THE EFFECT OF A SINGLE SESSION OF ACUTE
MINDFULNESS MEDITATION ON ENDOTHELIAL FUNCTION

by

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Abstract

Mindfulness meditation (MM) is associated with some improvements in cardiovascular health in addition to mental wellbeing. Vascular endothelial function, an indicator of overall cardiovascular health, is influenced strongly by sympathetic nervous activity that is altered by acute meditation. Only a few studies have noted that mindfulness improves the vascular endothelium after chronic mindfulness programs, however it is still unknown to what degree a single acute session of MM may affect endothelial function especially in young, healthy subjects. We hypothesized that an acute MM session would transiently improve endothelial function. Secondarily, we sought to characterize the meditative state using electroencephalography (EEG) to measure brain activity, heart rate variability (HRV) to measure autonomic activity, and MM questionnaires to assess subjective state mindfulness. Twelve healthy young adults (21 ± 2 years) with no meditation experience participated in two experimental visits each involving a 20-minute MM condition or an active control condition. We assessed endothelial function before and after each condition using reactive hyperemia-induced flow-mediated dilation (RH-FMD). We did not detect a change in endothelial function after MM or the active control (RH-FMD: p = 0.582), and MM did not stimulate an increase in EEG alpha power or HRV (alpha power: p = 0.568; HRV: p = 0.395). The subjective MM questionnaire revealed no change in state mindfulness after either condition (p = 0.800). We also correlated RH-FMD with both EEG alpha power and HRV during MM and found no significant relationships (RH-FMD versus alpha power: r² = 0.39, p = 0.054; RH-FMD versus HRV: r² = 0.39, p = 0.086). The secondary measures did not confirm that the subjects achieved the intended meditative state. It therefore remains unclear whether a successful MM can acutely improve endothelial function. The type of MM employed in an acute session may be the most critical factor for generating both cognitive and physiological effects in novice meditators. Further research is needed to determine whether a meditative state can transiently affect vascular endothelial function.
Co-Authorship

All chapters of this thesis were written by Darius Soo Lum, with supervision from Dr. Kyra Pyke. Darius Soo Lum was responsible for reviewing the literature, developing the research question and study design, coordinating data collection, running, and interpreting statistical analysis, and writing the manuscript. Dr. Pyke assisted with study design, interpretation of data and edited the thesis document. Dr. Pyke was the principal investigator on the research grant funding this study.
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Finally, to my family, without whose love and support I would not be where I am now. To my parents, **Cherry Ann and Patrick**, for the small task of bringing me up from childhood to adulthood, thank you for being only a short phone call or drive away and supporting me as I tried to find a way through the fog of life, and for continuing to do so forever. To my brothers, **Adrian and Tristan**, and to **Dianne**, thank you for all the laughs and games, road trips (whether it be to Burger Revolution or to the cabin). As we continue to get older, I know that we will always count on one another in all aspects.

To my grandfather, **Clive Inniss Sr.**, who ensured his children memorized this highly revered mantra that has endured for millennia – one that praises the divine light of consciousness in the cosmos, asks for wisdom, and expresses gratitude for life:

ॐ भूर्भुवः स्वः तत्सवितुरविष्णुः भगवः देवस्य धीमहि धियो यो न: प्रचोदयात् ॥

\[\text{oṁ bhūr bhuvah svah} \\
\text{tat savitur vareṇyaṁ} \\
\text{bhargo devasya dhīmahi} \\
\text{dhiyo yo naḥ pracodayāt} \]

– Rig Veda 3.62.10
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List of Abbreviations

AbsFMD – absolute RH-FMD
ANS -autonomic nervous system
BP – blood pressure
BWM - Buddhist Walking Meditation
CAD - coronary artery disease
CV - cardiovascular
CVD – cardiovascular disease
DBP – diastolic blood pressure
EEG – electroencephalography
eNOS - endothelial NO synthase
ET-1 – endothelin-1
%RH-FMD – RH-FMD expressed as the percent change from baseline
fMRI - functional magnetic resonance imaging
GSR – galvanic skin response
HF-HRV – high-frequency power heart rate variability
HPA - hypothalamic pituitary adrenal axis
HR – heart rate
HRV – heart rate variability
HRVB – heart rate variability biofeedback
MAAS – Mindful Attention Awareness Scale
MBCT - mindfulness-based cognitive therapy
MBSR – Mindfulness-based stress reduction
MEG - magnetoencephalography
MM – mindfulness meditation

MSNA - muscle sympathetic nervous activity

NO – nitric oxide

NOx – nitrite-nitrate

NormFMD – FMD normalized to the shear rate stimulus

PET - positron emission tomography

RCT - random control trial

RH-FMD – reactive hyperemia-induced flow-mediated dilation

RMSSD - root-mean-square standard deviation

ROS – reactive oxygen species

SBP - systolic blood pressure

SDNN - standard deviation of NN intervals

SNA – sympathetic nervous activity

SR – shear rate

SRAUC30 – shear rate area under the curve 30 seconds post-cuff release

TM – transcendental meditation

TMS – Toronto Mindfulness Scale
Chapter 1

Introduction

Meditation is a broad term for contemplative practices that commonly involve mental concentration for the purpose of developing desirable qualities and mental states. In one type known as mindfulness meditation (MM), the meditator trains their mind to let go of thoughts and/or focus solely on the present moment, thereby allowing deeper insight and eventual inner peace (Goleman & Davidson, 2017). Over several centuries, meditation developed independently in a variety of cultures around the world, though the contemporary versions of meditation are linked to ancient practices that were formulated in the ancient Indian subcontinent (Bodhi, 2011; Creswell, 2017). Through its secularization and export around the world, MM is now accessible to a global audience; Western researchers and scientists are increasingly studying MM as a result of its explosive popularity (Creswell, 2017).

MM has several physiological and cognitive effects that can be quantified to determine achievement and impact of a mindful state. Electroencephalography (a measure of electrical brain activity) quantifies the state of MM as an increase in alpha frequency power (Takahashi et al., 2005; Travis, 2019; Travis & Wallace, 1999; Wallace et al., 1971). Together with questionnaires - such as the Mindful Attention Awareness Scale (MAAS) and Toronto Mindfulness Scale (TMS) –cognitive measures can effectively measure the mindfulness experience. Indices of autonomic nervous system (ANS) activity are also useful for quantifying the physiological impact of MM. Since the ANS modifies cardiovascular variables, the MM-induced shift toward parasympathetic dominance (and away from sympathetic dominance) can be captured by measuring heart rate (HR), blood pressure (BP), and heart rate variability (HRV). Studies record a mild decrease in systemic BP and HR in both chronic and acute MM sessions (Bernardi et al., 2017; Ditto et al., 2006; Momeni et al., 2016). HRV increases with MM acutely, reflecting parasympathetic dominance, and this increased HRV correlates with lower BP and breathing...
rates which characterize the relaxation state generated by MM (Burg et al., 2012; Melville et al., 2012; Shearer et al., 2016).

Numerous cardiovascular studies centered on MM indicate reduced cardiovascular risk with chronic practice, and a reduced stress response activation with acute practice that is indicated through shifts in the autonomic nervous system (Bernardi et al., 2001; Bernardi et al., 2017; Davidson & Dahl, 2018; Ditto et al., 2006; Jayadevappa et al., 2007; Sullivan et al., 2009). Short- and long-term programs and MM interventions abound, and alongside these interventions are an exponentially rising number of scientific publications to corroborate the MM experience and its benefits (Creswell, 2017; Davidson & Dahl, 2018; Van Dam et al., 2018). Even acute sessions of MM may influence cardiovascular function in a positive manner (Ditto et al., 2006; Jones, 2013; Kim et al., 2005; Melville et al., 2012; Shearer et al., 2016) which makes assessing MM in conjunction with assessments of cardiovascular health and function an important direction for continued research.

The endothelium is a single-cell layer that lines the inside of blood vessels. It plays a vital role in controlling vascular tone and determining cardiovascular health overall and is a promising, underexplored potential connection between MM and cardiovascular health benefits (Halcox et al., 2002; Joannides et al., 1995; Mullen et al., 2001; Nabel et al., 1990). When stimulated by increased shear stress caused by blood flow moving through the vessel, the endothelial cells produce biomolecules (e.g., nitric oxide, NO) that enable relaxation of the surrounding smooth muscle resulting in vasodilation of the blood vessel known as flow-mediated dilation, or FMD (Cahill & Redmond, 2016; Joannides et al., 1995; Mullen et al., 2001; Palmer et al., 1988). If the endothelium becomes dysfunctional it suffers a reduced vasodilatory ability which is a precursor of atherosclerosis and a predictor of future cardiac events (Celemajer et al., 1992; Halcox et al., 2002). Researchers use several techniques to assess endothelial function with the most common being reactive hyperemia-induced FMD (RH-FMD) of the brachial artery. RH-FMD involves temporarily occluding the forearm using an inflatable cuff. This results in vasodilation of the forearm vasculature. When the occlusion is released, this increases brachial artery shear stress and
stimulates endothelium-driven vasodilation of the brachial artery (Celemajer et al., 1992). The non-invasive RH-FMD test is an accepted method that reflects the status of the endothelium (Thijssen et al., 2011).

Few studies to date have investigated the impact of MM on the endothelium. The studies that have explored MM and the endothelium considered both indirect and direct probes of endothelial function. Indirectly, measures of the biomolecules involved in endothelial function, (NO or the related nitrite-nitrate [NOx], reactive oxygen species [ROS]) may reflect the functional status of the endothelium; acute MM interventions indicate that NO levels increase while ROS levels decrease post-meditation which infers potential endothelial improvement (Im et al., 2015; Kim et al., 2005). With direct investigation of endothelial function, MM improved RH-FMD in long-term repeated meditative sessions over the course of a dozen weeks – though these results may be cofounded by the inclusion of light physical activity like walking or yoga (Gainey et al., 2016; Prakhinkit et al., 2014; Sivasankaran et al., 2006). These studies also did not report their conformity to established guidelines and consensus for the RH-FMD test (Thijssen et al., 2019), thereby limiting the interpretation of their RH-FMD results. It is unknown to what degree a single session of acute MM affects the endothelium, an important knowledge gap in our understanding of the acute physiological benefits of MM.

Endothelial function can be influenced by sympathetic nervous activity (SNA) meaning that increases in SNA can attenuate endothelial function which is represented by decreased RH-FMD (Hijmering et al., 2002; Thijssen et al., 2014). Measures of SNA in response to MM - such as galvanic skin response (GSR), norepinephrine, microneurography, and HRV – all indicate that SNA declines because of an acute MM intervention (Curiati et al., 2005; Das & Anand, 2012; Gabriely et al., 2020; Park et al., 2014; Pinter et al., 2012). Also, evidence suggests that a single mindfulness experience reduces oxidative stress (Im et al., 2015). Increased oxidative stress reduces NO bioavailability and RH-FMD (Bellien et al., 2012; Grebe et al., 2006; Guzik et al., 2000; Lin et al., 2008; Loffredo et al., 2007; Wassmann et al., 2002). Given the potential deleterious effects of increased SNA and oxidative stress on
RH-FMD, and the evidence that MM acutely reduces SNA and oxidative stress, it is reasonable to speculate that an acute bout of MM will transiently increase RH-FMD.

The primary purpose of the study detailed in Chapter 3 is to determine the effect of a single session of MM on endothelial function in young, healthy, novice meditators. This study will provide the first evidence of acute impact of MM on the endothelium. This will be accomplished by comparing RH-FMD after a 20-minute intervention of MM versus a 20-minute active control intervention. Secondarily, this study will determine if any correlations exist between changes in alpha power, HRV and RH-FMD before and after an acute MM intervention. This will provide some preliminary, hypothesis-generating insight regarding the mechanisms by which MM might influence endothelial function. Alpha power and HRV will also serve to quantify the MM state in a physiological sense, with self-report questionnaires acting as an inquiry into the participants’ subjective experience of MM.

The early effects of MM must be clarified so that we may understand its full timeline. Numerous studies have espoused the benefits of MM over longer periods of time, but there is no clear understanding of the physiological mechanisms that are first generated in singular sessions of meditation that compound over time to impart these important health benefits. In the case of endothelial function, any improvement in RH-FMD from single sessions of MM would be highly meaningful since it would mean that MM can provide some form of cardiovascular benefit to a wide range of people without necessarily having to devote weeks to meditation training programs. Conversely, no improvement in RH-FMD may instead indicate that despite shifts in cognitive activity and autonomic function that would indicate a more relaxed state, MM builds endothelial benefits over time rather than in a single session. Regardless, this study is a necessary stepping stone for pinpointing the effects of MM on the endothelium.

In summary, MM is explosively gaining recognition throughout society as a useful wellness practice that also provides several cardiovascular benefits over long-term sessions. MM has merit in acute sessions that result in meaningful cognitive, cardiovascular, and autonomic changes. However, in the case of endothelial function, it is unclear if there are any beneficial effects of acute MM. Quantifying MM
effects using self-report measures, EEG, and HRV will allow us to explore relationships between the physiological impact of mindfulness and endothelial function, as indexed by RH-FMD. It is imperative to evaluate the early stages of MM practice so that we can establish a baseline for its benefits, a necessary step for future investigations of MM practice and endothelial function.
Chapter 2

Literature Review

2.1 Introduction to meditation: brief history, definitions, and contemporary significance

Meditation is a family of practices that focuses on training attention and awareness. Meditation generates mental control and develops specific qualities: calmness, clarity, concentration, bhavana (mental cultivation) and a final samadhi or a blissful super consciousness state (Yogananda, 2014). Meditation is often associated with Hinduism and Buddhism, but numerous forms exist in many if not all major religions, spiritual, and secular practices around the world. Religious or spiritual associations are not essential for meditation practice despite its spiritual origins; this allows many people to use secularized forms of mediation to reap its benefits (Olex et al., 2013). In this review, the focus will be one specific form of meditation known as mindfulness meditation (MM) rooted in the Buddhist tradition.

