



Michigan Technological University
Create the Future Digital Commons @ Michigan Tech

Dissertations, Master's Theses and Master's Reports - Open

Dissertations, Master's Theses and Master's Reports

2011

Omega-3 fatty acids and the neurovascular responses to mental stress in humans

Christopher Elmer Schwartz
Michigan Technological University

Follow this and additional works at: <https://digitalcommons.mtu.edu/etds>


 Part of the [Biology Commons](#)

Copyright 2011 Christopher Elmer Schwartz

Recommended Citation

Schwartz, Christopher Elmer, "Omega-3 fatty acids and the neurovascular responses to mental stress in humans", Dissertation, Michigan Technological University, 2011.
<https://digitalcommons.mtu.edu/etds/194>

Follow this and additional works at: <https://digitalcommons.mtu.edu/etds>

 Part of the [Biology Commons](#)

**OMEGA-3 FATTY ACIDS AND THE NEUROVASCULAR RESPONSES
TO MENTAL STRESS IN HUMANS**

By

Christopher Elmer Schwartz

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(Biological Sciences)

MICHIGAN TECHNOLOGICAL UNIVERSITY

2011

© 2011 Christopher E. Schwartz

This dissertation, "Omega-3 Fatty Acids and the Neurovascular Responses to Mental Stress in Humans," is hereby approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY IN BIOLOGICAL SCIENCES.

Department of Biological Sciences

Signatures:

Dissertation Advisor _____

Jason R. Carter, PhD

Department Chair _____

K. Michael Gibson, PhD

Date _____

Table of Contents

List of Figures.....	vi
List of Tables.....	vii
Preface.....	viii
Acknowledgements	ix
Abbreviations.....	x
Abstract	xi
1. Chapter 1	1
1.1 Hypertension	1
1.2 Ways to Assess Autonomic Activity	3
1.2.1 Hemodynamic Measurements	3
1.2.2 Heart Rate Variability	4
1.2.3 Norepinephrine – Urine and Plasma	6
1.2.4 Norepinephrine – Regional Spillover	6
1.2.5 Microneurography	7
1.3 Neural Responses to Mental Stress	9
1.4 Cardiovascular Responses to Mental Stress.....	10
1.5 Mental Stress and Cardiovascular Disease	12
1.6 Treatment of Hypertension	13
1.7 Polyunsaturated Fatty Acids	14
1.8 Summary and Hypothesis.....	17
2. Chapter 2	19
2.1 Introduction	19
2.2 Methods	20
2.2.1 Subjects	20
2.2.2 Experimental Design	20

2.2.3 Measurements	21
2.2.4 Data Analysis	22
2.2.5 Statistical Analysis	22
2.3 Results	23
2.4 Discussion	28
2.5 Perspectives	31
2.6 Acknowledgments.....	31
3. Chapter 3	32
3.1 Introduction	32
3.2 Methods	34
3.2.1 Subjects.....	34
3.2.2 Experimental Protocol	34
3.2.3 Measurements	35
3.2.4 Data Analysis.....	37
3.2.5 Statistical Analysis	37
3.3 Results	38
3.3.1 Resting Baseline Measurements for Normotensive and Prehypertensive Subjects	38
3.3.2 Resting Measurements for Combined Non-Hypertensive Subjects	41
3.3.3 Hemodynamic Responses to Mental Stress in Normotensive and Prehypertensive Subjects	43
3.3.4 Sympathetic Nerve Responses to Mental Stress in Normotensive and Prehypertensive Subjects	46
3.3.5 Peripheral Vascular Responses to Mental Stress in Normotensive and Prehypertensive Subjects	47
3.3.6 Responses to Mental Stress for Combined Non-Hypertensive Subjects	51
3.4 Discussion	56

3.4.1 Resting Neurovascular Responses to Omega-3 Supplementation	56
3.4.2 Neurovascular Responses to Mental Stress Following Omega-3 Supplementation.....	57
4. Chapter 4	61
4.1 Summary	61
4.2 Future Directions – Nitric Oxide	62
4.3 Conclusion.....	63
References.....	64
Appendix A Raw Data Study 1	78
Appendix B Summary of Statistics Study 1	108
Appendix C Raw Data Study 2	115
Appendix D Summary of Statistics Study 2	145
Appendix E Permissions.....	173

List of Figures

Figure 1.1 A representation of two cardiac cycles.....	5
Figure 1.2 A representative neurogram and corresponding blood pressure	8
Figure 2.1 Hemodynamic responses to mental stress Study 1	25
Figure 2.2 MSNA responses to mental stress Study 1	25
Figure 2.3 Forearm vascular responses to mental stress Study 1	26
Figure 2.4 Calf vascular responses to mental stress Study 1	27
Figure 3.1 Experimental protocol for Study 2	35
Figure 3.2 Resting MSNA responses in PHT and NT Study 2.....	42
Figure 3.3 Linear regressions of MSNA-BP relationship Study 2	43
Figure 3.4 Linear regressions of MSNA-HR relationship Study 2	44
Figure 3.5 Blood pressure responses to mental stress Study 2	45
Figure 3.6 Heart rate responses to mental stress Study 2.....	46
Figure 3.7 Forearm vascular responses to mental stress Study 2	49
Figure 3.8 Calf vascular responses to mental stress Study 2.....	50
Figure 3.9 Blood pressure responses to mental stress in combined groups Study 2.....	52
Figure 3.10 MSNA responses to mental stress in combined groups Study 2	53
Figure 3.11 Total MSNA responses to mental stress in combined groups Study 2	53
Figure 3.12 Forearm vascular responses to mental stress in combined groups Study 2	54
Figure 3.13 Calf vascular responses to mental stress in combined groups Study 2	55

List of Tables

Table 1.1 Examples of saturated and unsaturated fatty acids	16
Table 2.1 Resting hemodynamics for subjects in Study 1	23
Table 2.2 Baseline values for subjects in Study 1	24
Table 3.1 Resting hemodynamics in NT group for Study 2.....	39
Table 3.2 Resting hemodynamics in PHT group for Study 2	39
Table 3.3 Baseline hemodynamics in NT group for Study 2	40
Table 3.4 Baseline hemodynamics in PHT group for Study 2.....	40
Table 3.5 Baseline values for combined groups for Study 2.....	41
Table 3.6 Changes in MSNA during mental stress in normotensives Study 2.....	47
Table 3.7 Changes in MSNA during mental stress in prehypertensives Study 2.....	48
Tables A.1 – A.30 Tables of raw data from Study 1 for all subjects	78-107
Tables B.1 – B.31 Tables of statistical analyses from Study 1 for all subjects.....	108-114
Tables C.1 – C.28 Tables of raw data from Study 2 for all subjects	115-144
Tables D.1 – D.100 Tables of statistical analyses from Study 2 for all subjects....	145-172

Preface

The article presented in chapter 2 of this thesis is **used with permission from the American Physiological Society**, as a collaborative work with co-authors Dr. John Durocher, Dr. Jason Carter. All data collection was conducted in the laboratory of Dr. Carter at Michigan Technological University. My personal contribution to the article included recruitment, orientation, conduction, data analysis and statistical analysis of the study. I had a significant role in the authorship of the Introduction, Methods, and Results sections, with a smaller but significant contribution to the Discussion section.

Acknowledgements

This dissertation is a testament not only to perseverance and hard work, but also to the constant support, advice and assistance I have received from many people along the way. First and foremost, I must thank my Lord Jesus Christ for the grace he has bestowed upon me. Through thick and thin, He is my rock. Second, I deeply thank and appreciate the mentorship and guidance I have received from my advisor, Dr. Jason Carter. The patience he has shown me through particularly through the process of composing this thesis, but also during my time working as his research assistant, was second to none. I will continue carry on the skills and knowledge he has passed down to me wherever my career takes me. Third, I thank my committee members for all their patience and guidance.

I would also like to thank my family; Dad, Mom, Becky, Joe, Eric, David, Johanna, Emily, Andrew, Jacob, Mary, Elsa, Carrie, all my brothers/sisters-in-law, and my nieces and nephews (especially my nieces and nephews) for your never-ceasing love and support. The Lord is my rock, but you all are the ground on top of that rock.

Thank you to my friends for all your advice and encouragement. Thank you to Rick for always being there, supporting me, and putting up with me during our endless hours of training. A special thanks goes to Agustin, Lilia and Leila, as well as Israel, Judy, Manu, Micah and Ty, and Matt, Heather, Kaisa, Ava and Lewis. I simply would not be the same person I am today without you. Thank you to Sikhu for always lending an ear, and helping me push through to the end. Thank you to Louisa and Jesse for letting me stay with them over the summer, and always providing awesome training days. Stephanie, G, Raman, Tejal, Jim, Andi, Mark, Lauren, Rob, Shannon, Joel, Valerie, Sinan, Ana, Corey, Rachel, and all my friends, thank you for being awesome!

I wish to thank my friends and fellow students in our lab. I don't know how I could repay you all for the help you have given me in completing this thesis. John Durocher, John Lawrence, Jenna Klein, Sarah Stream, Huan Yang, Robert Larson, Kristen Reed, Michelle King, Ashley Yenior, and Jen Witting, thank you for all the help, particularly over the last few months. I wish every graduate student could have the support that you all gave to me. Finally, to Terry Anderson, a deep thank you for everything you have done, and for making this time as easy as possible. I appreciate you!

List of Abbreviations

ALA.	Alpha-linolenic acid
BP.	Blood pressure
CBF.	Calf blood flow
CVD.	Cardiovascular disease
CVR.	Calf vascular resistance
CVC.	Calf vascular conductance
DAP.	Diastolic arterial pressure
DHA.	Docosahexaenoic Acid
EPA.	Eicosapentaenoic Acid
FBF.	Forearm blood flow
FVR.	Forearm vascular resistance
FVC.	Forearm vascular conductance
HF.	High frequency component of heart rate variability
HR.	Heart rate
HRV.	Heart rate variability
LF.	Low frequency component of heart rate variability
MAP.	Mean arterial pressure
MSNA.	Muscle sympathetic nerve activity
NE.	Norepinephrine
NT.	Normotension
NO.	Nitric Oxide
PHT.	Prehypertension
PUFA.	Polyunsaturated fatty Acid
RRI.	R-R Interval
SAP.	Systolic arterial pressure

Abstract

Hypertension is the most prevalent form of cardiovascular disease (CVD) in the world, and is known to increase the risk for developing other diseases. Recently, the American Heart Association introduced a new classification of blood pressure, prehypertension (PHT). The criteria for PHT include a systolic of 120-139 mmHg and/or a diastolic blood pressure of 80-89 mmHg. It has been observed that individuals with PHT have a higher risk of developing hypertension later in life. Therefore, it is important to understand the mechanisms contributing to PHT in order to possibly prevent hypertension. Omega-3 fatty acids found in fish oils have been suggested as a means of lowering blood pressure. However, little is known on the effects of fish oil in PHT humans. Therefore we conducted two studies. In **Study 1** we investigated PHT and normotensive (NT) individuals during a mental stress task. Mental stress is known to contribute to the development of hypertension. In **Study 2** PHT and NT subjects were placed in an eight week double-blind placebo controlled study in which subjects consumed 9g/day of either fish oil or placebo (olive oil) in addition to their regular diets. Subjects were tested during a resting baseline (seated and supine), 5 minutes of a mental stress task, and 5 minutes of recovery both pre and post supplementation. We measured arterial pressure (AP), heart rate (HR), muscle sympathetic nerve activity (MSNA), and forearm and calf vascular responses. In **Study 1** PHT demonstrated augmented AP and blunted vasodilation during mental stress, but MSNA did not change. In **Study 2**, fish oil did not directly influence blood pressure, MSNA or vascular responses to mental stress. However, it became clear that fish oil had an effect on some but not all subjects (both PHT and NT). Specifically, subjects who experienced a reduced blood pressure response to fish oil also demonstrated a decrease in MSNA and HR during mental stress. Collectively, the investigations in this dissertation had several novel findings. First, PHT individuals demonstrate an augmented pressor and blunted vascular response to mental stress, a response that may be contributing to the development of hypertension. Second, fish oil does not consistently lower resting blood pressure, but the interindividual responses may be related to MSNA. Third, fish oil attenuated the heart rate and MSNA responses and to mental stress in both PHT and NT. In conclusion, we found that there are both similarities and differences in the way PHT and NT individuals respond to mental stress and fish oil.

Chapter 1

Literature Review

1.1 Hypertension

Over 80 million Americans are diagnosed with at least one form of cardiovascular disease (CVD). The most prevalent form of CVD is hypertension, affecting 76.4 million Americans and as many as 1 billion people worldwide (Chobanian *et al.*, 2003). Cardiovascular related mortality, such as ischemic heart disease and stroke, increase dramatically when a comorbidity of high blood pressure is present. Additionally, 7.1 million people die from hypertension related causes annually (Brundtland, 2002; Chobanian *et al.*, 2003).

The concept that the blood circulates in the body was first suggested in the early 1600s by William Harvey (Harvey, 1628). At the time this was a giant step forward in the understanding of the human body and how it functions. Nearly 100 years later the first measurements of blood pressure were taken by Stephen Hales (Booth, 1977), who measured blood pressure from an artery of a horse using a nine-foot glass tube. The technique of analyzing blood pressure was later refined and modified by Jean Poiseuille (1828) and Carl Ludwig (1847) to essentially the method we use today (Norris, 1917). However, even before measurements of arterial pressure were conducted there seemed to be an understanding of a type of “hard pulse disease,” which we now know as hypertension (Esunge, 1991).

The classification of hypertension is considered to be a systolic arterial blood pressure ≥ 140 mmHg and/or a diastolic arterial blood pressure ≥ 90 mmHg (Chobanian *et al.*, 2003). Keith *et al.* (1974) found that there was a direct relationship with increased blood pressure and incidence of cardiovascular disease. Furthermore, risk of developing cardiovascular disease increases 2-fold when systolic arterial blood pressure is 130-139 mmHg and/or diastolic arterial blood pressure is 85-89 mmHg (Vasan *et al.*, 2001). These findings initiated the introduction of a new classification known as prehypertension. Having a systolic blood pressure of 120-139 mmHg and/or diastolic blood pressure of 80-89 mmHg is now classified as “prehypertensive.” Prehypertension is associated with an increased risk of developing hypertension later on in life (Vasan *et al.*, 2001; Moreira *et al.*, 2008). The reasons for this increased risk remain unclear.

Arterial pressure is closely regulated by the nervous system. Control of whole body arterial blood pressure is done through a negative feedback mechanism known as the arterial baroreflex. Modified stretch receptors located in the aortic arch, carotid sinuses and the cardiopulmonary blood vessels detect elastic changes in the arterial wall. Once a stretch is detected the baroreceptors send afferent nerve signals back to the area of the brain known as the medulla, more specifically the nucleus tractus solitarius. A reflex mechanism is then triggered sending efferent sympathetic and parasympathetic nerve signals down to the cardiovascular system to either increase or decrease blood pressure, respectively.

It has been recognized that arterial baroreceptors reset during hypertension. This effect is seen in studies comparing arterial blood pressure oscillations in normotensive and hypertensive subjects. Both groups responded similarly to vasoactive drugs, with the hypertensive group operating at a higher blood pressure compared to normotensive controls (Wallin *et al.*, 1973). Mechanisms behind the resetting of the baroreflex remain in question, but overactive sympathetic nerve activity remains a primary suspect.

With the introduction of new techniques to test sympathetic nerve activity (i.e. microneurography and norepinephrine (NE) spillover) studies of borderline and mild hypertensive individuals suggest that augmented sympathetic nerve activity contributed to hypertension (FitzGerald *et al.*, 1981; Anderson *et al.*, 1989). Urine and plasma NE have also been shown to be elevated in established hypertension (Esler & Nestel, 1973; Goldstein, 1983). Collectively, these findings provided important insight into the differences between hypertension and normal healthy humans. Similar discrepancies began to appear between these populations during physiological and psychological stimuli.

Sympathetic nerve activity increases during a mental challenge in most individuals (Anderson *et al.*, 1987; Tidgren & Hjemdahl, 1989; Wallin *et al.*, 1992; Carter *et al.*, 2005; Carter & Ray, 2009), and the concurrent increase in blood pressure and nerve activity does not follow the classic inverse relationship between sympathetic vasoconstrictor activity and arterial pressure seen at rest (Wallin *et al.*, 1973; Durocher *et al.*, 2011). This altered neurovascular responses to stress has been suggested as a possible mechanism contributing to the development of hypertension (Esler *et al.*, 2008).

Therefore, understanding how to assess autonomic nerve activity, and how neural and cardiovascular respond to mental stress is clinically relevant.

1.2 Measuring Autonomic Nerve Activity

1.2.1 Hemodynamic Measurements

Perhaps the oldest and most commonly used assessment of sympathetic and parasympathetic activity is done by examining changes in hemodynamic measurements (i.e. arterial pressure and heart rate). The autonomic nervous system innervates the heart and the blood vessels affecting both heart rate and blood pressure. During physiological and psychological stress, sympathetic nerves increase heart rate and blood pressure (Barcroft, 1946). For example, during exercise sympathetic nerve activation causes heart rate to increase and blood vessels in non-active areas (i.e., gut, kidneys, etc.) to constrict resulting in an increase in blood pressure (Rushmer *et al.*, 1959). Sympathetic nerves innervate the heart causing an increase in heart rate (measured in beats per minute), stroke volume (i.e. the amount of blood per minute), and cardiac output (i.e. the amount of blood that exits the heart per minute). Constriction of the vascular wall increases total peripheral resistance which increases blood pressure (Shepherd, 1987). Arterial pressure is a measure of the relationship between cardiac output and total peripheral resistance, the equation of which is shown below (Heymans, 1958):

$$\text{Mean Arterial Pressure} = \text{Cardiac Output} \times \text{Total Peripheral Resistance}$$

This direct relationship allows for analysis of changes in pressure as direct changes in the cardiovascular system, which in turn infers changes in autonomic control. In such a way, an increase in heart rate and blood pressure is an indication of sympathetic nerve stimulation and parasympathetic withdrawal, and likewise a decrease represents parasympathetic activation and sympathetic withdrawal. Therefore, monitoring changes in hemodynamic measurements gives us an indirect, but informative, method of analyzing autonomic function.

There are several positive aspects of hemodynamic assessment of autonomic nerve activity. These measurements are non-invasive and easy to conduct. Heart rate is

easily measured using electrocardiography, and requires relatively inexpensive equipment to acquire and analyze. Similarly, blood pressure can be measured using a simple, inexpensive sphygmomanometer. Modern methods for recording these variables can now be done virtually anywhere, including remotely so subjects do not have to be confined to a laboratory setting, and are free to move about while their hemodynamics are being monitored continuously. However, there are limitations to these measurements. One limitation is that there is a high amount of variability between subjects. Hemodynamics are influenced by many factors (i.e. activity, time of day, anxiety, etc.), and reproducibility is not always accurate because of these conditions (Parati *et al.*, 1988). Another limitation is that hemodynamic are indirect measurements of autonomic activity, and subject to the methods used to stimulate them. These limitations make it difficult to support hemodynamic measurements as a sole measure of autonomic activity. However, taken in combination with other measurements, hemodynamics can provide an important picture of whole body peripheral nerve function.

1.2.2 Heart Rate Variability – Spectral Analysis

Heart rate, as stated above, is an important hemodynamic measurement that indirectly depicts autonomic nerve function. The sympathetic and parasympathetic branches of the peripheral nervous system innervate the heart, thus affecting the increase and decrease of heart rate, respectively. This reciprocal relationship between sympathetic and parasympathetic branches is known as sympathovagal balance (Malliani *et al.*, 1991). Early analysis of heart rate variability looked primarily at the individual variability between resting heart rate measurements (Malmo & Shagass, 1949). However, more recent analyses of the neural control of heart rate has been made possible with more advanced computer and mathematic capabilities using a method known as power spectral analysis of heart rate variability.

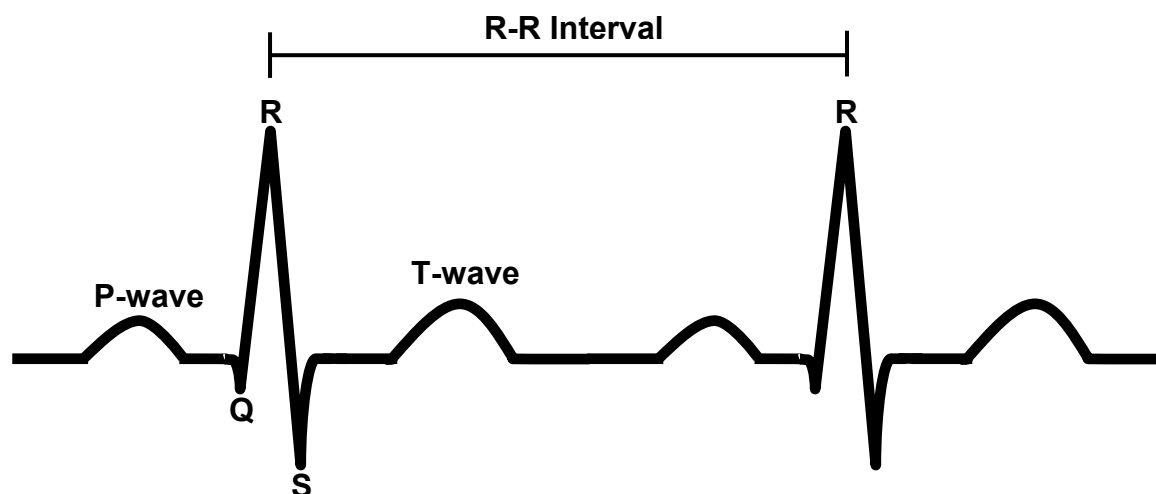


Figure 1.1. A representation of two cardiac cycles as seen in a typical electrocardiogram recording. The P-wave represents atrial depolarization, the QRS complex represents ventricular depolarization, and the T-wave represents ventricular repolarization. The time-distance, in milliseconds, between consecutive R-waves is known as the R-R Interval (RRI).

This technique utilizes mathematical equations to analyze changes in the R-R intervals (RRI) of each cardiac cycle. A representative model of two cardiac cycles is depicted in figure 1.1. Analysis of oscillations in RRIs throughout the time series can be reported as frequency and amplitude, and are compared to determine strength of autonomic activity (Pagani *et al.*, 1986; Malliani *et al.*, 1991). For example, an increase in the RRI would suggest influence from the vagus (parasympathetic) nerve, and a decrease in RRI would signify sympathetic control over heart rate. High frequency (HF) oscillations (~ 0.25 Hz) are representative of primarily parasympathetic nerve activity from the vagus nerve. Low frequency (LF) oscillations (~ 0.1 Hz) demonstrate both sympathetic and parasympathetic activity (Pomeranz *et al.*, 1985; Pagani *et al.*, 1986). The relationship between both peripheral nervous system branches on heart rate control is further represented by the ratio between the two frequencies, known as the LF/HF ratio (Pagani *et al.*, 1986). Power spectral analysis can serve as an important tool in interpreting autonomic control of cardiovascular function.

There are several limitations associated with heart rate variability. Similar to standard hemodynamics, power spectral analysis is bound to multiple external factors affecting heart rate and blood pressure. Additionally, there is speculation as to the accuracy of the use of computational analysis as a means of measuring autonomic

activity instead of direct recordings (Eckberg, 1997). Nevertheless, power spectral analysis of heart rate variability has given us valuable insight into the neural mechanisms contributing to cardiovascular function, and when used in conjunction with other techniques can provide a more complete measure of neural-cardiovascular interactions.

1.2.3 Norepinephrine – Urine and Plasma

Measurements of catecholamine levels in blood (i.e. plasma NE and epinephrine) and urine are commonly used to assess sympathetic activity. Early research in cats demonstrated a correlation with sympathetic nerve firing and NE release into the blood (Brown & Gillespie, 1957). Engelman et al. (1970) performed an enzymatic assay to analyze catecholamines in human plasma and urine, and were able to isolate circulating norepinephrine. The technique provided a more specific method of assessing sympathetic activity. Sympathetic nerves primarily excrete NE as their neurotransmitter. Molecules exit the pre-synaptic neuron and enter the synaptic space where NE can either bind to the post-synaptic neuron, be metabolized by catechol-O-methyltransferase, be absorbed into the circulation, or be taken back into the pre-synaptic neuron (Hertting, 1964). Any amount of NE not used or recycled is dissipated and taken up into the blood stream, and further filtered by the kidneys into the urine. It is in these two areas (plasma and urine) that NE can provide a measure of whole body sympathetic activity (Hjemdahl, 1984). However, the use of plasma or blood samples only provides a small, singular measurement of overall activity. Additionally, a high level of plasma NE does not always indicate a high level of nerve activity (Goldstein 2003). Acute sympathetic measurements are not measurable with this method, and the amount of NE absorbed into the blood is a small fraction (5-10%) of total neurotransmitter excreted at the nerve synapses (Grassi & Esler, 1999). Though plasma and urine NE measurements give us an idea of whole body sympathetic activation, the picture is not complete, and does not provide a means of continual measurement of neural activity.

1.2.4 Norepinephrine – Regional Spillover

The limitations that plasma and urine NE measurements pose brought about the introduction and implementation of a technique for measuring plasma NE kinetics, also

known as NE spillover rate (Esler *et al.*, 1989). This process uses the infusion of radioactive-labeled NE to measure the appearance rate of NE in the plasma. The spillover is acquired by measuring the relationship between the radio-labeled NE infusion rate and the appearance of radioactive NE in the plasma (Esler *et al.*, 1979; Esler *et al.*, 1989). The benefit of NE spillover compared to a simple plasma sampling is that through use of arterial or venous catheter, plasma uptake of NE can be measured in real-time at specific regions not otherwise accessible (i.e. cardiac and renal regions) (Esler *et al.*, 1984a). The measurement of NE kinetics is currently the only technique used in humans that can measure activity in deep, region specific tissue beds. However, NE spillover has its drawbacks. Spillover measures overall sympathetic outflow via NE measurements, but the exact source of the NE is unknown. Sympathetic nerves release the neurotransmitter NE, but this same molecule is released by the adrenal gland as well, and the spillover technique is not able to determine the source of NE (Esler *et al.*, 1984a). Furthermore, this technique is not a direct measurement of sympathetic nerve activity. Although there are drawbacks and it is only used in a few laboratories throughout the world, NE spillover is recognized as a robust and superior method of measuring sympathetic tone.

1.2.5 Microneurography

A major advancement in our ability to measure sympathetic nerve activity occurred in the late 1960s. Direct peripheral muscle nerves were successfully recorded in humans by using a tungsten needle with a microelectrode at the tip, and inserted into a nerve fascicle to obtain multi-unit discharge of activity (Hagbarth & Vallbo, 1967; Vallbo & Hagbarth, 1967; Hagbarth & Vallbo, 1969). This technique, termed microneurography, was refined and found to be a very useful tool in measuring sympathetic nerve activity (Figure 1.2) (Delius *et al.*, 1973). Moreover, microneurography is currently the only direct method of measuring sympathetic nerve activity in humans (Grassi & Esler, 1999). The microneurographic technique measures post-ganglionic multi-fiber efferent sympathetic nerve activity to skeletal muscle beds, referred to as muscle sympathetic nerve activity (MSNA) (Vallbo *et al.*, 2004). MSNA is typically measured as integrated bursts of activity per minute. MSNA bursts are coupled to the cardiac cycle, and if a burst of activity is going to occur, it will do so in one cardiac cycle

(Delius *et al.*, 1972). Therefore, reporting MSNA as bursts per 100 heartbeats is also an accepted quantification. Total MSNA activity is another measurement commonly reported. Total MSNA takes into account not only the burst activity per minute but also the amplitude and area underneath each integrated burst (Sundlof & Wallin, 1978). Because MSNA is most commonly measured as multifiber nerve recordings, multiple integrated sympathetic firings demonstrate varying amplitudes of firing strength. To account for this, analysis of total activity is employed using a baseline normalization procedure.

Microneurography has several strong attributes. First, it is currently the only direct method of assessing sympathetic neural activity in humans. Second it correlates strongly with other measures of autonomic activity such as the NE spillover technique (Wallin *et al.*, 1981). Another advantage is that it allows for continuous measurement of

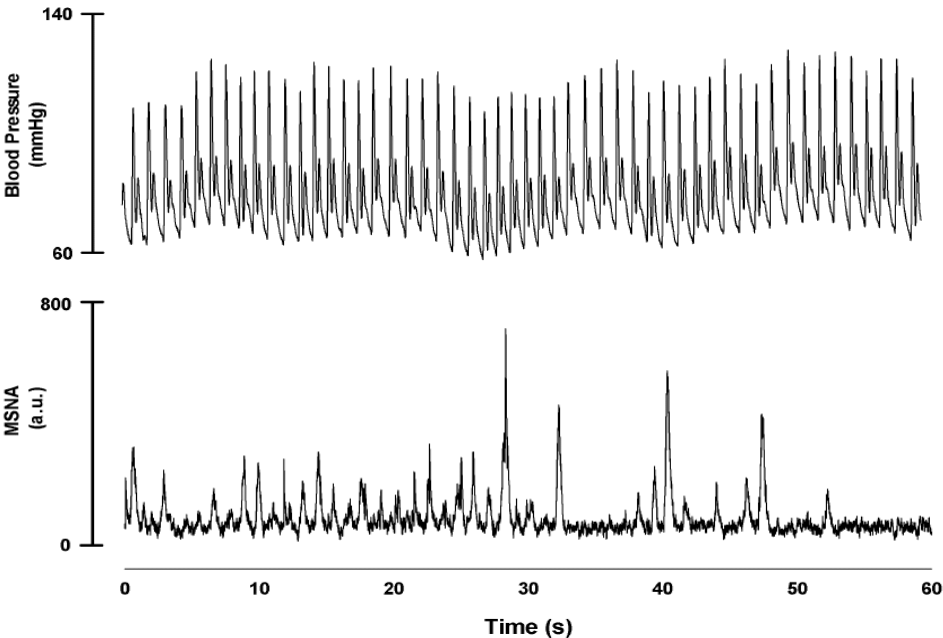


Figure 1.2. A representative neurogram and the corresponding blood pressure for one minute of baseline. Notice the relatively close relationship between oscillations in blood pressure and nerve activity. MSNA, muscle sympathetic nerve activity; a.u., arbitrary units

activity during different interventions. Techniques such as plasma/urine NE measurements only take isolated samples, thus providing a non-continuous measurement of sympathetic activity. However, there are disadvantages associated with microneurography. The technique is mildly invasive and can only be done by trained individuals. It is highly variable between individuals, though it remains highly reproducible when tested over a period of time, even up to years later (Sundlof & Wallin, 1977). Another disadvantage is that the technique is sensitive to movement, and the electrode can become dislodged easily. Therefore, it is only possible to use microneurography in a laboratory setting, with subjects typically in the supine or seated positions. Despite these limitations, microneurography, along with norepinephrine spillover, remains the “gold-standard” for assessing sympathetic nerve activity.

