very distressed

If you **DISAGREE** for the following questions please also indicate how much you are generally distressed by this situation

(1 = I am not at all distressed; 2 = I am somewhat distressed; 3 = I am distressed; 4 = I am very distressed).


Agree ________  Disagree ________

1 ________ 2 ________ 3 ________ 4 ________
Not at all distressed  Somewhat distressed  Distressed  Very distressed

21. Considering all my efforts and achievements, I receive the respect and prestige I deserve at work.

Agree ________  Disagree ________

1 ________ 2 ________ 3 ________ 4 ________
Not at all distressed  Somewhat distressed  Distressed  Very distressed

114
22. Considering all my efforts and achievements, my work prospects are adequate.

<table>
<thead>
<tr>
<th>Agree</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 ______</td>
</tr>
<tr>
<td>3</td>
<td>4 ______</td>
</tr>
</tbody>
</table>

Not at all distressed | Somewhat distressed | Distressed | Very distressed

23. Considering all my efforts and achievements, my salary/income is adequate.

<table>
<thead>
<tr>
<th>Agree</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 ______</td>
</tr>
<tr>
<td>3</td>
<td>4 ______</td>
</tr>
</tbody>
</table>

Not at all distressed | Somewhat distressed | Distressed | Very distressed

Please indicate to what extent (1 = strongly disagree; 2 = disagree; 3 = agree; 4 = strongly agree) you personally agree or disagree with these statements.

| 24. I usually take criticism very seriously. | 1   2   3   4 |
| 25. I am fueled by ambition. | 1   2   3   4 |
| 26. Even the slightest interruption bothers me. | 1   2   3   4 |
| 27. If something needs to be done right I'd better do it myself. | 1   2   3   4 |
28. I enjoy proving certain people wrong
29. Always being a little better or faster than others is sort of a game to me.
30. I can get very upset when someone keeps me from what I'm supposed to be doing.
31. I can get very upset with others more often than I should.
32. I get easily overwhelmed by time pressures at work.
33. I start thinking about work problems as soon as I get up in the morning.
34. I get angry with myself when I can't completely resolve a problem at work.
35. I don't let others do my work.
36. I get especially frustrated when my work is not properly appreciated.
37. I can get furious if someone doesn't understand me the first time.
38. When I get home, I can easily relax and forget all about work.
39. People close to me say I sacrifice too much for my job.
40. I only feel successful when I perform better than I expected.
41. Other people have confidence in my ability to handle difficult tasks.
42. I do everything possible to be in control.
43. My family or private life comes first, then work.
<table>
<thead>
<tr>
<th>44. I get furious when anybody questions my competence.</th>
<th>1 2 3 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>45. I don't usually get annoyed when my work routine is interrupted.</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>46. I always want more than I can get.</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>47. Work is usually still on my mind when I go to bed.</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>48. The slightest compliment really boosts my confidence.</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>49. I don't feel angry when others do better than me.</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>50. Every once in a while, I like it when others keep me from working.</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>51. I am always mentally prepared to do what needs to be done next.</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>52. If I put off something that needs to be done today, I'll have trouble sleeping at night.</td>
<td>1 2 3 4</td>
</tr>
</tbody>
</table>
Derogatis Stress Profile

### Instructions

Below are a series of statements that describe the way some people feel about themselves. Please read each statement carefully and circle the number to the right to indicate the extent to which the statement is true of you. Consider yourself as you typically behave or feel. Circle only one number for each statement and do not skip any items. If you change your mind, erase your first selection carefully. Read the example below before beginning.