Starting approximately a half century ago, meditation went from a questionable and marginal topic of scientific research to one of curious interest, saturating scientific literature and public news media in an exponential growth trajectory (Creswell, 2017; Van Dam et al., 2018). One of the great difficulties in meditation research has been defining meditation and its subtypes. Although there have been numerous attempts at standardizing mindfulness (Bishop et al., 2006; Bodhi, 2011; Grabovac et al., 2011; Hölzel et al., 2011; Shapiro et al., 2006), researchers debate the meaning of mindfulness, a conundrum that has implications for scientific study design (Van Dam et al., 2018).

Dahl et al. (2015) proposed a novel classification system for meditation types: Attentional family, constructive family, and deconstructive family. Mindfulness is considered a member of the attentional and the deconstructive families, as it focuses on one-pointed awareness, but also is “deconstructive” of thoughts and emotions by first noticing them, uncovering the processes that inform them, and questioning their validity, all in the present moment.
In the Buddhist sense, MM has its roots in the fifth century BCE, during which the Buddha’s teachings (known as the Dharma) extolled his revelation (enlightenment): suffering is inherent to life, but there is a way to overcome it. The Buddha’s Noble Eightfold Path to overcome suffering places special emphasis on mindfulness (Right Mindfulness; samma sati) by placing it as the seventh step on the Path, in between Right Effort and Right Concentration. From the Pāli Canon on the systematic practice of mindfulness, the Buddha states that there are four steps to mindfulness:

Here, a monk dwells contemplating the body in the body ... feelings in feelings ... mind in mind ... phenomena in phenomena, ardent, clearly comprehending, mindful, having removed covetousness and displeasure in regard to the world. (Satipatthana Samyutta SN 47.1)

From this statement, we can deduce that mindfulness involves objective contemplation of the physical body followed by a subjective turn to observation of mental phenomena in the present moment (Bodhi, 2011). The classification of MM by Dahl et al. (2015) parallels the Buddha’s discourse above. One of the most important scrutinies of meditation research is the consistency of the study design with the author’s specified definition (Van Dam et al., 2018). Thus, the present review will refer to MM studies that adhere to the modern and classical definitions above. Western adaptation of MM involves sessions lasting five minutes to an hour in seated/supine positions, in a quiet and distraction-free environment with a varying focus on the body, breath and/or passively letting go of thoughts, while especially avoiding thoughts that dwell on past or future events.

Physical and psychological wellbeing are the goals of secularized MM in the West; the popularity of MM has lead to society integrating the practice in numerous institutions: clinical treatments, corporate workplaces, schools, prisons, and the military (Creswell, 2017). The surging interest makes quality research on MM important so that clear conclusions can be drawn regarding its objective effects. However, there are many challenges in research on meditation design, in addition to the semantics debate on the definition of the construct discussed above (Davidson & Dahl, 2018; Van Dam et al., 2018).
**Definitions of skilled expertise.** The level of expertise - as well as the definition of the expertise - required to elicit measurable effects during a study design vary from study to study (Van Dam et al., 2018). A novice meditator may be defined as someone who has never attempted meditation [e.g. Zeidan et al. (2015); Zeidan et al. (2010)], or a novice may be a casual meditator, having accumulated relatively minimal lifetime hours of meditation and may meditate daily for a short 5-10 minute session or more infrequently in a home setting [e.g. Kemper et al. (2015)]. In comparison, adepts or experts may belong to formal practice centres or religious institutions [e.g. Peng et al. (2004); Lutz et al. (2004)]. Adepts or experts may also belong to Tibetan lineages - where meditation training in a monastic setting begins in adolescence and continues everyday, morning and evening, throughout a lifetime - or in the West where experts may have attended long-term meditation centres or programs in addition to continued home practices daily over years (Goleman & Davidson, 2017).

**Duration, intensity, and spacing of practice.** Researchers conduct mindfulness interventions in different ways. The widely used Mindfulness-Based Stress Reduction (MBSR) program is a gold-standard in long-term meditation studies, with a total of 20-26 hours of formal meditation training spread throughout eight weeks (Creswell, 2017; Kabat-Zinn, 1990). MM intensity in a session could vary from acute practices done during everyday chores (e.g. mindfulness while folding clothes or washing the dishes) to formal meditation (i.e. seated in an quiet space with the sole purpose of meditating) and finally to the extreme of long-term Vipassana in the tradition of the master U Ba Khin where speaking and moving is forbidden for hours (Dahl et al., 2015; Goleman & Davidson, 2017). Acute or short term interventions vary in their duration as some studies choose one time interventions of 20-60 minutes [e.g. Kim et al. (2005); Bernardi et al. (2001); Bernardi et al. (2017)] whereas some studies choose similarly short meditation sessions spread over three days [e.g. Momeni et al. (2016)]. The results from study designs can vary regardless of the duration, intensity or spacing selected, leading to unanswered questions regarding what might be the most effective choice of intervention (Davidson & Dahl, 2018; Goleman & Davidson, 2017).
In summary

Researchers struggle with standardizing MM, where they must fit it into the model of a modern intervention that acknowledges parameters such as duration, intensity, and frequency. Since meditation studies are so variable in their design, relevant studies are summarized in Table 1 so that it is clear how MM is identified in this review as defined by the Buddha and as classified by Dahl et al. (2015). MM in this review is defined as the contemplation of an object in the present moment (body and/or breath, but not including qualities like love or kindness) and letting go of thoughts.

Table 1: Classification of meditation types

<table>
<thead>
<tr>
<th>Practice</th>
<th>Key Features</th>
<th>Classification *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body scan meditation</td>
<td>• Exploration of the physical sensations in specific body parts (Bernardi et al., 2017);</td>
<td>Attentional family;</td>
</tr>
<tr>
<td></td>
<td>• Focus on bodily sensations and breath (Azam et al., 2015)</td>
<td>focused attention on the body. Included in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>definition of MM.</td>
</tr>
<tr>
<td>Buddhist walking meditation</td>
<td>• Concentration on the movement or position of the arms while waking (Gainey et al., 2016);</td>
<td>Attentional family;</td>
</tr>
<tr>
<td></td>
<td>• Combination of awareness while sitting and awareness while walking</td>
<td>focused attention on the body but involves movement. Included in definition of MM.</td>
</tr>
<tr>
<td>Compassion or loving-kindness</td>
<td>• Cultivation of a state of unconditional readiness to help living beings. No concentration on particular objects, memories or images (Lutz et al., 2004).</td>
<td>Constructive family; orientation on relations and values. Distinct from MM, not included.</td>
</tr>
<tr>
<td><strong>Koukundo</strong></td>
<td>• Relaxing stretches and self-message with deep breathing and focus on the lower abdomen (Im et al., 2015)</td>
<td>Attentional family; focused attention on the body but involves movement. Included in definition of MM</td>
</tr>
<tr>
<td>Mantra meditation</td>
<td>• Breathing in tandem with prayer or “om mani padme hum” mantra (Bernardi et al., 2001); • Breathing paced at 6 breaths/min with mantra repetition aloud (Bernardi et al., 2017); • Silent mental repetition of mantras with no specific breathing instructions (Steinhubl et al., 2015)</td>
<td>Attentional family; focused attention on the body. Included in definition of MM.</td>
</tr>
<tr>
<td><strong>MBSR</strong></td>
<td>• Long-term guided practices, with mindfulness retreats, utilizing body scan, stretching, and discussions on stress management (Kabat-Zinn, 1990)</td>
<td>Deconstructive family and attentional family; self-inquiry and insight. Included in definition of MM.</td>
</tr>
<tr>
<td>Transcendental Meditation</td>
<td>• Transcending levels of consciousness through the subtle thinking level to a state of full self-awareness then back to more active levels while releasing stress; cycling through these phases numerous times (Travis &amp; Wallace, 1999) • Perception of thoughts or sound without concentrating on them. Experiencing a finer or</td>
<td>Experiential fusion; no single object attention. Undermines attentional or deconstructive families. Distinct from MM, not included.</td>
</tr>
</tbody>
</table>
more creative level of thinking (Wallace et al., 1971)

Vipassana meditation

- Allows thoughts to arise and be examined without judgement, then allows thoughts to fade with no emotional attachment (Krygier et al., 2013)

Deconstructive family; breaks down mental constructs with insight.

Intensive form of MM, included in definition of MM.

Zen meditation

- Concentration by counting the breath; allowing thoughts to pass (Takahashi et al., 2005);
- Awareness on “just breathing” (Cysarz & Büssing, 2005);
- Focus on the “Zen Chakra” located in the brain (Wu & Lo, 2008)

Attentional family; focused attention on the body. Included in definition of MM.

*According to classifications by Dahl et al. (2015).

2.2 How do we know if a ‘mindful’ state has been achieved?

2.2.1 Electroencephalography (EEG)

The simplest way to assess the achievement of mindfulness is via a self-report questionnaire such as the Mindful Awareness Attention Scale or the Toronto Mindfulness Scale (Bishop et al., 2006; Brown & Ryan, 2003; Lau et al., 2006; Van Dam et al., 2018). A more objective/physiological means of assessment is via electroencephalography (EEG). EEG is a non-invasive measurement of spatiotemporal information of electrical brain activity that provides millisecond resolution of brain activation patterns during tasks or the absence thereof (Cacioppo et al., 2007; Cohen, 2011; Travis, 2019). In brief, the
electrical discharge of individual neurons is summated so that the difference in voltage of thousands to millions of neurons in synchrony can be detected by electrodes on the scalp as wave potentials or oscillations (Klein & Thorne, 2006; Nunez, 1981). When a neuron population is firing together as a unit, the oscillations increase in amplitude or frequency. The firing of different neuronal units are characteristics of different brain functions or states (Niedermeyer et al., 2011; Whittingstall & Logothetis, 2009). Traditionally encountered frequency bands from slowest to fastest include: delta rhythms (0-4 Hz), theta rhythms (4-7 Hz), alpha rhythms (8-13 Hz), beta rhythms (14 -30 Hz), and gamma rhythms [>30 Hz] (Niedermeyer et al., 2011). Table 2 outlines a generalized comparison of the common frequency bands and the characteristic brain functions they are associated with in normal EEG recordings.

**Table 2:** EEG frequency band comparison.

Adapted from Thammasan et al. (2016).

<table>
<thead>
<tr>
<th>Band</th>
<th>Frequency range (Hz)</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta</td>
<td>0-4</td>
<td>Deep sleep</td>
</tr>
<tr>
<td>Theta</td>
<td>4-8</td>
<td>Consciousness slips</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drowsiness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unfocused and undirected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Creative inspiration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deep meditation</td>
</tr>
<tr>
<td>Alpha1</td>
<td>8-10</td>
<td>Relaxed awareness &amp; eye closing</td>
</tr>
<tr>
<td>Alpha2</td>
<td>10-12</td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>14-30</td>
<td>Active thinking, Attention</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Motor behaviour</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Focusing on the outside world</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(eyes open)</td>
</tr>
</tbody>
</table>
The data recorded by the EEG electrodes provide a good overview of brain activity over time, which is an advantage over other measures of neural activity, e.g. functional magnetic resonance imaging (fMRI) or magnetoencephalography [MEG] (Cohen, 2011). EEG is weaker in the spatial domain since researchers must interpret what brain areas might be active, whereas fMRI can directly visualize active areas (Srinivasan, 1999). Interference from EEG artifacts (unintended muscular contractions, eye blinks, environmental noise, electromyographic or electrocardiographic interferences) must be filtered out to prevent misinterpretation (Niedermeyer et al., 2011). Looking at technical aspects briefly: EEG setup can be time consuming, but it is relatively less expensive, it does not involve invasive techniques nor radioligands like positron emission tomography (PET) scans and is far more mobile than fMRI machines (Vespa et al., 1999; Yasuno et al., 2008).

### 2.2.2 EEG and meditation studies

EEG power increases in the alpha and (less frequently) the theta frequency bands during meditation (Takahashi et al., 2005; Travis, 2019; Travis & Wallace, 1999; Wallace et al., 1971). There are two narrow bands of alpha activity: alpha2 (10-12 Hz) and alpha1 (8-10 Hz). Alpha2 activity is associated with mindfulness and is characterized by decreased posterior cortex blood flow and a phenomenon where irrelevant processes are deactivated and sensory perception is sharpened when the eyes are closed (Ergenoglu et al., 2004). Many studies do not identify the alpha 1 and 2 frequency bands specifically (Travis, 2019). A meta-analysis by Lomas et al. (2015) specifically indicates that alpha2 power is the more characteristic frequency band (Ergenoglu et al., 2004; Travis, 2019). Table 3 summarizes MM studies that measured alpha power using EEG.
2.3 In Summary

Both self-report measures and EEG are popular means of assessing state MM non-invasively and *in vivo*. EEG is a popular tool for meditation studies as it has several advantageous that are technically or methodologically convenient in addition to being analytically useful due to its ability to track MM over time. State MM is characterized by EEG alpha power in many studies.
Table 3: State/trait mindfulness and EEG changes with MM. Alpha1 or alpha2 reported if possible.