1.3 Neural Responses to Mental Stress

The advancement in our ability to measure autonomic nerve activity has allowed us to further investigate the effect of psychological stress in humans. Mental stress consistently increases heart rate, blood pressure and forearm blood flow, which are closely linked with the sympathetic and parasympathetic nervous systems (Blair *et al.*, 1959; Barcroft *et al.*, 1960). Simultaneous neuroendocrine responses can also be observed during this cardiovascular response to mental stress (Hjemdahl *et al.*, 1984). Ultimately, markers for established psychological distress were identified as an increase in heart rate and force contraction, skeletal muscle vasodilation, and increased circulating catecholamines (Herd, 1991).

Epinephrine is strongly linked to the mental stress response. When compared to non-psychological stimuli, such as the cold pressor test, mental stress causes a greater increase in heart rate, skeletal muscle vasodilation and increased circulating epinephrine. This is compared to no increase in epinephrine during a physical stressor like the cold pressor test (LeBlanc *et al.*, 1979). Notably, norepinephrine levels were greater during cold pressor test compared to mental stress. Because norepinephrine is closely linked with the sympathetic nervous system, these findings provided early evidence that a combination of circulating catecholamine and sympathetic nerve responses were causing the physiological changes during mental stress.

Circulating plasma norepinephrine has been closely linked with sympathetic nerve activity (Wallin, 1984). Today, the most common means of measuring sympathetic nerve activity include plasma or urine norepinephrine sampling, radioactive labeled norepinephrine spillover from cardiac and renal sympathetic beds, and microneurographic measurements of MSNA from the peroneal nerve. Plasma norepinephrine measurements have been shown to increase during mental stress (Dimsdale & Moss, 1980; Jones *et al.*, 1996). However, in one study mental stress increased plasma norepinephrine in older, but not young, subjects (Ng *et al.*, 1994). Norepinephrine spillover has also been shown to increase during a mental challenge (Goldstein *et al.*, 1987; Lindqvist *et al.*, 1993; Wilkinson *et al.*, 1998). MSNA recordings during mental stress typically demonstrate increases in sympathetic nerve activity (Anderson *et al.*, 1987; Hjemdahl *et al.*, 1989; Wallin *et al.*, 1992; Carter *et al.*, 2005; Carter & Ray, 2009), but recent work highlights substantial variability regarding the MSNA responses to mental stress (Carter & Ray, 2009).

1.4 Cardiovascular Responses to Mental Stress

The reaction of the body to stress is a compensatory mechanism. The human body is placed under stress constantly throughout any given day. Psychological and emotional stressors, in particular, are known to place a great amount of strain on both mental and physiological parameters in the human body. In “The Expression of the Emotions in Man and Animals” Charles Darwin describes a general state of expression (Darwin, 1886). The common response in animals is an activated nervous system, muscle trembles, a combination of emotions such as rage, joy and terror. Further observation of fear and astonishment resulted in multiple changes to a biological system, including increases in heart rate, pale skin, muscle trembles, widely open eyes and mouth (Darwin, 1886). Years later, Walter Cannon would describe these same responses as an “emotional experience” (Cannon, 1914). Additionally, Cannon and others discovered specific physiological changes that occur during emotional stimuli, most notably the release of adrenaline (i.e. epinephrine) from the adrenal glands (Newton, 1930). He also noted a specific interaction with the physiological system and “sympathin” (Cannon, 1933). We now refer to sympathin as noradrenaline (i.e. norepinephrine), the main neurotransmitter released from sympathetic neurons. This

hormone response causes a response similar to that directed by nervous system, which includes pupil dilation, blood vessel constriction and rapid heart rate (Cannon, 1914).

Cannon introduced the term “fight or flight” to describe the sympathetic nervous system’s reaction to stressors (Cannon, 1927). These findings introduced an important idea to our current views of multiple physiological systems’ responses to emotional stress. The autonomic nervous system is comprised of the parasympathetic and sympathetic nervous systems, and is a primary controller of the cardiovascular system. However, as Cannon and others discovered, there is an interaction in adrenergic activity from the nervous and endocrine systems (Cannon, 1933). More specifically, the autonomic nervous system and adrenal (medulla and cortex) responses work in parallel. However, Hans Selye is widely credited with highlighting a clinical reason for studying neuroendocrine responses to psychological stress.

The “General Adaptation Syndrome” was first proposed by Hans Selye (Selye, 1936). Selye discovered, by accident, that psychological stress to rats resulted in specific physiological responses, specifically an enlargement of the adrenal glands and increased ulcers. This sparked a whole new line of “stress” research. Investigations into the general response to different stressors such as cold, injury, exercise, drugs and others were conducted producing what Selye termed the general adaptation syndrome. Responses to many different types of stress results in “systemic damage”, and originate in the hypothalamus which can influence processes throughout the body including the adrenal glands, heart, and vascular system (Selye & Fortier, 1950).

In years to follow, Brod et al. (1959) reported that a mental stress task such as mental arithmetic caused similar physiological response as emotional stress or fear. Notably heart rate and blood pressure increased, and interestingly blood flow increased to peripheral muscles and decreased in the renal vascular bed. Forearm vasodilation during mental stress has been observed repeatedly in multiple studies (Barcroft & Edholm, 1945; Blair *et al.*, 1959; Rusch *et al.*, 1981; Dietz *et al.*, 1994; Carter *et al.*, 2005) and has led to the description of a classic “defense reaction” (Herd, 1991).

Peripheral blood flow in forearm and calf muscular beds are common measurements during a mental challenge. When blood flow to the peripheral muscles was studied during post-hemorrhagic fainting, there was a marked vasodilation at which the point when faint occurred (Barcroft & Edholm, 1945). Interestingly, it has been

observed that fainting also occurs during emotional distress, and studies began to incorporate mental and emotional stress as a means of analyzing cardiovascular responses during faint (Roddie, 1977).

Forearm vasodilation consistently increases during mental and emotional stress (Blair *et al.*, 1959; Barcroft *et al.*, 1960; Rusch *et al.*, 1981; Dietz *et al.*, 1994; Kuipers *et al.*, 2008). Calf blood flow has been found to increase in some studies (Blair *et al.*, 1959; Kuipers *et al.*, 2008), but not all (Rusch *et al.*, 1981). The proposed mechanisms contributing to vasodilation in the limbs include sympathetic mediated adrenergic responses, nitric oxide (NO) release from the endothelium, and circulating catecholamines.

The response to mental and emotional stress observed in these investigations fits with Cannon's "fight or flight" description. However, it has become clear that mental stress reactivity is more complicated than simply preparing the body for fight or flight. Observing and analyzing this stress response has become crucial to our understanding of how mental and emotional stress contributes to the risk of cardiovascular disease. With the improvements in technology it has become easier to measure changes in heart rate, blood flow, and autonomic nerve activity. This has been crucial in our understanding of the how neurological and humoral responses to stress influence health in humans.

1.5 Mental Stress and Cardiovascular Disease

It has been noted that an acute mental stress can adversely affect cardiovascular health. One example of this is seen in the increased number of cardiac related deaths following a traumatic event such as a major earthquake (Leor *et al.*, 1996). Additionally, electrocardiogram recordings during an earthquake event have shown that heart rate significantly increased in patients. Spectral analysis of this response suggested an increased sympathetic nerve response and reduced parasympathetic tone (Huang *et al.*, 2001). Furthermore, mental and emotional stress are closely linked with cardiovascular diseases such as myocardial ischemia (Deanfield *et al.*, 1984), endothelial dysfunction (Spieker *et al.*, 2002), atherosclerosis (Lynch *et al.*, 1997) and hypertension (Esler *et al.*, 2003). Perhaps the most well known study to linking stress and risk for cardiovascular disease was performed by Timio *et al.* (1988). They found that cloistered nuns, secluded

from society and living a very controlled and non-stressful lifestyle, did not experience the typical rise in blood pressure often seen with increasing age (Timio *et al.*, 1988). Further, it has been suggested that chronic mental stress leads to a rise in blood pressure over time (Esler *et al.*, 2008). The mechanisms by which mental stress affects cardiovascular disease are not fully understood, but a clear link exists, particularly in hypertension.

1.6 Treatment of Hypertension

Hypertension has been linked to a number of cardiovascular-related deaths. Therefore, early treatment and/or prevention of high blood pressure has become a primary focus in clinical care. Treating hypertension through pharmacological or non-pharmacological methods can reduce the risk of stroke, myocardial infarction, and heart failure (Neal *et al.*, 2000). Current treatment of hypertension is primarily focused on pharmacological intervention. Common types of anti-hypertensive medications include diuretics, ACE inhibitors, α and β -blockers and calcium channel blockers (Epstein, 2010). Most patients require at least two medications to lower their blood pressure (Cushman *et al.*, 2002; Epstein, 2010). It is evident that cost of treatment for hypertension can escalate quickly. The American Heart Association recently projected that the cost of hypertension treatment will increase from \$130.4 billion in 2008 to \$200.3 billion in 2030 (Heidenreich *et al.*, 2011). Therefore, it is prudent to investigate alternate methods of treatment and prevention of high blood pressure.

The most recent joint report on hypertension currently lists lifestyle modifications as the only non-pharmacological treatment plan (Chobanian *et al.*, 2003). Lifestyle modifications include weight loss, diet and exercise. The effects of omega-3 fatty acids, found in fatty fish or extracted from certain plants, have intrigued many patients and clinicians as a potential non-pharmacological approach to blood pressure control.

Supplementation with omega-3 fatty acids has been shown to reduce blood pressure in hypertensive but not normotensive individuals (Appel *et al.*, 1993; Morris *et al.*, 1993). There is also some debate as to the dosage needed to observe a blood pressure lowering effect. Studies have found that doses are only effective in very high amounts, such as 15 g/day (Knapp & FitzGerald, 1989). Other studies suggest that moderate amounts are capable of lowering blood pressure in those with mild-

hypertension (Appel *et al.*, 1993; Morris *et al.*, 1993). Additionally, Howe *et al.* (1991) found that fish oil was most beneficial in lowering blood pressure when combined with a low sodium diet in spontaneously hypertensive rats. It is suggested that when combined with other non-pharmaceutical methods, such as lifestyle and dietary modifications, omega-3 fatty acid supplementation may be beneficial in lowering blood pressure, particularly in mildly hypertensive individuals (Howe, 1995). Prevention of hypertension through omega-3 fatty acid supplementation, particularly in prehypertensive individuals who are at higher risk for development of hypertension, seems possible.

1.7 Polyunsaturated Fatty Acids

Throughout history there have been home remedies that claim to be cure-all drugs. These non-pharmaceutical and “natural” medicines have always been popular among individuals and main-stream media. Some of these medicinal tools have proven useless and sometimes dangerous, yet others have been effective. In particular, dietary modifications have been viewed as an obvious measure in disease prevention. Certain populations throughout the world have strikingly lower rates of cardiovascular disease (CVD) than in the United States or Western Europe, and diet is believed to contribute heavily to these differences. One such group that has shown remarkably low incidence of CVD is the Inuit Culture in Alaska and Greenland (Bang *et al.*, 1971). Analysis of the Inuit diet revealed a much higher consumption of polyunsaturated fatty acids (PUFAs), specifically from marine oils, when compared to their Danish counterparts (Bang *et al.*, 1980). These findings led to both interest and research on fish oil, and its effect on health and cardiovascular risk.

Recently, public awareness of PUFA health benefits has dramatically increased. Since the studies conducted by Bang and colleagues (1971 & 1980), the idea that fish oil could influence CVD has grown considerably. However, the mechanisms by which these “healthy” fats influence human physiology remain unclear. There are many suggested benefits of increasing PUFA consumption into the American diet, but one benefit, lowering blood pressure, seems to be at the forefront of public opinion.

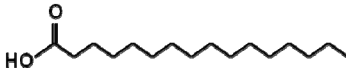


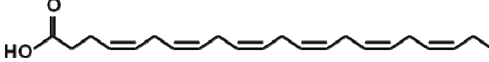
It is well-documented that unsaturated fats have more health benefits when compared to saturated fatty acids. The difference between PUFA's and saturated fatty acids is found in their molecular structure. Saturated fatty acids contain single bonds

between carbon molecules. Unsaturated fats are organic molecules containing one or more double bond in the general fatty acid structure; therefore, PUFAs are unsaturated fats with two or more double bonds. Table 1.1 shows a list of common saturated and unsaturated fats.

The most common PUFAs in a typical diet are omega-3 and omega-6 fatty acids. Omega-3 fatty acids include α -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-6 fatty acids include arachidonic acid and linoleic acid. The differences between these two types of PUFAs are determined by the location of the first double bond in their chemical structures. Omega-3 fatty acids are named as such because the sight of the first double bond in the fatty acid chain occurs at the 3rd carbon. Comparatively, omega-6 fatty acids first double bond is found at the 6th carbon. This seemingly small difference in structure appears to have dramatic implications on metabolism and integration in the body.

It has been suggested that the risk of developing cardiovascular disease decreases as the ratio of omega-6 to omega-3 fatty acids decreases, with the ideal ratio being 1:1. Currently, the typical American diet consumes a 15:1 omega-6/omega-3 ratio (Simopoulos, 2008). It has been hypothesized that a lack of omega-3 fatty acids in the diet may be contributing to the high incidence of cardiovascular disease found in the United States. This has led to a strong push for the general population to incorporate more foods containing omega-3 fatty acids in them, such as fish, flax seed, certain nuts and vegetable oils (Kris-Etherton *et al.*, 2003). The omega-3 fatty acids EPA and DHA are commonly found in marine animals. These are essential fatty acids, meaning they cannot be synthesized by the human body. The beneficial effects marine oils in reducing cardiovascular disease are believed to be through improved lipoprotein composition, endothelial and smooth muscle function, atherosclerotic plaque build-up, inflammatory mediators and peripheral nervous system function. Three of these, anti-inflammatory response, endothelial proliferation and the peripheral nervous system, are believed to be the primary contributors to lowering arterial blood pressure (Das, 2000; Abeywardena & Head, 2001).

Table 1.1. Examples of saturated and unsaturated fatty acids.

Name	Formula*	Structure
<i>Saturated</i>		
Palmitic Acid	16:0	
<i>Unsaturated</i>		
Arachidonic Acid	20:4 (n-6)	
Eicosapentaenoic Acid (EPA)	20:5 (n-3)	
Docosahexaenoic Acid (DHA)	22:6 (n-3)	

Formulas presented as number of carbons in the chain:number of double bonds (carbon at which the first double bond occurs); n-3, omega-3 fatty acid; n-6, omega-6 fatty acid

EPA and DHA are “less” inflammatory, more so than anti-inflammatory. The structures of omega-3 fatty acids, and products from their breakdown and integration into biological cells are the first processes that lead to their influence on the inflammation process. The source of PUFA’s health benefits lies in the multiple double bonds in the chemical structures. A greater number of double bonds lead to an increased fluidity in the cell membranes (De Caterina *et al.*, 1998; Hashimoto *et al.*, 1999). This altered membrane structure triggers the formation of specific types of cytokines and inflammatory mediators such as prostaglandins, tumor necrosis factor and interleukins (Endres *et al.*, 1989; De Caterina *et al.*, 2000). These molecules are inflammatory triggering products, but the properties of the structures of the mediators derived from EPA have anti-inflammatory properties compared to arachidonic acid. For example, arachidonic acid, an omega-6 fatty acid, is the precursor to active inflammation mediators prostaglandin PG₂ and thromboxane TXB₂. The omega-3 fatty acid EPA is the precursor to PG₃ and TXB₃ which are less active inflammatory agents (Terano *et al.*, 1986). In this way, omega-3 fatty acids are less-inflammatory when compared to their

omega-6 counterparts. Additionally, changes in the inflammatory response may have an effect on localized endothelial function.

Peripheral vasculature is highly influenced by endothelial function. Fish oils are suggested to improve endothelial function. Endothelial tissue can contribute to local vasodilation as well as constriction. For example, angiotensin II is a known vasoconstrictor while nitric oxide is a vasodilator. It is believed that an imbalance in these vasoactive actions is one of the causes of endothelial dysfunction and hypertension. Vasoactive agents such as angiotensin II, nitric oxide, endothelial derived hyperpolarizing factor, eicosanoids and endothelin are believed to be influenced by EPA and DHA (Gibbons, 1997; Harris *et al.*, 1997; Noll *et al.*, 1997).

Another potential mechanism, and the primary focus of this thesis, is that fish oil decreases blood pressure through the parasympathetic and sympathetic nervous systems. Although the exact mechanisms for these effects are unclear, it has been suggested that omega-3 fatty acids have a direct influence on peripheral nervous system function.

1.8 Summary and Hypotheses

The purpose of this dissertation is two-fold. First, we aim to compare the neurovascular responses to mental stress in prehypertensive and normotensive humans. Second, the effects of omega-3 fatty acid supplementation will be used to determine if there is a possible health benefit to fish oil supplementation in regards to blood pressure regulation and prevention of cardiovascular disease, specifically hypertension. The link between mental stress and possible mechanisms in the development of hypertension has led us to propose the following hypotheses.

Hypothesis 1: Previously, it has been shown that young borderline hypertensive subjects demonstrated an augmented pressor response during mental stress compared to normotensives (Santangelo *et al.*, 1989; Matsukawa *et al.*, 1991; Jern *et al.*, 1995). Mechanisms suggested to contribute to the augmented pressor response in borderline hypertensives include a blunted forearm vasodilation response (Santangelo *et al.*, 1989) and augmented MSNA responses to mental stress when compared to normotensives (Matsukawa *et al.*, 1991). The neural and vascular responses to mental stress have not been examined collectively in these populations. Additionally, based on recent evidence

regarding MSNA responses to mental stress (Carter & Ray, 2009), the results reported by (Matsukawa *et al.*, 1991) may not be representative of the majority of the population. Therefore, in Study 1 we aimed to comprehensively investigate blood pressure, heart rate, MSNA and vascular responses to mental stress. **We hypothesized that young prehypertensive males would elicit an augmented blood pressure and MSNA response, but a blunted vasodilation response to mental stress compared to normotensives.**

Hypothesis 2: Psychological stress is a known contributor to the development of hypertension (Esler *et al.*, 2008). Though the mechanisms underlying the development of this cardiovascular disease are not fully understood, it has been suggested that omega-3 fatty acid supplementation via fish oil may contribute to the prevention and treatment of hypertension (Appel *et al.*, 1993; Morris *et al.*, 1993; Mori, 2006). Studies investigating omega-3 fatty acids and mental stress have suggested blunted sympathetic activity (i.e., epinephrine) as a mechanism by which blood pressure may be lowered (Delarue *et al.*, 2003). However, direct measurements of sympathetic neural activity have not been investigated during mental stress, and no studies have examined prehypertensive responses to mental stress following omega-3 supplementation. **In Study 2 we hypothesized that supplementation with omega-3 fatty acids, specifically fish oil, would lower resting blood pressure in prehypertensive subjects compared to normotensive. Furthermore, we hypothesized that fish oil would blunt the blood pressure and MSNA, and augment the vascular responses to mental stress in these same prehypertensive individuals compared to normotensives.**

Chapter 2

Neurovascular Responses to Mental Stress in Prehypertensive Humans

The material contained in this chapter was previously published in the Journal of Applied Physiology

Schwartz, CE, Durocher, JJ, Carter, JR. (2011). Neurovascular responses to mental stress in prehypertensive humans. *Journal of Applied Physiology* **10**, 76-82. Doi: 10.1152/jappphysiol.00912.2010

2.1 Introduction

Mental stress has been linked to several cardiovascular diseases, including hypertension (Esler *et al.*, 2003; Esler *et al.*, 2008). Hypertensive and prehypertensive individuals, as well as normotensives with a family history of hypertension, have all demonstrated an augmented pressor response to mental stress (Falkner *et al.*, 1979; Santangelo *et al.*, 1989; Matsukawa *et al.*, 1991; Widgren *et al.*, 1992; Jern *et al.*, 1995). Although this hypertensive response is well documented, few studies have examined the mechanisms underlying this response.

Mental stress consistently induces forearm vasodilation (Blair *et al.*, 1959; Barcroft *et al.*, 1960; Roddie, 1977; Carter *et al.*, 2005), and evidence suggests that prehypertension blunts this response (Santangelo *et al.*, 1989). The mechanism(s) responsible for this attenuated response remain unresolved, but an augmented sympathetic neural response has been suggested. Specifically, Matsukawa *et al.* (Matsukawa *et al.*, 1991) reported that MSNA responses to mental stress were augmented in borderline hypertensives when compared to normotensives. Unfortunately, concurrent vascular responses were not assessed (Matsukawa *et al.*, 1991) and MSNA responses to mental stress are highly variable (Carter & Ray, 2009).

Therefore, the present study aims to determine both forearm vascular and MSNA responses to mental stress in prehypertensive and normotensive adults. We hypothesize that prehypertension will blunt forearm vasodilation and augment MSNA responses to mental stress. Understanding the mechanisms responsible for the augmented pressor response to mental stress is clinically relevant, and may lead to better intervention strategies to help prevent, or at least delay, the development of prehypertension and/or hypertension.

2.2 Methods

2.2.1 Subjects

35 healthy men (18 normotensive, age 23 ± 2 yrs; 17 prehypertensive, age 22 ± 1 yrs) participated in the study. Normotensive subjects were defined as having a resting systolic pressure less than 120 mmHg and diastolic pressure less than 80 mmHg. Prehypertensive subjects were defined as having a resting systolic pressure of 120-139 mmHg and/or a diastolic pressure of 80-89 mmHg. This is consistent with current blood pressure classifications (Chobanian *et al.*, 2003). Subject exclusion criteria included smoking, diabetes, use of blood pressure medication, and autonomic dysfunction. Normotensive (24 ± 1 kg/m²) and prehypertensive (26 ± 1 kg/m²) subjects had similar body mass indices ($P=0.13$). Subjects were asked to refrain from exercise, caffeine and alcohol for 12 hours prior to being tested. This experimental protocol was approved by the Michigan Technological University Institutional Review Board (Approval Protocol No. M0172), and all participants signed an informed consent form.

2.2.2 Experimental Design

Subjects reported to the laboratory on the three consecutive days. Testing occurred at the same time of day to avoid diurnal fluctuations in autonomic measurements. Resting blood pressures were measured in the seated position three times after 5 minutes of rest on each of the three consecutive days, and reported as the mean. Height and weight were recorded following the resting blood pressure readings on the third day. After resting measurements were taken on day three, subjects were instrumented for the mental stress autonomic function test, which included 5 minutes of supine rest (baseline), 5 minutes of mental stress (mental arithmetic), and 5 minutes of supine rest (recovery). Mental arithmetic consisted of subtracting the number 6 or 7 from a 2-3 digit number continuously as investigators encouraged the subject to respond quickly. The 2-3 digit number was changed every 5-10 seconds. MSNA, heart rate (HR), beat-to-beat blood pressure, forearm and calf blood flow were recorded throughout the protocol.

2.2.3 Measurements

Arterial blood pressure was measured using two techniques. Resting arterial blood pressure was measured three times (separated by approximately one minute intervals) over three consecutive days using an automated sphygmomanometer and reported as a mean value (i.e., 9 readings over 3 days). Beat-to-beat arterial blood pressure was recorded continuously via Finometer (Finapres Medical Systems, Amsterdam, The Netherlands) during the mental stress protocol (i.e., baseline, mental stress, and recovery). The Finometer accurately determines relative changes in arterial blood pressure, but should not be used to determine absolute values. Therefore, the Finometer was used to determine precise changes in arterial blood pressure that occurred during mental stress, while the sphygmomanometer allowed us to compare baseline arterial blood pressures (Table 1). Arterial blood pressures are expressed as systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressures. Three day average MAP is calculated using the classic formula $MAP = DAP + 0.333 (SAP - DAP)$. Supine baseline measurements of MAP are generated by each cardiac cycle waveform produced by the Finometer. HR was recorded with the automated sphygmomanometer during the 3 consecutive days of blood pressure monitoring, and with a three-lead electrocardiogram during the mental stress protocol.

Forearm and calf blood flow were measured using venous occlusion plethysmography. Changes in forearm and calf blood flow were measured via mercury-in-silastic strain gauges placed around the subject's forearm and calf at the point of greatest circumference. Cuffs were placed around the subjects left wrist, upper arm, thigh and ankle. The wrist and ankle cuffs were inflated to 220 mmHg to occlude blood flow to the hand and foot, while the upper arm and thigh cuffs were inflated to 60 mmHg for 8 seconds and deflated for 7 seconds (i.e. 15 second blood flow intervals).

Multifiber recordings of MSNA were made by inserting a tungsten microelectrode into the peroneal nerve of a resting leg. A reference electrode was inserted subcutaneously 2-3 cm from the recording electrode. Both electrodes were connected to a differential preamplifier, and then to an amplifier (total gain of 80,000) where the nerve signal was band-pass filtered (700-2000 Hz), and integrated (time constant, 0.1) to obtain a mean voltage display of nerve activity. Satisfactory recordings of MSNA were defined by spontaneous, pulse synchronous bursts that increased during end-expiratory

apnea, and did not change during auditory stimulation. A loss or shift of the neurogram during mental stress prevented analysis of MSNA data in 14 subjects; thus we report MSNA burst frequency responses to mental stress in a total of 21 subjects (10 normotensive, 11 prehypertensive). Total MSNA responses to mental stress are presented for 19 subjects (9 normotensive, 10 prehypertensive).

2.2.4 Data Analysis

Data were imported and analyzed in the WinCPRS software program (Absolute Aliens, Turku, Finland). R-waves were detected and marked in the time series. Bursts of MSNA were automatically detected on the basis of amplitude using a signal-to-noise ratio of 3:1, within a 0.5 s search window centered on a 1.3 s expected burst peak latency from the previous R-wave. Potential bursts were displayed and edited by one investigator. The average burst area occurring during baseline was normalized to a mean value of 100. MSNA was expressed as bursts per minute, bursts per 100 heart beats, and total MSNA (i.e., the sum of the normalized burst areas per minute).

Forearm and calf blood flows were analyzed as percent change, and used to calculate vascular resistance and vascular conductance. Vascular resistance was calculated as MAP divided by limb blood flow, while vascular conductance was calculated as the reciprocal (i.e., limb blood flow divided by MAP).

2.2.5 Statistical Analysis

All data were analyzed statistically using commercial software (SPSS 15.0, SPSS Inc., Chicago, Illinois, USA). A two-way repeated measures ANOVA was utilized to determine if changes in MSNA, SAP, DAP, MAP, HR, forearm and calf vascular resistance and conductance occurred during baseline and mental stress, and across trial groups (normotensive and prehypertensive). Post-hoc analyses were performed using least significant difference pairwise comparisons. Resting variables were compared using independent t-tests. Means were considered significantly different when $P < 0.05$.

All results are expressed as mean \pm SE. Hemodynamic variables are presented as 5 min averages in results text and 1 min averages in figures to provide more detail on differences between groups. Although there were group differences in forearm vascular responses, data are only presented as 5 min averages as this was more conducive to

the sampling technique (venous occlusion plethysmography). Finally, MSNA and calf vascular responses were not different between groups regardless of analysis (i.e., minute by minute vs. 5 min averages), so data are presented as 5 min averages.

2.3 Results

Table 2.1 reports seated resting blood pressure and heart rate values taken over three consecutive days. Resting SAP, DAP, and MAP were greater in prehypertensive than normotensive subjects, while resting HR was not different across groups. Table 2.2 reports supine baseline MSNA, along with limb blood flow, vascular resistance, and vascular conductance to the forearm and calf. Forearm blood flow was significantly greater in prehypertensive compared to normotensive subjects, while all other baseline variables were not different between groups (Table 2.2).

Figure 2.1 demonstrates that increases in SAP ($\Delta 9 \pm 2$ mmHg vs. $\Delta 14 \pm 2$ mmHg; $P < 0.05$), DAP ($\Delta 8 \pm 1$ mmHg vs. $\Delta 11 \pm 1$ mmHg; $P < 0.05$) and MAP ($\Delta 10 \pm 1$ mmHg vs. $\Delta 14 \pm 1$ mmHg; $P < 0.05$) during mental stress were significantly greater in prehypertensive compared to normotensive subjects (condition x group interaction for Δ SAP and Δ MAP were significant at $P < .05$, condition x group interaction for Δ DAP was considered significant at $P = .065$).

Table 2.1. Resting hemodynamics for normotensive and prehypertensive subjects.

Variable	Normotensive				Prehypertensive			
	Day 1	Day 2	Day 3	Mean	Day 1	Day 2	Day 3	Mean
SAP (mmHg)	113 \pm 2	112 \pm 1	111 \pm 1	112 \pm 1	127 \pm 2*	126 \pm 2*	127 \pm 2*	127 \pm 2*
DAP (mmHg)	65 \pm 2	64 \pm 2	64 \pm 2	64 \pm 1	71 \pm 2*	71 \pm 2*	70 \pm 2*	71 \pm 2*
MAP (mmHg)	81 \pm 1	80 \pm 1	80 \pm 1	80 \pm 1	90 \pm 2*	89 \pm 2*	89 \pm 2*	89 \pm 2*
HR (beats/min)	69 \pm 3	68 \pm 2	68 \pm 2	68 \pm 2	75 \pm 3	74 \pm 3	76 \pm 3	75 \pm 3

Values are mean \pm SE (n = 18 for normotensive, and n = 17 for prehypertensive). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial blood pressure; HR, heart rate; *Significantly different ($P < 0.05$) from corresponding normotensive value. Measurements were recorded after 5 minutes of seated rest at the same time of day over three consecutive days with an automated sphygmomanometer.

Table 2.2. Baseline values for normotensive and prehypertensive subjects.

Variable	Normotensive	Prehypertensive	P value
MSNA (bursts/min)	10 ± 2	11 ± 2	P=0.78
MSNA (bursts/100 hb)	17 ± 3	16 ± 3	P=0.88
FBF (mL/100mL/min)	2.5 ± 0.3	3.4 ± 0.3*	P=0.03
FVR (mmHg/mL/100mL/min)	35 ± 2	30 ± 4	P=0.28
FVC (mL/100mL/min/mmHg)	0.03 ± 0.003	0.04 ± 0.004	P=0.13
CBF (mL/100mL/min)	2.1 ± 0.2	2.4 ± 0.2	P=0.25
CVR (mmHg/mL/100mL/min)	42 ± 3	42 ± 4	P=0.99
CVC (mL/100mL/min/mmHg)	0.03 ± 0.002	0.03 ± 0.002	P=0.80

Values are mean ± SE. MSNA, muscle sympathetic nerve activity (n = 15 for normotensive and n = 16 for prehypertensive); FBF, forearm blood flow (n = 17 for normotensive and n = 16 for prehypertensive); FVR, forearm vascular resistance; FVC, forearm vascular conductance; CBF, calf blood flow (n = 17 for normotensive and n = 15 for prehypertensive); CVR, calf vascular resistance; CVC, calf vascular conductance; *Significantly different between groups.

Figure 2 demonstrates that mental stress significantly increased MSNA from baseline when expressed as bursts per minute (normotensive: 11 ± 2 to 18 ± 3 bursts/min, P<0.05; prehypertensive: 11 ± 3 to 18 ± 3 bursts/min, P<0.05) or total MSNA (normotensive: 3918 ± 718 to 10064 ± 1295 a.u., P<0.05; prehypertensive: 5591 ± 1360 to 13417 ± 2684 a.u., P<0.05). In contrast to blood pressure, MSNA responses to mental stress were not different between groups (Figure 2.2).