**TO WHAT EXTENT IS THE STATEMENT TRUE OF YOU?**

<table>
<thead>
<tr>
<th>Statement</th>
<th>Rating (1-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I feel there is never enough time to get things done.</td>
<td></td>
</tr>
<tr>
<td>2. I rarely have feelings of being tapped or caught in life.</td>
<td></td>
</tr>
<tr>
<td>3. I feel rules were made to be broken.</td>
<td></td>
</tr>
<tr>
<td>4. I take some time out almost every day just to relax.</td>
<td></td>
</tr>
<tr>
<td>5. I laugh easily.</td>
<td></td>
</tr>
<tr>
<td>6. My job provides me many opportunities for challenging and satisfying activities.</td>
<td></td>
</tr>
<tr>
<td>7. When I am on vacation with my family I don't have as much fun as I think I should.</td>
<td></td>
</tr>
<tr>
<td>8. I get into frequent arguments.</td>
<td></td>
</tr>
<tr>
<td>9. I rarely feel tense and under pressure.</td>
<td></td>
</tr>
<tr>
<td>10. I rarely exercise.</td>
<td></td>
</tr>
<tr>
<td>11. I feel no interest in things.</td>
<td></td>
</tr>
<tr>
<td>12. I would like to be with my family more, but I can never seem to find the time.</td>
<td></td>
</tr>
<tr>
<td>13. I never worry about being a “workaholic”.</td>
<td></td>
</tr>
<tr>
<td>14. I believe that if you don't beat the other guy to the punch, he will beat you.</td>
<td></td>
</tr>
<tr>
<td>15. I never sit still for very long.</td>
<td></td>
</tr>
<tr>
<td>16. I am not very good at telling funny stories or jokes.</td>
<td></td>
</tr>
<tr>
<td>17. I get great pleasure from the people I work with.</td>
<td></td>
</tr>
<tr>
<td>18. I have a satisfying sex life.</td>
<td></td>
</tr>
<tr>
<td>19. I have no problems with control of my temper.</td>
<td></td>
</tr>
<tr>
<td>20. I am usually worried about something.</td>
<td></td>
</tr>
<tr>
<td>21. I smoke too much.</td>
<td></td>
</tr>
<tr>
<td>22. I rarely feel lonely.</td>
<td></td>
</tr>
<tr>
<td>23. When I eat, I usually take my time.</td>
<td></td>
</tr>
<tr>
<td>24. I frequently say I am going to spend less time on work, but I don't seem to be able to.</td>
<td></td>
</tr>
<tr>
<td>25. Most things I do I see as a challenge.</td>
<td></td>
</tr>
<tr>
<td>26. I am not very interested in hobbies or sports.</td>
<td></td>
</tr>
<tr>
<td>27. I seem to be more focused on the future than the present.</td>
<td></td>
</tr>
<tr>
<td>28. My full range of talents are not utilized on my job.</td>
<td></td>
</tr>
<tr>
<td>29. I have a good relationship with my wife/husband (or unmarried partner).</td>
<td></td>
</tr>
<tr>
<td>30. Sometimes I just feel like hitting somebody.</td>
<td></td>
</tr>
<tr>
<td>31. I rarely feel nervous or uptight.</td>
<td></td>
</tr>
<tr>
<td>32. I am good physical shape.</td>
<td></td>
</tr>
<tr>
<td>33. I sometimes have feelings of worthlessness.</td>
<td></td>
</tr>
<tr>
<td>34. I rarely feel pressurized for time.</td>
<td></td>
</tr>
<tr>
<td>35. The more things I achieve in life the less I seem to enjoy them.</td>
<td></td>
</tr>
<tr>
<td>Q.</td>
<td>Statement</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>36.</td>
<td>I tend to be impatient</td>
</tr>
<tr>
<td>37.</td>
<td>I sometimes just &quot;turn off&quot; of work and get involved in other things.</td>
</tr>
<tr>
<td>38.</td>
<td>Sex is an important part of my life.</td>
</tr>
<tr>
<td>39.</td>
<td>I am frequently frustrated in my work.</td>
</tr>
<tr>
<td>40.</td>
<td>Interacting with my family and friends is a great source of enjoyment.</td>
</tr>
<tr>
<td>41.</td>
<td>I rarely have angry thoughts about people.</td>
</tr>
<tr>
<td>42.</td>
<td>When I know I have something unpleasant to do I worry about it for a long time.</td>