<table>
<thead>
<tr>
<th>Author(s) (Year)</th>
<th>Group Condition</th>
<th>MM Exposure</th>
<th>Results / Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ahani et al. (2014)</td>
<td>RCT; n=34 novices (no experience), age 55-75, reported stress.</td>
<td>6 weeks of MM: one weekly 60min session of body awareness.</td>
<td>Increased alpha2, beta, theta power in different scalp locations versus control.</td>
</tr>
<tr>
<td>Barnhofer et al. (2007)</td>
<td>RCT; n=34 adults who have had an episode of major depression with suicidal ideation</td>
<td>MBCT, n=10, versus treatment-as-usual, n=12, for 8 weeks.</td>
<td>Alpha power activation was maintained in MBCT. Declined in treatment-as-usual group.</td>
</tr>
<tr>
<td>Bing-Canar et al. (2016)</td>
<td>Undergraduate students, n=44.</td>
<td>Mindfulness versus active control for approximately 15min.</td>
<td>Alpha2 power increased in the MM group versus active control.</td>
</tr>
<tr>
<td>Colgan et al. (2019)</td>
<td>Single case n=1, participant undergoing brain surgery, experienced meditator.</td>
<td>Active control versus MM (breath awareness).</td>
<td>Increased alpha power versus active control. Less consistent theta, beta, and high gamma activity.</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Meditation Type</td>
<td>Findings</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------------------------</td>
<td>------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Dunn et al. (1999)</td>
<td>Student volunteers n=10</td>
<td>Breath concentration meditation versus MM versus relaxation control. 15min each.</td>
<td>MM increased theta, alpha2, and beta power versus breath concentration meditation. Breath concentration meditation and MM versus relaxation control increased beta and alpha, while decreasing delta and theta power.</td>
</tr>
<tr>
<td>Hinterberger et al. (2014)</td>
<td>Highly experienced meditators n=30, non-experienced n=20</td>
<td>Idiosyncratic meditation (participants’ own form of MM) for 20-30min followed by: thoughtless emptiness (TE), focused attention (FA) and open monitoring (OM), 2 minutes each.</td>
<td>TE decreased alpha2 and delta activity compared to the other types, but only in the experienced meditators, indicating that the TE state may be different from other mentally focused states.</td>
</tr>
<tr>
<td>Murata et al. (2004) *</td>
<td>N=22 adults, non-experienced</td>
<td>Zen meditation: focused concentration on the breath</td>
<td>Increased alpha coherence (degree of similarity between frequency oscillations in two sensors) in the frontal area.</td>
</tr>
<tr>
<td>Study</td>
<td>Group Details</td>
<td>Intervention</td>
<td>Summary</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------</td>
<td>---------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Steinhubl et al. (2015)*</td>
<td>n=20 experienced (&lt; 840min over last three months), n=20 non-experienced.</td>
<td>Week-long meditation retreat: daily 24-26min session of silent mantra meditation, followed by relaxation and guided breathing for a total of 60-90min. Measurements on the first and last days only.</td>
<td>Alpha activity increased in both groups</td>
</tr>
<tr>
<td>Takahashi et al. (2005)*</td>
<td>RCT; n=20 undergraduate students, non-experienced</td>
<td>Su-soku (Zen) meditation: focused concentration on the breath.</td>
<td>Increased alpha1 and theta power in the frontal area versus control. No difference with alpha2.</td>
</tr>
<tr>
<td>Travis and Wallace (1999)*</td>
<td>n=22 experienced meditators.</td>
<td>TM (silent mantra meditation) for 10min, vs. eyes closed rest for 10min</td>
<td>No significantly different alpha power between conditions, but higher alpha coherence (degree of similarity between frequency oscillations in two sensors) in TM</td>
</tr>
</tbody>
</table>
Wallace et al. n=36 20-30min of TM. Increase power in alpha1 waves and occasional theta waves.

*(1971)*

*Not described as mindfulness within the study but contains relevant measures and results common to most meditation studies.*

**Abbreviations:** EEG, electroencephalography; RCT, random control trial; MBCT, mindfulness-based cognitive therapy; MBSR, Mindfulness-Based Stress Reduction; TM, transcendental meditation.
2.4 Autonomic and cardiovascular changes associated with MM practice

The scientific interest in the influence of meditation on cardiovascular health is growing in part due to growing interest in novel and inexpensive secondary interventions for patients and those at risk of cardiovascular disease [CVD] (Levine et al., 2017). The American Heart Association in their 2017 statement on meditation conceded that meditation may have possible benefit for cardiovascular risk, reviewing numerous long-term studies on their efficacy and quality (Levine et al., 2017). Numerous studies now document instances where elevated sympathetic stimulation is not only coincident with poor CV health, but a cause of it. Animal and human studies show increased chronic sympathetic activity is linked to cardiac and hemodynamic stresses: arrhythmia, high blood pressure, atherosclerosis development, and sudden cardiac death (Hedblad et al., 2001; Hjalmarson, 1998; Huang et al., 2001; La Rovere et al., 2001; Metra et al., 2000). The underpinning physiology of how MM may improve cardiovascular function is likely rooted in the relationship between autonomic function and its efferent control of the heart and vasculature (Olex et al., 2013).

2.4.1 The autonomic nervous system and MM

A typical autonomic response to MM is a reduction in sympathetic activity when measured by galvanic skin response (GSR), norepinephrine, or muscle sympathetic nervous activity [MSNA] (Das & Anand, 2012; Gabriely et al., 2020; Park et al., 2014). In a long-term MBSR intervention in patients with attention deficit hyperactivity disorder, GSR increased in the control group post-intervention after watching a stressful video, whereas the MM group had a non-significant decrease, indicating that MM may lead to a less reactive stress response (Gabriely et al., 2020). A 30-minute mantra meditation also resulted in significantly reduced GSR in healthy young people compared to a control group (Das & Anand, 2012). Though no study has measured norepinephrine levels to determine sympathetic activation in acute meditation interventions, one heart patient study did measure norepinephrine after 12 weeks of daily 30-minute MM and found reduced levels compared to a control group (Curiati et al., 2005). Finally,
in chronic kidney disease patients, a 14-minute mindfulness intervention reduced MSNA recorded by microneurography versus an active control group involving health education (Park et al., 2014).

### 2.4.2 Heart rate variability and MM

Heart rate variability (HRV) indexes autonomic function by measuring the variations of heart rate over time (Olex et al., 2013). It is also a predictor of cardiovascular risk and mortality where low variability is associated with increased risk (Bruyne et al., 1999; Tsuji et al., 1996). The simplest measurement methods of HRV determine variability between adjacent QRS complexes over time (Electrophysiology, 1996). Table 4 summarizes some time-based HRV parameters. Many MM studies also measure frequency-based HRV and report that high frequency power (HF-HRV) increases with MM or that low/high frequency ratio decreases. Increased HF-HRV indicates parasympathetic activity, whereas low/high frequency ratio indicates sympatho-vagal balance (Murata et al., 2004; Takahashi et al., 2005). However, interpreting frequency-based HRV data is controversial, so time-based HRV will be the focus in this review. Increased values in time-based HRV parameters indicate a shift towards parasympathetic activation, whereas low HRV values indicate a shift to sympathetic activation (Electrophysiology, 1996).

**Table 4:** Time-based HRV analyses

Adapted from Wu and Lo (2008) and Electrophysiology (1996).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean HR</td>
<td>beats/min</td>
<td>Mean heart rate</td>
</tr>
<tr>
<td>SDNN</td>
<td>ms</td>
<td>Standard deviation of NN intervals</td>
</tr>
<tr>
<td>RMSSD</td>
<td>ms</td>
<td>Root-Mean-Square of the differences between adjacent NN intervals</td>
</tr>
<tr>
<td>NN50 count</td>
<td></td>
<td>Number of adjacent NN intervals differing by more than 50ms in the entire recording.</td>
</tr>
</tbody>
</table>
Seventy-four students took the Wechsler Adult Intelligence Scale-IV (a cognitive stress challenge) after four weekly sessions of either MM or a study break with dogs; standard deviation of NN intervals (SDNN) was higher in the MM group despite the stress test (Shearer et al., 2016). In a within-subjects 15-minute MM versus active control intervention with novice meditators, SDNN HRV increased significantly in meditation compared to active control (Melville et al., 2012). In another study of 23 participants with no meditation experience, self-reported mindfulness (assessed with the Kentucky Inventory of Mindfulness Skills and the Mindful Attention and Awareness Scale) was positively correlated with SDNN and root-mean-square standard deviation [RMSSD] (Burg et al., 2012). Table 5 summarizes MM studies that measured HRV in the time domain.

2.4.3 Blood pressure and MM

Acute meditation can reduce blood pressure (Melville et al., 2012). Autonomic function is well known to mediate blood pressure through the arterial baroreflex (Cowley et al., 1973). Controlled breathing at slower breathing rates increases the magnitude of carotid baroreceptor activation which when stimulated decreases heart rate and blood pressure (Bernardi et al., 2002; Radaelli et al., 2004b). In an acute study, immediately after slow breathing, baroreflex sensitivity increased while blood pressure was reduced by a similar degree in both hypertensive and control subjects because of cardiac parasympathetic activation and sympathetic inhibition (Joseph et al., 2005; Montano et al., 1998). The acute meditative effect on blood pressure is likely through slower breathing or, at least, less spontaneous breathing that has its strongest modulation of heart rate at breathing rates of six breaths per minute (Bernardi et al., 2002; Cysarz & Büssing, 2005; Joseph et al., 2005). Increased HRV is negatively correlated with lower
breathing rates and blood pressure, further indicating a shift towards parasympathetic activity (Ditto et al., 2006; Peng et al., 2004).

Long-term interventions like MBSR can also reduce blood pressure, however, there appears to be a more dramatic effect in individuals who already exhibit a higher baseline blood pressure such as hypertensive patients (Abbott et al., 2014; Bernardi et al., 2002; Joseph et al., 2005; Olex et al., 2013; Park et al., 2014). It is important to be aware that MM studies involve control groups that exhibit reduced BP simply because they are sitting or resting quietly. Some studies report a decrease in BP after MM that was greater than in quietly seated controls, specifically: an isolated systolic reduction (Bernardi et al., 2017), and a systolic and diastolic reduction that was greatest in female participants (Ditto et al., 2006). Overall, changes in blood pressure appear to be moderate with meditation and are most apparent in long-term studies or in chronic disease (Momeni et al., 2016), whereas in the short-term, changes in blood pressure return to baseline post-intervention (Melville et al., 2012).

2.5 In summary

Autonomic control of cardiovascular variables is affected by MM. Both hormonal (norepinephrine) indicators of sympathetic activation and measurement of direct nerve activity indicate that sympathetic activity decreases following exposure to MM. MM increases time domain HRV, indicating a shift towards parasympathetic activation. Changes in autonomic function may contribute to modest decreases in BP post MM, although these changes may be more relevant following longer interventions in groups with elevated baseline BP.
Table 5: Summary of relevant studies on autonomic nervous activity indexed by time-domain HRV during MM.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Group Condition</th>
<th>Intervention</th>
<th>Results / Conclusions</th>
<th>MM effect on HRV interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burg et al. (2012)</td>
<td>Undergraduate students, n=23</td>
<td>Approximately 18min of MBE assessment of ability to remain mindful of the breath</td>
<td>SDNN and RMSSD were positively correlated with the MBE assessment.</td>
<td>Parasympathetic increase</td>
</tr>
<tr>
<td>Mankus et al. (2013)</td>
<td>Undergraduate students with high (n=22) or low (n=45) generalized anxiety</td>
<td>MM for 3 hours.</td>
<td>MSD in the high anxiety group was greater than the low anxiety group after MM.</td>
<td>Parasympathetic increase</td>
</tr>
<tr>
<td>Melville et al. (2012)</td>
<td>Sedentary office workers n=20</td>
<td>15min interventions of yoga or guided meditation plus control group.</td>
<td>Yoga increased HR, while meditation decreased HR vs control. Yoga and meditation decreased respiration, and increased SDNN vs control. DBP and SBP decreased during meditation at one timepoint only.</td>
<td>Parasympathetic increase</td>
</tr>
<tr>
<td>Shearer et al. (2016)</td>
<td>RCT; Undergraduate students</td>
<td>MM in 4 weekly sessions for 1hr, active control (dog interactions), versus inactive control.</td>
<td>SDNN increased significantly versus dog group or control.</td>
<td>Parasympathetic increase</td>
</tr>
</tbody>
</table>

**Abbreviations:** HRV, heart rate variability; SDNN, standard deviation of NN intervals; RMSSD, root-mean-square of the differences between NN intervals; MSD, mean of the absolute value of the difference between successive inter-beat intervals; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MBE, Mindful Breathing Exercise.
2.6 The vascular endothelium and its relation to MM

The vascular endothelium is a layer of single cells lining blood vessels and it plays an important role in hemodynamic regulation and vascular structure (Cahill & Redmond, 2016). Endothelial cells contribute to vasoregulation by sensing the frictional force exerted by the flow of blood (shear stress). The cells transduce the mechanical signal and activate a biochemical response synthesizing and releasing different relaxing factors, including nitric oxide (NO), that cause the smooth muscle surrounding the blood vessel to relax, increasing the diameter of the vessel [flow-mediated dilation; FMD] (Furchgott & Zawadzki, 1980). The failure of the endothelium to induce vasodilation in response to increased shear stress is a precursor to the development of atherosclerosis and coronary artery disease (Furchgott & Zawadzki, 1980; Nabel et al., 1990; Pohl et al., 1986; Takase et al., 1998).

Only three studies (Table 6) have assessed endothelial function in response to MM, and all three of them used reactive hyperemia-induced flow-mediated dilation (RH-FMD) as an index of endothelial function. Gainey and colleagues (2016) studied 27 subjects with type II diabetes (aged 40-75) who completed a Buddhist Walking Meditation (BWM) or walking exercise training for 12 weeks. RH-FMD was assessed before and after the intervention (Gainey et al., 2016). BWM and walking exercise significantly increased RH-FMD compared to pre-intervention levels, however, no significant difference in RH-FMD improvement between the walking and BWM conditions was reported. Though, pulse wave velocity (a measure of arterial stiffness and another index of endothelial function) only increased in the BWM group (Gainey et al., 2016). Breaking from usual RH-FMD report guidelines, Gainey and colleagues (2016) did not report a measure of the shear stress stimulus (which can affect the degree of vasodilation especially if it varies considerably between individuals); this makes their results more difficult to interpret conclusively.

In a study by Prakhinkit and colleagues (2014), elderly adults with depression symptoms but no CVD completed BWM, walking exercise or control interventions for 12 weeks. RH-FMD improved in
both the walking and BWM groups but not the sedentary control group post-intervention compared to pre-intervention values; they reported no significant differences in RH-FMD between BWM and walking exercise, however, RH-FMD was only significantly higher than post-control in the Buddhist walking mediation group (Prakhinkit et al., 2014). This study also measured NO concentration which can indicate improved endothelial function. NO levels increased post-intervention compared to pre-intervention for BWM and walking exercise only, not the sedentary control group (Prakhinkit et al., 2014). Walking itself may have presented a confound in interpreting the lone effects of MM on the endothelium.