Figure 2.3 represents the forearm blood flow responses during mental stress in normotensive and prehypertensive subjects. Although mental stress increased forearm blood flow in both groups, increases were significantly blunted in prehypertensive subjects (Δ 116 ± 16% vs. Δ 62 ± 11%; P<0.01). Likewise, decreases in forearm vascular resistance (Δ -41 ± 4% vs. Δ -18 ± 5%; P<0.01) and increases in conductance (Δ 95 ± 14% vs. Δ 37 ± 8%; P<0.001) during mental stress were blunted in prehypertensive subjects. In contrast, calf blood flow (Δ 43 ± 11% vs. Δ 36 ± 6%), resistance (Δ -12 ± 5% vs. Δ -8 ± 4%) and conductance (Δ 29 ± 10% vs. Δ 19 ± 5%) responses during mental stress were similar between groups (Figure 2.4).

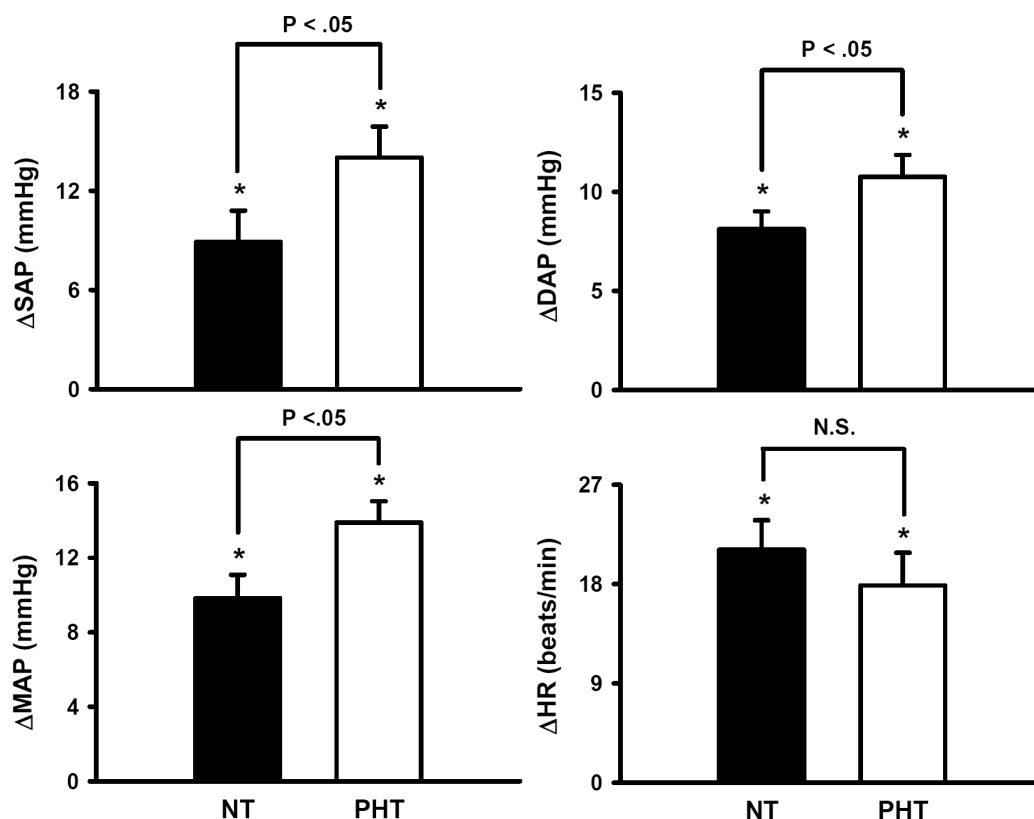


Figure 2.1. Changes in SAP, DAP, MAP and HR during 5 minutes of mental stress in normotensive, NT (n=18) and prehypertensive, PHT (n=16) subjects. Mental stress elicited a significant pressor response in both groups, and this response was augmented in PHT subjects. Mental stress increased HR in both NT and PHT subjects, and there was no significant difference between groups. *P<0.05 from corresponding NT value.

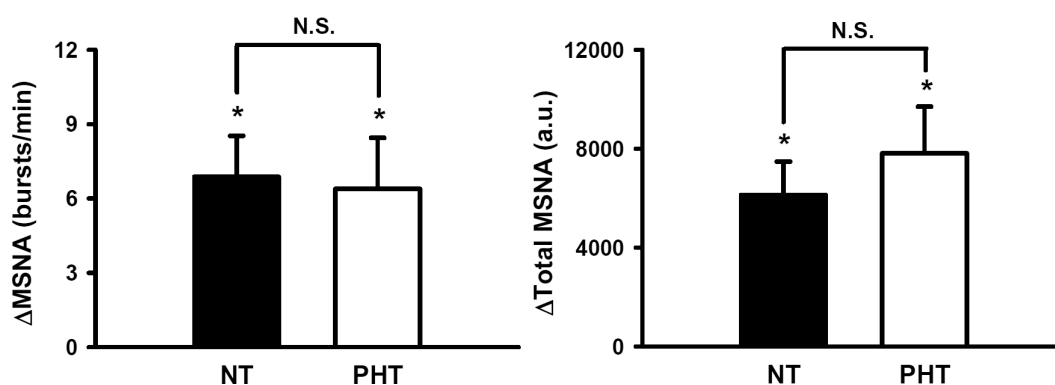


Figure 2.2. Changes in MSNA bursts per minute and total MSNA during 5 minutes of mental stress in normotensive (NT) and prehypertensive (PHT) subjects. Mental stress increased MSNA burst frequency and total MSNA in both groups. These responses were not significantly different between groups (burst frequency: time \times group, P=0.43; total MSNA: time \times group, P=0.29). *P<0.05 from baseline; N.S., no significance; a.u., arbitrary units per minute.

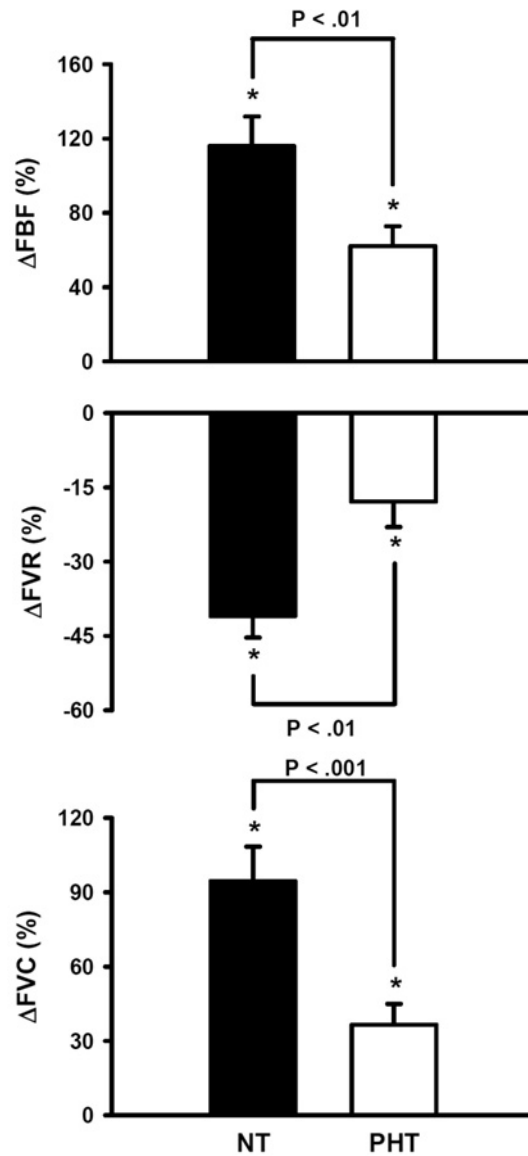


Figure 2.3. Changes in forearm blood flow (FBF), vascular resistance (FVR) and vascular conductance (FVC) during 5 minutes of mental stress. Mental stress elicited forearm vasodilation in both groups, but these responses were blunted in prehypertensive, PHT (n=15) compared to normotensive, NT (n=17) subjects (time \times group = $P < 0.01$, all). * $P < 0.05$ from baseline.

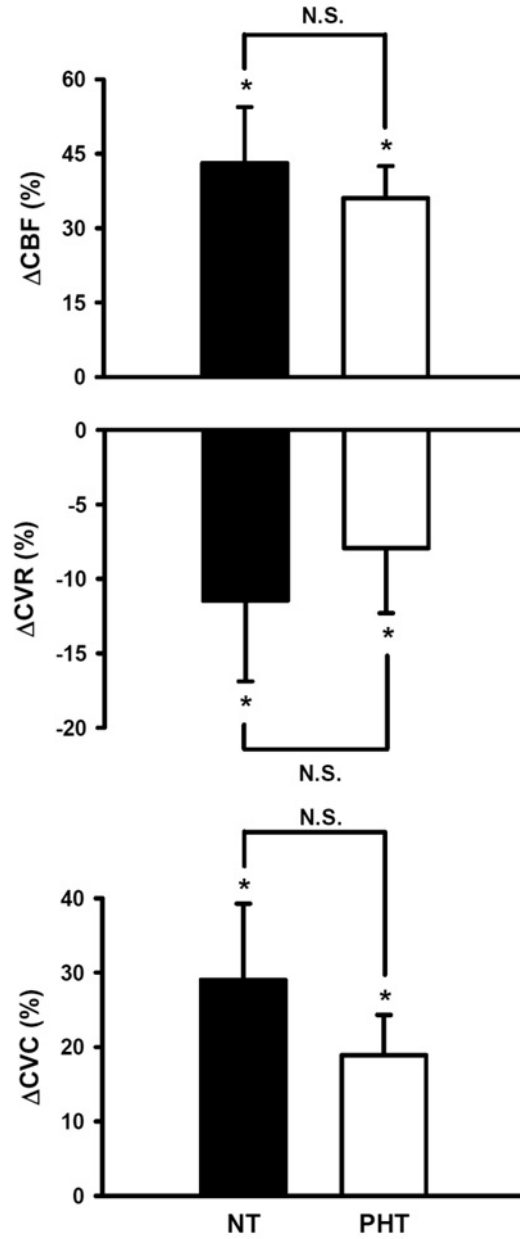


Figure 2.4. Changes in calf blood flow (CBF), vascular resistance (CVR) and vascular conductance (CVC) during 5 minutes of mental stress. Mental stress elicited calf vasodilation in normotensive, NT (n=17) and prehypertensive, PHT (n=14) groups, and these responses were not different between groups (time \times group, $P > 0.20$, all). * $P < 0.05$ from baseline, N.S., no significance.

2.4 Discussion

The present study compared neurovascular responses to mental stress in prehypertensive and normotensive subjects, and our data reveal three major findings. First, mental stress elicited an augmented pressor response in prehypertensive subjects. Second, this augmented pressor response was associated with an attenuated forearm vasodilation. These findings are consistent with previous work (Santangelo *et al.*, 1989; Matsukawa *et al.*, 1991; Jern *et al.*, 1995). Third, prehypertension did not alter MSNA responses to mental stress. This finding is novel and provides new mechanistic insight into the well documented, yet poorly understood, link between mental stress and hypertension. Collectively, our data indicate that blunted forearm vasodilation, not augmented MSNA, contributes to a more dramatic increase of arterial blood pressure during mental stress in prehypertensive subjects.

Several studies have shown augmented blood pressure responses to mental stress in prehypertensive compared to normotensive subjects (Santangelo *et al.*, 1989; Matsukawa *et al.*, 1991; Jern *et al.*, 1995). Similar results have also been seen in subjects with a family history of hypertension (Widgren *et al.*, 1992; Noll *et al.*, 1996). Furthermore, an augmented pressor response to stress in normotensives may even help predict the development of hypertension (Matthews *et al.*, 2004; Esler *et al.*, 2008). In the present study, we report an augmented pressor response to mental stress in prehypertensive subjects. Thus, our findings are consistent with previous studies and strengthen the rationale to determine potential mechanisms that may be responsible for this augmented response.

Matsukawa *et al.* (Matsukawa *et al.*, 1991) reported an elevated blood pressure response to mental stress in borderline hypertensives, and suggested that the difference was likely due to a decrease in MSNA in normotensives and no change in the borderline hypertensives. However, recent evidence (Carter & Ray, 2009) suggests that the findings of Matsukawa *et al.* (Matsukawa *et al.*, 1991) may not represent typical MSNA responses to mental stress. Specifically, a recent retrospective analysis of 82 neurograms from normotensive subjects indicated that nearly 90% of subjects demonstrated an increase or no change in MSNA, while ~10% were classified as “negative” MSNA responders ($\leq \Delta -3$ bursts/min) (Carter & Ray, 2009). Therefore, the findings of Matsukawa *et al.* (Matsukawa *et al.*, 1991), which reported a significant

sympathoinhibition of MSNA during mental stress in normotensive subjects, may not represent a normal distribution with regards to typical MSNA responses to mental stress. The present study demonstrates similar increases of MSNA during mental stress in normotensive and prehypertensive patients. We attribute differences between our data and the findings of Matsukawa et al. (Matsukawa *et al.*, 1991) to the inherent variability of MSNA responses to mental stress in humans (Carter & Ray, 2009). However, it is important to note that in the present study, 10 of 10 normotensive subjects and 10 of 11 prehypertensive subjects demonstrated an increase or no change in MSNA during mental stress. These ratios are consistent with a recent large-scale study that reported MSNA either increases or does not change during mental stress in approximately 90% of adults (Carter & Ray, 2009). Therefore, we are confident in our data and conclude that MSNA responses to mental stress are similar in normotensive and prehypertensive populations.

Whereas prehypertension did not alter MSNA responses to mental stress, it did alter forearm vascular responses. Specifically, prehypertension blunted the classic forearm vasodilatory response associated with mental stress. Blunted forearm vascular responses to physiological stressors are associated with increased incidence or risk of hypertension (Takeshita *et al.*, 1982; Santangelo *et al.*, 1989; Boutcher *et al.*, 2009). For example, young normotensive individuals with a family history of hypertension display a reduction in peak forearm blood flow (Boutcher *et al.*, 2009) and an increased forearm vascular resistance during (Takeshita *et al.*, 1982) and after (Boutcher *et al.*, 2009) reactive hyperemia. Moreover, borderline hypertensives demonstrate increased forearm vascular resistance and MAP during mental stress (Santangelo *et al.*, 1989). Thus the present study, which demonstrates blunted forearm vascular responses to mental stress in prehypertensive subjects, is consistent with previous work (Santangelo *et al.*, 1989) and offers new insights by demonstrating that the blunted forearm vascular response to mental stress is not accompanied by altered MSNA.

A recent study reports similar MSNA responses to mental stress in the arm and leg (Carter *et al.*, 2005), making it reasonable to assume that other mechanisms beyond MSNA may be responsible for the blunted forearm vasodilation to mental stress in our prehypertensive subjects. However, it should be noted that other studies have reported a divergence in arm and leg MSNA (Anderson *et al.*, 1987) and decreases of arm MSNA

(Halliwill *et al.*, 1997) during mental stress. We recognize the absence of arm MSNA data as a limitation to the present study, but do not believe this lessens the impact of the data. Our primary focus remains on mechanisms responsible for the augmented pressor response to mental stress in prehypertensive subjects, not the mechanisms underlying forearm vasodilation. Both leg MSNA and forearm vascular conductance have been independently proposed as potential mechanisms contributing to the augmented pressor response, and the present study advanced our knowledge by demonstrating that blunted forearm vasodilation, not augmented MSNA, is a primary contributor. This is important as it had previously been assumed that augmented MSNA was a likely contributor (Matsukawa *et al.*, 1991). However, we recognize that other potential mechanisms might include vasoconstriction to non-muscular beds, including renal, splanchnic and skin (Brod, 1963; Wallin *et al.*, 1973; Tidgren & Hjerdahl, 1989; Kuipers *et al.*, 2008); future work will have to address these vascular beds.

As previously noted, passive MSNA withdrawal has been suggested as a potential mechanism for the forearm vasodilation associated with mental stress (Halliwill *et al.*, 1997), but this remains debatable (Carter *et al.*, 2005) and other mechanisms have been proposed (Dietz *et al.*, 1994; Lindqvist *et al.*, 1996; Cardillo *et al.*, 1997). Specifically, both nitric oxide (Dietz *et al.*, 1994; Cardillo *et al.*, 1997; Cardillo *et al.*, 1998) and circulating epinephrine (Lindqvist *et al.*, 1996) have been shown to contribute to forearm vasodilation during mental stress. Of interest to the present study, Cardillo *et al.* (Cardillo *et al.*, 1998) reported that nitric oxide mediated vasodilation during mental stress was attenuated in hypertensives, and this could be due to possible endothelial dysfunction. We did not assess nitric oxide levels in the current study, but it seems reasonable to speculate that the blunted forearm vasodilation during mental stress in prehypertensive subjects may be related to altered nitric oxide responses.

Although mental stress does not typically modulate calf vasculature (Rusch *et al.*, 1981; Carter *et al.*, 2005), calf vasodilation has been observed during mental stress (Kuipers *et al.*, 2008). Therefore, the modest, yet significant, calf vasodilation reported in the present study is reasonable and consistent with prior work. Importantly, calf vasodilatory responses to mental stress were similar in normotensive and prehypertensive subjects. Thus, prehypertension appears to elicit divergent limb vascular responses to mental stress. The clinical consequence of this divergent vascular

response remains unclear, but it is consistent with other studies reporting divergent limb vascular responses during physiological stressors in both animals (Wilson *et al.*, 2006) and humans (Monahan & Ray, 2002; Lawrence *et al.*, 2010).

In conclusion, the present study demonstrates that prehypertension elicits a more dramatic pressor response to mental stress when compared to normotensive subjects. This augmented pressor response appears to be related to blunted forearm vasodilation, but not augmented MSNA. These findings provide new insight into the complex relationship between mental stress and hypertension.

2.5 Perspectives

Despite attempts to recruit young, prehypertensive women, we were only successful in the recruitment of 17 prehypertensive men. Accordingly, we caution the extrapolation of the present data to women. A growing body of evidence suggests that the relations between MSNA and arterial blood pressure regulation differ in men and women (Joyner *et al.*, 2010), and we cannot speculate how these differences might translate to the present findings. Although young, otherwise healthy women have a lower incidence of prehypertension than their young, male counterparts (Izzo, 2007), they have a much higher incidence of anxiety and panic disorder (Westenberg & Liebowitz, 2004). The long term impact of these more 'chronic' stressors on arterial blood pressure remains unclear, but evidence suggests that such chronic stressors are linked to essential hypertension via autonomic mechanisms (Esler *et al.*, 2008). Whereas the present study focused on neurovascular responses to acute mental stress in prehypertensive men, future investigations might focus on not only prehypertensive women, but also chronic stress.

2.6 Acknowledgements

The authors thank Jenna Klein, Ashley Yenior, Kristen Reed, Huan Yang and Sarah Stream for their assistance in data collection and analysis for this project. We also thank all the subjects for their participation and cooperation. This project was supported by National Institutes of Health grants HL-088689 and HL-098676.

Chapter 3

Omega-3 Fatty Acids and the Neurovascular Responses to Mental Stress in Humans

3.1 Introduction

Omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in fish oil, have been suggested as a possible non-pharmaceutical treatment of cardiovascular disease (CVD), including hypertension (Burr, 1989; Morris *et al.*, 1993; Marchioli *et al.*, 2002; Thies *et al.*, 2003; Yamagishi *et al.*, 2008). Currently, over 76 million people suffer from high blood pressure (Roger *et al.*, 2011). Hypertension has been shown to increase the risk of stroke, ischemic heart disease and cardiac related mortality (Kannel, 1975; MacMahon *et al.*, 1990; van den Hoogen *et al.*, 2000). A small reduction in blood pressure may have clinical significance on the impact on cardiovascular health (Collins *et al.*, 1990).

Fish oil has been reported to lower blood pressure in hypertensive individuals (Appel *et al.*, 1993; Morris *et al.*, 1993; Geleijnse *et al.*, 2002). However, omega-3 fatty acids demonstrate mixed effects in mild hypertension (Levinson *et al.*, 1990; Singer, 1990; Morris *et al.*, 1993), and do not appear to have a hypotensive influence on individuals with normal blood pressures (Appel *et al.*, 1993; Morris *et al.*, 1993). Collectively, it appears that fish oil may have an increasingly preventative effect as resting blood pressure increases.

Chronic mental stress is a known contributor to hypertension (Esler *et al.*, 2008), and typically elicits augmented neural and cardiovascular responses (Cannon, 1927; Blair *et al.*, 1959; Barcroft *et al.*, 1960; Carter & Ray, 2009). Augmented sympathetic nerve activity (Esler *et al.*, 1986; Egan *et al.*, 1987; Anderson *et al.*, 1989) and altered peripheral vascular function are two proposed mechanisms contributing to the development of hypertension. Animal studies demonstrate reduced blood pressure, cardiac sympathetic activity and sympathoadrenal responses, as well as improved vascular compliance, following omega-3 supplementation (Bayorh *et al.*, 1989; Chin-Dusting *et al.*, 1998; Nishimura *et al.*, 2000; Rousseau-Ralliard *et al.*, 2009). However, investigations in humans are less clear. Fish oil has demonstrated both decreases and no change in resting blood pressure (Appel *et al.*, 1993; Morris *et al.*, 1993) and

sympathetic nerve activity (Delarue *et al.*, 2003; Monahan *et al.*, 2004). Additionally, vascular responses in humans following omega-3 supplementation appear to be improved (Kenny *et al.*, 1992; Mori *et al.*, 2000; Khan *et al.*, 2003). However, few studies have investigated the effect of fish oil in humans during a psychological challenge.

Delarue *et al.* (2003) reported that circulating catecholamines, an indirect measure of sympathetic neural activity, were reduced during mental stress after fish oil supplementation. More recently, Monahan *et al.* (2004) measured muscle sympathetic nerve activity (MSNA) responses following omega-3 supplementation and demonstrated augmented responses to physiological stressors, although mental stress was not investigated. Borderline hypertensive subjects have demonstrated augmented MSNA as well as augmented norepinephrine responses to mental stress (Eliasson, 1985; Anderson *et al.*, 1989; Matsukawa *et al.*, 1991). Therefore, investigating the effects of fish oil on MSNA during mental stress may provide information as to the beneficial effects of omega-3 fatty acids on the development of hypertension.

Recently, the National Heart, Lung and Blood Institute and the American Heart Association introduced prehypertension as a new category of blood pressure classification. These individuals demonstrate a resting blood pressure in between normal and high categories, and represent a population at higher risk of developing hypertension (Vasan *et al.*, 2001; Moreira *et al.*, 2008). Early intervention, such as fish oil supplementation, may play an important role in preventing the onset of hypertension (Fuchs, 2010). However, the mechanisms by which fish oils act in reducing blood pressure in prehypertensive subjects remain unclear.

Therefore, the aim of this study was to investigate MSNA and vascular responses to mental stress before and after omega-3 supplementation in prehypertensive and normotensive individuals. We hypothesized that fish oil would 1) lower resting blood pressure and MSNA in prehypertensive individuals, 2) blunt the blood pressure and MSNA responses to mental stress in the prehypertensive group, and 3) augment vasodilatory responses to mental stress in prehypertensive individuals compared to normotensive. Identifying the mechanism(s) by which omega-3 fatty acids lower blood pressure may help us further understand the development of hypertension, and provide a possible preventative and/or therapeutic measure against the onset of cardiovascular disease.

3.2 Methods

3.2.1 Subjects

Thirty-eight normotensive subjects (18 men, 20 women) age 24 ± 1 yrs and 29 prehypertensive subjects (28 men, 1 woman) age 24 ± 1 yrs participated in this study. Normotension was classified as a resting systolic blood pressure less than 120 mmHg and a resting diastolic blood pressure of 80 mmHg. Prehypertension was classified as a resting systolic blood pressure of 120-139 mmHg and/or a diastolic blood pressure of 80-89 mmHg (Chobanian *et al.*, 2003). All subjects signed an informed consent and refrained from caffeine, alcohol and exercise for at least 12 hours before testing. Exclusion criteria for participants included smoking, diabetes, autonomic dysfunction, and use of blood pressure medication. This study was approved by the Michigan Technological University Institutional Review Board (Approval Protocol No. M0172).

3.2.2 Experimental Protocol

All subjects were randomly assigned into this double-blind, placebo controlled investigation, and were tested before (pre) and after (post) 8 weeks of fish oil or placebo (olive oil) supplementation. Subjects reported to the Integrative Physiology Laboratory at Michigan Technological University at the same time of day for three consecutive days during the pre- and post-treatment sessions. The protocol for both pre- and post-treatments are outlined in Figure 3.1.

Upon completion of the pre-treatment testing, subjects were randomly assigned into the fish oil or placebo (olive oil) group. Participants ingested 9 grams/day of fish oil pills (1.6g EPA, 1.1g DHA) or 9 grams/day of placebo pills for 8 weeks. A pill diary and regular email and phone reminders were issued to the subjects to track compliance according to the study terms. Subjects were asked to maintain current diet and exercise habits during the 8 week supplementation period. After 8 weeks of treatment, subjects returned to the laboratory for post-treatment testing, and completed the same protocol outlined above (Fig.3.1). Two subjects in the prehypertensive group were unable to complete the post autonomic testing procedure, but did complete the seated resting blood pressure measurements. Therefore, autonomic testing during mental stress is reported for 27 prehypertensive subjects.

Day 1	Day 2	Day 3
Seated Resting Blood Pressure	Seated Resting Blood Pressure	Seated Resting Blood Pressure + Mental Stress Protocol*

*** Mental Stress Protocol**



Figure 3.1. The experimental protocol for pre and post testing for subjects. Testing protocols were identical pre and post fish oil or placebo supplementation and were separated by 8 weeks.

3.2.3 Measurements

Heart rate was measured using a 3-lead electrocardiogram. Arterial blood pressure was measured utilizing two different methods. Seated resting blood pressures were obtained using the IntelliSense® automated sphygmomanometer (Model HEM-907XL, Omron Healthcare, Inc., Bannockburn, IL). A cuff was placed around the upper arm in line with the brachial artery and inflated to approximately 200mmHg to occlude blood flow to the arm. Pressure was then slowly released allowing for systolic and diastolic arterial pressures to be recorded by the automated sphygmomanometer. Seated resting recordings were taken 3 times each day, separated by 1 minute between readings and following 5 minutes of seated rest. Importantly, all pre- and post-treatment blood pressure measurements were recorded at the same time of day to limit diurnal blood pressure variations as a potential confounder (Richards *et al.*, 1986). Beat to beat arterial pressure was recorded using the Finometer (Finapres Medical Systems, Amsterdam, The Netherlands) on the middle finger throughout autonomic testing. The Finometer is an ideal technique for measuring beat-to-beat changes in arterial pressure, but is not accurate and reproducible in measuring absolute values. Therefore, we used the seated resting brachial arterial pressure as absolute resting values and the Finometer for recording relative changes during mental stress and recovery.

Multifiber recordings of MSNA were recorded using the microneurography technique. Briefly, a tungsten microelectrode was inserted into the common peroneal nerve located in the popliteal region of the knee or at the base of the fibular head of the lower leg. A reference electrode was inserted subcutaneously 2-3 centimeters away from the recording electrode. Both electrodes were connected to a preamplifier and amplifier. The signal was amplified 80,000 times, band-pass filtered (700-2000 Hz) and integrated (time constant, 0.1s) to obtain a mean voltage display of nerve activity. MSNA was determined by observing spontaneous multifiber bursts of activity, and confirmed by having the subject perform end-expiratory apnea with a resultant increase in MSNA. The signal was distinguished from skin sympathetic nerve activity by performing auditory stimulation with no subsequent neural reaction. A shift in the neurogram during mental stress prevented recordings in 18 normotensive (11 fish oil, 7 placebo) and 17 prehypertensive (9 fish oil, 8 placebo) individuals. Therefore, nerve recordings are reported in only 30 (20 normotensive, 10 prehypertensive) subjects for the mental stress portion of the study.

Arm and leg blood flow were measured using venous occlusion plethysmography (D.E. Hokanson, Inc., Bellevue, WA). Occlusion cuffs were placed around the left upper arm, wrist, thigh, and ankle. The wrist and ankle cuffs were inflated to 220mmHg in order to temporarily prevent circulation to the hand and foot. The arm and thigh cuffs were then inflated to 60mmHg for 7 seconds and deflated for 8 seconds (i.e. 4 readings per minute). Inflation of the collecting cuffs allows arterial blood flow into the forearm and calf, but prevents venous flow return. Strain gauges were placed around the greatest circumference of the forearm and calf allowing for direct measurements of changes in arm and leg circumference. Technical issues and artifact during mental stress prevented the analysis of forearm and calf blood flow in several subjects. We were able to record forearm measurements in 29 fish oil subjects and 26 placebo subjects, while in the calf we recorded measurements in 28 fish oil and 25 placebo subjects.

3.2.4 Data Analysis

Data were recorded using WinDaq/Pro data acquisition software (DATAQ Instruments Inc., Akron, Ohio), and imported into WinCPRS (Absolute Aliens, Turku, Finland) software package for analysis. R-waves from the electrocardiogram were

detected and marked in the time series. Integrated bursts of MSNA were detected as a 3:1 burst-noise ratio within a search window of 0.5 seconds based on an average expected burst peak latency of 1.3 seconds following the previous R-wave. Spontaneous bursts of MSNA were normalized to the average burst size during baseline, and designated a value of 100 arbitrary units. Sympathetic bursts of activity were expressed as burst frequency (bursts/minute), burst incidence (bursts/100 heartbeats) and total activity (arbitrary units). Total MSNA was determined as the burst activity multiplied by the average normalized area under the burst. This analysis accounts for the change in the size of the burst per minute. Blood flow data were analyzed using the NIVP3 software (D.E. Hokanson, Inc., Bellevue, WA). Percent changes in flow per time were analyzed in both the forearm and calf. Absolute limb blood flow, as well as limb vascular resistance and conductance were reported. Vascular resistance was calculated as the mean arterial pressure divided by the absolute blood flow measurement, whereas vascular conductance was calculated as the reciprocal of resistance (i.e. blood flow divided by mean arterial pressure).

3.2.5 Statistical Analysis

Data are presented as mean \pm SE and analyzed using commercial software SPSS 18.0 (SPSS Chicago, IL). Effects of fish oil and placebo at rest and mental stress were analyzed in the normotensive and prehypertensive groups, respectively. Resting values were compared using a 1-between (fish oil vs. placebo) by 1-within (pre vs. post) repeated measures ANOVA. Statistically significant differences were analyzed post-hoc using paired t-tests. Significance was determined as $P < 0.05$.

The mental stress condition was analyzed using a 1-between (fish oil vs. placebo) by 2-within (pre vs. post; baseline vs. mental stress) repeated measures ANOVA. Those analyses that showed a significant ($P < 0.05$) interaction were further analyzed post-hoc using a paired t-test when comparing pre vs. post within groups as well as independent t-tests when analyzing between groups. Prehypertensive and normotensive groups were analyzed separately in order to better compare individual group responses to fish oil or placebo supplementation. These groups were further combined into one group, and analyzed using a 1-between (fish oil vs. placebo) by 2-within (pre vs. post; baseline vs. mental stress) repeated measures ANOVA. Any

interactions that were significantly different were further analyzed post-hoc using a paired t-test.