</tr>
<tr>
<td>43.</td>
<td>I don't take antacids for heartburn or gas.</td>
</tr>
<tr>
<td>44.</td>
<td>I usually have plenty of energy.</td>
</tr>
<tr>
<td>45.</td>
<td>I enjoy being under pressure and doing a good job on many projects at the same time.</td>
</tr>
<tr>
<td>46.</td>
<td>I really look forward to my vacations.</td>
</tr>
<tr>
<td>47.</td>
<td>I make a serious effort to achieve a balance between work and fun.</td>
</tr>
<tr>
<td>48.</td>
<td>It is not difficult for me to unwind after work.</td>
</tr>
<tr>
<td>49.</td>
<td>I really believe it is lonely at the top.</td>
</tr>
<tr>
<td>50.</td>
<td>Doing my job gives me a good feeling about myself.</td>
</tr>
<tr>
<td>51.</td>
<td>I have a good balance between family activities and work activities.</td>
</tr>
<tr>
<td>52.</td>
<td>I get easily annoyed or irritated.</td>
</tr>
<tr>
<td>53.</td>
<td>I frequently have the feeling that something bad is going to happen to me.</td>
</tr>
<tr>
<td>54.</td>
<td>I believe having good health is important than anything.</td>
</tr>
<tr>
<td>55.</td>
<td>Sometimes I feel hopeless about the future.</td>
</tr>
<tr>
<td>56.</td>
<td>When I am driving the car, I almost never rush through traffic.</td>
</tr>
<tr>
<td>57.</td>
<td>Every day must get something tangible accomplished or I don't feel good about myself.</td>
</tr>
<tr>
<td>58.</td>
<td>I feel the most important thing in life is that you achieve something with it.</td>
</tr>
<tr>
<td>59.</td>
<td>The idea of meditation or relaxation training has not had much appeal for me.</td>
</tr>
<tr>
<td>60.</td>
<td>I believe you can get a lot of help from others in getting the job done in life.</td>
</tr>
<tr>
<td>61.</td>
<td>There are significant parts of my job that are honestly dull and boring.</td>
</tr>
<tr>
<td>62.</td>
<td>I don't interact much with friends or neighbors.</td>
</tr>
<tr>
<td>63.</td>
<td>I rarely check my face during conversation.</td>
</tr>
<tr>
<td>64.</td>
<td>I rarely let things get me anxious or tense because I know they always get worked out somewhere.</td>
</tr>
<tr>
<td>65.</td>
<td>I am very careful about my diet.</td>
</tr>
<tr>
<td>66.</td>
<td>I sometimes have thoughts of ending my life.</td>
</tr>
<tr>
<td>67.</td>
<td>When I have an appointment I rarely arrive late or at the last minute.</td>
</tr>
<tr>
<td>68.</td>
<td>Once I get started on a project, I don't like to stop until I am finished.</td>
</tr>
<tr>
<td>69.</td>
<td>I believe competition builds character and is good for you.</td>
</tr>
<tr>
<td>70.</td>
<td>I have trouble relaxing.</td>
</tr>
<tr>
<td>71.</td>
<td>I believe life is a struggle and you don't get anything for free out of it.</td>
</tr>
<tr>
<td>72.</td>
<td>When I wake up in the morning, I really look forward to going to work.</td>
</tr>
<tr>
<td>73.</td>
<td>I really enjoy going to parties and meeting people.</td>
</tr>
<tr>
<td>74.</td>
<td>If someone expresses a stupid idea, I rarely publicly disagree.</td>
</tr>
<tr>
<td>75.</td>
<td>Sometimes I feel tense and anxious for no apparent reason.</td>
</tr>
<tr>
<td>76.</td>
<td>I take tranquilizers to relax or sleep.</td>
</tr>
<tr>
<td>77.</td>
<td>I rarely blame myself unduly for things that go wrong.</td>
</tr>
</tbody>
</table>

Please indicate what you believe your current level of stress to be by placing an "X" on the line below.

- Totally Free of Stress
- Extremely Highly Stressed

Also, please assign a number from 0 to 100 where 0 is Totally Free of Stress and
100 is Extremely Highly Stressed in the space provided.

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