One other study utilized RH-FMD but did so in a six-week intervention of Yoga and meditation with coronary artery disease (CAD) patients and non-CAD individuals (Sivasankaran et al., 2006). Thirty-three subjects underwent 15 minutes of MM, 15 minutes of yogic breathing, 20 minutes of relaxation, and 40 minutes of yoga asana postural exercises. RH-FMD significantly improved in CAD patients compared to pre-yoga intervention levels (Sivasankaran et al., 2006). One limitation to this study is that the authors did not report shear rate stimulus. The effect of MM is obscured by Yoga exercise in this study (like BWM in the previously mentioned studies), so it remains difficult to confirm whether MM alone improves RH-FMD. These studies were also long-term, and do not provide insight regarding how MM will affect RH-FMD in a single session.

2.6.1 Vasoactive substances and MM

As mentioned previously, NO is produced by endothelial cells when shear stress activates the signal transduction pathways necessary for vasodilation (Cooke & Dzau, 1997; Joannides et al., 1995; Palmer et al., 1988). Endothelial dysfunction is characterized by a reduction in endothelium-derived NO. In one hypothesis, reduced NO may be associated with dysfunction of endothelial NO synthase (eNOS) such that the enzyme produces superoxides when NO precursors (L-arginine and tetrahydrobiopterin) are in disequilibrium (Briones & Touyz, 2010; Cosentino et al., 1998; Landmesser et al., 2003). Reactive oxygen species (ROS), which can be produced by both endothelial cells and vascular smooth muscle
cells, are implicated in disrupting the NO signaling pathway by reducing the bioavailability of NO; a scenario that is substantially exacerbated in disease states (Guzik et al., 2000; Harrison, 1997; Wassmann et al., 2002). Even in healthy individuals, acute dysfunction can occur because of oxidative stress after high-fat or high-glycemic meals but is rescued by the addition of antioxidants (Esposito et al., 2003; Mah et al., 2013; Tsai et al., 2004). Thus, the activities of NO and ROS are of importance to endothelial function.

Although not direct assessments of endothelial function, studies have examined the impact of MM on NO bioavailability and ROS (Table 6). If MM increases NO bioavailability or decreases ROS, that may promote improved endothelial function as described above. In comparison to non-meditators, experienced Zen meditators (approximately 56 months of practice) had reduced ROS and increased nitrate-nitrite (NOx) concentrations after a single 60 minute session (Kim et al., 2005). Important to note in this study is that the measurements of NOx were reported and compared post-intervention between non-meditators and Zen meditators only. We cannot determine based on their report whether NOx or ROS changed with the MM intervention from their baseline levels before any intervention was applied.

Another study that measured NO and ROS did so before and after an acute session of Kouksundo, (a Korean mind-body practice that involves mindful breath as well as stretching). Both ROS and NO were lower post Kouksundo versus pre-intervention, as was sympathetic release of dopamine and norepinephrine (Im et al., 2015). There was no control group in this study. The finding that NO reduced after a meditative practice was unexpected. The authors describe it as a beneficial outcome since extraordinarily high levels of NO can be detrimental (Brown, 2010) however it is unclear how this reduction would relate to a direct measure of endothelial function.
Table 6: MM studies that have measured endothelial function, nitric oxide levels, or oxidative stress.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Group Condition</th>
<th>Intervention</th>
<th>Results / Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gainey et al. (2016)</td>
<td>Type II diabetes patients, n=23</td>
<td>Randomized into walking group or Buddhist walking meditation group. 30min/session, 3 times/week.</td>
<td>RH-FMD increased in both BWM and walking group. PMV increased only in BWM.</td>
</tr>
<tr>
<td>Prakhinkit et al. (2014)</td>
<td>Elderly adults aged 60-90 years, n=45</td>
<td>Randomization into walking group, control, and Buddhist walking meditation group. 3 times/week, 20-30min</td>
<td>RH-FMD and NO levels improved in both walking groups. RH-FMD only significantly higher than post-control in the Buddhist walking meditation group.</td>
</tr>
<tr>
<td>Sivasankaran et al. (2006)</td>
<td>N=33, 30% with CAD and 70% without</td>
<td>6 weeks of yoga and MM for 90min, 3 times/week.</td>
<td>Reductions in SBP and DBP, HR, BMI. RH-FMD improved only in the participants with CAD.</td>
</tr>
<tr>
<td>Kim et al. (2005)</td>
<td>Experienced Zen meditators n=20 and non-experienced control group n=20</td>
<td>60min of Zen meditation, focus on the breath.</td>
<td>Meditation group had lower lipid peroxidation and higher NOx levels than the control group, post-intervention.</td>
</tr>
</tbody>
</table>
Im et al. (2015)  Trainees of *Kouksundo*  90min, breathing MM accounted for 45min of the session.
n=57  Reduced levels of NO and ROS. Increased epinephrine. Decreased norepinephrine and dopamine.

**Abbreviations:** SBP, systolic blood pressure; DBP, diastolic blood pressure; FMD, flow-mediated dilation; RH-FMD, reactive hyperemia-induced flow-mediated dilation; NO, nitric oxide; HR, heart rate; BMI, body-mass index; CAD, coronary artery disease; NOx, nitrate-nitrite concentrations; ROS, reactive oxygen species; BWM, Buddhist walking meditation; PWV, pulse wave velocity.
2.6.2 Putative mechanisms by which MM may have a positive impact on endothelial function

There are indications that SNA influences endothelial function in healthy individuals. Diurnal variation in sympathetic activation (higher in the morning) is speculated to at least partially explain why RH-FMD is blunted in the early morning upon waking, and why CV events occur in patients in the morning (Kario et al., 2004; Otto et al., 2004). Acute sympathetic activation using lower body negative pressure resulted in attenuated RH-FMD responses; an effect abolished with alpha-adrenergic blockade by phentolamine, indicating that SNA reduces endothelial function (Hijmering et al., 2002). Another surrogate marker of endothelial function known as reactive hyperemia peripheral arterial tonometry (RH-PAT) was also found to be negatively correlated with MSNA recorded via microneurography (Sverrisdóttir et al., 2010). Similarly, another study determined that RH-FMD is blunted by sympathetic activation using lower body negative pressure (Thijssen et al., 2014). However, RH-FMD attenuation is not a consistent response to all methods of activating SNA (Dyson et al., 2006).

As previously mentioned, decreased SNA occurs in response to MM - measured directly by microneurography, GSR, and decreased norepinephrine levels, and indirectly by HRV. Therefore, MM may improve RH-FMD by altering autonomic tone to favour increased parasympathetic and decreased sympathetic activity. This is supported by the observation of Pinter and colleagues that time-based HRV parameters (RR interval, pNN50 and RMSSD) were all significantly positively correlated with RH-FMD in healthy subjects (Pinter et al., 2012).

Both sympathetic activation and activation of the hypothalamic pituitary adrenal axis (HPA) are involved in the physiological stress response. Cortisol is the effector hormone of the HPA axis, and elevations in cortisol may have a negative impact on endothelial function (Broadley et al., 2005; Mangos et al., 2000). When cortisol is blocked in Cushing’s syndrome (where excess glucocorticoid excretion causes endothelial dysfunction), the result is an improvement in endothelial function (RH-FMD) compared to pre-treatment (Akaza et al., 2010). In healthy participants subjected to mental stress, blocked
cortisol production also leads to improved endothelial function (as well as baroreflex sensitivity) compared to placebo (Broadley et al., 2005).

Several MM studies have assessed cortisol levels. Gainey et al. (2016) found that their BWM intervention decreased cortisol post-intervention compared to pre-intervention, and also compared to the walking exercise group. Prakhinkit et al. (2014) measured cortisol which they found to decrease post-BWM, and decrease compared to sedentary control; walking exercise without meditation did not alter cortisol which was similar to Gainey et al., (2016). Im et al. (2015) found that Kouksundo decreased cortisol significantly compared to pre-intervention levels. Decreased cortisol may contribute to improved vascular function, but whether acute MM on its own (i.e., without walking, yoga or Kouksundo) results in reduced cortisol is unclear from these studies.

2.7 In summary

The vascular endothelium is vital to proper regulation of blood flow and pressure, and its dysfunction is a first step towards the development of CVD (Cahill & Redmond, 2016). Researchers and clinicians acknowledge MM as a secondary therapy to improve cardiovascular function (Levine et al., 2017). However, any role of MM in endothelial function improvement is unclear because MM studies that directly measure the impact of MM on endothelial function are sparse and complicated by the inclusion of exercise. There is evidence that MM influences autonomic function, NO/ROS equilibrium, and cortisol both acutely and longitudinally in a way that would promote improved endothelial function. Additional research is required to determine the nature and mechanisms of both the acute and long-term impact of MM on endothelial function.

2.8 Conclusion

MM is quickly gaining popularity in the West among the public and academia (Van Dam et al., 2018). MM results in increased self-reported mindfulness and changes in EEG activity, most notably an
increase in alpha power. This review presented the small number of studies that directly investigated vascular endothelial function as an outcome in MM interventions. These few longitudinal interventions included movement (e.g., walking or yoga) in combination with MM therefore the isolated impact of MM remains unclear. Studies to date used RH-FMD as an index of endothelial function - but no group has measured RH-FMD in response to a single acute and isolated (i.e., without movement) MM session. This review also summarized supporting studies that build a theoretical rationale regarding how mindfulness meditation might lead to an improvement in vascular endothelial function: specifically, via its effects on autonomic function, NO and ROS and cortisol.
Chapter 3

The effect of a single session of acute mindfulness meditation on endothelial function

3.1 Introduction

Mindfulness is a family of practices rooted in Buddhist tradition that involves conscious cognitive attention on the breath, body, sensations, and/or mental state (Amarasekera & Chang, 2019). While focused on psychological or spiritual development, mindfulness meditation (MM) has well-established physiological effects (Olex et al., 2013). Electroencephalography (EEG) is a well-established tool used to document the distinct brainwave patterns that occur during meditation; EEG patterns correlate with cardiorespiratory and autonomic nervous system measures in real-time (Ahani et al., 2014; Colgan et al., 2019; Steinhubl et al., 2015). Evidence suggests that chronic MM is associated with reduced cardiovascular risk as demonstrated in large cohort studies of mindfulness-based interventions in patients with cardiac disease (Davidson & Dahl, 2018; Jayadevappa et al., 2007; Sullivan et al., 2009). Even acute bouts of MM result in a rebalancing of the autonomic nervous system, with evidence of reduced sympathetic and increased parasympathetic activity: lower blood pressure and increased heart rate variability [HRV] (Bernardi et al., 2017; Ditto et al., 2006). Casual meditators have a higher nitrite-nitrate (NO\textsubscript{x}) blood concentration and lower levels of oxidative stress than non-meditators (Kim et al., 2005) while a single session of a Korean mind-body practice (Kouksundo) increased NO\textsubscript{x} and lowered oxidative stress (Im et al., 2015). Therefore, meditation practices, including mindfulness, lead to physiological changes that are relevant to cardiovascular health and function.

Despite the growing body of evidence that supports the benefits of MM (Azam et al., 2015; Barnes et al., 2008; Bernardi et al., 2017; Ditto et al., 2006; Mankus et al., 2013; Melville et al., 2012; Momeni et al., 2016; Park et al., 2014; Peng et al., 2004; Peressutti et al., 2012; Phongsuphap et al., 2008;
Shearer et al., 2016; Van Der Zwan et al., 2015; Wu & Lo, 2008; Zeidan et al., 2010), the evidence has yet to explain the exact mechanisms by which MM may influence cardiovascular function, particularly in the short-term. One important aspect of cardiovascular function that may benefit from meditation is the vascular endothelium. The endothelium plays a central role in controlling vascular tone, and endothelial dysfunction contributes to the development of cardiovascular disease (Joannides et al., 1995; Nabel et al., 1990; Pohl et al., 1986). To date, only three studies (Gainey et al., 2016; Prakhinkit et al., 2014; Sivasankaran et al., 2006) have investigated the impact of meditation on endothelial function. While they found a significant improvement, these studies used long-term meditation interventions that were also paired with either walking or yoga, which may have masked the sole effects of meditation practice on the endothelium. No study to date has determined the isolated acute impact of a single session of MM on endothelial function.

Several documented physiological effects of MM are likely to have a beneficial effect on endothelial function. For example, endothelial function can be transiently attenuated by elevated sympathetic activity and reduced nitric oxide bioavailability (an important vasodilator) caused by increased oxidative stress (Campese et al., 2004; Clapp et al., 2004; Hijmering et al., 2002; Victor et al., 1987). In contrast, acute meditation results in a decrease in sympathetic nervous activity (Bernardi et al., 2001; Raupach et al., 2008; Zeidan et al., 2010). In addition, there is evidence that a single mindfulness exercise increases NOx and reduces oxidative stress (Im et al., 2015). It is therefore reasonable to hypothesize that a single acute session of meditation will result in a transient improvement in endothelial function.

The primary purpose of this study is to determine the effect of a single session of MM on vascular endothelial function in healthy novice meditators. Secondarily, changes in HRV (as an index of autonomic function) and EEG from pre- to post-MM will be examined in relation to changes in endothelial function to determine if correlations exist. Such correlations would form the basis of future investigations of specific causal, mechanistic links between MM and changes in endothelial function.
Exploring the acute effects of MM on novice meditators will improve our understanding of how the effects of meditation manifest over time. Understanding the earliest effects of MM in a single session in novice meditators allows us to determine the benefits that casual, self-driven meditation may impart. If endothelial function benefits are apparent immediately, this suggests that MM may offer benefits to a wide range of people who cannot commit to a standard, lengthy meditation training program.

### 3.2 Materials and Methods

#### 3.2.1 Study participants and screening

Thirteen young healthy participants (age 18-29) who had little to no experience (no more than 5-10 minutes daily and less than 100 hours in a lifetime) with meditation were recruited for the study. RH-FMD data for one participant was lost due to technical issues with the ultrasound recording, therefore the data of the twelve remaining participants is reported. Meditation experience was defined similarly to Kemper et al. (2015): inexperienced meditators have less than 100 hours of lifetime practice, experienced meditators have 300 hours or greater. During a screening visit before the commencement of the study, potential participants were asked to complete a medical screening questionnaire in which they reported history of cardiovascular diseases, as well as all medications. They also had their blood pressure assessed (BpTru BPM-100, BpTRU Medical Devices, Coquitlam, BC, Canada). Any participants with hypo- or hypertension, reported cardiovascular disease or medications were excluded. Sample characteristics including weight, sex, and height were recorded. A measure of level of trait mindfulness was recorded during the screening session using the Mindful Attention Awareness Scale (MAAS). The eligible participants were introduced to the protocol which involved a preliminary ultrasound test to ensure that a clear artery image and blood velocity signal could be recorded.