Mauchly's test of sphericity was performed for all ANOVA analyses in order to test for differences between variances. A significant ($P < 0.05$) result led to the use the Huynh-Feldt (sphericity values greater than 0.75) or Greenhouse-Geisser (sphericity values less than 0.75) correction factors.

3.3 Results

3.3.1 Resting Baseline Measurements for Prehypertensive and Normotensive Subjects

Resting blood pressure and heart rate values for normotensive and prehypertensive groups are reported in Tables 3.1 and 3.2 respectively. There were no differences in SAP, DAP, MAP or HR following fish oil or placebo supplementation. Resting forearm and calf vascular measurements are presented in Tables 3.3 and 3.4 for normotensive and prehypertensive groups, respectively. FBF, FVR, FVC, CVR and CVC all showed a significant time effect (pre vs. post) in the normotensive group, but these responses were not different between fish oil and placebo groups. All limb blood flow measurements in the prehypertensive group were not statistically altered by either fish oil or placebo.

Resting MSNA measurements are represented in Fig. 3.2. In the normotensive group, MSNA burst frequencies were not significantly different following either fish oil or placebo supplementation (fish oil, 11 ± 2 to 10 ± 1 bursts/min; placebo, 10 ± 2 to 13 ± 2 bursts/min; time, $P=0.73$; time x drug interaction, $P=0.16$). Similarly, MSNA incidences were not different following either fish oil or placebo supplementation (fish oil, 18 ± 3 to 16 ± 2 bursts/100 heart beats; placebo, 17 ± 2 to 19 ± 3 bursts/100 heart beats; time, $P=0.86$; time x drug interaction, $P=0.34$). Similar findings were observed in the prehypertensive individuals (Fig. 3.2).

Table 3.1. Resting hemodynamics before and after 8 weeks of fish oil supplementation or placebo in normotensive adults.

Variable	Pre-Treatment				Post -Treatment			
	Day 1	Day 2	Day 3	Ave	Day 1	Day 2	Day 3	Ave
Normotensive, Fish Oil Group (n=19):								
SAP (mmHg)	110 ± 2	109 ± 2	110 ± 2	110 ± 1	109 ± 1	107 ± 2	106 ± 3	107 ± 2
DAP (mmHg)	65 ± 1	67 ± 1	66 ± 1	66 ± 1	67 ± 1	67 ± 1	66 ± 1	66 ± 1
MAP (mmHg)	80 ± 1	81 ± 1	80 ± 1	80 ± 1	81 ± 1	80 ± 1	75 ± 4	80 ± 1
HR (beats/min)	71 ± 2	70 ± 2	72 ± 3	71 ± 2	69 ± 3	69 ± 3	75 ± 3	71 ± 2
Normotensive, Placebo Group (n=19):								
SAP (mmHg)	108 ± 2	107 ± 2	105 ± 2	107 ± 2	107 ± 2	106 ± 2	108 ± 2	107 ± 2
DAP (mmHg)	66 ± 2	65 ± 2	65 ± 1	65 ± 1	68 ± 1	64 ± 1	67 ± 1	66 ± 1
MAP (mmHg)	80 ± 1	79 ± 1	78 ± 1	79 ± 1	81 ± 1	78 ± 1	80 ± 2	80 ± 1
HR (beats/min)	74 ± 3	73 ± 2	75 ± 2	74 ± 2	76 ± 3	73 ± 3	78 ± 2	76 ± 2

Values are mean ± SE. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial blood pressure; HR, heart rate. All time and time × drug interactions were P>0.05.

Table 3.2. Resting hemodynamics before and after 8 weeks of fish oil supplementation or placebo in prehypertensive adults.

Variable	Pre-Treatment				Post -Treatment			
	Day 1	Day 2	Day 3	Ave	Day 1	Day 2	Day 3	Ave
Prehypertensive, Fish Oil Group (n=15):								
SAP (mmHg)	127 ± 2	126 ± 2	126 ± 1	127 ± 1	126 ± 2	124 ± 1	126 ± 2	125 ± 2
DAP (mmHg)	68 ± 3	69 ± 3	67 ± 3	68 ± 2	70 ± 2	68 ± 2	65 ± 2	68 ± 2
MAP (mmHg)	88 ± 2	88 ± 2	87 ± 2	88 ± 2	88 ± 2	87 ± 1	85 ± 1	87 ± 1
HR (beats/min)	77 ± 4	73 ± 3	76 ± 4	73 ± 2	71 ± 3	74 ± 3	72 ± 2	73 ± 2
Prehypertensive, Placebo Group (n=14):								
SAP (mmHg)	128 ± 3	126 ± 2	127 ± 2	126 ± 2	123 ± 2	122 ± 2	125 ± 2	123 ± 2
DAP (mmHg)	73 ± 2	74 ± 2	74 ± 2	74 ± 2	73 ± 2	73 ± 2	74 ± 2	74 ± 2
MAP (mmHg)	91 ± 2	91 ± 2	92 ± 2	92 ± 1	90 ± 2	89 ± 2	91 ± 2	90 ± 2
HR (beats/min)	74 ± 3	71 ± 3	73 ± 3	76 ± 3	72 ± 4	74 ± 3	76 ± 4	74 ± 3

Values are mean ± SE. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial blood pressure; HR, heart rate. All time and time × drug interactions were P>0.05.

Table 3.3. Baseline values for normotensive subjects pre and post fish oil or placebo supplementation.

Variable	Fish Oil		Placebo		Time Interaction	Time x Drug Interaction
	PRE	POST	PRE	POST		
FBF (units)	2.5 ± 0	2.8 ± 0	2.2 ± 1	2.5 ± 1	0.05	0.81
FVR (mmHg/units)	45 ± 5	40 ± 5	49 ± 5	41 ± 5*	0.02	0.68
FVC (100*units/mmHg)	2.7 ± 0	3.2 ± 0*	2.4 ± 0	2.9 ± 0*	0.01	0.95
CBF (units)	2.0 ± 0	2.2 ± 0	2.2 ± 0	2.3 ± 0	0.14	0.70
CVR (mmHg/units)	54 ± 4	45 ± 4*	46 ± 5	40 ± 3*	0.01	0.56
CVC (100*units/mmHg)	2.1 ± 0	2.4 ± 0*	2.5 ± 0	2.8 ± 0*	0.01	0.93

Values are mean ± SE. MSNA, muscle sympathetic nerve activity (n = 16 for fish oil and n = 17 for placebo); FBF, forearm blood flow (n = 18 for fish oil and n = 18 for placebo); FVR, forearm vascular resistance (n = 18 for fish oil and n = 18 for placebo); FVC, forearm vascular conductance (n = 18 for fish oil and n = 18 for placebo); CBF, calf blood flow (n = 16 for fish oil and n = 16 for placebo); CVR, calf vascular resistance (n = 16 for fish oil and n = 16 for placebo); CVC, calf vascular conductance (n = 16 for fish oil and n = 16 for placebo) *Significantly different from pre value for fish oil or placebo, respectively.

Table 3.4. Baseline values for prehypertensive subjects pre and post fish oil or placebo supplementation.

Variable	Fish Oil		Placebo		Time Interaction	Time x Drug Interaction
	PRE	POST	PRE	POST		
FBF (units)	3.9 ± 0	3.5 ± 0	3.2 ± 0	3.4 ± 1	0.80	0.37
FVR (mmHg/units)	27 ± 3	30 ± 3	33 ± 3	37 ± 6	0.21	0.75
FVC (100*units/mmHg)	4.1 ± 0	3.9 ± 0	3.4 ± 0	3.7 ± 1	0.96	0.55
CBF (units)	2.8 ± 0	3.0 ± 0	2.4 ± 0	2.5 ± 0	0.48	0.82
CVR (100*mmHg/units)	36 ± 3	35 ± 4	42 ± 2	44 ± 5	0.92	0.57
CVC (units/mmHg)	3.0 ± 0	3.4 ± 0	2.5 ± 0	2.7 ± 0	0.16	0.67

Values are mean ± SE. MSNA, muscle sympathetic nerve activity (n = 11 for fish oil and n = 10 for placebo); FBF, forearm blood flow (n = 14 for fish oil and n = 13 for placebo); FVR, forearm vascular resistance (n = 14 for fish oil and n = 13 for placebo); FVC, forearm vascular conductance (n = 14 for fish oil and n = 13 for placebo); CBF, calf blood flow (n = 14 for fish oil and n = 11 for placebo); CVR, calf vascular resistance (n = 14 for fish oil and n = 11 for placebo); CVC, calf vascular conductance (n = 14 for fish oil and n = 11 for placebo).

3.3.2 Resting Measurements for Combined Non-Hypertensive Subjects

We combined normotensive and prehypertensive groups into one non-hypertensive group in order to increase statistical power of our analyses. Combining the normotensive and prehypertensive groups yielded no differences in blood pressure or muscle sympathetic nerve activity responses after fish oil or placebo. Resting values for the combined groups are represented in Table 3.5. There were no significant interactions between fish oil and placebo supplementation groups at rest.

Table 3.5. Baseline values for combined non-hypertensive subjects pre and post fish oil or placebo supplementation.

	Fish Oil		Placebo		Interaction P-Value
	PRE	POST	PRE	POST	
SAP (mmHg)	117 ± 2	115 ± 2	115 ± 2	114 ± 2	0.53
DAP (mmHg)	67 ± 1	67 ± 1	69 ± 1	69 ± 1	0.79
MAP (mmHg)	84 ± 1	83 ± 1	84 ± 1	84 ± 2	0.67
HR (beats/min)	72 ± 2	72 ± 2	75 ± 2	75 ± 2	0.82
MSNA (bursts/min)	11 ± 1	10 ± 1	12 ± 1	12 ± 2	0.72
MSNA (bursts/100hb)	17 ± 2	16 ± 2	18 ± 2	18 ± 2	0.98
FBF (units)	3.1 ± 0.2	3.1 ± 0.2	2.6 ± 0.2	2.8 ± 0.3	0.51
FVR (mmHg/units)	37 ± 3	35 ± 3	42 ± 3	40 ± 3	0.86
FVC (100*units/mmHg)	3.3 ± 0.3	3.5 ± 0.3	2.7 ± 0.2	2.4 ± 0.3	0.60
CBF (units)	2.4 ± 0.2	2.6 ± 0.2	2.3 ± 0.1	2.4 ± 0.2	0.67
CVR (100*mmHg/units)	46 ± 3	40 ± 3	45 ± 3	42 ± 3	0.50
CVC (units/mmHg)	2.5 ± 0.2	2.9 ± 0.2	2.4 ± 0.1	2.8 ± 0.2*	0.73

Values are mean ± SE (n = 34 for fish oil, and n = 33 for placebo unless noted). SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial blood pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity (n = 27 for fish oil and n = 27 for placebo); FBF, forearm blood flow (n = 32 for fish oil and n = 31 for placebo); FVR, forearm vascular resistance (n = 32 for fish oil and n = 31 for placebo); FVC, forearm vascular conductance (n = 32 for fish oil and n = 31 for placebo); CBF, calf blood flow (n = 30 for fish oil and n = 27 for placebo); CVR, calf vascular resistance (n = 30 for fish oil and n = 27 for placebo); CVC, calf vascular conductance (n = 30 for fish oil and n = 27 for placebo).

We further investigated the relationships between blood pressure changes and MSNA changes during rest, and these are presented as figure 3.3 and 3.4. A significant correlation was found between the changes in MAP and MSNA burst frequency following fish oil supplementation ($R=0.354$, $P=0.03$) compared to placebo ($R= -0.036$, $P=0.86$). Likewise, a correlation was observed when comparing changes in MAP and MSNA burst incidence following fish oil ($R=0.325$, $P=0.05$) and placebo ($R=-0.062$, $P=0.758$) supplementation. Similarly, a significant change was seen in the change in MSNA bursts per minute ($R=-.466$, $P=.007$), and MSNA bursts per 100 heart beats ($R=-.429$, $P=.013$) when compared to the resting HR before supplementation.

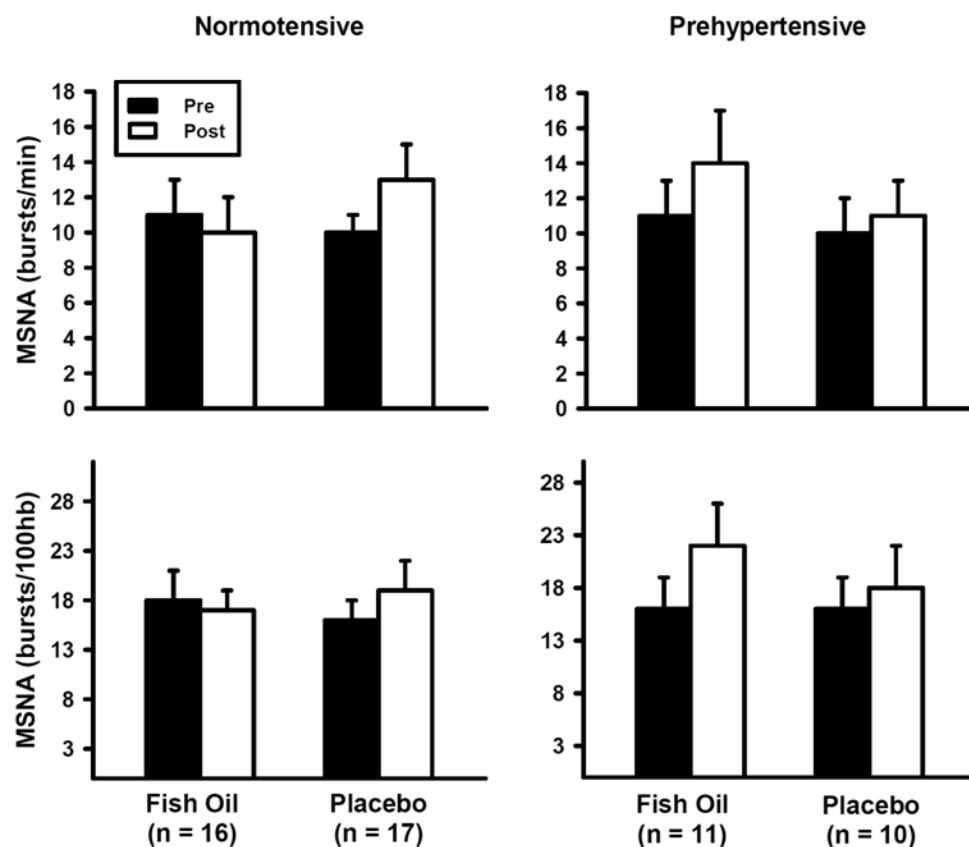


Figure 3.2. Resting MSNA pre and post fish oil and placebo supplementation in normotensive and prehypertensive groups. There was no significance between pre and post treatments.

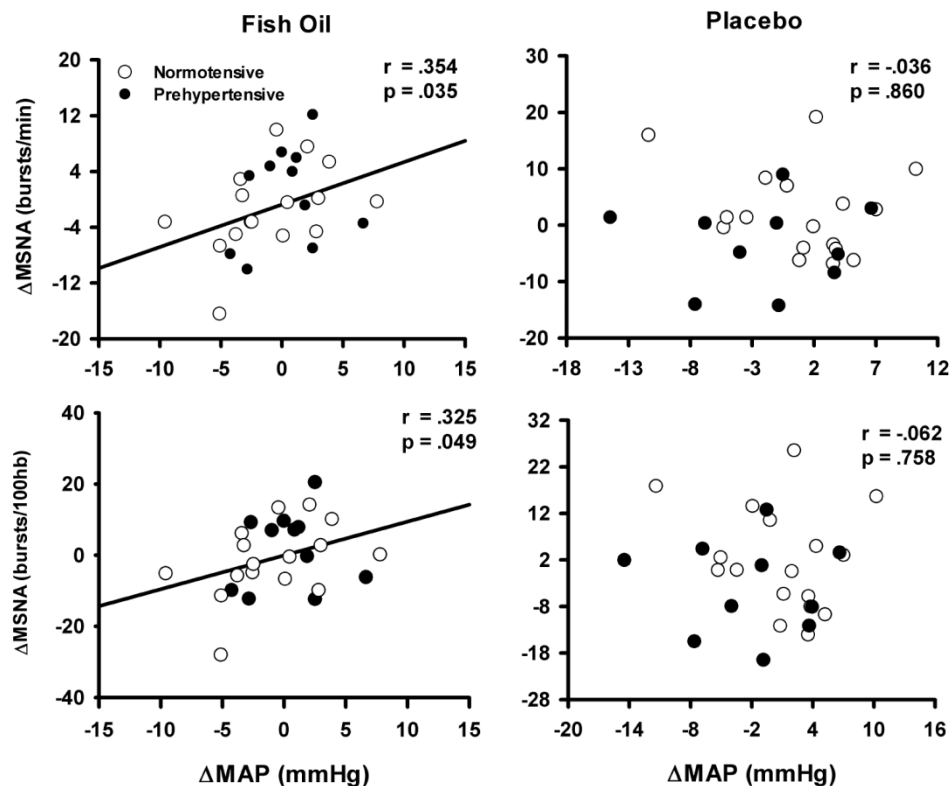


Figure 3.3. Linear regressions of combined non-hypertensive subjects following fish oil or placebo supplementation. Significance was considered as $P < 0.05$

3.3.3 Hemodynamic Responses to Mental Stress in Prehypertensive and Normotensive Subjects

Blood pressure responses to mental stress in normotensive and prehypertensive subjects are represented in figure 3.4. In normotensives, mental stress significantly increased SAP (fish oil, $\Delta 12 \pm 2$ to $\Delta 13 \pm 2$ mmHg; placebo, $\Delta 9 \pm 1$ to $\Delta 8 \pm 2$ mmHg; $P < .001$), DAP (fish oil, $\Delta 10 \pm 1$ to $\Delta 11 \pm 1$ mmHg; placebo, $\Delta 8 \pm 1$ to $\Delta 8 \pm 1$ mmHg; $P < .001$), MAP (fish oil, $\Delta 12 \pm 1$ to $\Delta 13 \pm 1$ mmHg; placebo, $\Delta 10 \pm 1$ to $\Delta 9 \pm 1$ mmHg; $P < .001$), but these responses were similar across supplementation groups (time x condition x drug interactions, $P > .202$).

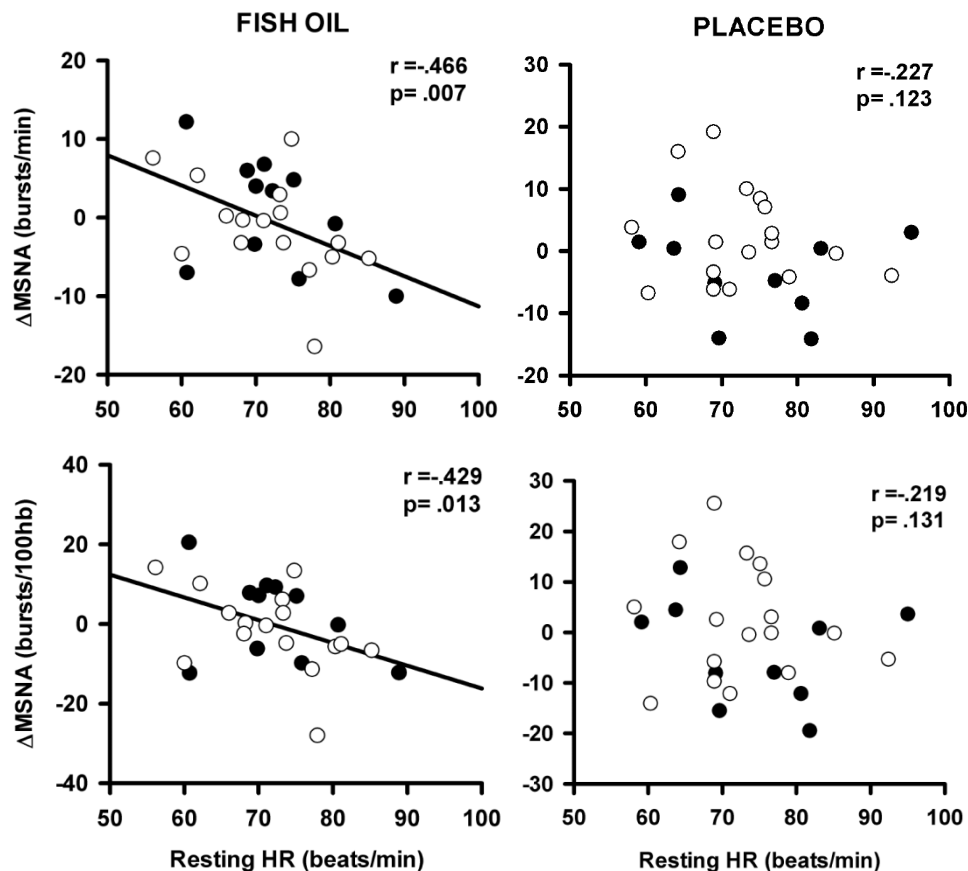


Figure 3.4. Linear regressions between change in MSNA and resting HR following omega-3 supplementation in combined non-hypertensive subjects following fish oil or placebo supplementation. Significance was considered as $P < 0.05$

Similarly in prehypertensives, mental stress significantly increased SAP (fish oil, $\Delta 15 \pm 3$ to $\Delta 14 \pm 3$ mmHg; placebo, $\Delta 14 \pm 3$ to $\Delta 14 \pm 4$ mmHg; $P < .001$), DAP (fish oil, $\Delta 11 \pm 2$ to $\Delta 10 \pm 1$ mmHg; placebo, $\Delta 11 \pm 1$ to $\Delta 10 \pm 2$ mmHg; $P < .001$), MAP (fish oil, $\Delta 15 \pm 2$ to $\Delta 13 \pm 2$ mmHg; placebo, $\Delta 14 \pm 2$ to $\Delta 13 \pm 2$ mmHg; $P < .001$) in both pre and post trials. These responses were similar across supplementation groups (time x condition x drug interactions, $P > .388$).

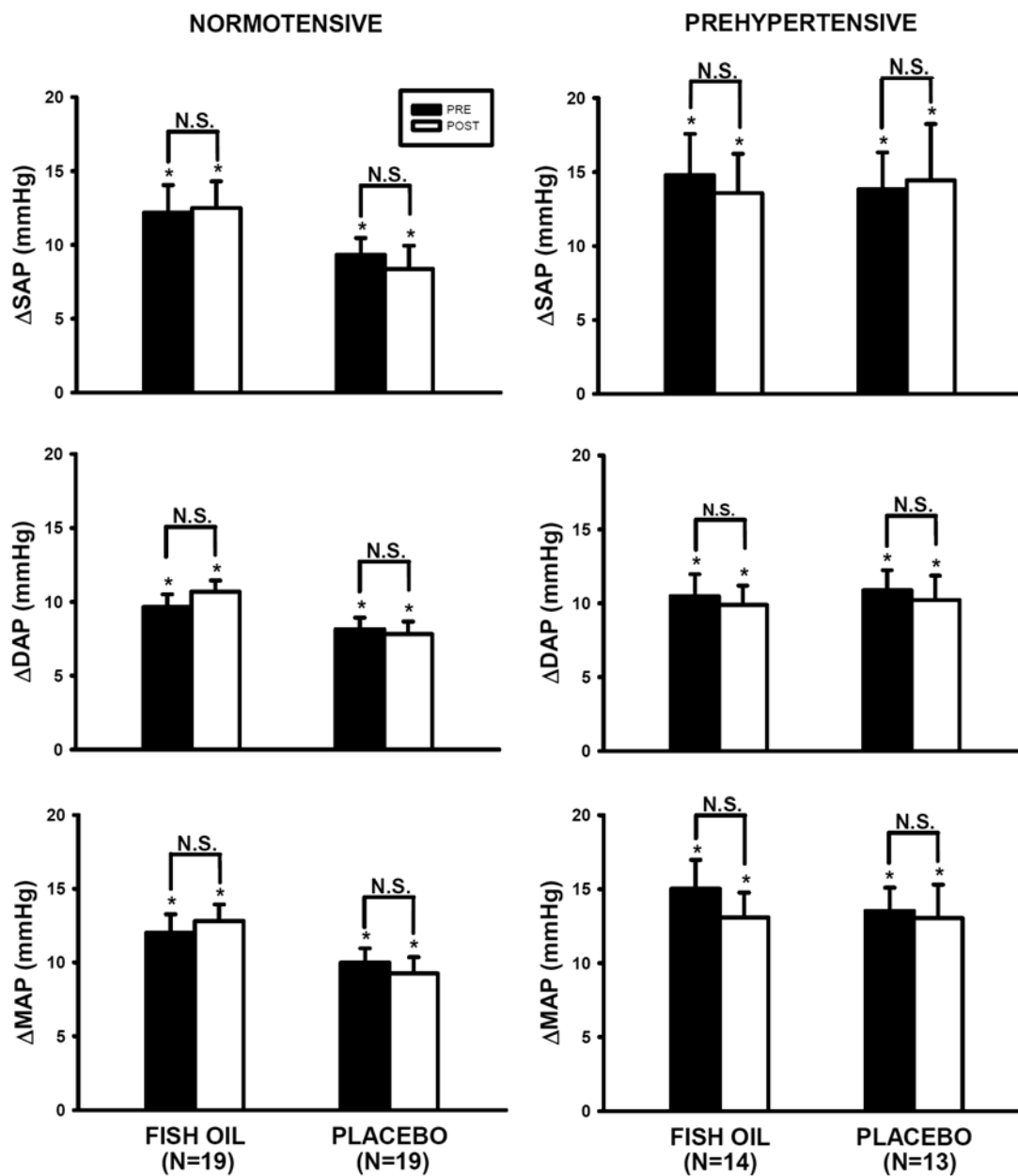


Figure 3.5. Blood pressure responses to mental stress in normotensive and prehypertensive subjects pre and post fish oil or placebo supplementation. *Significantly increased responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

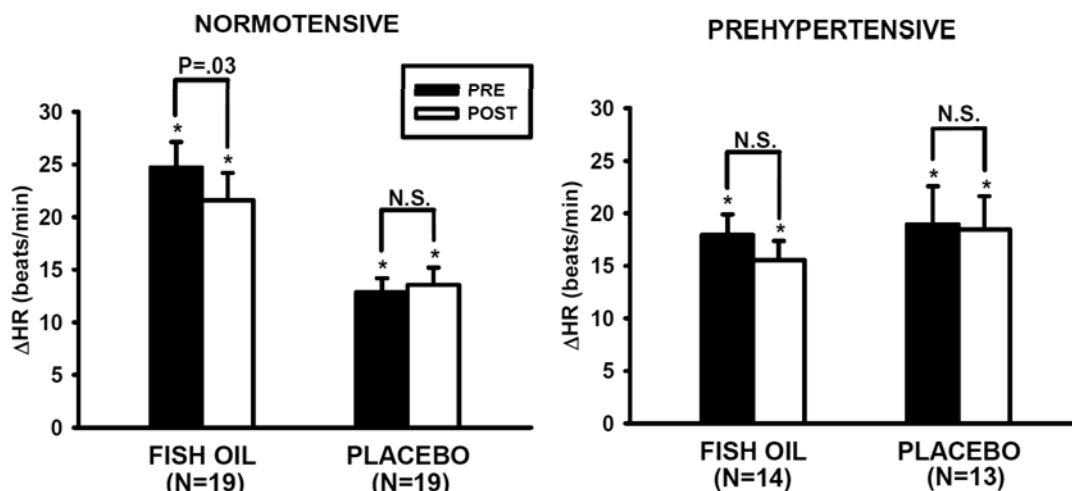


Figure 3.6. Heart rate responses to mental stress in normotensive and prehypertensive subjects pre and post fish oil or placebo supplementation. *Significantly increased responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

Heart rate responses to mental stress are shown in figure 3.6. Mental stress significantly increased HR in normotensives (fish oil, $\Delta 23 \pm 2$ and $\Delta 22 \pm 3$ beats/min; placebo, $\Delta 13 \pm 1$ and $\Delta 14 \pm 2$ beats/min; $P < .001$, pre and post respectively) groups, but this response was blunted post fish oil compared to placebo (time x condition x drug interaction, $P = .015$). In prehypertensives, HR responses to mental stress were significantly increased (fish oil, $\Delta 18 \pm 2$ and $\Delta 16 \pm 2$ beats/min; placebo, $\Delta 19 \pm 4$ and $\Delta 19 \pm 3$ beats/min; $P < .001$, pre and post respectively), but were not significantly different between fish oil and placebo groups (time x condition x drug interaction, $P = .377$).

3.3.4 Sympathetic Nerve Responses to Mental Stress for Normotensive and Prehypertensive Subjects

MSNA responses to mental stress in normotensive (Table 3.6) and prehypertensive (Table 3.7) subjects are presented below. Neurograms were obtained in only a total of 20 normotensive subjects (8 fish oil, 12 placebo), and 9 prehypertensive (4 fish oil, 5 placebo) subjects. This number was not enough to justify statistical analysis due to lack of power for a time x condition x drug comparison. Therefore data are presented in table format. However, review of the data appears to indicate a difference in total MSNA in the fish oil group but not in the placebo group for both normotensive and prehypertensive subjects. Therefore, we pooled the normotensive and

prehypertensive groups together and analyzed them as a combined group. Data are presented in section 3.3.6.

3.3.5 Peripheral Vascular Responses to Mental Stress in Prehypertensive and Normotensive Subjects

Forearm blood flow responses to mental stress in normotensive and prehypertensive subjects are presented in figure 3.7. In normotensives, mental stress elicited statistically similar increases in FBF (fish oil, $\Delta 109.1 \pm 16\%$ and $\Delta 104.1 \pm 18\%$; placebo, $\Delta 58.9 \pm 11\%$ and $\Delta 54.1 \pm 10\%$; $P < .001$, pre and post respectively), decreases in FVR (fish oil, $\Delta -37.9 \pm 5\%$ and $\Delta -35.6 \pm 5\%$; placebo, $\Delta -25.6 \pm 5\%$ and $\Delta -20.1 \pm 5\%$; $P < .001$, pre and post respectively), and increases in FVC (fish oil, $\Delta 86.0 \pm 14\%$ and $\Delta 78.4 \pm 16\%$; placebo, $\Delta 45.1 \pm 10\%$ and $\Delta 39.3 \pm 9\%$; $P < .001$, pre and post respectively). Forearm vascular responses to mental stress were similar in both supplement groups (time x condition x drug interaction, $P > .653$).

In prehypertensive subjects, mental stress elicited statistically similar increases in FBF (fish oil, $\Delta 71.8 \pm 14\%$ and $\Delta 50.4 \pm 13\%$; placebo, $\Delta 67.6 \pm 17\%$ and $\Delta 45.6 \pm 15\%$; $P < .013$, pre and post respectively), decreases in FVR (fish oil, $\Delta -18.1 \pm 7\%$ and $\Delta -12.6 \pm 8\%$; placebo, $\Delta -23.6 \pm 7\%$ and $\Delta -14.1 \pm 6\%$, $P < .05$, pre and post respectively), and increases in FVC (fish oil, $\Delta 48.9 \pm 13\%$ and $\Delta 30.8 \pm 11\%$; placebo, $\Delta 45.4 \pm 14\%$ and $\Delta 31.2 \pm 14\%$; $P < .05$). Similar to normotensive subjects, there were no significant differences between supplement groups (time x condition x drug interaction, $P > .697$).