#### 3.2.2 Experimental Design
Each participant attended one session of MM and one active control session on separate visits. Whether the MM or control intervention was experienced on the first visit was counterbalanced between participants. As per recommendations by Thijssen et al. (2019), participants were asked to abstain from moderate to vigorous physical activity, caffeine, and alcohol for at least 24 hours before their visit, and to fast for 12 hours prior to their visit. The visits were approximately 93 minutes in length and were conducted in the same temperature-controlled room (20°C) at the same time of day within a participant (Figure 1).

**Mindfulness Meditation.**

The participants laid supine while listening to a 20-minute guided mindfulness meditation from the Healthy Minds app (Center for Healthy Minds, Madison, WI, USA; [https://hminnovations.org/meditation-app](https://hminnovations.org/meditation-app)). This session, entitled “Mindful Breathing”, was developed by the Center for Healthy Minds which established breath counting as a generator of state mindfulness that correlated with self-report measures of mindfulness (Levinson et al., 2014). Breath counting has also existed as a Buddhist MM tool well-established over 1,500 years ago (Levinson et al., 2014). The meditation directed the participant to focus on counting the length of the breath without specific breath timings. The guidance also focused on the quality of the breath, guiding the participant to feel the breath as it enters and exits the body, as well as the movement of the abdomen, diaphragm, and chest. The depth of each breath is not the focus of the guided meditation, so participants were free to inhale and exhale to whatever depth was most comfortable.

**Active Control.**

The participants laid supine while listening to an 18-minute Technology, Entertainment, Design (TED) Talk entitled “How to learn any language in six months” by Chris Lonsdale ([https://ed.ted.com/on/GOA9dfE2#digdeeper](https://ed.ted.com/on/GOA9dfE2#digdeeper)). This audio was chosen as it was used as an active control in the Lin et al. (2019) study on neurocognitive mechanisms of MM. The participants were only asked to
focus on the educational material in the audio. There were no additional instructions given regarding how
the participant should breathe or move.

**Figure 1: Protocol Timeline**

Abbreviations: Electroencephalogram (EEG); heart rate variability (HRV); reactive hyperemia low-mediated
dilation (RH-FMD); blood pressure (BP) discrete measurement with BPTru; continuous blood pressure (contBP)
with finometer; and Toronto Mindfulness Scale (TMS). Intervention on a given visit was either mindfulness
meditation (MM) or active control (AC). Whether the MM or AC intervention was experienced on the first visit was
counterbalanced between participants. Total time for the session is approximately 93 minutes including initial
instrumentation.

### 3.2.3 Experimental Measures

**Mindfulness.**

The MAAS, which was administered during participant screening, is a 15-item questionnaire
designed to assess trait mindfulness (Brown & Ryan, 2003). The MAAS determines a participant’s initial
propensity towards self-awareness and mindfulness. The Toronto Mindfulness Scale (TMS) is a 13-item
questionnaire designed to assess state mindfulness after an experience of mindfulness; the assessment
defines a subjective awareness of self, internal states and feelings, and one’s surroundings (Bishop et al.,
2006; Lau et al., 2006; Van Dam et al., 2018). The TMS was administered to participants immediately
following the 20-minute intervention (MM or active control).
**Heart rate, HRV, and blood pressure.**

Heart rate (HR) was captured continuously throughout the protocol using electrocardiogram (ECG) electrodes, two placed on the chest and one on the abdomen. ECG recordings were captured in LabChart Pro (ADInstruments, Colorado Springs, CO, USA). Blood pressure (BP) was assessed on the right arm with an automatic BP device that takes 6 consecutive recordings of BP approximately 5 minutes before the first baseline RH-FMD and once again before the second post-intervention RH-FMD. BP was also recorded continuously using an automated blood pressure device (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands) during the initial 20-minute resting baseline period and the 20-min intervention period; this data was recorded in LabChart for analysis.

**Electroencephalography.**

Electroencephalography (EEG) was recorded using the Muse™ (RRID:SCR_014418; InteraXon Inc., Toronto, ON, Canada) before and during the intervention. Muse™ is “a portable scalp EEG system that can be used to measure brain activity. It is battery powered and has four active electrodes located at 10-20 coordinates TP9, AF7, AF8, and TP10” (Muse, RRID:SCR_014418). The EEG data was collected with a third-party app called Mind Monitor via Bluetooth connection (https://mind-monitor.com/#page-top). Mind Monitor exported the EEG data in a common separated values (CSV) file that was uploaded to a secure server for analysis.

**Reactive hyperemia-induced flow-mediated dilation.**

Reactive hyperemia-induced flow-mediated dilation (RH-FMD) was performed using brachial artery occlusion. An occlusion cuff was placed on the left forearm at the antecubital fossa, distal to the ultrasound measurement site. After 1 min of baseline recording the cuff was inflated to 250 mmHg. The occlusion was released after 5 minutes to induce reactive hyperemia.

**Artery diameter and blood velocity.**

Brachial artery diameter was measured continuously during the RH-FMD test using two-dimensional ultrasound in B mode, while blood velocity was measured by Doppler ultrasound at an
insonation angle of 68° (12 MHz; Vivid i2; GE Medical Systems, Mississauga, ON, Canada). As described in the procedure by Pyke and Jazuli (2011), baseline brachial artery diameter was recorded for 1 minute before the cuff is inflated to 250mmHg for 5 minutes. The recording resumed 1 minute before the cuff was released and continued for an additional 3 minutes. RH-FMD trials were conducted before and immediately following the 20-minute intervention. The ultrasound images were recorded with a frame-grabber (Epiphan Systems Inc., Ottawa, ON, Canada) and saved separately as AVI files using Camtasia Studio (TechSmith, Okemos, MI, USA). Blood velocity was captured using a Multigon 500P TCD (Multigon Industries, Yonkers, NY, USA) which captures the Doppler shift frequency spectrum to determine mean blood velocity. The velocity output was captured in real-time and saved in LabChart Pro for analysis.

3.2.4 Data Analysis

HRV and BP.

Heart rate variability (HRV) was assessed using an HRV analysis module within LabChart Pro to calculate time-based HRV in milliseconds for the 20-minute rest period and for the 20-minute intervention period. The HRV module determined the standard deviation of normal-normal (SDNN; also known as SDRR) R wave intervals as recommended by Electrophysiology (1996). Systolic and diastolic BP before each FMD trial pre- and post- intervention were determined from BPTru measures as the average of the last 5 of 6 discrete BP readings. Continuous finometer mean arterial pressure (MAP) was averaged into one-minute time bins. Finometer MAP assesses change in BP accurately, but is less accurate in determining absolute values (Pickering et al., 2005). The finometer MAP was corrected to the MAP determined from the BPTru values as described in Lew et al. (2021).

Brachial artery blood velocity.

Blood velocity was compiled within LabChart offline in 3-second average time bins; this was done for the one-minute baseline period, one-minute occlusion before cuff release, and 3 minutes post
cuff release. The mean blood velocity profile was obtained for use in the calculation for shear rate as
described below.

**Brachial artery diameter.**

Each 5-minute AVI video capture of the brachial artery was analyzed offline using automated
edge detection and a wall tracking software (Encoder FMD and Bloodflow v.3.0.3; Reed Electronics,
Perth, WA, Australia). A region of interest was placed around the highest quality portion of the B-mode
longitudinal image of the artery; this allowed the software to track the walls of the vessel at a frequency of
30 Hz. The diameter data was compiled into 3-second time bins, as described in Pyke and Jazuli (2011).
The investigator was blinded to trial and condition during diameter analysis.

**Shear rate and RH-FMD.**

Shear rate (an estimate of shear stress) was determined using the 3-second average time bins of
blood velocity and diameter calculated as mean blood velocity divided by diameter. Area under the curve
of the shear rate (SRAUC) during the 30 seconds following cuff release (SRAUC30) was used to
characterize the stimulus for RH-FMD. RH-FMD was characterized as a percent change in diameter
(%RH-FMD) from baseline and an absolute change in diameter from baseline (AbsFMD). To account for
shear rate stimulus differences between trials or conditions, the SRAUC was added to the FMD analysis
as a covariate.

**Electroencephalography.**

The CSV file generated by Mind Monitor is the product of a Fast Fourier Transform (FFT) that
decomposes raw EEG signals into log-transformed absolute power spectral density (PSD) commonly in
the -1; +1 range expressed in an arbitrary unit, µV^2/Hz. The automatically generated data displays log-
transformed PSD for each of the four electrode sensors grouped by frequency band (alpha, beta, theta,
delta, gamma). The mean alpha power was averaged from the four sensors and reported for the 20-minute
periods during resting baseline and during the intervention.
3.2.5 Statistical Analysis

A linear mixed model (LMM) with a compound symmetry co-variance structure was conducted with IBM SPSS Statistics 27 (IBM Corp., Armonk, NY). A two-factor LMM with time (pre- versus post-intervention) and condition (mindfulness versus active control) as factors was used for all cardiovascular variables, EEG, and all FMD variables. Subjective measures were compared using a paired samples t-test. Data is displayed as mean ± standard deviation. Linear regression analysis was conducted to assess the relationship between change in SDNN and change in RH-FMD as well as the relationship between change in EEG alpha power and change in RH-FMD; the changes in HRV and alpha power were calculated as intervention values minus baseline values, similarly, the change in RH-FMD was calculated as post-intervention minus pre-intervention values. Alpha power was chosen for correlation because alpha power is the most common indicator of MM (Ergenoglu et al., 2004; Travis, 2019).

No previous study has examined the acute impact of MM on FMD, so it was difficult to estimate effect size for the purpose of determining an appropriate sample size. The only studies investigating the impact of MM on FMD were several weeks and included mild exercise (Gainey et al., 2016; Prakhinkit et al., 2014). These studies reported an average improvement in FMD of ~5.4% from pre to post MM intervention. To detect a change half this size (2.7%) as significant from pre to post intervention with typical variability in our laboratory (SD 2.2%) (King & Pyke, 2020; Tremblay et al., 2019), G*Power 3.1.9.4 (Faul et al., 2009) indicates that a sample size of 8 is needed with an alpha level of 0.05 and power set at 0.8. We placed our target sample size at 12 participants.
3.3 Results

3.3.1 Participant Characteristics

Participant characteristics are reported in Table 7. Participants did not differ in the 7-day sleep quality recall questionnaire between the AC and MM (\(p = 0.339\)) experimental visits.

Table 7: Participant Characteristics

<table>
<thead>
<tr>
<th>Sex</th>
<th>MAAS score</th>
<th>Lifetime MM Experience (hrs)</th>
<th>Sleep Quality score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n)</td>
<td>5</td>
<td>4.13 ± 4.36</td>
<td>AC 6.92 ± 1.17</td>
</tr>
<tr>
<td>Female (n)</td>
<td>7</td>
<td></td>
<td>MM 6.67 ± 1.16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.5 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172.8 ± 9.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD. MAAS = Mindful Attention Awareness Scale, AC = Active Control, MM = Mindfulness Meditation. No significant difference found in Sleep Quality between conditions, \(p = 0.339\).

3.3.2 State mindfulness and EEG alpha power

The TMS (Toronto Mindfulness Scale) score, capturing state mindfulness following the intervention was not significantly different between conditions (AC 31.58 ± 6.26; MM 32.17 ± 9.74, \(p = 0.800\)). EEG analysis identified a significant effect of time where pre-intervention alpha power (0.710 a.u. ± 0.237) was greater than post-intervention alpha power (0.646 a.u. ± 0.217, \(p = 0.020\)). There was also a significant effect of condition where AC alpha power (0.720 a.u. ± 0.228) was greater than MM (0.639 ± 0.224, \(p = 0.016\)), with no significant time by condition interaction (Figure 2B).
3.3.3 HR, HRV and MAP

There was a significant effect of time on HR where pre-intervention HR (62.26 bpm ± 5.08) was greater than post-intervention HR (60.60 bpm ± 4.86, \( p = 0.011 \); Figure 3A). There was no effect of time, condition, or interaction between factors for HRV measured as SDNN or MAP (Figure 3B and C).

3.3.4 RH-FMD parameters
One RH-FMD trial was excluded from the analysis due to poor data capture. There was no effect of time or condition on baseline SR or diameter (Table 8). There was a significant effect of condition on SRAUC30 such that the stimulus was higher in the MM condition (Table 8).

There was no effect of time or condition on %RH-FMD (Figure 4) or absolute FMD (Table 8). SRAUC30 was added as a covariate to %RH-FMD, and this did not change the results.
A) 

![Graph showing HR (beats per min) with rest intervention.](image)

- **HR (beats per min)**
- **AC**
- **MM**

- **T:** $p = 0.011$
- **Cond:** $p = 0.713$
- **T×Cond:** $p = 0.703$

B) 

![Graph showing SDNN (ms) with rest intervention.](image)

- **SDNN (ms)**
- **AC**
- **MM**

- **T:** $p = 0.378$
- **Cond:** $p = 0.903$
- **T×Cond:** $p = 0.395$
Figure 3. A) HR, B) HRV measured as SDNN, and C) MAP.

N = 12 for all. The 20 min average HR, HRV and MAP measured during the 20 min rest period and during the 20 min MM and AC interventions. HR = Heart Rate, SDNN = standard deviation of NN intervals (normal R wave to normal R wave interval), MAP = Mean Arterial Pressure. AC = Active Control, MM = Mindfulness Meditation. T = effect of time, Cond = effect of condition, T×Cond: time by condition interaction. (*) indicates significant effect of time. Error bars reflect SD.
Table 8: RH-FMD characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre</th>
<th>Post</th>
<th>$p$ - values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline SR (s$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>12.29 ± 4.69</td>
<td>11.48 ± 5.71</td>
<td>Time: $p = 0.290$</td>
</tr>
<tr>
<td>MM</td>
<td>14.44 ± 11.39</td>
<td>11.89 ± 7.22</td>
<td>Condition: $p = 0.640$</td>
</tr>
<tr>
<td>SRAUC30 (s$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>2436 ± 560</td>
<td>2512 ± 601</td>
<td>Condition: $p = 0.014$ *</td>
</tr>
<tr>
<td>MM</td>
<td>2935 ± 773</td>
<td>2818 ± 856</td>
<td>Time × Condition: $p = 0.562$</td>
</tr>
<tr>
<td>Baseline diameter (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>0.349 ± 0.053</td>
<td>0.342 ± 0.048</td>
<td>Condition: $p = 0.152$</td>
</tr>
<tr>
<td>MM</td>
<td>0.355 ± 0.054</td>
<td>0.357 ± 0.048</td>
<td>Time × Condition: $p = 0.107$</td>
</tr>
<tr>
<td>AbsFMD (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>0.021 ± 0.010</td>
<td>0.020 ± 0.012</td>
<td>Condition: $p = 0.634$</td>
</tr>
<tr>
<td>MM</td>
<td>0.022 ± 0.010</td>
<td>0.022 ± 0.011</td>
<td>Time × Condition: $p = 0.885$</td>
</tr>
</tbody>
</table>

All data are mean ± SD. Variables pre and post the MM and AC interventions. Significance indicated by (*), $p$ - value denotes main effects and interaction factors. AC = Active Control, MM = Mindfulness Meditation, SR = shear rate, SRAUC30 = area under the curve in the 30 seconds following cuff release, AbsFMD = absolute RH-FMD. N = 12 for all table measures.
Figure 4. %RH-FMD.

N = 12. Percent RH-FMD values pre and post the MM and AC interventions. %RH-FMD = reactive-hyperemia flow-mediated dilation expressed as the percent change from baseline AC = Active Control, MM = Mindfulness Meditation. T = effect of time, Cond = effect of condition, T×Cond: time by condition interaction. Error bars reflect SD.
3.3.5 Relationships (ΔAlpha Power and ΔSDNN versus Δ%RH-FMD)

ΔAlpha power was positively related to the Δ%RH-FMD in the MM condition \( (r^2 = 0.44, p = 0.019; \text{Figure 5A}) \), but not in the AC condition \( (r^2 = 0.139, p = 0.232; \text{Figure 5B}) \). Cook’s distance test revealed two outliers in the MM condition, and two outliers in the AC condition. When the outliers were removed, the strength of the relationship weakened and lost significance for the MM condition, while the relationship remained nonsignificant for the AC condition (MM. \( r^2 = 0.39, p = 0.054 \); AC. \( r^2 = 0.203, p = 0.192 \); \text{Figure 5A and 5B}, respectively) In contrast, ΔSDNN was not related to the Δ%RH-FMD in the MM condition \( (r^2 = 0.20, p = 0.147; \text{Figure 5C}) \), however these variables were positively related in the AC condition \( (r^2 = 0.40, p = 0.027; \text{Figure 5D}) \). Cook’s distance test revealed one outlier in the MM condition, and no outliers in the AC condition. When the outlier was removed, the relationship remained nonsignificant for the MM condition \( (r^2 = 0.29, p = 0.086; \text{Figure 5C}) \).
Figure 5. A) \( \Delta \alpha \) Power versus \( \Delta \% \text{RH-FMD} \) after MM, B) \( \Delta \alpha \) Power versus \( \Delta \% \text{RH-FMD} \) after AC, C) \( \Delta \text{SDNN} \) versus \( \Delta \% \text{RH-FMD} \) after MM, and D) \( \Delta \text{SDNN} \) versus \( \Delta \% \text{RH-FMD} \) after AC.

\( N=12 \). Change in alpha power and SDNN (standard deviation of NN intervals) calculated as post- minus pre-intervention \%RH-FMD, and intervention minus rest values for alpha power and SDNN for each condition, AC (Active Control) or MM (Mindfulness Meditation). Change in \%RH-FMD is the difference between post-intervention and pre-intervention for each condition, AC or MM. Cook’s distance was calculated to identify outliers which have been circled in a red outline. Regression lines do not include outliers.
3.4 Discussion

This study is the first to explore the acute impact of a single session of MM on vascular endothelial function in young healthy adults with no previous meditation experience. We assessed endothelial function via RH-FMD in 12 participants before and after a single session MM intervention and a single session active listening control intervention on two separate days. We found that there was no impact of the chosen MM on RH-FMD; this was contrary to our hypothesis. Secondarily, the results also indicate that HRV, EEG alpha power and self-reported mindfulness were not increased by MM as predicted, and consequently no relationships were found between these variables and RH-FMD in the MM condition. Given that the secondary measures did not conform to the initial predictions, these results suggest that the chosen MM intervention was not effective at producing a meditative state. Thus, further research is required to provide a definitive answer to whether a single MM session that results in a meditative state can transiently improve vascular endothelial function.

3.4.1 Impact of MM on RH-FMD

We found no effect of time, condition, or interaction between factors for the primary variable, RH-FMD. The few previous studies that evaluated endothelial function using RH-FMD after MM reported findings contrary to the present study; Sivasankaran et al. (2006), Gainey et al. (2016) and Prakhinkit et al. (2014) all found improvement in RH-FMD after long-term MM interventions. However, whether the reported improvements in RH-FMD are due to MM per se is unclear, as these studies all included some form of exercise along with the MM, and exercise alone can improve RH-FMD (Clarkson et al., 1999; Goto et al., 2003; Hambreucht et al., 2003). In addition to the nature of the intervention (long term MM including exercise vs. single session MM with no exercise in the present study), differing adherence to RH-FMD methodological guidelines may have influenced the conflicting results. In the present study, RH-FMD was conducted in accordance with recent guidelines (Thijssen et al., 2011; Thijssen et al., 2019). The previous MM RH-FMD studies did not control training status and did not ensure a lack of participant exercise 24 hours before experimental visits; they did not control for the
impact of menstrual phase by consistently collecting data in female subjects during the early follicular phase of the menstrual cycle; and they did not account for the magnitude of the shear stress stimulus using SRAUC (Pyke & Tschakovsky, 2005; Pyke & Tschakovsky, 2007; Thijssen et al., 2019). Overall, our secondary results discussed below suggest that the MM intervention did not elicit a meditative state in our participants, and this may explain the lack of impact of MM on endothelial function.

3.4.2 Impact on EEG alpha power

Overall alpha power was lower post-intervention and there was an effect of condition wherein alpha power was higher during AC than MM. This finding is inconsistent with most studies that conclude that alpha power increases in a relaxed, closed eye, conscious and mentally attentive state such as mindfulness meditation as reviewed by (Cahn & Polich, 2006). However, consistent with the present findings, Cahn and Polich (2006) report that studies comparing TM or yogic meditation (which differ from, but involve, aspects of MM) with a relaxation control have consistently found no increases in alpha power with meditation (Corby et al., 1978; Jacobs & Lubar, 1989; Lehrer et al., 1980; Lehrer et al., 1983; Lou et al., 1999; Pagano & Warrenburg, 1983; Travis & Wallace, 1999). It is unclear why the MM in the present study resulted in alpha power results more consistent with TM or yogic meditation approaches. As suggested by Jacobs and Lubar (1989), it is possible that in the present study the initial level of relaxation was the critical factor in determining alpha power. The subjects may have been more relaxed at the start of the AC experimental visit resulting in higher alpha power pre-intervention and no further changes post-intervention. Participants overall may have become agitated with the length of the visit and still supine rest diminishing feelings of relaxation in both conditions. We also compared the highest minute of the baseline alpha power and the highest minute of the intervention alpha power to determine if transient changes existed during the MM and AC conditions, but analysis indicated no changes between conditions or over time (results not shown).

Subjective measures of mindfulness.
Subjects responded to the MAAS before the experiment to quantify their level of trait mindfulness. The MAAS indicated a trait mindfulness score 4.13 ± 0.69 out of the highest possible score of 6. According to Brown and Ryan (2003), the average MAAS score for a large sample of undergraduate students was 3.85 ± 0.68, while a large heterogenous sample of adults scored 3.97 ± 0.61. This suggests that the participants in the present study were within the average range of trait mindfulness for untrained meditators. Responses to the TMS after each intervention to quantify state mindfulness indicated no change with either intervention. This is contrary to our hypothesis but consistent with the alpha power findings. Together the subjective and cognitive measures indicate that the chosen MM did not induce a meditative state as expected.

3.4.3 Impact on HR, MAP and HRV

In this study, MM did not alter HR, MAP, or HRV, however overall, HR decreased over time. Previous meditation studies report mild, if any, changes in HR that are either an increase or a decrease when compared to baseline measurements (Melville et al., 2012; Peng et al., 2004). We speculate that the decrease in HR from pre- to post-intervention is due to the length of time the subjects spent in supine rest. MAP remained unchanged over time and did not differ between conditions. This was unexpected as previous studies have shown that acute meditation can lead to lower BP by increasing vagal activity as a result of a lowered breathing rate. (Joseph et al., 2005; Li et al., 2018; Radaelli et al., 2004a; Raupach et al., 2008). However, the present study did not measure respiration, so it is unclear if respiration rate was reduced. Our primary analysis utilized the average MAP over the 20-minute baseline period vs. the intervention period. To determine whether there was a more transient impact of the interventions on MAP we compared the lowest minute of the baseline MAP and the lowest minute of the intervention MAP during the MM and AC conditions. This analysis still indicated that MAP remained constant between conditions and over time (results not shown).

Contrary to our hypothesis, HRV measured as SDNN, did not increase from baseline during the MM intervention. HRV is an index of autonomic function which usually increases with MM, indicating a
shift away from sympathetic activity toward parasympathetic activity after meditation (Azam et al., 2015; Cysarz & Büssing, 2005; Mankus et al., 2013; Olex et al., 2013). However, in the present study, HRV indicated no change in autonomic activity. The result conforms to the other measures of the physiological state of mindfulness in the present study; no change in HRV is consistent with the overall evidence that MM did not induce a meditative state in this study.

While slow deep breathing is usually associated with increased HRV and increased parasympathetic tone (Kim et al., 2016; Melville et al., 2012), one reason that MM might have failed to increase HRV is that, somewhat paradoxically, paced deep breathing can cause stress and no increase in HRV in participants who are not trained, thus opposing any relaxation by heightening or maintaining sympathetic activity (Khan et al., 2013; Kim et al., 2016; Sasaki & Maruyama, 2014). The present study used mindful breath counting, which was not explicitly paced, but which may have been stressful to participants. However, we do not have any clear evidence of stress due to breathing instruction and HRV was not different between the MM and AC conditions (AC did not involve breathing instruction).

3.4.4 Relationships between RH-FMD and secondary variables

We did not find a significant relationship between the change in RH-FMD and the change in alpha power following the removal of outliers. However, in the MM condition the relationship approached significance ($r^2=0.39$, $p=0.054$). This provides some evidence to suggest that those who most successfully experienced a meditative state (indicated by increased alpha power) may have experienced an improvement in endothelial function. This supports conducting further research with a different MM intervention which may be more successful in stimulating cognitive changes.

No significant relationship between change in RH-FMD and change in HRV was detected in the MM condition which was contrary to the initial hypothesis. However, we did find an unexpected positive relationship between change in RH-FMD and change in HRV in the AC condition. The range of change in HRV in the AC condition was greater than in the MM condition, and this may have supported the
detection of a relationship in the AC condition. It is unclear why the AC condition stimulated a wide range of HRV responses.

### 3.4.5 The MM intervention

The MM intervention used in the present study is based on the original Buddhist technique (that itself is based on more ancient principles established in ancient India as described in the *Yoga Sutras*) that includes breath counting (Patanjali, 2012). Breath counting involves counting each breath or counting the seconds within each inhale and exhale. Levinson et al. (2014) provided evidence that breath counting is a route to mindfulness by correlating the theorized consequences of mindfulness (improved counting accuracy, mood, non-attachment, lower mind wandering, and subjective questionnaire scores that indicate mindfulness) to breath counting among novice and trained meditators. The MM in the present study involved participants engaging in each breath to a count of three, then repeating, with occasional short breaks from the practice after every approximately five minutes. As mentioned above it is possible that in the present study the novice participants found the prolonged breaths stressful.

The few studies that have measured HRV and/or alpha power specifically during a breath counting meditation report an increase in those variables (Steinhubl et al., 2015; Takahashi et al., 2005). One difference between the present study and these two studies is that the former studies used more robust EEG measures; they collected other frequency bands (delta, beta, theta, in addition to alpha), used greater or different sensor placements that provide a greater resolution of cognitive activity, and were able to manually clean and analyze EEG data. The present study used the Muse EEG headset which did not enable us to apply a Fast Fourier Transform manually or clean the data for artifacts as this data processing was done automatically by the unit software prior to data output. A robust analysis of other frequency bands may have revealed a theta power increase which is also known to be related to greater mindfulness as some studies also report (Ahani et al., 2014; Dunn et al., 1999; Takahashi et al., 2005; Wallace et al., 1971). Additionally, these previous studies provided some minimal pretraining in meditation immediately before the intervention, so the participants were prepared for the breath counting which they performed.
while seated as opposed to lying supine in the present study. Finally, Steinhubl et al. (2015) and Takahashi et al. (2005) documented respiratory rates during their meditation interventions. As previously discussed, the MM in the present study may have influenced participant breathing inadvertently by causing stress with the emphasis on breath. However, without a measure of respiration it is difficult to ascertain how breathing may have been affected objectively and we did not ask for a subjective assessment. Nevertheless, the participants were introduced to the breath counting mediation for the first time during the experiment, and with no prior training, they may have struggled to maintain the meditation for a full 20 minutes. Even the study by Levinson et al. (2014) noted that the accuracy of participants in maintaining the breath counting improved after successive attempts while also improving subjective measures of mindfulness and reducing measures of mind wandering. Hence, this study may have benefited from some form of pretraining.