Table 3.6. Changes in MSNA during mental stress pre and post fish oil or placebo supplementation in normotensive subjects.

	Fish Oil		Placebo	
	PRE	POST	PRE	POST
Δ MSNA (bursts/min)	6 \pm 2	7 \pm 1	6 \pm 2	7 \pm 2
Δ MSNA (bursts/100hb)	5 \pm 4	6 \pm 2	5 \pm 2	5 \pm 2
Δ Total MSNA (arb. units)	9414 \pm 3145	4944 \pm 849	5547 \pm 1670	7463 \pm 2688

Values are mean \pm SE. MSNA, muscle sympathetic nerve activity (n = 8 for fish oil and n = 12 for placebo); Total MSNA (n = 7 for fish oil and n = 10 for placebo).

Table 3.7. Changes in MSNA during mental stress pre and post fish oil or placebo supplementation in prehypertensive subjects.

	Fish Oil		Placebo	
	PRE	POST	PRE	POST
Δ MSNA (bursts/min)	5 \pm 3	0 \pm 2	6 \pm 2	4 \pm 2
Δ MSNA (bursts/100hb)	3 \pm 4	-3 \pm 2	1 \pm 2	1 \pm 3
Δ Total MSNA (arb. units)	17349 \pm 6974	-745 \pm 1273	10143 \pm 2405	3402 \pm 2263

Values are mean \pm SE. MSNA, muscle sympathetic nerve activity (n = 4 for fish oil and n = 5 for placebo); Total MSNA (n = 3 for fish oil and n = 4 for placebo).

Figure 3.8 represents the calf vascular responses to mental stress in normotensive and prehypertensive subjects. In the normotensive group, mental stress significantly increased CBF (fish oil, Δ 53 \pm 12% and Δ 32.9 \pm 8%; placebo Δ 25.2 \pm 9% and Δ 22.7 \pm 10%; $P < .05$, pre and post respectively). Fish oil groups CVR responses to mental stress were mixed (fish oil, Δ -15.1 \pm 7% pre; $P < .04$, and Δ -6.5 \pm 5% post; $P = .243$), and placebo CVR responses did not change (fish oil, Δ -6.7 \pm 4% and Δ -2.3 \pm 6%; $P > .135$, pre and post respectively). CVC significantly increased fish oil (Δ 36.3 \pm 11% and Δ 16.1 \pm 7%; $P < .05$, pre and post respectively), but not placebo (Δ 14.6 \pm 8% and Δ 10.4 \pm 9%; $P > .074$, pre and post respectively). Fish oil supplementation blunted CBF ($P = .011$), CVR ($P = .004$) and CVC ($P = .007$) responses to mental stress in fish oil but not placebo.

Prehypertensive calf vascular responses to mental stress were mixed. CBF increased pre and post fish oil and placebo (Δ 29.9 \pm 9% pre, Δ 19.1 \pm 7% post, fish oil; Δ 23.4 \pm 7% pre, Δ 23 \pm 8% post, placebo; $P < .02$), but responses were not different pre vs. post in either group (time x condition x drug interaction, $P > .160$). CVR did not change during mental stress (Δ 3.1 \pm 9% pre, Δ 3.2 \pm 6% post, fish oil; Δ -0.6 \pm 5% pre, Δ -3.1 \pm 6% post, placebo; $P = .883$), nor was there a difference pre vs. post in either fish oil or placebo group (time x condition x drug interaction, $P = .781$). Similarly, CVC did not change during mental stress (Δ 12.1 \pm 8% pre, Δ 3.74 \pm 7% post, fish oil; Δ 6.2 \pm 6% pre, Δ 5.8 \pm 5% post, placebo; $P = .093$), and these responses were not different when comparing pre vs. post supplement (time x condition x drug interaction, $P = .233$).

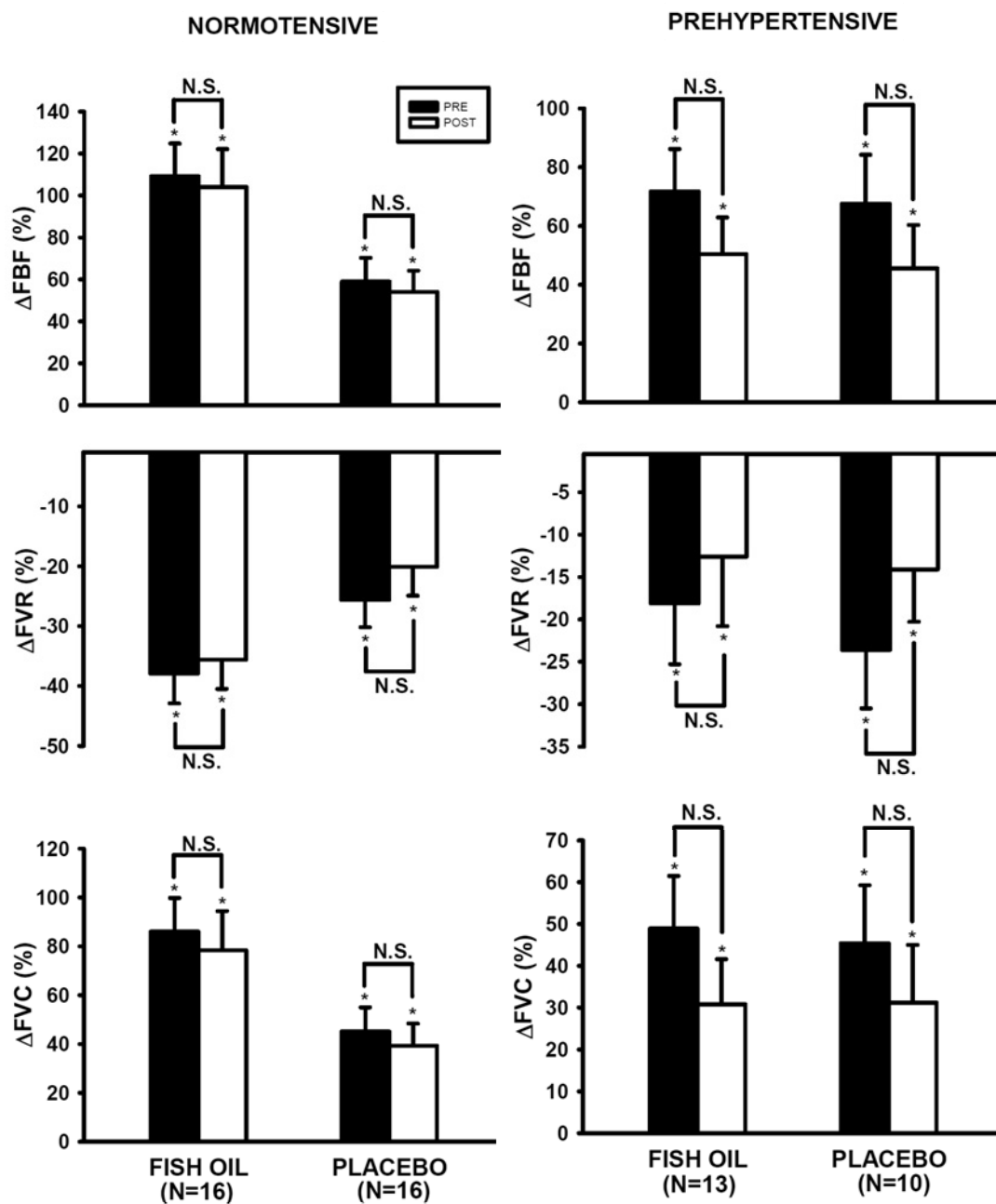


Figure 3.7. Changes (Δ) in forearm blood flow (FBF), vascular resistance (FVR) and vascular conductance (FVC) during mental stress in normotensive and prehypertensive subjects pre and post fish oil or placebo supplementation. *Significantly increased or decreased responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

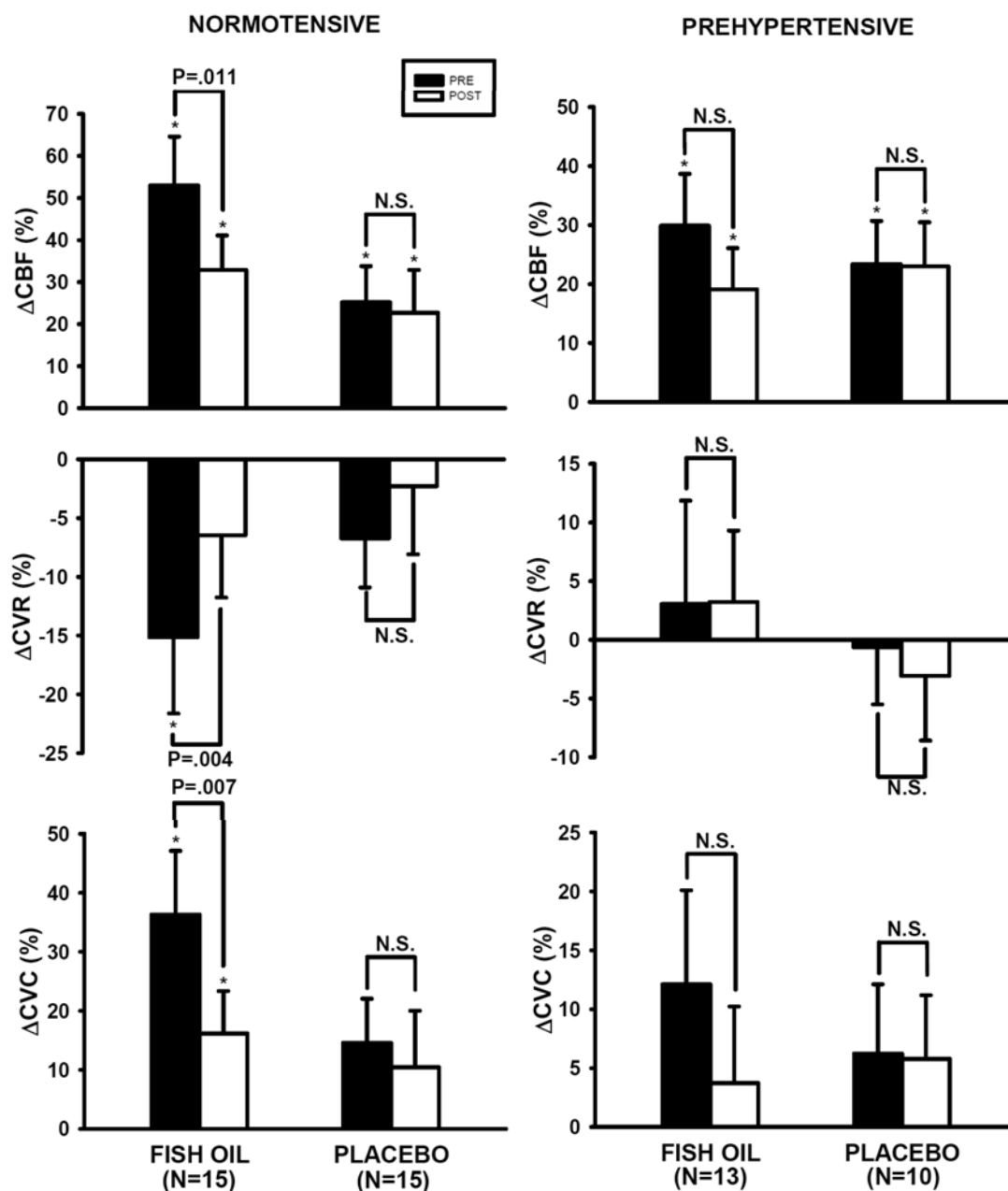


Figure 3.8. Changes (Δ) in calf blood flow (CBF), vascular resistance (CVR) and vascular conductance (CVC) during mental stress in normotensive and prehypertensive subjects pre and post fish oil or placebo supplementation. *Significantly increase or decrease responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

3.3.6 Responses to Mental Stress for Combined Non-Hypertensive Subjects

Combined blood pressure and heart rate responses to mental stress between fish oil and placebo trials are represented in figure 3.8. Mental stress significantly increased SAP (fish oil, $\Delta 13 \pm 2$ mmHg to $\Delta 13 \pm 2$ mmHg; placebo, $\Delta 11 \pm 1$ to $\Delta 11 \pm 2$ mmHg; $P < .001$), DAP (fish oil, $\Delta 10 \pm 1$ to $\Delta 10 \pm 1$ mmHg; placebo, $\Delta 9 \pm 1$ to $\Delta 9 \pm 1$ mmHg; $P < .001$), and MAP (fish oil, $\Delta 13 \pm 1$ to $\Delta 13 \pm 1$ mmHg; placebo, $\Delta 11 \pm 1$ to $\Delta 11 \pm 1$ mmHg; $P < .001$). However, blood pressure responses were not different following fish oil or placebo supplementation (time x condition x drug interaction; $P > .340$). HR responses to mental stress were attenuated following fish oil supplementation (fish oil, 21.8 ± 2 to $\Delta 19.0 \pm 2$ beats/min, $P = .002$), but not placebo ($\Delta 15.3 \pm 2$ to $\Delta 15.5 \pm 2$ beats/min, $P = .801$).

MSNA responses to mental stress are represented in figure 3.9. MSNA burst frequency (fish oil, $\Delta 6 \pm 2$ and $\Delta 5 \pm 1$ bursts/min; placebo, $\Delta 6 \pm 1$ and $\Delta 6 \pm 1$ bursts/min; $P < .001$, pre and post respectively) and MSNA burst incidence (fish oil, $\Delta 4 \pm 2$ and $\Delta 3 \pm 3$ bursts/100hb; placebo, $\Delta 4 \pm 2$ and $\Delta 4 \pm 2$ bursts/100hb; $P < .001$) significantly increased. However, there were no differences between supplementation groups (time x condition x drug interaction, $P > .700$). In contrast, total MSNA (figure 3.10) was reduced post fish oil ($\Delta 9628 \pm 2407$ to $\Delta 3830 \pm 1029$ a.u., $P = .029$), but not in the placebo group ($\Delta 6860 \pm 1463$ to $\Delta 6302 \pm 2042$ a.u., $P = .756$).

Combined forearm vascular responses to mental stress are shown in figure 3.12. Mental stress increased FBF (fish oil, $\Delta 92 \pm 11\%$ and $\Delta 80 \pm 11\%$; $\Delta 62 \pm 9\%$ and $\Delta 51 \pm 8\%$; $P < .001$, pre and post respectively), FVC (fish oil, $\Delta 69 \pm 10\%$ and $\Delta 57 \pm 11\%$; placebo, $\Delta 45 \pm 8\%$ and $\Delta 36 \pm 8\%$; $P < .001$, pre and post respectively), but there were no significant differences between groups (time x condition x drug interaction $P > .800$). Similarly, mental stress decreased FVR (fish oil, $\Delta -30 \pm 4\%$ and $\Delta -25 \pm 5\%$; placebo, $\Delta -25 \pm 4\%$ and $\Delta -18 \pm 4\%$; $P < .001$, pre and post respectively), but these responses were not significant between groups (time x condition x drug interaction $P = .733$).

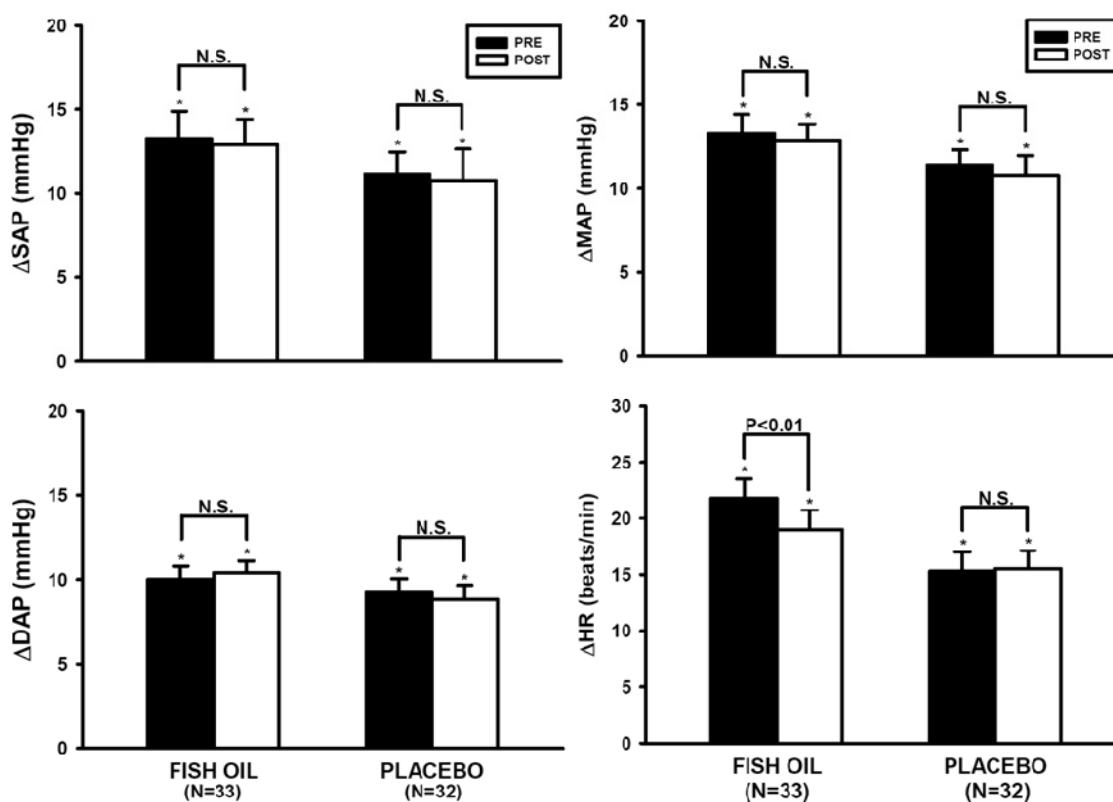


Figure 3.9. Changes (Δ) in systolic (SAP), diastolic (DAP), mean (MAP) arterial pressures and heart rate (HR) during mental stress in combined normotensive and prehypertensive (non-hypertensive) subjects pre and post fish oil or placebo supplementation. *Significantly increased responses during mental stress, $P<0.05$; N.S., no significance between pre and post trials.

Combined calf vascular responses to mental stress are shown in figure 3.13. Mental stress increased CBF (fish oil, $\Delta 42 \pm 8\%$ and $\Delta 27 \pm 6\%$; placebo, $\Delta 25 \pm 6\%$ and $\Delta 23 \pm 7\%$; $P<.002$, pre and post respectively). However, the fish oil group demonstrated a blunted calf vascular response during mental stress compared to placebo (time \times condition \times drug interaction, $P=.004$). Mental stress did not change CVR in either the fish oil ($\Delta -7 \pm 6\%$ and $\Delta -2 \pm 4\%$ pre and post respectively) or placebo ($\Delta -4 \pm 3\%$ and $\Delta -3 \pm 4\%$, pre and post respectively). Mental stress significantly increased CVC in the fish oil group ($\Delta 25 \pm 7\%$ and $\Delta 10 \pm 5\%$; $P<.05$, pre and post respectively), but only during the pre testing in the placebo group ($\Delta 11 \pm 5\%$ pre, $P<.012$, $\Delta 10 \pm 6\%$ post; $P<.147$). Fish oil significantly blunted the vascular conductance response ($P=.003$) in the calf compared to no change in the placebo group at rest (time \times condition \times drug interaction $P=.034$).

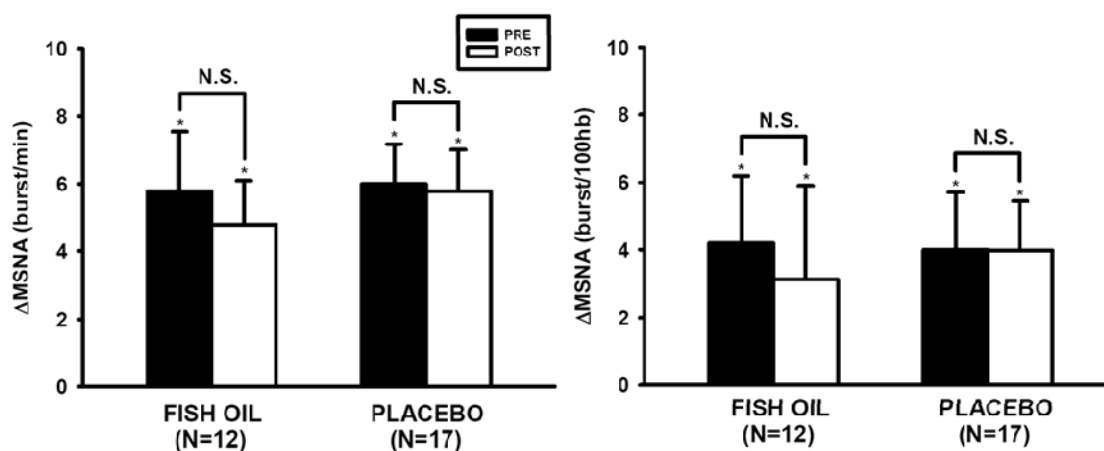


Figure 3.10. Changes (Δ) in muscle sympathetic nerve activity (MSNA), expressed as bursts per minute and bursts per 100 heartbeats, during mental stress in combined normotensive and prehypertensive (non-hypertensive) subjects pre and post fish oil or placebo supplementation. *Significantly increased responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

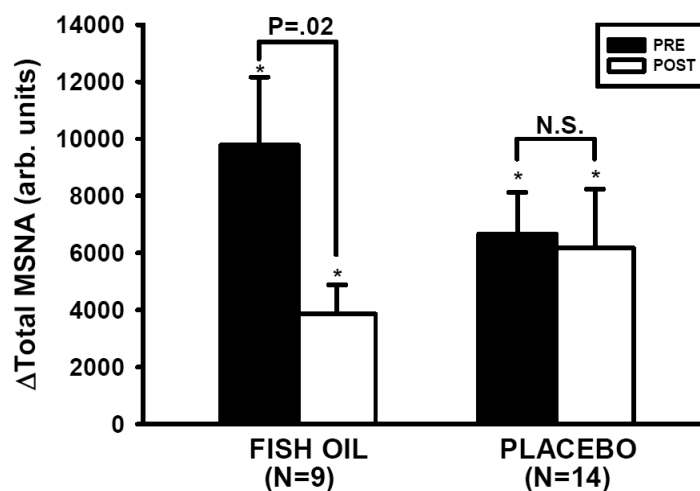


Figure 3.11. Changes (Δ) in muscle sympathetic nerve activity (MSNA), expressed as total activity, during mental stress in combined normotensive and prehypertensive (non-hypertensive) subjects pre and post fish oil or placebo supplementation. *Significantly increased responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

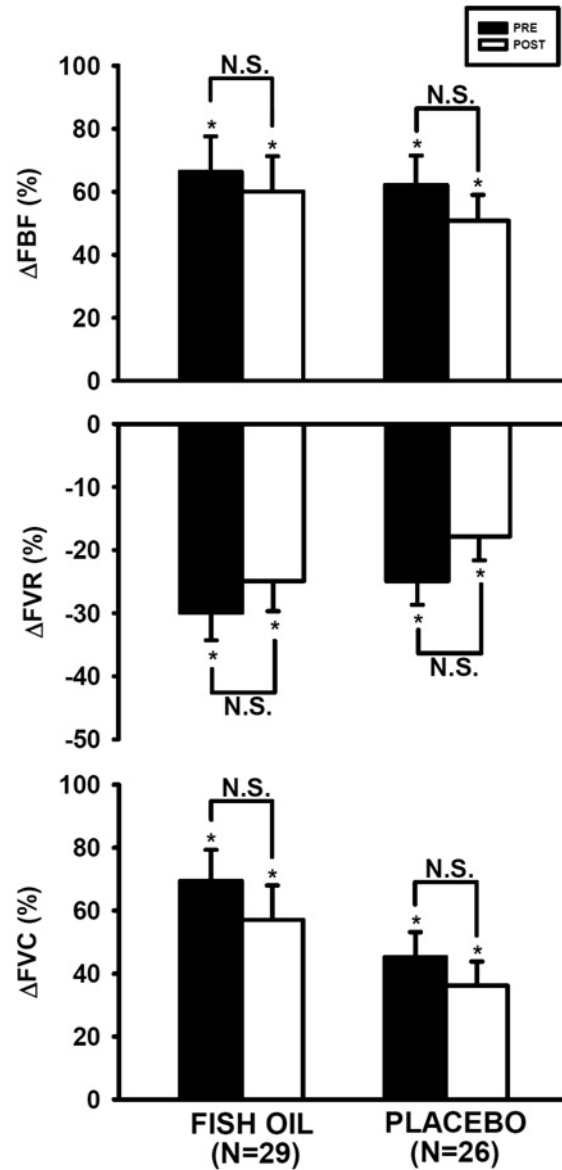


Figure 3.12. Changes (Δ) in forearm blood flow (FBF), vascular resistance (FVR) and vascular conductance (FVC) during mental stress in combined normotensive and prehypertensive (non-hypertensive) subjects pre and post fish oil or placebo supplementation. *Significantly increased or decreased responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

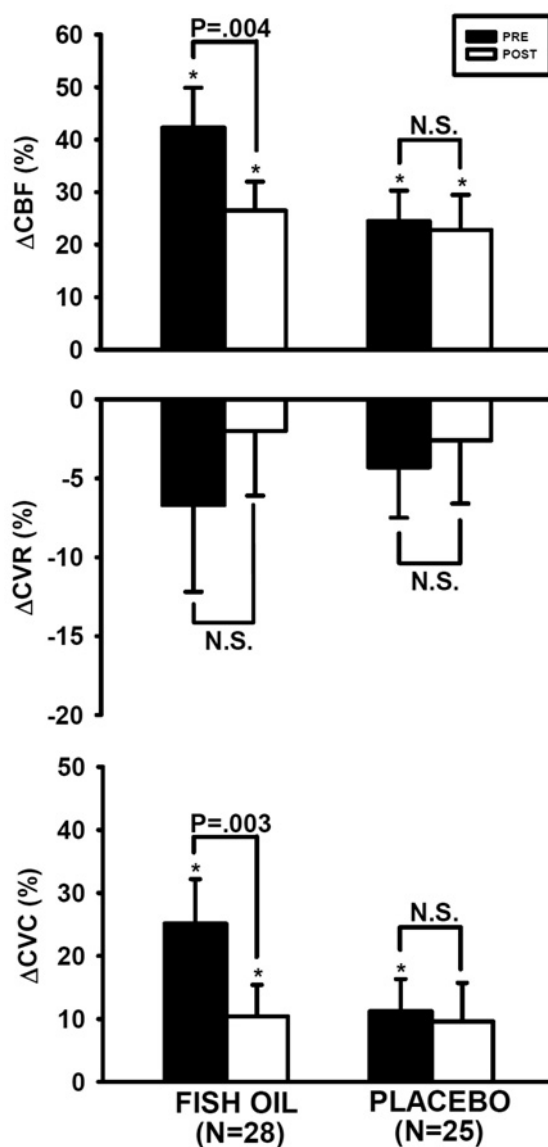


Figure 3.13. Changes (Δ) in calf blood flow (CBF), vascular resistance (CVR) and vascular conductance (CVC) during mental stress in combined normotensive and prehypertensive (non-hypertensive) subjects pre and post fish oil or placebo supplementation. *Significantly increased or decreased responses during mental stress, $P < 0.05$; N.S., no significance between pre and post trials.

3.4 Discussion

The current investigation offers several new and novel insights into the mechanistic influence of omega-3 fatty acids on neural and cardiovascular function. First, fish oil did not reduce that resting blood pressure or MSNA in either normotensive or prehypertensive individuals. However, further analysis of combined groups revealed a significant correlation between changes in arterial pressure and changes in MSNA following fish oil supplementation. This suggests that fish oil may have an influence on the sympathetic nervous system in individuals who experience a reduction in blood pressure following fish oil supplementation. Second, fish oil attenuated heart rate response during mental stress, and this effect may be mediated by the sympathetic nervous system (i.e., total MSNA). Third, omega-3 supplementation may improve peripheral vascular function at rest, but this does not appear to be specific to fish oils. Combined, these observations offer new information regarding the mechanistic affect omega-3s have on cardiovascular function at rest and during mental stress.

3.4.1 Resting Neurovascular Responses to Omega-3 supplementation

Fish oil has been suggested to lower blood pressure in hypertensive rather than normotensive individuals (Appel *et al.*, 1993; Morris *et al.*, 1993). This is the first study to investigate the effect of fish oil in prehypertension. Contrary to our original hypothesis, fish oil supplementation did not reduce resting blood pressure or resting MSNA in prehypertensive individuals. This finding is consistent with a prior study demonstrating no effect of fish oil on resting blood pressure, heart rate or MSNA in healthy normotensive individuals (Monahan *et al.*, 2004). However, we did observe a modest, yet significant, positive correlation between change in sympathetic nerve activity and change in blood pressure in the fish oil group. More specifically, subjects demonstrating a decrease of blood pressure after supplementation demonstrated a concomitant decrease in MSNA. Similarly, we found that a decrease in resting heart rate was significantly correlated with subject's resting blood pressure. It appears that fish oil may have a bradycardic influence as resting arterial pressure increases. Sympathetic nerve activity plays a strong role in both blood pressure regulation and heart rate. Our findings provide new evidence that fish oil may, in part, reduce blood pressure and heart rate via sympathetic neural mechanisms.

To date, only one study has investigated the effect of fish oil on MSNA. Monahan *et al.* (2004) reported that fish oil did not alter resting MSNA levels, however the relationship between changes in MSNA and changes in blood pressure or heart rate were not examined. It has been reported that resting heart rate decreases more dramatically in patients with elevated resting heart rates following fish oil supplementation (Mozaffarian *et al.*, 2005). Additionally, EPA and DHA have been reported to lower plasma norepinephrine in healthy volunteers (Hamazaki *et al.*, 2005), and improve heart rate variability measures in older adults (Mozaffarian *et al.*, 2008). These studies support our finding of altered autonomic function as a potential mechanism by which fish oil works to reduce cardiovascular risk. A large scale, longitudinal investigation on cardiovascular disease known as the Framingham study reported that elevated heart rate is linked with a higher incidence of cardiac mortality (Kannel *et al.*, 1987). Additionally, a decrease in heart rate may have significant implications in cardiovascular health (Jouven *et al.*, 2001). We suggest the sympathetic nervous system as a potential mechanism in the reduction of cardiovascular disease from fish oil. However, we recognize this is unlikely to be the only mechanism by which fish oil can play a role in cardiovascular disease prevention.

3.4.2 Neurovascular Responses to Mental Stress Pre and Post Omega-3 Supplementation

Fish oil has been suggested to reduce blood pressure through several mechanisms including altered endothelial function, reduced vascular restriction, and changes in the autonomic nervous system (Kannel *et al.*, 1987; Mori & Woodman, 2006). During mental stress, the classic physiological response is increased heart rate, blood pressure and sympathetic nerve activity with reduced vasoconstriction to the forearm. However the affect of omega-3 fatty acids on these neurovascular mechanisms has not been previously investigated.