3.4.6 Strengths and Limitations

By measuring the shear rate stimulus and adhering to recent methodological guidelines for the RH-FMD test, we were able to provide a clearer picture of endothelial function than previous MM studies. We combined cognitive and physiological measures into a single study, using subjective measures to quantify state mindfulness and trait mindfulness. This ensured that we could correlate cognitive measures with RH-FMD measures (a combination not previously attempted) and cardiovascular measures with RH-FMD measures after MM. In addition, by including an active control rather than a control condition devoid of activity, we were able to match the listening and attention component between the conditions, isolating the MM instruction component of the MM condition.

We did not include a direct measure of autonomic activity, but only an index using time-based HRV. Measures of cortisol, skin conductance, norepinephrine or microneurography would have provided more insight regarding stress response activation/autonomic activity. We did not include a measure of respiration or abdominal/thoracic movement. Measuring respiration would have indicated how well participants executed the breathing instruction during the MM. We also did not measure any metrics of
NO bioavailability or oxidative stress. It is therefore unclear whether the MM intervention impacted these variables, despite a lack of change in RH-FMD. Finally, the sample size of this study limited our ability to probe for individual response differences in the correlation analysis, or in alternate analyses.

3.4.7 Conclusion

We found no impact of a single session MM intervention on endothelial function as assessed by RH-FMD. We also found no impact of MM on alpha power HRV or subjective mindfulness suggesting that participants did not achieve a meditative state. Future research is needed to determine whether MM which elicits a physiologically and self report verified meditative state can transiently improve endothelial function. The present results suggest that the nature of the MM intervention is important as not all MM protocols may elicit mindfulness in novice meditators.
Chapter 4

General Discussion

Chapter 3 outlined the first study to investigate the acute impact of a single session of MM on endothelial function in healthy young adults. The results of this study indicated that endothelial function remains unaltered by MM. Further, there was no relationship between RH-FMD as an index of endothelial function and EEG alpha power or HRV during MM. The findings of this study indicate that the chosen MM protocol was ineffective at inducing a meditative state which may explain the lack of effect of the MM intervention on endothelial function. Therefore, we cannot conclude that acute MM results in any impact on endothelial function, but it may be possible to observe an effect if an effective MM measured physiologically and subjectively, is utilized in the future. The following sections of this chapter will discuss the methodological considerations and challenges of the study as well as discuss potential future directions to further explore this question.

4.1 Methodological Challenges

The current global health crisis made conducting pilot work impossible due to mandated lockdowns, lack of access to laboratory space, and dispersal of the potential study population. Hence, the following subsections explain some specific technical challenges that would have been identified during a pilot study, if not for the current global circumstances.

4.1.1 EEG Measurement

As previously described in Chapters 2 and 3, EEG is used to examine cognitive activity in many meditation interventions. EEG is a more objective measure of mindfulness than self-report measures and provides physiological quantification of the experience of mindfulness since it is the summation of neural/electrical brain activity (Cohen, 2011; Nunez, 1981; Travis, 2019). The alpha power frequency
band is most associated with the state of meditation, though some studies also report increases in theta power alongside alpha power (Takahashi et al., 2005; Travis, 2019; Travis & Wallace, 1999; Wallace et al., 1971).

We opted to use a measure of alpha power to confirm, and quantify, the state of MM and potentially establish a correlation between alpha power and RH-FMD. This was a challenge as our group had not previously assessed cognitive activity using EEG. We chose the Muse™ EEG headset as it was a commercially available device that was inexpensive and did not require a technically intensive or time-consuming setup. While the headset has been used in research applications, such as Krigolson et al. (2017) and Bird et al. (2019), there are some limitations to using consumer-grade EEG equipment; it lacks the resolution and precision that a larger, multi-sensor system could provide, and the output of these devices is simplified for layperson use (Acabchuk et al., 2021). In fact, Acabchuk and colleagues (2021) reported that the Muse™ system should not be used as proxy for other measures of mindfulness such as questionnaires since the output (built for consumers) does not correlate with validated subjective measures of mindfulness.

We did not use the consumer-tailored output in this study, but instead used a third-party application called Mind Monitor which provided more raw data from the headset. While this provided better quantifiable data for analysis, we were still restricted to the four sensors of the headset, and the automatically log-transformed data generated by Mind Monitor. Thus, we had little control over the data generated, and had to accept the output. Further, the quality of the Muse™ headset declined after the first six participants; sensors appeared to degrade over time and lose contact with the skin surface intermittently, a situation that was exacerbated with each use. We replaced the headset halfway through the experiment. We calculated the ratio of missing data cells to completed data cells, and found that overall, the malfunctioning initial headset captured 73% of the desired data (27% of cells missing data) by the end of its use in the experiment, with only one trial excluded. Since EEG alpha power was a secondary measurement and not the primary outcome variable, we concluded that despite the limitations of a
consumer-grade device, that this was acceptable for the purposes of this thesis. A future study may benefit from a more robust measure of cognitive activity using a higher-grade EEG system.

4.1.2 FMD Measurement and the MM Intervention

While one of the strengths of the study was to follow expert consensus on FMD measurement, it is possible that the constraints around correctly conducting RH-FMD interfered with the establishment of a meditative state. Thijssen et al. (2019) highly recommended that subjects rest for 15 minutes positioned in a supine posture such that the imaged brachial artery is at heart level. Our subjects rested in a supine position for at least 15 minutes as recommended by Electrophysiology (1996) before HRV measurement, and then remained supine during baseline EEG and HRV measurement for 20 minutes, leading to a total of 35 minutes before the first RH-FMD trial.

However, all the MM studies that we reference in this thesis (as described in Chapter 2) conducted their MM and control interventions with the subjects in a seated position. In fact, the audio instructions of the MM we utilized from the Healthy Minds app informs the subjects to take a seated position. We reasoned that the RH-FMD recommendations took precedence over the MM instructions. It is possible that by having the participants lay supine for the entirety of the protocol, including during the MM and AC conditions, we did not promote the meditative state effectively. More likely, by combining a mindful breath awareness type of meditation with a supine position, we created a condition more akin to yoga nidra than to MM. Yoga nidra is a discipline of meditation not previously discussed as it involves a state of relaxation that operates in the realm of deep sleep that Patañjali’s Yoga Sutras describe as a means of entering an enlightened state (Parker et al., 2013). Thus, it is not the same as mindfulness, but can involve similar techniques while in a supine position. Previous studies have shown that yoga nidra can increase both EEG alpha power and HRV just as we predicted with MM, though the fact that yoga nidra involves a sleeping state makes EEG delta power the most likely frequency to dominate cognitive activity especially in untrained novices (Markil et al., 2012; Parker et al., 2013). Regardless, the MM intervention
we chose to conduct in a supine position was neither a true session of yoga nidra nor MM, because of the mismatched combination of breath awareness and supine relaxation. Though we did not assess if the participants fell asleep or had high delta power dominance which indicates sleep, it is possible that body position is one reason the participants did not achieve a meditation state in all trials.

Conducting the RH-FMD test while seated may have brought the MM intervention more in line with a true MM session, as well as made this study more comparable to other MM interventions conducted by other studies. One study that followed the guidelines prescribed by Thijssen et al. (2011), reported that RH-FMD remained unaltered during a three-hour course of prolonged sitting, where brachial artery FMD as well as SRAUC showed no changes at the one, two and three hour mark when compared to baseline (Thosar et al., 2014). If a future study utilized a similar protocol to Thosar et al. (2014) the study would be able to capture RH-FMD while ensuring the most optimal conditions for creating the meditative state. Hence, by conducting the study in a seated posture, we may have been able to properly create a meditative condition.

4.1.3 Loss of Data

Over the course of the study, we experienced a few equipment failures in addition to the EEG quality loss. To capture artery diameter for RH-FMD calculations, ultrasound images were recorded using a frame-grabber (as outlined in Chapter 3) that failed during one collection. The data from that participant was excluded from the analysis but we still completed the study with a total of 12 participants as expected.

4.2 Future Directions

The study described in Chapter 3 is inconclusive on whether MM has a transient affect on endothelial function. Aside from the EEG challenges presented previously, I am able to propose several alterations to the study design that may improve the ability to detect an effect of MM should a study
pursue this question in the future. First, I discussed previously in this chapter that a more robust measure of cognitive brain activity using a non-commercial EEG system would be of benefit so the researcher could have more control and confidence in the output. I also described the possibility that a seated posture would be a better representation of the MM state that we did not achieve in the study.

Second, as alluded to in Chapter 3, other measures could have been included in the study to enhance the characterization of the meditative state. For instance, breathing rate and galvanic skin response may have provided additional information on autonomic activity during the MM and AC conditions. We utilized HRV as an index of autonomic function but relying on it as the sole index may not have been beneficial in this study without a measure of breathing to corroborate the HRV result (Cysarz & Büssing, 2005; Kim et al., 2016; Steinhubl et al., 2015).

Third, to truly determine whether endothelial function is affected by MM, a MM state must be achieved by the participants. A future study may endeavor to only include participants who have been identified through screening procedures as being capable of reaching the desired state in an acute session. This would ensure that we could test endothelial function with greater certainty that the participant would be cognitively and physiologically affected by MM. However, it is also possible that participants with no training in MM will not be able to generate the experience of mindfulness on-demand, so such a study would not be able to investigate on complete novices.

One of the most important lessons of this study in my opinion is that to truly investigate the physiological/cognitive effects of a behavioural experience on other physiological functions, that experience must elicit the desired physiological response in a quantifiable way. This is vital for researchers since much thought must be put into defining the meaning, quality, and measurement of the experience of mindfulness as Van Dam et al. (2018) describes, before we can test it against physiology or make claims that mindfulness can enhance human wellbeing. Dahl et al. (2015) explained some of the challenges that research on meditation has faced including difficulties defining mindfulness, and
difficulties standardizing duration, intensity or spacing of mindfulness practice. As I have described previously, this study encountered some of these challenges, thereby preventing us from cultivating the mindfulness state in our participants. Though the results were inconclusive regarding the effects of MM on endothelial function, the surging interest in Eastern practices like mindfulness among Western populations further underscores the need to understand its physical impacts (Van Dam et al., 2018).

4.3 MSc Thesis Journey

This was the first investigation of the acute impact of a single session of MM on endothelial function, and the first study in our lab to utilize measures of cognitive activity (EEG) alongside physiological measures. Therefore, an extensive amount of research and innovation was required to ensure that it was possible to carry out the study described in Chapter 3. No previous students have used EEG in our lab, and so I had to familiarize myself with literature from neuroscience and psychology disciplines with which I had no previous exposure. I was able to develop a protocol to collect and analyze this complex data, and I am proud that I had the opportunity to expand my own knowledge as well as lay the groundwork should a future student in this lab to use cognitive measures in their own investigations. This project gave me an appreciation for the importance of conducting research with as much control and design as possible to overcome limitations described in previous research, but also made me recognize that even a meticulous approach cannot eliminate limitations.

The COVID-19 pandemic made it difficult to complete my MSc and hampered my efforts to fine-tune my experimental protocol and improve my skills with ultrasound. It made conducting pilot work impossible and challenged recruitment of participants. Though I was able to meet the sample size originally set, there was great difficulty in doing so that certainly erased any possibility of a preceding pilot study. The loss of a full MSc experience, and the opportunity to push my project beyond what is described in this thesis, was extremely disappointing. The experience of conducting an MSc thesis under these circumstances posed challenges and delays that affected our ability to answer the research question
posed in this thesis, though the extended time away from the physical laboratory space enabled a deeper understanding of the literature presented in Chapter 2. Despite the challenges and lost time, investigating a topic like meditation has exposed me to a world of research, books, print magazines, art, training opportunities, and personal wellbeing that I never imagined existed. During the periods of lockdown that were imposed, I spent much of my time reading books written by experts on the subjects of meditation, yoga, and other Eastern philosophies from a scientific and humanities point of view to better understand the context of my thesis project. My worldview has certainly expanded alongside my appreciation for science and academic rigour. Though I am continuing to support science in industry as opposed to academia, I am confident there will be a time where I can contribute to this line of inquiry again with new insights. Certainly, my exposure to the crossroads of Eastern philosophy and Western modern scientific discovery has set me on trajectory I endeavor to follow for years to come. I would like to thank Dr. Kyra Pyke for opening the door to these possibilities.

4.4 Conclusion

The study described in Chapter 3 is the first to explore the acute impact of a single session of mindfulness meditation on vascular endothelial function in young healthy subjects. The results from Chapter 3 were inconclusive in answering this question with no change in RH-FMD identified after either a MM intervention or an AC intervention. However, the lack of evidence for the successful cultivation of a meditative state in the subjects indicates that there is still a possibility for MM to affect endothelial function, but this can only be ascertained if the subjects are able to achieve a meditative state in a single acute session. Further studies can use the results of this study as a basis to fine tune their approach and provide a definitive answer to whether or not MM has an acute effect on the vascular endothelium in particular, thereby contributing to physiological research on the ancient experience that is mindfulness meditation.


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Appendix A: Research Ethics Board Approval and Amendments
QUEEN’S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD (HSREB)

HSREB Delegated Amendment to Ethics Clearance

March 05, 2021

Dr. Kyra Ellen Pyke
School of Kinesiology and Health Studies
Queen’s University

TRAQ #: 6004461
Department Code: PHE-090-09
Study Title: “PHE-090-09 - Flow Mediated Dilation Variability: Reactive Hyperaemia vs. Exercise Induced Increases in Shear Stress (NSERC project title: Shear Stress and the Regulation of Human Arterial Function and Structure)”
Review Type: Delegated
Date Ethics Clearance Issued: March 05, 2021

Dear Dr. Pyke:

The Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed the amendment event form and is granting ethics clearance for the changes listed below:

<table>
<thead>
<tr>
<th>Document Name</th>
<th>Comments</th>
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<tr>
<td>Consent Form</td>
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<tr>
<td>Questionnaire</td>
<td>Medical Screening Form</td>
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<tr>
<td>Questionnaire</td>
<td>Sleep Quality Scale</td>
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Regards,

[Signature]

Albert F Clark, PhD
Chair, Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board

The HSREB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the international Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Product Regulations; Part 3 of the Medical Devices Regulations, and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP). Federalwide Assurance Number: FWA#: 00004184, IRB#: 00001173. HSREB members involved in the research project do not participate in the review, discussion or decision.
Appendix B: Consent Form

School of Kinesiology and Health Studies
Queen's University

Kyra E. Pyke, Ph.D., Principle Investigator
Study performed in Room 400 D, Kinesiology Building, 28 Division

CONSENT FORM
FOR RESEARCH PROJECT ENTITLED:

Flow mediated dilation variability : reactive hyperaemia vs. exercise induced increases in shear stress

This is an important form. Please read it carefully. It tells you what you need to know about participation in this study. If you agree to take part in this study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. 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 laboratory session directed by Dr. Kyra Pyke and Darius Soo Lum to evaluate the variability of the measured arterial response to patterns of increased blood flow before and after listening to and concentrating on an audio recording. A student investigator will read through this consent form with you and describe the procedures in detail and answer any questions you may have. These procedures have been reviewed for ethical compliance by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

The purpose of this laboratory session is to determine how different forms of restful concentration influence vascular function.