Currently, only one study has examined the cardiovascular effects of omega-3 fatty acids during mental stress in humans. Delarue *et al.* (2003) reported that the circulating plasma catecholamine responses were blunted during mental stress following fish oil supplementation (Delarue *et al.*, 2003). Mental stress is known to simultaneously increase norepinephrine and epinephrine (Esler *et al.*, 1984b). However, epinephrine

appears to increase more dramatically during psychological stimulation compared to norepinephrine (LeBlanc *et al.*, 1979; Dimsdale & Moss, 1980). The epinephrine response during mental stress has been linked to sympathetic activation of the adrenal medulla (Reims *et al.*, 2004). We did not analyze circulating catecholamines in the present study. However, MSNA is known to be a robust model of measuring direct sympathetic neural activity. Additionally, it has been demonstrated that MSNA correlates strongly with norepinephrine spillover analysis (Wallin *et al.*, 1992; Kingwell *et al.*, 1994).

Fish oil did not alter MSNA burst frequency or burst incidence responses to mental stress, but did reduce total MSNA. This divergent sympathetic response may be an important distinction in understanding potential mechanisms underlying the effect of fish oil on the sympathetic nervous system during stress. Previously, it has been suggested that sympathetic burst activity and total MSNA operate off of two separate central nervous system (CNS) inputs (Reims *et al.*, 2004). The generation of a burst is suggested to be controlled through a gated (i.e. on/off) mechanism, while the total MSNA appears to operate via graded input. Whereas this gated mechanism may operate off of one CNS input, the graded mechanism may respond to multiple CNS inputs (Kienbaum *et al.*, 2001; Keller *et al.*, 2006). Recently, Keller *et al.* (2006) demonstrated that whole body heating increased burst activity (i.e. via the gating mechanism), but did not affect the total MSNA response, supporting the theory presented by Kienbaum *et al.* (2001) that these two mechanisms of muscle sympathetic nerve activity operate on two separate CNS inputs. Using this information, Steinback *et al.* (2010) began to investigate the neuron recruitment strategies of the sympathetic nerves that cause a greater burst size (i.e. total MSNA). Steinback *et al.* (2010) showed that the neuron recruitment in muscle sympathetic nerves may follow a similar pattern as the recruitment of skeletal muscle fibers proposed by Henneman *et al.* (1965). This theory proposes that recruitment of fibers is graded based on size, where the smaller slower fibers are recruited first followed by bigger fibers as greater force or stimulation is required (Henneman *et al.*, 1965; Keller *et al.*, 2006). Collectively, physical stressors have demonstrated different mechanisms in increasing MSNA activity through burst activity (Steinback *et al.*, 2010) or through total MSNA (Keller *et al.*, 2006). Mental stress typically increases both burst activity and total MSNA (Anderson *et al.*, 1991; Callister *et al.*, 1992). Based on the previous findings of Kienbaum *et al.* (2001), this suggests that

multiple mechanisms are influencing CNS control over sympathetic nerve activity during mental stress. The current study suggests that fish oil blunts the total MSNA response to mental stress, but not the burst frequency. This infers that fish oil may be affecting sympathetic neuron recruitment more so than a gating mechanism.

The current study demonstrated a blunted heart rate response to mental stress in the fish oil group but not the placebo in combined subjects (i.e., normotensive and prehypertensive groups). There are several possible mechanisms that could contribute to a reduction in heart rate. One such mechanism is sympathetic withdrawal which we have demonstrated through blunted total MSNA. Another mechanism could be an increase in parasympathetic nerve activity. Previous research has suggested that parasympathetic tone increases following fish oil supplementation (Mozaffarian *et al.*, 2008) and it has been suggested that hypertensive individuals have reduced parasympathetic activation on the heart during mental stress (Das, 2000). The only method for indirectly measuring parasympathetic nerve activity is via spectral analysis of heart rate variability. One recent study found that fish oil did not affect HRV during physiological stressors in dogs (Billman & Harris, 2011). Though we analyzed heart rate variability, the results did not offer any clear insight into the effects of omega 3 fatty acids on parasympathetic activity in normotensive or prehypertensives during mental stress. Therefore the affect of omega-3 fatty acids on the parasympathetic nervous system during mental stress remains equivocal.

Finally, altered vascular function has also been proposed as a potential mechanism for the cardiovascular benefits associated with fish oil. In the present study we monitored forearm and calf vascular response to mental stress. Whereas the BP, HR and MSNA responses to mental stress between prehypertensive and normotensive (as well as combined subjects) showed a clear picture of the effects of fish oil, the peripheral vascular responses to mental stress post omega-3 supplementation were inconsistent. At rest, the combined non-hypertensive individuals demonstrated increased baseline forearm blood flow, but there was no difference between fish oil and placebo. In contrast to the forearm response, there were no significant changes in calf vascular function at rest. Moreover, vascular responses to mental stress did not change in response to fish oil or placebo. These findings provide inconclusive evidence as to the effects of omega-3 fatty acids on the peripheral vasculature, although it is clear that fish oil and placebo

appeared to not have any consistent influence on either forearm or calf vascular responses to mental stress. The selective improvement of omega-3 fatty acids on resting forearm vascular function remains unclear. The evidence that there was no difference between fish oil and placebo might suggest methodological flaws. The current study used olive oil as the placebo treatment, which is an omega-3 fatty acid. Olive oil is commonly used in placebo-based fish oil studies (Monahan *et al.*, 2004), but may have a beneficial effect on vascular function similar to that suggested by fish oil. It has been previously reported that olive oil may contribute to improvement in endothelial function, inflammation, and decrease the risk of cardiovascular disease (Ruediger *et al.*, 2004). To this point, most research using olive oil as a placebo in a fish oil based study have found fish oil to be more effective at lowering blood pressure, and therefore it is commonly used as a placebo. Our findings suggest that olive oil may exert some positive vascular adaptations, thus may not be an ideal placebo. More research comparing olive oil and fish oil appears warranted to establish the true mechanisms behind

In conclusion, we found that fish oil does not consistently decrease resting arterial blood pressure or MSNA in normotensive or prehypertensive humans. However, changes in arterial blood pressure are significantly correlated to changes in MSNA after fish oil, suggesting the sympathetic nervous system does play a role when a fish oil-induced hypotensive response is observed. Moreover, fish oil decreased heart rate and total MSNA responses to mental stress, suggesting fish oil may have positive health benefits regarding neural cardiovascular reactivity in humans. These findings provide new mechanistic insight into nonpharmacological approaches to treating and/or preventing hypertension and other cardiovascular conditions.

Chapter 4

Summary and Future Directions

4.1 Summary

Prehypertension is a relatively new classification of blood pressure. This moderate level of blood pressure has been linked to a heightened risk of future development of hypertension (Zhang *et al.*, 2006; Egan & Julius, 2008). However, the mechanisms responsible for this increased risk are not fully understood. Earlier research investigating borderline hypertensives indicates that the neurovascular system may play a key role in the development of cardiovascular disease (Mark, 1984; Anderson *et al.*, 1989; Santangelo *et al.*, 1989; Matsukawa *et al.*, 1991), and mental stress may potentiate this. However, these mechanisms have not been investigated in prehypertensive patients. It is becoming increasingly important to use preventative measures to decrease the increasing incidence of hypertension and cardiovascular disease. Therefore, we conducted two investigations to further our understanding of the mechanisms contributing to prehypertension (**Study 1**), and the role of non-pharmacological intervention via fish oil in this same population (**Study 2**).

In **Study 1** we investigated possible mechanisms contributing to prehypertension in young males including sympathetic nerve activity and peripheral vascular responses to mental stress. In **Study 2** we added omega-3 fatty acids from fish oil to the normal diet of young males and females in order to investigate the mechanisms behind the cardiovascular effects of fish oil on blood pressure and mental stress. We found several novel findings which may lead to a better understanding of prehypertension, hypertension and the role omega-3 fatty acids have on the cardiovascular system.

From **Study 1** we were able to demonstrate that young prehypertensive males experience augmented blood pressure responses to a mental stress task compared to their normotensive counterparts. These responses appear to be linked to a blunted peripheral vasodilation, not an increase in sympathetic nerve activity. **Study 2** demonstrated that fish oil had no discernable influence on cardiovascular and nervous system function at rest in prehypertensive or normotensive humans. However, further analysis of the data revealed that fish oil may have had a variable response such that individuals who experienced a decrease in blood pressure also demonstrated a concomitant decrease in MSNA. Moreover, fish oil reduced neural (i.e., total MSNA) and

cardiovascular (i.e., heart rate) reactivity to mental stress. Robust cardiovascular reactivity has been shown to increase the risk of future development of hypertension (Matthews *et al.*, 1993).

These are the first studies to collectively investigate the neurocardiovascular responses to mental stress both before and after fish oil supplementation in prehypertensive humans. We have several novel findings that further our understanding of prehypertension, hypertension and the possible role that fish oil might play in disease prevention. However, the novel discoveries also introduced more questions that could be answered in future investigations

4.2 Future Directions – Nitric Oxide

We have suggested that prehypertensive individuals demonstrate blunted vascular responses to mental stress, and these responses were not mediated through MSNA. Our study only measured whole limb changes blood flow during mental stress. We were unable to investigate other mechanisms that contribute to increased blood flow in the limbs, such as nitric oxide (NO). It has been demonstrated that vascular endothelial cells have the ability to secrete NO as a powerful local vasodilator (Radomski *et al.*, 1987; Shepherd & Katusic, 1991). The release of NO has been suggested to be mediated by both shear stress on the vascular wall, and by the nervous system, both of which can be affected by hypertension. In humans, mental stress has elicited a NO-mediated forearm vasodilatory response (Dietz *et al.*, 1994; Cardillo *et al.*, 1997), and a response that has been blunted hypertensive individuals (Cardillo *et al.*, 1998). Collectively, it is possible that nitric oxide could be playing an important role in the blunted responses we observe in prehypertensive individuals during mental stress. Interestingly, one mechanism by which omega-3 fatty acids has been suggested to lower blood pressure is through nitric oxide mediators (Harris *et al.*, 1997; De Caterina *et al.*, 2000). Future research should investigate the role of nitric oxide in peripheral vasodilation in prehypertension.

4.3 Conclusion

This dissertation reports novel and clinically relevant information regarding the prehypertensive population. Furthermore, we have comprehensively investigated the

role of fish oil plays on neurovascular function. It is clear that there are altered vascular responses to a psychological stress response in prehypertensives, and these responses may be contributing to the further development of hypertension. As public knowledge of prehypertension begins to grow, it will be important to use this classification as an early indicator for preventative care in order to reduce the risks of cardiovascular disease. Fish oil is one current method that is prescribed as a preventative mechanism. We report mixed results regarding the influence of fish oil on neurovascular control in humans. However, our findings suggest a potentially important interaction between fish oil, the sympathetic nervous system, and arterial blood pressure, especially regarding neural and hemodynamic responses to mental stress.

References

- Abeywardena MY & Head RJ. (2001). Longchain n-3 polyunsaturated fatty acids and blood vessel function. *Cardiovascular Research* **52**, 361-371.
- Anderson EA, Sinkey CA, Lawton WJ & Mark AL. (1989). Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. *Hypertension* **14**, 177-183.
- Anderson EA, Sinkey CA & Mark AL. (1991). Mental stress increases sympathetic nerve activity during sustained baroreceptor stimulation in humans. *Hypertension* **17**, III43-49.
- Anderson EA, Wallin BG & Mark AL. (1987). Dissociation of sympathetic nerve activity in arm and leg muscle during mental stress. *Hypertension* **9**, III114-119.
- Appel LJ, Miller ER, Seidler AJ & Whelton PK. (1993). Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. *Archives of Internal Medicine* **153**, 1429-1438.
- Bang HO, Dyerberg J & Nielsen AB. (1971). Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. *Lancet* **1**, 1143-1145.
- Bang HO, Dyerberg J & Sinclair HM. (1980). The composition of the Eskimo food in north western Greenland. *American Journal of Clinical Nutrition* **33**, 2657-2661.
- Barcroft H, Brod J, Hejl BZ, Hirsjarvi EA & Kitchin AH. (1960). The mechanism of the vasodilatation in the forearm muscle during stress (mental arithmetic). *Clinical Science* **19**, 577-586.
- Barcroft H & Edholm OG. (1945). On the vasodilatation in human skeletal muscle during post-haemorrhagic fainting. *Journal of Physiology (London)* **104**, 161-175.
- Barcroft J. (1946). The respiratory function of the blood; man under conditions of stress. *Journal of the Royal Institute of Public Health* **9**, 74-85.
- Bayorh MA, McGee L & Feuerstein G. (1989). Acute and chronic effects of eicosapentaenoic acid (EPA) on the cardiovascular system. *Research Communications in Chemical Pathology and Pharmacology* **66**, 355-374.
- Billman GE & Harris WS. (2011). Effect of dietary omega-3 fatty acids on the heart rate and the heart rate variability responses to myocardial ischemia or submaximal exercise. *American Journal of Physiology - Heart and Circulatory Physiology* **300**, H2288-2299.
- Blair DA, Glover WE, Greenfield AD & Roddie IC. (1959). Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. *Journal of Physiology (London)* **148**, 633-647.

Booth J. (1977). A short history of blood pressure measurement. *Proceedings of the Royal Society of Medicine* **70**, 793-799.

Boutcher YN, Park YJ & Boutcher SH. (2009). Vascular and baroreceptor abnormalities in young males with a family history of hypertension. *European Journal of Applied Physiology* **107**, 653-658.

Brod J. (1963). Haemodynamic basis of acute pressor reactions and hypertension. *British Heart Journal* **25**, 227-245.

BROD J, FENCL V, HEJL Z & JIRKA J. (1959). Circulatory changes underlying blood pressure elevation during acute emotional stress (mental arithmetic) in normotensive and hypertensive subjects. *Clinical Science* **18**, 269-279.

Brown GL & Gillespie JS. (1957). The output of sympathetic transmitter from the spleen of the cat. *Journal of Physiology (London)* **138**, 81-102.

Brundtland GH. (2002). From the World Health Organization. Reducing risks to health, promoting healthy life. *JAMA* **288**, 1974.

Burr ML. (1989). Fish and the cardiovascular system. *Progress in Food and Nutrition Science* **13**, 291-316.

Callister R, Suwarno NO & Seals DR. (1992). Sympathetic activity is influenced by task difficulty and stress perception during mental challenge in humans. *Journal of Physiology (London)* **454**, 373-387.

Cannon WB. (1914). The Interrelations of Emotions as Suggested by Recent Physiological Researches. *The American Journal of Psychology* **25**, 256-282.

Cannon WB. (1927). *Bodily Changes in Pain, Hunger, Fear and Rage*. D. Appleton and Company, New York and London.

Cannon WBar, A. (1933). Studies on Conditions of Activity in Endocrine Organs: Sympathin E and Sympathin I. *American Journal of Physiology* **104**, 557-574.

Cardillo C, Kilcoyne CM, Cannon RO & Panza JA. (1998). Impairment of the nitric oxide-mediated vasodilator response to mental stress in hypertensive but not in hypercholesterolemic patients. *Journal of the American College of Cardiology* **32**, 1207-1213.

Cardillo C, Kilcoyne CM, Quyyumi AA, Cannon RO & Panza JA. (1997). Role of nitric oxide in the vasodilator response to mental stress in normal subjects. *American Journal of Cardiology* **80**, 1070-1074.

Carter JR, Kupiers NT & Ray CA. (2005). Neurovascular responses to mental stress. *Journal of Physiology (London)* **564**, 321-327.

Carter JR & Ray CA. (2009). Sympathetic neural responses to mental stress: responders, nonresponders and sex differences. *American Journal of Physiology - Heart and Circulatory Physiology* **296**, H847-853.

Chin-Dusting JP, Jovanovska V, Kingwell BA, Du XJ & Dart AM. (1998). Effect of fish oil supplementation on aortic compliance in rats: role of the endothelium. *Prostaglandins Leukotrienes and Essential Fatty Acids* **59**, 335-340.

Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr. & Roccella EJ. (2003). Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* **42**, 1206-1252.

Collins R, Peto R, MacMahon S, Hebert P, Fiebach NH, Eberlein KA, Godwin J, Qizilbash N, Taylor JO & Hennekens CH. (1990). Blood pressure, stroke, and coronary heart disease. Part 2, Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet* **335**, 827-838.

Cushman WC, Ford CE, Cutler JA, Margolis KL, Davis BR, Grimm RH, Black HR, Hamilton BP, Holland J, Nwachuku C, Papademetriou V, Probstfield J, Wright JT, Jr., Alderman MH, Weiss RJ, Piller L, Bettencourt J & Walsh SM. (2002). Success and predictors of blood pressure control in diverse North American settings: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). *Journal of Clinical Hypertension (Greenwich)* **4**, 393-404.

Darwin C. (1886). *The Expression of the Emotions in Man and Animals*. D. Appleton and Company, New York, NY.

Das UN. (2000). Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? *Prostaglandins Leukotrienes and Essential Fatty Acids* **63**, 351-362.

De Caterina R, Bernini W, Carluccio MA, Liao JK & Libby P. (1998). Structural requirements for inhibition of cytokine-induced endothelial activation by unsaturated fatty acids. *Journal of Lipid Research* **39**, 1062-1070.

De Caterina R, Liao JK & Libby P. (2000). Fatty acid modulation of endothelial activation. *American Journal of Clinical Nutrition* **71**, 213S-223S.

Deanfield JE, Shea M, Kensett M, Horlock P, Wilson RA, de Landsheere CM & Selwyn AP. (1984). Silent myocardial ischaemia due to mental stress. *Lancet* **2**, 1001-1005.

Delarue J, Matzinger O, Binnert C, Schneiter P, Chiolero R & Tappy L. (2003). Fish oil prevents the adrenal activation elicited by mental stress in healthy men. *Diabetes and Metabolism* **29**, 289-295.

Delius W, Hagbarth KE, Hongell A & Wallin BG. (1972). Manoeuvres affecting sympathetic outflow in human muscle nerves. *Acta Physiologica Scandinavica* **84**, 82-94.

- Delius W, Wallin G & Hagbarth KE. (1973). Role of sympathetic nerve impulses in regulation of peripheral circulation. *Scandinavian Journal of Clinical and Laboratory Investigation. Supplement* **128**, 47-50.
- Dietz NM, Rivera JM, Eggenger SE, Fix RT, Warner DO & Joyner MJ. (1994). Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. *Journal of Physiology (London)* **480 (Pt 2)**, 361-368.
- Dimsdale JE & Moss J. (1980). Plasma catecholamines in stress and exercise. *JAMA* **243**, 340-342.
- Durocher JJ, Klein JC & Carter JR. (2011). Attenuation of sympathetic baroreflex sensitivity during the onset of acute mental stress in humans. *American Journal of Physiology - Heart and Circulatory Physiology* **300**, H1788-1793.
- Eckberg DL. (1997). Sympathovagal balance: a critical appraisal. *Circulation* **96**, 3224-3232.
- Egan B, Panis R, Hinderliter A, Schork N & Julius S. (1987). Mechanism of increased alpha adrenergic vasoconstriction in human essential hypertension. *Journal of Clinical Investigation* **80**, 812-817.
- Egan BM & Julius S. (2008). Prehypertension: risk stratification and management considerations. *Current Hypertension Reports* **10**, 359-366.
- Eliasson K. (1985). Borderline hypertension. Circulatory, sympatho-adrenal and psychological reactions to stress. *Acta Med Scand Suppl* **692**, 1-90.
- Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC & et al. (1989). The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *New England Journal of Medicine* **320**, 265-271.
- Engelman K & Portnoy B. (1970). A sensitive double-isotope derivative assay for norepinephrine and epinephrine. Normal resting human plasma levels. *Circulation Research* **26**, 53-57.
- Epstein BJ. (2010). Improving blood pressure control rates by optimizing combination antihypertensive therapy. *Expert Opinions in Pharmacotherapy* **11**, 2011-2026.
- Esler M, Eikelis N, Schlaich M, Lambert G, Alvarenga M, Dawood T, Kaye D, Barton D, Pier C, Guo L, Brenchley C, Jennings G & Lambert E. (2008). Chronic mental stress is a cause of essential hypertension: presence of biological markers of stress. *Clinical and Experimental Pharmacology and Physiology* **35**, 498-502.
- Esler M, Jackman G, Bobik A, Kelleher D, Jennings G, Leonard P, Skews H & Korner P. (1979). Determination of norepinephrine apparent release rate and clearance in humans. *Life Sciences* **25**, 1461-1470.

Esler M, Jennings G, Biviano B, Lambert G & Hasking G. (1986). Mechanism of elevated plasma noradrenaline in the course of essential hypertension. *Journal of Cardiovascular Pharmacology* **8 Suppl 5**, S39-43.

Esler M, Jennings G, Korner P, Blombery P, Sacharias N & Leonard P. (1984a). Measurement of total and organ-specific norepinephrine kinetics in humans. *American Journal of Physiology* **247**, E21-28.

Esler M, Jennings G & Lambert G. (1989). Measurement of overall and cardiac norepinephrine release into plasma during cognitive challenge. *Psychoneuroendocrinology* **14**, 477-481.

Esler M, Lambert G, Brunner-La Rocca HP, Vaddadi G & Kaye D. (2003). Sympathetic nerve activity and neurotransmitter release in humans: translation from pathophysiology into clinical practice. *Acta Physiologica Scandinavica* **177**, 275-284.

Esler MD, Jennings GL, Johns J, Burke F, Little PJ & Leonard P. (1984b). Estimation of 'total' renal, cardiac and splanchnic sympathetic nervous tone in essential hypertension from measurements of noradrenaline release. *Journal of Hypertension Supplement* **2**, S123-125.

Esler MD & Nestel PJ. (1973). High catecholamine essential hypertension: clinical and physiological characteristics. *Australian and New Zealand Journal of Medicine* **3**, 117-123.

Esunge PM. (1991). From blood pressure to hypertension: the history of research. *Journal of the Royal Society of Medicine* **84**, 621.

Falkner B, Onesti G, Angelakos ET, Fernandes M & Langman C. (1979). Cardiovascular response to mental stress in normal adolescents with hypertensive parents. Hemodynamics and mental stress in adolescents. *Hypertension* **1**, 23-30.

FitzGerald GA, Hossmann V & Dollery CT. (1981). Norepinephrine release in essential hypertension. *Clinical Pharmacology and Therapeutics* **30**, 164-171.

Fuchs FD. (2010). Prehypertension: the rationale for early drug therapy. *Cardiovascular Therapy* **28**, 339-343.

Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR & Kok FJ. (2002). Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *Journal Hypertension* **20**, 1493-1499.

Gibbons GH. (1997). Endothelial function as a determinant of vascular function and structure: a new therapeutic target. *American Journal of Cardiology* **79**, 3-8.

Goldstein DS. (1983). Arterial baroreflex sensitivity, plasma catecholamines, and pressor responsiveness in essential hypertension. *Circulation* **68**, 234-240.

- Goldstein DS, Eisenhofer G, Sax FL, Keiser HR & Kopin IJ. (1987). Plasma norepinephrine pharmacokinetics during mental challenge. *Psychosomatic Medicine* **49**, 591-605.
- Grassi G & Esler M. (1999). How to assess sympathetic activity in humans. *Journal of Hypertension* **17**, 719-734.
- Hagbarth KE & Vallbo AB. (1967). Mechanoreceptor activity recorded percutaneously with semi-microelectrodes in human peripheral nerves. *Acta Physiologica Scandinavica* **69**, 121-122.
- Hagbarth KE & Vallbo AB. (1969). Single unit recordings from muscle nerves in human subjects. *Acta Physiologica Scandinavica* **76**, 321-334.
- Halliwill JR, Lawler LA, Eickhoff TJ, Dietz NM, Nauss LA & Joyner MJ. (1997). Forearm sympathetic withdrawal and vasodilatation during mental stress in humans. *Journal of Physiology (London)* **504 (Pt 1)**, 211-220.
- Hamazaki K, Itomura M, Huan M, Nishizawa H, Sawazaki S, Tanouchi M, Watanabe S, Hamazaki T, Terasawa K & Yazawa K. (2005). Effect of omega-3 fatty acid-containing phospholipids on blood catecholamine concentrations in healthy volunteers: a randomized, placebo-controlled, double-blind trial. *Nutrition* **21**, 705-710.
- Harris WS, Rambjor GS, Windsor SL & Diederich D. (1997). n-3 fatty acids and urinary excretion of nitric oxide metabolites in humans. *American Journal of Clinical Nutrition* **65**, 459-464.
- Harvey W. (1628). *On the Motion of the Heart and Blood in Animals*. P.F. Collier & Son, 1909-14, New York. Published online in 2001, www.bartelby.com/38/3.
- Hashimoto M, Hossain S, Yamasaki H, Yazawa K & Masumura S. (1999). Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. *Lipids* **34**, 1297-1304.
- Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khara A, Lloyd-Jones DM, Nelson SA, Nichol G, Orenstein D, Wilson PW & Woo YJ. (2011). Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* **123**, 933-944.
- Henneman E, Somjen G & Carpenter DO. (1965). Excitability and inhibitability of motoneurons of different sizes. *J Neurophysiol* **28**, 599-620.
- Herd JA. (1991). Cardiovascular response to stress. *Physiological Reviews* **71**, 305-330.
- Hertting G. (1964). The Fate of Norepinephrine (Ne) at the Sympathetic Nerve Endings. *Acta Neurovegetativa* **26**, 267-270.

- Heymans C. (1958). Baroreceptor and chemoceptor reflexes in monkeys. *Circulation Research* **6**, 567-569.
- Hjemdahl P. (1984). Inter-laboratory comparison of plasma catecholamine determinations using several different assays. *Acta Physiologica Scandinavica. Supplementum* **527**, 43-54.
- Hjemdahl P, Fagius J, Freyschuss U, Wallin BG, Daleskog M, Bohlin G & Perski A. (1989). Muscle sympathetic activity and norepinephrine release during mental challenge in humans. *American Journal of Physiology* **257**, E654-664.
- Hjemdahl P, Freyschuss U, Juhlin-Dannfelt A & Linde B. (1984). Differentiated sympathetic activation during mental stress evoked by the Stroop test. *Acta Physiologica Scandinavica. Supplementum* **527**, 25-29.
- Howe PR. (1995). Can we recommend fish oil for hypertension? *Clinical and Experimental Pharmacology and Physiology* **22**, 199-203.
- Howe PR, Lungershausen YK, Rogers PF, Gerkens JF, Head RJ & Smith RM. (1991). Effects of dietary sodium and fish oil on blood pressure development in stroke-prone spontaneously hypertensive rats. *Journal of Hypertension* **9**, 639-644.
- Huang JL, Chiou CW, Ting CT, Chen YT & Chen SA. (2001). Sudden changes in heart rate variability during the 1999 Taiwan earthquake. *American Journal of Cardiology* **87**, 245-248, A249.
- Izzo JL, Jr. (2007). Prehypertension: demographics, pathophysiology, and treatment. *Current Hypertension Reports* **9**, 264-268.
- Jern S, Bergbrant A, Hedner T & Hansson L. (1995). Enhanced pressor responses to experimental and daily-life stress in borderline hypertension. *Journal of Hypertension* **13**, 69-79.
- Jones PP, Spraul M, Matt KS, Seals DR, Skinner JS & Ravussin E. (1996). Gender does not influence sympathetic neural reactivity to stress in healthy humans. *American Journal of Physiology* **270**, H350-357.
- Jouven X, Zureik M, Desnos M, Guerot C & Ducimetiere P. (2001). Resting heart rate as a predictive risk factor for sudden death in middle-aged men. *Cardiovascular Research* **50**, 373-378.
- Joyner MJ, Charkoudian N & Wallin BG. (2010). Sympathetic nervous system and blood pressure in humans: individualized patterns of regulation and their implications. *Hypertension* **56**, 10-16.
- Kannel WB. (1975). Role of blood pressure in cardiovascular disease: the Framingham Study. *Angiology* **26**, 1-14.

- Kannel WB, Kannel C, Paffenbarger RS & Cupples LA. (1987). Heart rate and cardiovascular mortality: the Framingham Study. *American Heart Journal* **113**, 1489-1494.
- Keith NM, Wagener HP & Barker NW. (1974). Some different types of essential hypertension: their course and prognosis. *American Journal of the Medical Sciences* **268**, 336-345.
- Keller DM, Cui J, Davis SL, Low DA & Crandall CG. (2006). Heat stress enhances arterial baroreflex control of muscle sympathetic nerve activity via increased sensitivity of burst gating, not burst area, in humans. *Journal of Physiology (London)* **573**, 445-451.
- Kenny D, Warltier DC, Pleuss JA, Hoffmann RG, Goodfriend TL & Egan BM. (1992). Effect of omega-3 fatty acids on the vascular response to angiotensin in normotensive men. *American Journal of Cardiology* **70**, 1347-1352.
- Khan F, Elherik K, Bolton-Smith C, Barr R, Hill A, Murrie I & Belch JJ. (2003). The effects of dietary fatty acid supplementation on endothelial function and vascular tone in healthy subjects. *Cardiovascular Research* **59**, 955-962.
- Kienbaum P, Karlsson T, Sverrisdottir YB, Elam M & Wallin BG. (2001). Two sites for modulation of human sympathetic activity by arterial baroreceptors? *Journal of Physiology (London)* **531**, 861-869.
- Kingwell BA, Thompson JM, Kaye DM, McPherson GA, Jennings GL & Esler MD. (1994). Heart rate spectral analysis, cardiac norepinephrine spillover, and muscle sympathetic nerve activity during human sympathetic nervous activation and failure. *Circulation* **90**, 234-240.
- Knapp HR & FitzGerald GA. (1989). The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. *New England Journal of Medicine* **320**, 1037-1043.
- Kris-Etherton PM, Harris WS & Appel LJ. (2003). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology* **23**, e20-30.
- Kuipers NT, Sauder CL, Carter JR & Ray CA. (2008). Neurovascular responses to mental stress in the supine and upright postures. *Journal of Applied Physiology* **104**, 1129-1136.
- Lawrence JE, Klein JC & Carter JR. (2010). Menstrual cycle elicits divergent forearm vascular responses to vestibular activation in humans. *Autonomic Neuroscience* **154**, 89-93.
- LeBlanc J, Côté J, Jobin M & Labrie A. (1979). Plasma catecholamines and cardiovascular responses to cold and mental activity. *Journal of Applied Physiology* **47**, 1207-1211.

Leimbach WN, Jr., Wallin BG, Victor RG, Aylward PE, Sundlof G & Mark AL. (1986). Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. *Circulation* **73**, 913-919.

Leor J, Poole WK & Kloner RA. (1996). Sudden cardiac death triggered by an earthquake. *New England Journal of Medicine* **334**, 413-419.

Levinson PD, Iosiphidis AH, Saritelli AL, Herbert PN & Steiner M. (1990). Effects of n-3 fatty acids in essential hypertension. *American Journal of Hypertension* **3**, 754-760.

Lindqvist M, Davidsson S, Hjemdahl P & Melcher A. (1996). Sustained forearm vasodilation in humans during mental stress is not neurogenically mediated. *Acta Physiologica Scandinavica* **158**, 7-14.