Benefits For You: There are no direct benefits to you by participating in this study.

Description of Experiment and Risks:

There is a remote possibility that during your research activities you could come into contact with someone with COVID-19. If this highly unlikely event were to occur, we are required by the Public Health Unit to retain on file your email
address or phone number to share with them for contact tracing purposes.

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. An upper arm cuff will be placed on the right arm and will also be inflated periodically. This procedure is similar to what is experienced when blood pressure is taken at the doctor’s office.

  **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.
ELECTROENCEPHALOGRAPHY: Electrical activity in the brain is measured using electrodes attached to headband placed around the head. Headphones connected to a mobile device enables the concentrative/contemplative task to be carried out, while the headband measures electrical activity of the brain.

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

MINDFULNESS MEDITATION OR AUDIO LISTENING TASK: Mindfulness meditation involves concentration/one-point focus on a single object. Headphones will be placed in your ear so that instructions from the Healthy Minds Program App can be played from a mobile device. The meditation will involve concentration on the breath and will last for twenty minutes. Conversely, the audio listening task will involve listening to a TEDTalk for approximately twenty minutes.

RISKS: These techniques are non-invasive and pose no risk.

POST-MEDITATION QUESTIONNAIRE: The Toronto Mindfulness Scale is a thirteen-item questionnaire that assesses a person’s experiences after meditation. It takes approximately five minutes to rate each question on a scale from 0-4.

RISKS: This poses no risk

MEDITATION SCREENING QUESTIONNAIRE: The Mindful Attention Awareness Scale is a 15-item questionnaire that measures mindfulness across several general areas of life. It will take approximately five minutes to rate each question on a scale from 1-6.

RISKS: This poses no risk.

7 DAY PHYSICAL ACTIVITY RECALL: This is a questionnaire that will ask you to report your physical activity levels over the past 7 days.

RISKS: This poses no risk

Sleep Quality Scale: This is a scale upon which to rank your sleep quality over the last 7 days. Sleep quality can impact our vascular measures and Electroencephalography measures.

RISKS: This poses no risk

How long will it take?

On an initial visit you will be asked to lie down while we will use ultrasound to get
an image of the artery in your upper arm to make sure that we can get clear pictures. You will also be asked to fill out a medical screening form and answer some questions about physical activity. We will also measure your height and weight and blood pressure. These questions and measures will allow us to determine whether or not you meet the study inclusion and exclusion criteria. You will also be asked to fill out a questionnaire that assesses your level of mindful awareness. This visit will take approximately 20-30 min.

☐ Each of two experimental visits will take 90-120 min. While lying down, instrumentation for heart rate, blood pressure, blood flow/ultrasound, and EEG will occur. After a 20 min rest period, a cuff around your lower arm will be inflated while measurement of your upper arm brachial artery blood flow and diameter occurs. The cuff will be released after 5 min. You will then perform either mindfulness meditation or the other audio listening task for 20 min. Once complete you will answer a brief questionnaire about the experience. After, the cuff will be inflated again, and an identical measurement of the brachial artery will occur again.

☐ 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 try to get a good night’s sleep before the experimental visits, to not exercise for 24 hours, or drink alcohol or caffeine during the 12 hours prior to the experimental visits. Also, we ask that you have your last meal or snack a minimum of 6 hours preceding the experiments (fasting for at least the 6 hours prior to your visit start time – water consumption during this period is fine). Please consume a meal/snack that is typical for you and repeat the same meal/snack prior to each visit (we will ask you to describe this meal after experimental visit 1 and 2). Please avoid wearing of makeup, especially on the forehead, as these can damage the EEG headset. You should empty your bladder immediately prior to starting the test. When the testing is finished, we will have you sit in the laboratory for a short time to allow you to readjust to the upright posture. 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.
Safety Precautions:

- We will 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 is likely to allow you to quickly recover.

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 a $40 gift certificate to thank you for your participation in the three experimental visits of this study (a $20 certificate will be provided for each completed visit if you are unable to complete both).

Freedom to Withdraw

Your participation in this laboratory session is voluntary. You may refuse to participate or you may discontinue participation at any time without penalty and without affecting your future academic or other commitments in the School of Kinesiology and Health Studies.

Participant 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 206, Kinesiology building, 28 Division St.
Queen’s University, Kingston, ON, K7L 3N6
Tel: (613) 533-6000, ext, 79631

Student Investigators:
Darius Soo Lum d.soolum@queensu.ca;
Rm 400D, Kinesiology building 28 Division St.
Queen’s University, Kingston, ON, K7L 3N6; Tel: (613) 533-6000, ext, 79377

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;
Tel: 1-844-535-2988 email HSREB@queensu.ca

By signing this consent form, I am indicating that I agree to participate in this study.

______________________  _________________________
Participant Signature   Person obtaining consent Signature

______________________  _________________________
Participant Name (please print)  Person obtaining consent Name (please print)

______________________  _________________________
Date (day/month/year)   Date (day/month/year)
Appendix C: Medical Questionnaire for Research Study

School of Kinesiology and Health Studies

MEDICAL QUESTIONNAIRE FOR RESEARCH STUDY

Flow mediated dilation variability: reactive hyperaemia vs. exercise induced increases in shear stress
(consent v. 31; Impact of resting concentration)

Faculty Investigator:
Kyra E. Pyke, PhD, School of Kinesiology and Health Studies
Graduate student investigator: Darius Soo Lum

To the study participant: Please answer all questions of this form.
SECTION 1: PERSONAL DATA (please print)

Participant number: ____________________________________________

Year of birth: _________________________________________________

Date filling out form:___________________________________________

SECTION 2: MEDITATION EXPERIENCE

1. Have you attempted meditation practice previously? (Circle one): YES NO

2. If you answered yes to the question 1, do you meditate daily? (Circle one):

   YES

   NO

3. If you answered yes to question 1, please estimate:

   a. The number of hours in your lifetime you have spent meditating: ______ hrs
   b. The number of minutes you spend meditating in one session: _____ min

SECTION 2: MEDICAL HISTORY

1. Do you have, or have you ever had, problems with any of the following?

   Yes   No

   i. Heart or blood vessels       ____   ____

   (these might include but are not limited to: heart attack, stroke, heart murmurs, angina, coronary artery disease, high blood pressure, high cholesterol,
congenital heart disease, any heart operation, bleeding or clotting disorders)

ii. Nerves or brain

iii. Breathing or lungs

iv. Hormones, thyroid, or diabetes

v. Muscles, joints, or bones

vi. Other (please list) ________________________________________________

2. Please list the diagnosis or/or briefly describe any problems identified in #1

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________

Are you presently taking any medications? If yes, please list.

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________

____________________________________________________________________

3. Do you have any allergies to adhesive tape or latex?
11. Do you currently smoke? ______ yes/no

If previous smoker date of last cigarette__________________ (year, month)

For women only: Hormonal fluctuations during the menstrual cycle can impact vascular function. We need to schedule your two visits so that they are in the same phase of your menstrual cycle, specifically, days 1-5 of the menstrual phase.

Please answer the following questions (i - vi) regarding your menstrual cycle history:

i. Are you currently having menstrual periods?

   ___ No (skip rest of form).
   ___ Yes
   Date of the start of last menstrual period __________

ii. Have you given birth in the last 12 months?

   ___ No
   ___ Yes (skip rest of form).

iii. Are you currently taking oral or any other form of hormonal contraceptives (e.g. intrauterine devices)?

   ___ Yes Brand ______________________________
   ___ No

iv. Currently, what is the average duration of your menstrual cycle (A full cycle goes from the start of menstrual flow [menses] to the start of the next menstrual flow [menses])? The average cycle length is 28 days.

   ___ days.
v. How many days do you typically experience menstrual flow each cycle? Please check the correct response below:

- 0 days
- 1 day
- 2 days
- 3 days
- 4 days
- 5+ days

vi. Please estimate the number of menstrual cycles you have had in the past 12 months:

______ (number) menstrual cycles.

SECTION 3: DEMOGRAPHIC QUESTIONS

1. What was your assigned sex at birth? (Check ONE only):
   - Male
   - Female
   - Undetermined

2. Which best describes your current gender identity? (Check ONE only):
   - Male or primarily masculine
   - Female or primarily feminine
   - Indigenous or other cultural gender minority identity (e.g. Two-Spirit)
   - Non-binary
   - Not listed (e.g. gender fluid)

3. Which of the following BEST describes your racial or ethnic group? (Check ONE only):
   - White (European decent)
   - Black (African, Afro-Caribbean, African-American decent)
   - East/Southeast Asian (e.g. Chinese, Korean, Japanese, Cambodian, Vietnamese)
   - Latino (Latin American, Hispanic decent)
   - Middle Eastern (Arab, Persian, West Asian decent, e.g. Afghan, Egyptian, Iranian)
   - South Asian (South Asian decent e.g. East Indian, Pakistani, Bangladeshi,
Sri Lankan)
☐ Other (please briefly describe)______________________________
Appendix D: Mindfulness Questionnaires

Day-to-Day Experiences

Instructions: Below is a collection of statements about your everyday experience. Using the 1-6 scale below, please indicate how frequently or infrequently you currently have each experience. Please answer according to what really reflects your experience rather than what you think your experience should be. Please treat each item separately from every other item.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Almost Always</td>
<td>Very Frequently</td>
<td>Somewhat Frequently</td>
<td>Somewhat Infrequently</td>
<td>Very Infrequently</td>
<td>Almost Never</td>
</tr>
</tbody>
</table>

<p>| I could be experiencing some emotion and not be conscious of it until some time later. | 1 | 2 | 3 | 4 | 5 | 6 |
| I break or spill things because of carelessness, not paying attention, or thinking of something else. | 1 | 2 | 3 | 4 | 5 | 6 |
| I find it difficult to stay focused on what’s happening in the present. | 1 | 2 | 3 | 4 | 5 | 6 |
| I tend to walk quickly to get where I’m going without paying attention to what I experience along the way. | 1 | 2 | 3 | 4 | 5 | 6 |
| I tend not to notice feelings of physical tension or discomfort until they really grab my attention. | 1 | 2 | 3 | 4 | 5 | 6 |
| I forget a person’s name almost as soon as I’ve been told it for the first time. | 1 | 2 | 3 | 4 | 5 | 6 |
| It seems I am “running on automatic,” without much awareness of what I’m doing. | 1 | 2 | 3 | 4 | 5 | 6 |
| I rush through activities without being really attentive to them. | 1 | 2 | 3 | 4 | 5 | 6 |
| I get so focused on the goal I want to achieve that I lose touch with what I’m doing right now to get there. | 1 | 2 | 3 | 4 | 5 | 6 |
| I do jobs or tasks automatically, without being aware of what I’m doing. | 1 | 2 | 3 | 4 | 5 | 6 |
| I find myself listening to someone with one ear, doing something else at the same time. | 1 | 2 | 3 | 4 | 5 | 6 |</p>
<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>I drive places on ‘automatic pilot’ and then wonder why I went there.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I find myself preoccupied with the future or the past.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I find myself doing things without paying attention.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I snack without being aware that I’m eating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MAAS Scoring

To score the scale, simply compute a mean of the 15 items. Higher scores reflect higher levels of dispositional mindfulness.
**Toronto Mindfulness Scale**

*Note:* In contrast to the FFMS, the TMS scale is intended to measure "state-like" experiences *during* mediation, rather than "trait-like" cognitive dispositions that might reflect the cognitive consequences of meditative practice.

We are interested in what you just experienced. Below is a list of things that people sometimes experience. Please read each statement. Please indicate the extent to which you agree with each statement. In other words, how well does the statement describe what you just experienced, just now?

<table>
<thead>
<tr>
<th>Rate</th>
<th>Item #</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>1</td>
<td>I experienced myself as separate from my changing thoughts and feelings.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>I was more concerned with being open to my experiences than controlling or changing them.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>I was curious about what I might learn about myself by taking notice of how I react to certain thoughts, feelings, or sensations.</td>
</tr>
<tr>
<td></td>
<td>I experienced my thoughts more as events in my mind than as a necessarily accurate reflection of the way things ‘really’ are.</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>I was curious to see what my mind was up to from moment to moment.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>I was curious about each of the thoughts and feelings that I was having.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>I was receptive to observing unpleasant thoughts and feelings without interfering with them.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>I was more invested in just watching my experiences as they arose, than in figuring out what they could mean.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I approached each experience by trying to accept it, no matter whether it was pleasant or unpleasant.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>I remained curious about the nature of each experience as it arose.</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>I was aware of my thoughts and feelings without overidentifying with them.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>I was curious about my reactions to things.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>I was curious about what I might learn about myself by just taking notice of what my attention gets drawn to.</td>
<td></td>
</tr>
</tbody>
</table>
Scoring the Toronto Mindfulness Scale

The TMS measures two different aspects of mindfulness. In order to determine your score, calculate the following subtotals:

<table>
<thead>
<tr>
<th></th>
<th>Sum of Item #s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Curiosity</strong></td>
<td>((3 + 5 + 6 + 10 + 12 + 13))</td>
</tr>
<tr>
<td><strong>De-Centering</strong></td>
<td>((1 + 2 + 4 + 7 + 8 + 9 + 11))</td>
</tr>
</tbody>
</table>

*All items are positively keyed.*