Lindqvist M, Kahan T, Melcher A & Hjemdahl P. (1993). Cardiovascular and sympatho-adrenal responses to mental stress in primary hypertension. *Clinical Science (London)* **85**, 401-409.

Lynch J, Krause N, Kaplan GA, Salonen R & Salonen JT. (1997). Workplace demands, economic reward, and progression of carotid atherosclerosis. *Circulation* **96**, 302-307.

MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyer A & Stamler J. (1990). Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* **335**, 765-774.

Malliani A, Pagani M, Lombardi F, Furlan R, Guzzetti S & Cerutti S. (1991). Spectral analysis to assess increased sympathetic tone in arterial hypertension. *Hypertension* **17**, III36-42.

Malmö RB & Shagass C. (1949). Variability of heart rate in relation to age, sex and stress. *Journal of Applied Physiology* **2**, 181-184.

Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi MG, Geraci E, Levantesi G, Maggioni AP, Mantini L, Marfisi RM, Mastrogiuseppe G, Mininni N, Nicolosi GL, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C & Valagussa F. (2002). Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* **105**, 1897-1903.

Mark AL. (1984). Structural changes in resistance and capacitance vessels in borderline hypertension. *Hypertension* **6**, III69-73.

Matsukawa T, Gotoh E, Uneda S, Miyajima E, Shionoiri H, Tochikubo O & Ishii M. (1991). Augmented sympathetic nerve activity in response to stressors in young borderline hypertensive men. *Acta Physiologica Scandinavica* **141**, 157-165.

Matthews KA, Katholi CR, McCreath H, Whooley MA, Williams DR, Zhu S & Markovitz JH. (2004). Blood pressure reactivity to psychological stress predicts hypertension in the CARDIA study. *Circulation* **110**, 74-78.

Matthews KA, Woodall KL & Allen MT. (1993). Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension* **22**, 479-485.

Monahan KD & Ray CA. (2002). Limb neurovascular control during altered otolithic input in humans. *Journal of Physiology (London)* **538**, 303-308.

Monahan KD, Wilson TE & Ray CA. (2004). Omega-3 fatty acid supplementation augments sympathetic nerve activity responses to physiological stressors in humans. *Hypertension* **44**, 732-738.

Moreira LB, Fuchs SC, Wiehe M, Gus M, Moraes RS & Fuchs FD. (2008). Incidence of hypertension in Porto Alegre, Brazil: a population-based study. *Journal of Human Hypertension* **22**, 48-50.

Mori TA. (2006). Omega-3 fatty acids and hypertension in humans. *Clinical and Experimental Pharmacology and Physiology* **33**, 842-846.

Mori TA, Watts GF, Burke V, Hilme E, Puddey IB & Beilin LJ. (2000). Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation* **102**, 1264-1269.

Mori TA & Woodman RJ. (2006). The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. *Current Opinion in Clinical Nutrition and Metabolic Care* **9**, 95-104.

Morris MC, Sacks F & Rosner B. (1993). Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* **88**, 523-533.

Mozaffarian D, Geelen A, Brouwer IA, Geleijnse JM, Zock PL & Katan MB. (2005). Effect of fish oil on heart rate in humans: a meta-analysis of randomized controlled trials. *Circulation* **112**, 1945-1952.

Mozaffarian D, Stein PK, Prineas RJ & Siscovick DS. (2008). Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. *Circulation* **117**, 1130-1137.

Neal B, MacMahon S & Chapman N. (2000). Effects of ACE inhibitors, calcium antagonists, and other blood-pressure-lowering drugs: results of prospectively designed overviews of randomised trials. Blood Pressure Lowering Treatment Trialists' Collaboration. *Lancet* **356**, 1955-1964.

Newton HF, Zwemer, R.L., Cannon, W.B. (1930). Studies on the Conditions of Activity in Endocrine Organs: The Mystery of Emotional Acceleration of the Denervated Heart after Exclusion of Known Humoral Accelerators. *American Journal of Physiology* **96**, 377-391.

- Ng AV, Callister R, Johnson DG & Seals DR. (1994). Sympathetic neural reactivity to stress does not increase with age in healthy humans. *American Journal of Physiology* **267**, H344-353.
- Nishimura M, Nanbu A, Komori T, Ohtsuka K, Takahashi H & Yoshimura M. (2000). Eicosapentaenoic acid stimulates nitric oxide production and decreases cardiac noradrenaline in diabetic rats. *Clinical and Experimental Pharmacology and Physiology* **27**, 618-624.
- Noll G, Tschudi M, Nava E & Luscher TF. (1997). Endothelium and high blood pressure. *International Journal of Microcirculation: Clinical and Experimental* **17**, 273-279.
- Noll G, Wenzel RR, Schneider M, Oesch V, Binggeli C, Shaw S, Weidmann P & Luscher TF. (1996). Increased activation of sympathetic nervous system and endothelin by mental stress in normotensive offspring of hypertensive parents. *Circulation* **93**, 866-869.
- Norris GW. (1917). *Blood-pressure*. Lea & Febiger, Philadelphia and New York.
- Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E & et al. (1986). Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circulation Research* **59**, 178-193.
- Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Groppelli A, Pedotti A, Zanchetti A & Mancia G. (1988). Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension* **12**, 214-222.
- Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ & et al. (1985). Assessment of autonomic function in humans by heart rate spectral analysis. *American Journal of Physiology* **248**, H151-153.
- Radomski MW, Palmer RM & Moncada S. (1987). Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *British Journal of Pharmacology* **92**, 181-187.
- Reims HM, Fossum E, Høieggen A, Moan A, Eide I & Kjeldsen SE. (2004). Adrenal medullary overactivity in lean, borderline hypertensive young men. *American Journal of Hypertension* **17**, 611-618.
- Richards AM, Nicholls MG, Espiner EA, Ikram H, Cullens M & Hinton D. (1986). Diurnal patterns of blood pressure, heart rate and vasoactive hormones in normal man. *Clinical and Experimental Hypertension. Part A, Theory and Practice* **8**, 153-166.
- Roddie IC. (1977). Human responses to emotional stress. *Irish Journal of Medical Science* **146**, 395-417.
- Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Greenlund KJ, Hailpern SM, Heit JA, Ho PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH,

Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, McDermott MM, Meigs JB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Rosamond WD, Sorlie PD, Stafford RS, Turan TN, Turner MB, Wong ND & Wylie-Rosett J. (2011). Heart disease and stroke statistics--2011 update: a report from the American Heart Association. *Circulation* **123**, e18-e209.

Rousseau-Ralliard D, Moreau D, Guillard JC, Raederstorff D & Grynberg A. (2009). Docosahexaenoic acid, but not eicosapentaenoic acid, lowers ambulatory blood pressure and shortens interval QT in spontaneously hypertensive rats in vivo. *Prostaglandins Leukotrienes and Essential Fatty Acids* **80**, 269-277.

Ruediger H, Seibt R, Scheuch K, Krause M & Alam S. (2004). Sympathetic and parasympathetic activation in heart rate variability in male hypertensive patients under mental stress. *Journal of Human Hypertension* **18**, 307-315.

Rusch NJ, Shepherd JT, Webb RC & Vanhoutte PM. (1981). Different behavior of the resistance vessels of the human calf and forearm during contralateral isometric exercise, mental stress, and abnormal respiratory movements. *Circulation Research* **48**, 1118-130.

Rushmer RF, Smith O & Franklin D. (1959). Mechanisms of cardiac control in exercise. *Circulation Research* **7**, 602-627.

Santangelo K, Falkner B & Kushner H. (1989). Forearm hemodynamics at rest and stress in borderline hypertensive adolescents. *American Journal of Hypertension* **2**, 52-56.

Selye H. (1936). A Syndrome produced by diverse nocuous agents. *Nature* **138**, 32.

Selye H & Fortier C. (1950). Adaptive reaction to stress. *Psychosomatic Medicine* **12**, 149-157.

Shepherd JT. (1987). Circulatory response to exercise in health. *Circulation* **76**, VI3-10.

Shepherd JT & Katusic ZS. (1991). Endothelium-derived vasoactive factors: I. Endothelium-dependent relaxation. *Hypertension* **18**, III76-85.

Simopoulos AP. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine (Maywood)* **233**, 674-688.

Singer P. (1990). Blood pressure-lowering effect of mackerel diet. *Klinische Wochenschrift* **68 Suppl 20**, 40-48.

Spieker LE, Hurlimann D, Ruschitzka F, Corti R, Enseleit F, Shaw S, Hayoz D, Deanfield JE, Luscher TF & Noll G. (2002). Mental stress induces prolonged endothelial dysfunction via endothelin-A receptors. *Circulation* **105**, 2817-2820.

- Steinback CD, Salmanpour A, Breskovic T, Dujic Z & Shoemaker JK. (2010). Sympathetic neural activation: an ordered affair. *Journal of Physiology (London)* **588**, 4825-4836.
- Sundlof G & Wallin BG. (1977). The variability of muscle nerve sympathetic activity in resting recumbent man. *Journal of Physiology (London)* **272**, 383-397.
- Sundlof G & Wallin BG. (1978). Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *Journal of Physiology (London)* **274**, 621-637.
- Takehita A, Imaizumi T, Ashihara T, Yamamoto K, Hoka S & Nakamura M. (1982). Limited maximal vasodilator capacity of forearm resistance vessels in normotensive young men with a familial predisposition to hypertension. *Circulation Research* **50**, 671-677.
- Terano T, Salmon JA, Higgs GA & Moncada S. (1986). Eicosapentaenoic acid as a modulator of inflammation. Effect on prostaglandin and leukotriene synthesis. *Biochemical Pharmacology* **35**, 779-785.
- Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC & Grimble RF. (2003). Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* **361**, 477-485.
- Tidgren B & Hjemdahl P. (1989). Renal responses to mental stress and epinephrine in humans. *American Journal of Physiology* **257**, F682-689.
- Timio M, Verdecchia P, Venanzi S, Gentili S, Ronconi M, Francucci B, Montanari M & Bichisao E. (1988). Age and blood pressure changes. A 20-year follow-up study in nuns in a secluded order. *Hypertension* **12**, 457-461.
- Vallbo AB & Hagbarth KE. (1967). Impulses recorded with micro-electrodes in human muscle nerves during stimulation of mechanoreceptors and voluntary contractions. *Electroencephalography and Clinical Neurophysiology* **23**, 392.
- Vallbo AB, Hagbarth KE & Wallin BG. (2004). Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *Journal of Applied Physiology* **96**, 1262-1269.
- van den Hoogen PC, Feskens EJ, Nagelkerke NJ, Menotti A, Nissinen A & Kromhout D. (2000). The relation between blood pressure and mortality due to coronary heart disease among men in different parts of the world. Seven Countries Study Research Group. *New England Journal of Medicine* **342**, 1-8.
- Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB & Levy D. (2001). Impact of high-normal blood pressure on the risk of cardiovascular disease. *New England Journal of Medicine* **345**, 1291-1297.

- Wallin BG. (1984). Muscle sympathetic activity and plasma concentrations of noradrenaline. *Acta Physiologica Scandinavica. Supplementum* **527**, 21-24.
- Wallin BG, Delius W & Hagbarth KE. (1973). Comparison of sympathetic nerve activity in normotensive and hypertensive subjects. *Circulation Research* **33**, 9-21.
- Wallin BG, Esler M, Dorward P, Eisenhofer G, Ferrier C, Westerman R & Jennings G. (1992). Simultaneous measurements of cardiac noradrenaline spillover and sympathetic outflow to skeletal muscle in humans. *Journal of Physiology (London)* **453**, 45-58.
- Wallin BG, Sundlöf G, Eriksson BM, Dominiak P, Grobecker H & Lindblad LE. (1981). Plasma noradrenaline correlates to sympathetic muscle nerve activity in normotensive man. *Acta Physiologica Scandinavica* **111**, 69-73.
- Westenberg HG & Liebowitz MR. (2004). Overview of panic and social anxiety disorders. *Journal of Clinical Psychiatry* **65 Suppl 14**, 22-26.
- Widgren BR, Wikstrand J, Berglund G & Andersson OK. (1992). Increased response to physical and mental stress in men with hypertensive parents. *Hypertension* **20**, 606-611.
- Wilkinson DJ, Thompson JM, Lambert GW, Jennings GL, Schwarz RG, Jefferys D, Turner AG & Esler MD. (1998). Sympathetic activity in patients with panic disorder at rest, under laboratory mental stress, and during panic attacks. *Archives of General Psychiatry* **55**, 511-520.
- Wilson TD, Cotter LA, Draper JA, Misra SP, Rice CD, Cass SP & Yates BJ. (2006). Vestibular inputs elicit patterned changes in limb blood flow in conscious cats. *Journal of Physiology (London)* **575**, 671-684.
- Yamagishi K, Iso H, Date C, Fukui M, Wakai K, Kikuchi S, Inaba Y, Tanabe N & Tamakoshi A. (2008). Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. *Journal of the American College of Cardiology* **52**, 988-996.
- Zhang Y, Lee ET, Devereux RB, Yeh J, Best LG, Fabsitz RR & Howard BV. (2006). Prehypertension, diabetes, and cardiovascular disease risk in a population-based sample: the Strong Heart Study. *Hypertension* **47**, 410-414.

Appendix A

Table A.1. Raw data for subject characteristics in normotensive group for Study 1.

Subject	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m²)	Gender
1	25	176	100	32	Male
2	18	173	71	24	Male
3	19	177	60	19	Male
4	23	179	66	21	Male
5	20	167	63	23	Male
6	18	185	98	29	Male
7	39	168	60	21	Male
8	19	196	106	28	Male
9	20	185	60	18	Male
10	19	177	75	24	Male
11	22	184	80	24	Male
12	18	170	63	22	Male
13	20	174	79	26	Male
14	23	161	70	27	Male
15	22	181	72	22	Male
16	19	179	91	28	Male
17	40	184	89	26	Male
18	30	170	77	27	Male

BMI; Body Mass Index

Table A.2. Raw data for subject characteristics in prehypertensive group for Study 1.

Subject	Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m²)	Gender
1	32	184	97	29	Male
2	19	178	83	26	Male
3	20	178	84	27	Male
4	22	178	84	27	Male
5	21	176	77	25	Male
6	21	179	87	27	Male
7	23	183	92	27	Male
8	21	179	67	21	Male
9	19	185	81	24	Male
10	18	166	85	31	Male
11	20	189	92	26	Male
12	20	174	95	31	Male
13	23	177	76	24	Male
14	21	184	84	25	Male
15	21	173	82	27	Male
16	28	189	86	24	Male
17	20	174	81	27	Male

BMI; Body Mass Index

Table A.3 Raw data for seated resting measurements in normotensive group for Study 1.

Subject	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (beats/min)
1	116.3	61.6	79.8	71.0
2	116.3	62.1	80.2	74.8
3	107.8	64.2	78.7	82.8
4	110.0	57.0	74.7	60.0
5	108.6	68.2	81.7	73.6
6	116.4	76.2	89.6	68.9
7	103.3	65.0	77.8	68.9
8	117.4	69.6	85.5	63.9
9	109.0	56.0	73.7	58.1
10	109.3	63.4	78.7	47.7
11	113.6	65.6	81.6	72.3
12	111.7	65.2	80.7	68.0
13	103.4	66.2	78.6	60.3
14	115.7	62.9	80.5	78.9
15	106.0	51.6	69.7	73.3
16	119.0	65.0	83.0	59.4
17	111.0	67.2	81.8	77.9
18	116.2	71.4	86.3	73.3

SAP; Systolic Arterial Pressure

DAP; Diastolic Arterial Pressure

MAP; Mean Arterial Pressure

HR; Heart Rate

Table A.4. Raw data for seated resting measurements in prehypertensive group for Study 1

Subject	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (beats/min)
1	120.3	66.4	84.4	60.6
2	132.9	77.0	95.6	69.6
3	118.0	81.0	93.3	81.8
4	125.0	71.0	89.0	84.0
5	139.9	77.4	98.2	71.4
6	139.7	84.4	102.8	59.1
7	133.0	66.0	88.3	77.0
8	124.7	69.1	87.6	80.7
9	121.5	67.5	85.5	68.6
10	122.4	72.8	89.3	95.0
11	122.0	63.6	83.1	64.3
12	121.2	78.8	92.9	95.0
13	133.4	73.8	93.7	72.3
14	123.6	69.6	87.6	88.9
15	121.9	50.2	74.1	55.7
16	128.6	69.0	88.9	75.7
17	125.6	62.4	83.5	71.1

SAP; Systolic Arterial Pressure

DAP; Diastolic Arterial Pressure

MAP; Mean Arterial Pressure

HR; Heart Rate

Table A.5. Raw data for 5 minute average measurements of systolic arterial pressure as measured by the finometer in normotensive group for Study 1.

Subject	Base	MS	Rec
1	138.5	158.3	154.3
2	133.9	145.4	134.9
3	115.5	114.2	104.1
4	108.4	132.6	110.6
5	127.7	135.2	130.9
6	124.0	134.0	117.1
7	123.1	124.0	112.7
8	121.7	124.0	120.8
9	122.1	133.3	131.0
10	120.6	118.8	124.9
11	128.5	144.7	135.3
12	127.1	124.3	129.7
13	118.6	121.5	118.6
14	133.8	147.7	137.9
15	116.0	131.7	108.0
16	129.1	133.1	132.9
17	129.6	137.2	127.8
18	120.6	139.1	132.3

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.6. Raw data for 5 minute average measurements of systolic arterial pressure as measured by the finometer in prehypertensive group for Study 1.

Subject	Base	MS	Rec
1	124.8	135.7	129.3
2	141.2	150.1	146.9
3	138.6	145.0	133.2
4	107.3	130.8	122.7
5	141.5	157.1	145.4
6*	--	--	--
7	122.1	152.1	131.4
8	129.7	155.6	135.1
9	123.7	134.8	131.0
10	121.7	123.7	121.7
11	117.7	129.4	114.5
12	110.1	118.2	112.4
13	136.4	146.4	141.7
14	118.8	138.9	123.8
15	123.6	137.3	129.1
16	129.2	144.2	132.8
17	144.2	155.9	141.7

* Subject data not collected due to Finometer equipment malfunction during protocol.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.7. Raw data for 5 minute average measurements of diastolic arterial pressure as measured by the finometer in normotensive group for Study 1.

Subject	Base	MS	Rec
1	78.3	94.4	87.0
2	76.7	88.1	76.5
3	79.8	85.2	76.1
4	60.7	71.9	59.9
5	67.4	76.2	68.5
6	72.1	76.2	69.9
7	66.8	74.2	65.2
8	71.9	75.0	74.1
9	71.1	80.5	76.2
10	71.6	74.9	75.0
11	68.7	79.6	72.3
12	73.4	78.9	74.5
13	73.5	79.1	75.0
14	83.2	95.4	86.3
15	68.5	80.9	67.4
16	68.8	72.4	70.3
17	71.5	77.8	71.6
18	72.8	82.4	76.7

Table A.8. Raw data 5 minute average measurements of diastolic arterial pressure as measured by the finometer in prehypertensive group for Study 1.

Subject	Base	MS	Rec
1	74.7	81.7	75.9
2	74.1	83.6	73.8
3	81.9	92.9	82.4
4	80.8	92.1	78.5
5	75.6	87.5	77.6
6*	--	--	--
7	71.4	94.3	73.2
8	70.9	87.0	72.7
9	71.9	83.1	73.2
10	70.0	73.6	70.4
11	64.4	72.4	65.9
12	66.8	76.4	67.3
13	84.5	89.2	88.4
14	71.8	82.3	73.7
15	66.3	79.4	69.9
16	70.2	80.8	72.7
17	68.4	79.4	69.4

* Subject data not collected due to Finometer equipment malfunction during protocol.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.9. Raw data 5 minute average measurements of mean arterial pressure as measured by the finometer in normotensive group for Study 1.

Subject	Base	MS	Rec
1	97.2	116.6	109.5
2	98.5	110.7	98.6
3	93.5	97.5	88.2
4	76.3	93.1	77.7
5	88.6	98.9	91.2
6	89.2	94.6	86.3
7	87.5	92.6	83.7
8	87.3	90.9	89.2
9	87.7	99.1	94.9
10	85.8	90.2	90.9
11	89.1	103.6	94.3
12	91.1	95.7	93.5
13	89.1	95.0	91.1
14	101.2	116.2	105.7
15	82.2	99.4	80.6
16	88.2	93.3	90.9
17	90.9	98.9	91.7
18	90.4	104.5	97.6

Base; Baseline

MS; Mental Stress

Rec; Recovery

Appendix A.10. Raw data for 5 minute average measurements of mean arterial pressure as measured by the finometer in prehypertensive group for Study 1.

Subject	Base	MS	Rec
1	90.4	101.0	93.1
2	97.9	110.3	100.3
3	100.6	111.9	100.6
4	90.7	107.6	93.9
5	97.5	115.4	101.1
6*	--	--	--
7	90.0	114.0	93.8
8	90.1	111.3	93.2
9	90.1	102.4	93.4
10	83.8	89.5	85.1
11	79.5	90.4	81.3
12	80.8	91.6	82.3
13	102.3	111.9	107.5
14	85.5	101.4	89.2
15	84.0	99.4	88.9
16	89.7	103.5	93.5
17	88.6	101.9	88.7

* Subject data not collected due to Finometer equipment malfunction during protocol.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.11. Raw data for 5 minute average measurements of heart rate as measured by the finometer in normotensive group for Study 1.

Subject	Base	MS	Rec
1	67.7	94.9	75.5
2	74.6	126.2	79.7
3	65.7	98.1	65.1
4	51.1	73.8	50.4
5	64.1	80.6	63.3
6	68.8	73.5	69.5
7	61.6	76.4	59.2
8	57.2	60.1	57.5
9	48.8	74.8	49.5
10	47.7	72.9	48.6
11	66.7	91.8	67.5
12	62.0	92.6	61.7
13	52.9	69.9	53.5
14	58.0	74.3	57.8
15	58.2	75.7	57.4
16	54.6	72.8	58.6
17	60.8	68.0	61.6
18	64.0	88.1	67.0

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.12 Raw data for 5 minute average measurements of heart rate as measured by the finometer in prehypertensive group for Study 1.

Subject	Base	MS	Rec
1	51.2	64.8	53.4
2	71.0	87.9	67.7
3	72.0	83.7	76.0
4	81.2	88.3	77.1
5	67.4	84.3	65.0
6	49.6	73.6	54.0
7	71.2	128.9	80.9
8	80.3	114.2	83.2
9	62.0	73.8	64.6
10	71.9	92.7	78.8
11	52.9	70.6	58.0
12	77.4	88.9	77.4
13	69.1	86.6	70.0
14	79.1	86.2	78.6
15	51.8	66.4	54.5
16	63.5	72.1	65.8
17	63.5	76.1	60.9

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.13. Raw data for 5 minute average measurements of muscle sympathetic nerve activity (MSNA) bursts/min in normotensive group for Study 1.

Subject	Base	MS	Rec
1	8.6	12.4	6.4
2	2.6	--	--
3	--	--	--
4	6.6	12.6	11.6
5	6.2	24.2	7.8
6	15.6	21.2	19.2
7	9.2	21.0	16.4
8	6.0	--	--
9	4.0	5.8	3.2
10	--	--	--
11	--	--	--
12	8.8	11.8	13.2
13	16.0	17.0	28.0
14	10.6	--	--
15	4.0	14.0	9.2
16	10.4	--	--
17	29.4	37.2	29.6
18	8.2	--	--

Missing data is due to either loss of nerve recordings during protocol, or unsuccessful location of nerve activity.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.14. Raw data for 5 minute average measurements of MSNA bursts/min in prehypertensive group for Study 1.

Subject	Base	MS	Rec
1	7.0	--	--
2	27.0	31.7	--
3	25.2	29.2	28.8
4	11.4	32.4	17.0
5	--	--	--
6	3.4	--	--
7	8.2	19.0	23.4
8	3.8	9.6	6.2
9	2.8	6.2	6.0
10	7.2	--	--
11	18.0	--	--
12	7.0	--	--
13	10.8	12.6	15.2
14	17.0	11.8	17.8
15	8.0	13.6	13.0
16	4.5	9.0	7.3
17	6.4	20.3	--

Missing data is due to either loss of nerve recordings during protocol, or unsuccessful location of nerve activity.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.15. Raw data for 5 minute average measurements of MSNA bursts per 100 heart beats in normotensive group for study 1.

Subject	Base	MS	Rec
1	12.7	13.2	8.4
2	3.5	--	--
3	--	--	--
4	12.9	17.9	23.1
5	9.7	30.2	12.4
6	23.1	29.1	28.3
7	15.0	27.7	28.0
8	10.9	--	--
9	8.3	7.8	6.5
10	--	--	--
11	--	--	--
12	14.2	12.8	21.5
13	30.4	24.4	52.8
14	18.4	--	--
15	6.96	18.6	16.1
16	19.1	--	--
17	48.6	54.6	48.2
18	12.9	--	--

Missing data is due to either loss of nerve recordings during protocol, or unsuccessful location of nerve activity.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.16. Raw data for 5 minute average measurements of MSNA bursts per 100 heart beats in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	13.7	--	--
2	38.7	36.8	--
3	35.0	35.1	38.0
4	14.1	36.9	22.0
5	--	--	--
6	7.0	--	--
7	11.7	14.2	29.1
8	4.8	8.4	7.4
9	4.6	8.4	9.4
10	10.5	--	--
11	34.2	--	--
12	9.1	--	--
13	15.7	14.5	21.8
14	21.7	13.8	22.7
15	15.4	20.6	23.8
16	7.2	12.5	11.2
17	10.1	26.1	--

Missing data is due to either loss of nerve recordings during protocol, or unsuccessful location of nerve activity.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.17. Raw data for 5 minute average measurements of total MSNA (arbitrary units) in normotensive group for study 1.

Subject	Base	MS	Rec
1	4665.4	12323.9	4687.6
2	2265.5	--	--
3	--	--	--
4	3346.8	12917.3	9870.0
5	3143.7	16129.0	2970.2
6	7878.0	8252.9	6569.8
7	2350.2	7873.3	3258.2
8	2185.5	--	--
9	1921.1	4301.4	1921.2
10	--	--	--
11	--	--	--
12	4209.9	6218.5	4504.8
13	6464.1	13705.9	11897.5
14	6906.8	--	--
15	1286.6	8854.8	3526.3
16	4874.6	--	--
17	--	--	--
18	3072.2	--	--

Missing data is due to either loss of nerve recordings during protocol, or unsuccessful location of nerve activity.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.18. Raw data for 5 minute average measurements of total MSNA (arbitrary units) in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	3919.4	--	--
2	15174.3	25679.3	--
3	9522.4	26003.6	9210.4
4	4552.6	22021.3	7797.7
5	--	--	--
6	2048.1	--	--
7	--	--	--
8	2427.1	7951.7	2560.0
9	1349.4	4434.6	4417.1
10	2875.2	--	--
11	9547.2	--	--
12	3173.9	--	--
13	4089.3	14199.4	6910.5
14	8623.5	9246.8	9207.2
15	4086.3	7066.5	5199.3
16	1722.9	3605.4	2304.8
17	4365.1	13956.6	--

Missing data is due to either loss of nerve recordings during protocol, or unsuccessful location of nerve activity.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.19. Raw data for 5 minute average measurements of forearm blood flow (mL/100mL/min) in normotensive group for study 1.

Subject	Base	MS	Rec
1	2.78	4.01	2.87
2	2.41	2.19	2.06
3	2.02	2.74	2.04
4	--	--	--
5	3.21	5.34	3.27
6	2.63	3.14	2.75
7	2.20	3.78	2.41
8	3.88	5.80	3.93
9	1.84	2.28	2.28
10	3.04	3.57	3.37
11	1.64	2.08	1.65
12	--	--	--
13	1.74	2.91	1.51
14	2.05	2.61	1.90
15	1.59	2.60	1.84
16	1.01	1.42	1.00
17	3.60	3.05	3.11
18	2.92	6.23	2.90

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.20. Raw data for 5 minute average measurements of forearm blood flow (mL/100mL/min) in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	2.49	4.68	2.53
2	2.69	3.54	2.47
3	2.25	3.24	2.65
4	--	--	--
5	4.21	6.91	3.96
6	1.28	3.09	1.29
7	5.34	10.77	6.17
8	3.72	8.37	3.66
9	2.65	3.87	3.38
10	2.40	5.15	2.88
11	3.61	6.15	3.54
12	4.38	7.50	4.00
13	3.43	4.36	3.90
14	5.93	5.64	5.79
15	3.68	4.88	3.92
16	2.91	3.36	2.48
17	4.15	5.22	3.19

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.21. Raw data for 5 minute average measurements of forearm vascular resistance (mmHg/mL/100mL/min) in normotensive group for study 1.

Subject	Base	MS	Rec
1	16.99	12.19	16.32
2	24.21	11.11	24.96
3	33.66	12.66	28.28
4	37.69	13.29	39.22
5	44.76	20.22	50.66
6	40.18	25.39	31.57
7	33.84	28.18	37.46
8	--	--	--
9	48.97	25.98	56.82
10	49.85	28.39	51.41
11	46.85	31.43	62.82
12	41.18	16.75	49.94
13	55.22	27.33	40.40
14	41.67	29.03	44.41
15	47.10	26.13	53.49
16	48.08	30.23	44.59
17	29.26	31.63	28.62
18	30.98	18.01	33.73

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.22. Raw data for 5 minute average measurements of forearm vascular resistance (mmHg/mL/100mL/min) in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	36.57	24.07	37.17
2	37.92	34.36	43.98
3	44.86	36.57	38.45
4	--	--	--
5	23.34	18.45	25.87
6	--	--	--
7	16.87	10.67	15.34
8	24.49	15.15	26.01
9	34.72	27.66	30.63
10	35.11	17.80	29.72
11	23.21	17.57	23.02
12	18.51	12.92	20.60
13	29.98	26.22	28.01
14	14.47	18.20	15.44
15	23.14	20.44	22.96
16	31.28	36.07	37.94
17	21.38	20.81	27.85

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.23. Raw data for 5 minute average measurements of forearm vascular conductance (mL/100mL/min/mmHg) in normotensive group for study 1.

Subject	Base	MS	Rec
1	0.060	0.084	0.062
2	0.042	0.096	0.041
3	0.030	0.084	0.037
4	0.027	0.078	0.026
5	0.022	0.064	0.020
6	0.025	0.041	0.032
7	0.030	0.036	0.027
8	--	--	--
9	0.020	0.042	0.018
10	0.020	0.039	0.020
11	0.022	0.035	0.016
12	0.025	0.063	0.021
13	0.018	0.037	0.026
14	0.024	0.035	0.023
15	0.021	0.040	0.019
16	0.021	0.034	0.022
17	0.034	0.033	0.036
18	0.032	0.060	0.030

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.24. Raw data for 5 minute average measurements of forearm vascular conductance (mL/100mL/min/mmHg) in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	0.028	0.047	0.027
2	0.028	0.032	0.025
3	0.022	0.029	0.026
4	--	--	--
5	0.043	0.061	0.039
6	--	--	--
7	0.059	0.094	0.066
8	0.041	0.076	0.039
9	0.029	0.037	0.036
10	0.029	0.057	0.034
11	0.045	0.069	0.044
12	0.054	0.082	0.049
13	0.034	0.039	0.036
14	0.069	0.055	0.065
15	0.044	0.049	0.044
16	0.032	0.033	0.027
17	0.047	0.052	0.036

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.25. Raw data for 5 minute average measurements of calf blood flow (mL/100mL/min) in normotensive group for study 1.

Subject	Base	MS	Rec
1	3.18	4.34	--
2	3.58	4.50	3.50
3	2.18	4.71	2.00
4	1.21	1.20	1.12
5	1.79	1.98	2.12
6	1.94	2.28	2.07
7	1.52	1.32	1.58
8	2.51	3.17	2.27
9	1.66	3.83	1.73
10	1.84	3.10	1.92
11	1.40	2.44	1.23
12	--	--	--
13	3.02	3.46	2.26
14	1.80	2.41	1.76
15	2.38	2.77	1.42
16	1.86	2.36	1.66
17	1.60	1.72	1.56
18	1.70	4.11	2.32

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.26. Raw data for 5 minute average measurements of calf blood flow (mL/100mL/min) in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	2.78	4.01	2.87
2	2.41	2.19	2.06
3	2.02	2.74	2.04
4	--	--	--
5	3.21	5.34	3.27
6	2.63	3.14	2.75
7	2.20	3.78	2.41
8	3.88	5.80	3.93
9	1.84	2.28	2.28
10	3.04	3.57	3.37
11	1.64	2.08	1.65
12	--	--	--
13	1.74	2.91	1.51
14	2.05	2.61	1.90
15	1.59	2.60	1.84
16	1.01	1.42	1.00
17	3.60	3.50	3.11

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.27. Raw data for 5 minute average measurements of calf vascular resistance (mmHg/mL/100mL/min) in normotensive group for study 1.

Subject	Base	MS	Rec
1	31.03	32.37	--
2	27.66	26.09	30.37
3	43.80	26.03	44.69
4	63.18	82.52	70.01
5	49.94	51.17	43.25
6	46.00	43.00	41.78
7	58.77	72.71	56.56
8	35.00	29.06	40.28
9	53.14	30.72	55.10
10	46.66	32.05	47.79
11	65.85	43.86	78.13
12	--	--	--
13	29.54	27.59	41.58
14	56.41	48.90	61.41
15	34.71	36.27	58.14
16	47.48	39.88	55.91
17	57.38	57.52	59.33
18	54.10	28.00	42.52

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.28. Raw data for 5 minute average measurements of calf vascular resistance (mmHg/mL/100mL/min) in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	32.54	28.64	32.48
2	41.44	52.62	49.10
3	49.91	42.67	49.57
4	--	--	--
5	30.77	21.98	31.12
6	--	--	--
7	41.22	33.78	39.25
8	23.27	21.87	23.85
9	49.26	45.69	41.68
10	27.68	25.86	25.40
11	49.30	43.84	49.50
12	--	--	--
13	58.92	46.26	71.28
14	41.84	39.78	47.12
15	53.54	40.38	49.55
16	89.62	81.05	96.48
17	24.72	31.31	28.62

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.29. Raw data for 5 minute average measurements of calf vascular conductance (mL/100mL/min/mmHg) in normotensive group for study 1.

Subject	Base	MS	Rec
1	0.033	0.037	--
2	0.036	0.041	0.035
3	0.023	0.048	0.023
4	0.016	0.013	0.014
5	0.020	0.020	0.023
6	0.022	0.024	0.024
7	0.017	0.014	0.019
8	0.029	0.035	0.025
9	0.019	0.039	0.018
10	0.021	0.034	0.021
11	0.016	0.024	0.013
12	--	--	--
13	0.034	0.036	0.025
14	0.018	0.021	0.017
15	0.029	0.028	0.018
16	0.021	0.025	0.018
17	0.018	0.017	0.017
18	0.019	0.040	0.024

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Table A.30. Raw data for 5 minute average measurements of calf vascular conductance (mL/100mL/min/mmHg) in prehypertensive group for study 1.

Subject	Base	MS	Rec
1	0.031	0.040	0.031
2	0.025	0.020	0.021
3	0.020	0.024	0.020
4	--	--	--
5	0.033	0.046	0.032
6	--	--	--
7	0.024	0.033	0.026
8	0.043	0.053	0.042
9	0.020	0.022	0.024
10	0.036	0.040	0.040
11	0.021	0.023	0.020
12	--	--	--
13	0.017	0.026	0.014
14	0.024	0.025	0.021
15	0.019	0.026	0.021
16	0.011	0.014	0.011
17	0.041	0.035	0.035

Missing data is due to either artifact during protocol, or mechanical issues with the equipment.

Base; Baseline

MS; Mental Stress

Rec; Recovery

Appendix B

Table B.1. Baseline comparisons between normotensive (nt) and prehypertensive (pht) groups for Study 1. Independent t-tests are presented as two-tailed significance.

Variable	Mean Diff.	Std. Error	95% Conf. Interval		Sig.	N nt/pht
			Lower	Upper		
Age (yrs)	-1.29	1.82	-5.00	2.41	.482	18/17
BMI (kg/m ²)	1.75	1.12	-.523	4.03	.126	18/17
SAP (mmHg)	14.97	1.98	10.94	18.99	.000	18/17
DAP (mmHg)	6.23	2.36	1.44	11.03	.012	18/17
MAP (mmHg)	9.14	1.92	5.23	13.04	.000	18/17
HR (beats/min)	6.24	3.50	-.869	13.36	.083	18/17
MSNA (bursts/min)	.735	2.55	-4.48	5.95	.775	15/16
MSNA (bursts/100hb)	-.612	3.96	-8.72	7.49	.878	15/16
FBF (mL/100mL/min)	.922	.393	.120	1.72	.026	17/16
FVR (mmHg/mL/100mL/min)	-4.91	4.46	-14.00	4.18	.279	17/16
FVC (mL/100mL/min/mmHg)	.0074	.0048	-.0023	.017	.129	17/16
CBF (mL/100mL/min)	.308	.259	-.222	.837	.245	17/15
CVR (mmHg/mL/100mL/min)	-.565	5.00	-10.80	9.67	.990	17/15
CVC (mL/100mL/min/mmHg)	.001	.003	-.006	.008	.753	17/15

Table B.2. Repeated Measures ANOVA of changes in 5 minute average Δ SAP (mmHg) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.050

Table B.3. Post-hoc paired t-test of changes in Δ SAP (mmHg) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	8.90	8.07	4.89	12.92	.000	18
Prehypertensive	14.01	7.47	10.00	18.00	.000	16

Table B.4. Post-hoc independent t-test of changes in Δ SAP (mmHg) base to mental stress.

Group	Mean Diff.	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Normotensive vs. Prehypertensive	5.11	2.68	-.344	10.57	.033

Table B.5. Repeated Measures ANOVA of changes in 5 minute average Δ DAP (mmHg) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.065

Table B.6. Post-hoc paired t-test of changes in Δ DAP (mmHg) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	8.12	3.77	6.25	9.99	.000	18
Prehypertensive	10.76	4.43	8.40	13.12	.000	16

Table B.7. Post-hoc independent t-test of changes in Δ DAP (mmHg) base to mental stress.

Group	Mean	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Normotensive vs. Prehypertensive	2.64	1.41	-.225	5.50	.035

Table B.8. Repeated Measures ANOVA of changes in 5 minute average Δ MAP (mmHg) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.017

Table B.9. Post-hoc paired t-test of changes in Δ MAP (mmHg) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	9.83	5.33	7.18	12.48	.000	18
Prehypertensive	13.90	4.56	11.46	16.33	.000	16

Table B.10. Post-hoc independent t-test of changes in Δ MAP (mmHg) base to mental stress.

Group	Mean	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Normotensive vs. Prehypertensive	4.07	1.71	.577	7.56	.017

Table B.11. Repeated Measures ANOVA of changes in 5 minute average Δ HR (beats/min) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.195

Table B.12. Post-hoc paired t-test of changes in Δ HR (beats/min) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	21.12	11.30	15.50	26.74	.000	18
Prehypertensive	17.49	12.49	10.84	24.15	.000	16

Table B.13. Repeated Measures ANOVA of changes in 5 minute average Δ MSNA (bursts/min) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.479

Table B.14. Post-hoc paired t-test of changes in Δ MSNA (bursts/min) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	6.88	5.23	3.14	10.62	.001	10
Prehypertensive	6.39	6.84	1.80	10.98	.005	11

Table B.15. Repeated Measures ANOVA of changes in 5 minute average Δ Total MSNA (arbitrary units) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.244

Table B.16. Post-hoc paired t-test of changes in Δ Total MSNA (arbitrary units) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	6145.7	4015.7	3058.9	9232.4	.001	9
Prehypertensive	7825.2	5980.2	3547.3	12103.2	.002	10

Table B.17. Repeated Measures ANOVA of changes in 5 minute average Δ FBF (%) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.005

Table B.18. Post-hoc paired t-test of changes in Δ FBF (%) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	116.03	65.68	82.26	149.8	.000	17
Prehypertensive	62.18	42.55	39.51	84.85	.000	16

Table B.19. Post-hoc independent t-test of changes in Δ FBF (%) base to mental stress.

Group	Mean	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Normotensive vs. Prehypertensive	53.84	19.15	14.58	93.11	.005

Table B.20. Repeated Measures ANOVA of changes in 5 minute average ΔFVR (%) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.001

Table B.21. Post-hoc paired t-test of changes in ΔFVR (%) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	-40.97	18.10	-50.27	-31.66	.000	17
Prehypertensive	-17.85	20.02	-28.93	-6.77	.002	15

Table B.22. Post-hoc independent t-test of changes in ΔFVR (%) base to mental stress.

Group	Mean	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Normotensive vs. Prehypertensive	-23.12	6.73	-36.87	-9.36	.001

Table B.23. Repeated Measures ANOVA of changes in 5 minute average ΔFVC (%) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.001

Table B.24. Post-hoc paired t-test of changes in ΔFVC (%) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	94.47	57.57	64.87	124.07	.000	17
Prehypertensive	36.53	32.60	18.48	54.59	.000	15

Table B.25. Post-hoc independent t-test of changes in ΔFVC (%) base to mental stress.

Group	Mean	Std. Error	95% Confidence Interval		Sig.
			Lower	Upper	
Normotensive vs. Prehypertensive	57.93	16.30	24.41	91.46	.000

Table B.26. Repeated Measures ANOVA of changes in 5 minute average ΔCBF (%) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.302

Table B.27. Post-hoc paired t-test of changes in ΔCBF (%) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	43.14	46.58	19.19	67.09	.001	17
Prehypertensive	36.08	24.92	22.28	49.88	.000	15

Table B.28. Repeated Measures ANOVA of changes in 5 minute average ΔCVR (%) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.005
Condition x Group	.313

Table B.29. Post-hoc paired t-test of changes in ΔCVR (%) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	-11.47	22.36	-22.96	.029	.025	17
Prehypertensive	-7.93	16.39	-17.39	1.54	.047	14

Table B.30. Repeated Measures ANOVA of changes in 5 minute average Δ CVC (%) in Study 1, 2 condition vs. 2 groups (Normotensive/Prehypertensive), presented as one-tailed significance.

Source	Base – Mental Stress
Condition	.000
Condition x Group	.210

Table B.31. Post-hoc paired t-test of changes in Δ CVC (%) base to mental stress.

Group	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Normotensive	29.03	42.24	7.31	50.75	.006	17
Prehypertensive	18.90	20.25	7.21	30.59	.002	14

Appendix C

Table C.1. Anthropometric data for the normotensive group in study 2

Sub- ject	Gend er	Drug	Pre Supplementation				Post Supplementation			
			Age yr	Height cm	Weight kg	BMI kg/m ²	Age yr	Height cm	Weight kg	BMI kg/m ²
1	M	FO	25	176	99.5	32	25	176	100.5	33
2	F	PL	39	176	79.5	26	39	175	79.5	26
3	M	FO	18	173	71.0	24	19	173	72.0	24
4	F	FO	18	166	61.0	22	18	166	62.0	22
5	M	FO	19	177	60.0	19	19	177	60.5	19
6	F	PL	18	161	68.0	26	18	162	69.0	26
7	F	PL	18	163	56.0	21	18	163	57.0	21
8	F	PL	19	174	68.0	22	19	174	69.5	23
9	F	FO	20	169	69.5	24	20	169	72.0	25
10	F	PL	21	152	60.5	26	21	157	61.5	25
11	M	FO	23	179	66.0	21	23	181	66.0	20
12	F	FO	20	168	62.5	22	20	169	64.0	22
13	F	FO	21	160	57.0	22	21	163	57.5	22
14	F	FO	20	155	52.0	22	20	154	52.0	22
15	F	FO	23	171	55.0	19	23	171	56.5	19
16	F	FO	22	149	62.5	28	22	149	64.0	29
17	F	PL	18	161	65.5	25	18	161	65.5	25
18	F	FO	21	163	57.5	22	22	163	55.5	21
19	F	PL	21	166	74.0	27	21	166	73.0	26
20	M	PL	20	167	63.0	23	20	168	62.5	22
21	M	PL	18	185	97.5	28	19	185	101.5	30
22	M	PL	39	168	60.0	21	39	167	60.5	22
23	M	PL	20	185	60.0	18	20	185	60.0	18
24	M	FO	19	177	75.0	24	19	177	74.0	24
25	F	PL	20	163	57.0	21	20	163	56.0	21
26	M	FO	22	184	80.0	24	22	186	77.0	22
27	M	FO	18	170	62.5	22	19	168	63.0	22
28	M	PL	20	174	79.0	26	20	171	80.0	27
29	M	PL	23	161	69.8	27	23	171	71.0	24
30	M	PL	22	181	72.0	22	22	182	72.0	22
31	F	PL	21	173	72.0	24	21	171	72.0	25
32	F	FO	21	169	77.6	27	21	169	79.0	28
33	M	FO	40	184	89.0	26	40	186	87.0	25
34	M	FO	30	170	77.0	27	30	170	77.5	27
35	F	PL	52	178	77.5	24	52	179	78.0	24
36	F	FO	53	163	62.5	24	54	162	62.5	24
37	M	PL	19	170	76.0	26	19	170	74.5	26
38	M	PL	25	173	81.5	27	25	173	80.0	27

M, Male; F, Female; FO, Fish Oil; PL, Placebo; BMI, Body Mass Index

Table C.2. Anthropometric data for prehypertensive group in Study 2

Subj- ect	Gen- der	Drug	Pre Supplementation				Post Supplementation			
			Age yr	Height cm	Weight kg	BMI kg/m ²	Age yr	Height cm	Weight kg	BMI kg/m ²
39	M	FO	32	184	97.0	29	32	184	97.0	29
40	M	PL	19	178	82.5	26	19	179	82.5	26
41	M	PL	20	178	84.0	27	20	178	82.0	26
42	M	FO	21	176	77.0	25	21	175	79.0	26
43	M	PL	21	179	87.0	27	21	180	87.5	27
44	M	PL	23	183	91.5	27	23	183	92.5	28
45	M	FO	21	179	66.5	21	21	179	67.0	21
46	M	FO	19	185	81.0	24	19	184	85.0	25
47	M	PL	18	166	85.0	31	18	166	77.5	28
48	M	PL	20	189	92.0	26	21	189	91.0	25
49	M	PL	20	174	95.0	31	20	174	96.0	32
50	M	FO	23	177	75.5	24	23	172	75.0	25
51	M	FO	21	184	83.5	25	22	184	82.0	24
52	M	FO	20	174	80.5	27	20	174	82.0	27
53	M	FO	26	185	95.0	28	26	186	97.5	28
54	M	FO	20	185	113.0	33	20	184	112.5	33
55	M	PL	31	184	91.0	27	31	183	91.0	27
56	M	PL	52	192	102.5	28	52	192	101.5	28
57	M	FO	33	173	97.0	32	34	172	96.5	33
58	M	FO	19	176	87.0	28	20	176	84.0	27
59	F	PL	50	164	81.0	30	50	164	82.0	30
60	M	PL	18	176	90.5	29	18	175	92.0	30
61	M	FO	18	176	75.5	24	18	176	77.0	25
62	M	PL	20	188	79.5	22	21	183	88.0	26
63	M	FO	23	170	92.0	32	23	170	92.0	32
64	M	FO	22	170	103.5	36	22	171	102.5	35
65	M	PL	19	172	67.5	23	19	172	66.5	22
66	M	FO	21	189	109.5	31	21	189	110.0	31
67	M	PL	19	179	87.5	27	19	179	86.5	27

M, Male; F, Female; FO, Fish Oil; PL, Placebo; BMI, Body Mass Index

Table C.3. Raw data for seated resting blood pressure (mmHg) and heart rate (beats/min) for normotensive group in study 2.

Subject	Pre supplementation				Post supplementation			
	SAP	DAP	MAP	HR	SAP	DAP	MAP	HR
1	116.3	61.6	79.8	71.0	114.8	63.0	80.3	78.9
2	104.0	66.0	78.7	75.1	104.2	63.0	76.7	70.8
3	116.3	62.1	80.2	74.8	115.1	62.0	79.7	64.1
4	117.9	70.0	86.0	80.3	109.9	68.3	82.2	80.4
5	107.8	64.2	78.7	82.8	110.8	74.1	86.3	89.6
6	104.1	68.0	80.0	90.6	107.3	68.0	81.1	95.7
7	102.7	66.2	78.4	79.4	98.8	66.3	77.1	76.8
8	99.1	57.8	71.6	68.9	101.8	61.8	75.1	68.3
9	105.0	64.0	77.7	66.0	106.1	67.9	80.6	65.6
10	97.0	67.0	77.0	71.0	100.0	66.7	77.8	71.6
11	110.0	57.0	74.7	60.0	107.0	62.7	77.5	64.6
12	106.0	64.9	78.6	68.2	114.9	72.1	86.4	71.3
13	92.2	59.1	70.1	62.1	96.2	62.9	74.0	58.2
14	106.7	70.3	82.4	85.2	103.7	71.9	82.5	84.6
15	110.9	68.3	82.5	73.2	107.4	64.9	79.1	75.6
16	104.9	65.7	78.8	73.7	97.8	65.4	76.2	65.2
17	105.1	68.3	80.6	85.1	101.7	62.0	75.2	72.8
18	99.6	72.9	81.8	81.1	90.2	63.2	72.2	87.9
19	105.2	66.2	79.2	69.2	100.4	61.0	74.1	60.9
20	108.6	68.2	81.7	73.6	109.4	70.7	83.6	65.9
21	116.4	76.2	89.6	68.9	122.6	80.9	94.8	85.1
22	103.3	65.0	77.8	68.9	107.4	66.2	79.9	73.6
23	109	56.0	73.7	58.1	110.6	61.7	78.0	67.8
24	109.3	63.4	78.7	47.7	109.6	60.9	77.1	47.7
25	95.9	64.3	74.8	76.6	90.7	61.7	71.4	76.6
26	113.6	65.6	81.6	72.3	111.5	69.7	83.6	67.3
27	111.7	65.2	80.7	68	104.8	64.9	78.2	72.8
28	103.4	66.2	78.6	60.3	106.8	69.8	82.1	67.3
29	115.7	62.9	80.5	78.9	121.2	65.8	84.3	79.0
30	106	51.6	69.7	73.3	116.7	61.6	80.0	86.4
31	105.2	63.3	77.3	75.7	106.0	62.6	77.1	71.0
32	111.8	66.4	81.5	77.2	100.7	64.3	76.4	69.6
33	111	67.2	81.8	77.9	108.4	60.8	76.7	75.6
34	116.2	71.4	86.3	73.3	108.0	70.6	83.1	72.0
35	113.2	75.8	88.3	64.2	100.2	65.2	76.9	63.3
36	113.3	72.2	85.9	56.1	118.1	72.9	88.0	58.6
37	114.3	60.4	78.4	76.6	110.7	72.7	85.4	91.1
38	118.6	66	83.5	92.4	111.6	71.2	84.7	94.8

SAP, systolic arterial pressure
DAP, diastolic arterial pressure
MAP, mean arterial pressure
HR, heart rate

Table C.4. Raw data for seated resting blood pressure (mmHg) and heart rate (beats/min) for prehypertensive group in study 2

Subject	Pre supplementation				Post supplementation			
	SAP	DAP	MAP	HR	SAP	DAP	MAP	HR
39	120.3	66.4	84.4	60.6	123.4	68.6	86.9	60.9
40	132.9	77.0	95.6	69.6	127.2	68.4	88.0	54.9
41	118.0	81.0	93.3	81.8	119.4	79.0	92.5	78.4
42	139.9	77.4	98.2	71.4	125.3	69.7	88.2	64.0
43	139.7	84.4	102.8	59.1	121.2	71.9	88.3	58.2
44	133.0	66.0	88.3	77.0	125.2	63.9	84.3	80.3
45	124.7	69.1	87.6	80.7	128.7	69.9	89.5	89.2
46	121.5	67.5	85.5	68.6	123.2	68.9	87.0	69.9
47	122.4	72.8	89.3	95.0	131.1	78.4	96.0	90.6
48	122.0	63.6	83.1	64.3	118.4	64.6	82.5	62.0
49	121.2	78.8	92.9	95.0	121.6	82.7	95.7	90.9
50	133.4	73.8	93.7	72.3	125.1	73.9	91.0	66.7
51	123.6	69.6	87.6	88.9	121.6	66.3	84.7	86.3
52	125.6	62.4	83.5	71.1	124.3	63.0	83.4	70.6
53	127.9	66.6	87.0	69.8	132.2	74.4	93.7	77.8
54	128.3	66.7	87.2	70.0	128.4	67.9	88.1	77.3
55	124.6	68.4	87.1	80.6	123.7	74.3	90.8	75.4
56	121.9	72.0	88.6	69.1	124.9	76.4	92.6	72.9
57	125.2	79.9	95.0	75.1	126.0	78.0	94.0	75.0
58	129.3	66.2	87.2	68.8	129.4	67.9	88.4	71.3
59	115.7	82.7	93.7	80.5	111.8	76.1	88.0	77.9
60	124.3	63.1	83.5	63.7	114.0	58.0	76.7	51.9
61	122.6	76.8	92.1	96.3	115.7	65.6	82.3	78.6
62	131.2	70.7	90.9	68.7	132.2	72.7	92.5	77.2
63	126.0	65.2	85.5	75.8	117.4	63.1	81.2	71.2
64	134.1	70.7	91.8	68.5	135.2	69.4	91.3	69.0
65	133.2	78.7	96.9	78.5	132.6	82.0	98.9	81.1
66	123.1	45.4	71.3	60.7	123.2	49.1	73.8	61.9
67	127.0	79.8	95.5	83.1	121.9	80.8	94.5	84.6

SAP, systolic arterial pressure
DAP, diastolic arterial pressure
MAP, mean arterial pressure
HR, heart rate

Table C.4. Raw data for 5 minute average systolic arterial pressure (mmHg) as measured by the finometer for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	138.5	158.3	154.3	125.0	148.3	137.4
2	113.5	126.4	116.4	134.9	148.8	138.3
3	133.9	145.4	134.9	137.9	151.6	130.1
4	128.8	147.6	140.6	137.2	149.4	143.6
5	115.5	114.2	104.1	125.1	122.2	141.9
6	131.3	138.2	133.9	103.5	110.5	107.1
7	123.4	128.7	129.6	121.6	124.9	128.0
8	113.8	120.6	97.4	124.1	137.0	119.4
9	125.7	138.0	127.0	115.6	139.0	121.8
10	129.0	139.0	117.1	124.3	131.1	131.3
11	108.4	132.6	110.6	125.3	146.3	118.0
12	132.9	153.4	138.6	131.8	147.9	138.1
13	118.4	130.3	123.5	116.0	139.2	121.5
14	137.1	147.2	140.0	119.8	138.5	--
15	112.8	121.1	116.6	120.0	128.8	120.1
16	115.5	133.8	124.6	117.5	126.4	120.8
17	116.8	125.6	123.9	120.1	136.9	129.3
18	130.9	149.5	137.5	114.9	123.2	118.5
19	133.8	139.7	135.7	119.0	131.7	124.0
20	127.7	135.2	130.9	126.1	125.6	130.5
21	124.0	134.0	117.1	126.7	128.4	124.9
22	123.1	124.0	112.7	119.1	115.3	103.3
23	122.1	133.3	131.0	112.1	107.9	111.5
24	120.6	118.8	124.9	121.4	117.3	133.8
25	120.5	132.5	128.1	121.0	128.0	128.4
26	128.5	144.7	135.3	112.2	125.6	115.5
27	127.1	124.3	129.7	114.9	121.3	126.7
28	118.6	121.5	118.6	106.5	114.2	107.8
29	133.8	147.7	137.9	113.4	125.7	116.0
30	116.0	131.7	108.0	118.5	136.6	128.1
31	134.5	134.8	138.7	126.9	130.6	130.6
32	127.3	131.7	128.8	133.6	141.1	136.6
33	129.6	137.2	127.8	118.7	131.8	117.4
34	120.6	139.1	132.3	123.4	137.2	132.9
35	124.4	138.8	130.5	87.8	100.7	91.6
36	148.0	164.6	159.1	135.6	147.5	139.0
37	130.2	148.8	129.0	111.4	129.5	112.5
38	112.0	125.1	129.1	105.2	117.8	115.0

Missing data point was due to power outage and loss of recovery data.

Table C.5. Raw data for 5 minute average systolic arterial pressure (mmHg) as measured by the finometer for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	124.8	135.7	129.3	135.8	142.1	139.2
40	141.2	150.1	146.9	138.2	150.1	150.0
41	138.6	145.0	133.2	123.2	129.1	123.4
42	141.5	157.1	145.4	140.9	155.9	132.2
43	122.6	--	124.5	--	--	--
44	122.1	152.1	131.4	134.1	176.5	129.6
45	129.7	155.6	135.1	114.1	149.1	119.6
46	123.7	134.8	131.0	131.8	134.3	135.4
47	121.7	123.2	122.2	121.6	122.4	116.3
48	117.7	129.4	114.5	111.6	124.6	117.5
49	110.1	118.2	112.4	119.6	125.3	120.5
50	136.4	146.4	141.7	134.7	145.9	141.2
51	118.8	138.9	123.8	111.4	124.2	128.1
52	144.2	155.9	141.7	119.8	143.6	121.0
53	164.9	187.8	168.2	153.2	169.4	156.0
54	119.8	142.2	136.1	130.1	136.5	133.0
55	127.9	143.0	126.6	119.8	124.2	115.4
56	135.0	148.8	131.2	135.5	140.8	139.1
57	141.5	154.7	144.7	128.6	142.5	134.8
58	139.1	135.8	137.0	138.5	144.1	145.4
59	165.5	199.9	166.1	144.1	182.7	156.7
60	126.7	139.3	133.0	132.1	135.2	134.7
61	136.6	148.0	140.5	--	--	--
62	144.0	154.5	148.2	151.3	171.6	162.6
63	138.9	150.4	140.4	121.2	134.3	120.1
64	138.4	175.1	146.7	136.1	164.7	151.4
65	139.3	151.7	146.8	146.8	156.0	157.2
66	150.6	149.4	161.4	156.0	155.8	157.3
67	138.5	152.8	148.3	120.1	147.1	144.1

Missing data points are due to subject withdrawal and equipment error.

Table C.6. Raw data for 5 minute average diastolic arterial pressure (mmHg) as measured by the finometer for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	78.3	94.4	87.0	73.5	86.7	77.8
2	76.4	89.1	76.2	75.9	88.3	76.8
3	76.7	88.1	76.5	74.8	87.9	74.4
4	80.3	94.5	80.5	72.7	86.6	71.3
5	79.8	85.2	76.1	83.0	91.2	95.2
6	79.9	84.6	79.7	68.5	74.5	70.1
7	74.9	79.3	78.2	68.8	72.2	71.3
8	75.4	83.9	70.6	74.3	87.2	73.2
9	73.7	82.1	73.6	60.9	80.1	66.7
10	75.0	84.3	68.9	67.8	74.8	71.0
11	60.7	71.9	59.9	68.3	80.8	64.4
12	71.3	84.4	72.4	65.0	76.7	67.4
13	59.8	74.3	63.1	58.0	73.2	62.4
14	75.9	84.3	78.5	66.8	78.9	--
15	66.2	72.6	63.5	70.8	78.4	72.4
16	66.5	76.1	68.4	65.2	72.0	66.1
17	62.1	72.5	67.0	63.3	78.6	69.3
18	74.0	88.2	77.8	61.3	71.2	59.9
19	73.8	81.8	73.0	61.8	71.5	63.8
20	67.4	76.2	68.5	67.4	73.9	69.2
21	72.2	76.2	69.9	76.4	77.6	75.6
22	66.8	74.2	65.2	64.4	69.4	61.0
23	71.1	80.5	76.2	71.7	76.6	75.5
24	71.6	74.9	75.0	67.6	75.4	75.8
25	69.5	76.0	73.9	68.9	72.8	70.6
26	68.7	79.6	72.3	63.1	73.4	66.2
27	73.4	78.9	74.5	68.4	77.8	75.5
28	73.5	79.1	75.0	64.8	72.6	68.1
29	83.2	95.4	86.3	68.2	77.8	69.5
30	68.5	80.9	67.4	59.9	71.9	63.7
31	74.7	75.9	77.8	69.3	73.6	71.0
32	75.0	79.4	76.1	76.3	83.5	78.4
33	71.5	77.8	71.6	65.2	73.8	66.6
34	72.8	82.4	76.7	72.6	81.4	76.7
35	70.4	76.9	73.4	67.9	78.4	79.5
36	72.4	83.1	78.5	62.1	70.0	64.3
37	73.4	87.8	74.3	66.9	76.3	68.0
38	70.5	78.9	78.8	64.5	71.1	70.5

Missing data point was due to power outage and loss of recovery data.

Table C.7. Raw data for 5 minute average diastolic arterial pressure (mmHg) as measured by the finometer for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	74.7	81.7	75.9	79.9	85.9	81.9
40	74.1	83.6	73.8	73.5	83.7	78.1
41	81.9	92.9	82.4	68.1	73.5	67.5
42	75.6	87.5	77.6	68.1	80.3	67.2
43	67.5	--	71.7	--	--	--
44	71.4	94.3	73.2	69.2	91.1	69.9
45	70.9	87.0	72.7	62.9	81.8	66.1
46	71.9	83.1	73.2	70.8	76.6	74.1
47	70.0	73.2	70.9	67.0	71.5	64.8
48	64.4	72.4	65.9	69.0	78.1	71.3
49	66.8	76.4	67.3	70.2	77.6	70.3
50	84.5	89.2	88.4	76.7	85.0	81.0
51	71.8	82.3	73.7	63.5	72.4	74.7
52	68.4	79.4	69.4	65.0	76.4	67.5
53	97.6	106.4	91.3	82.6	96.5	85.8
54	63.6	81.3	75.1	63.2	75.5	69.9
55	72.1	83.0	71.1	68.2	73.5	69.1
56	72.6	79.8	71.2	74.5	79.0	76.1
57	82.4	90.2	83.3	75.2	82.0	78.3
58	73.1	77.9	75.2	76.4	80.6	79.4
59	88.7	106.6	88.0	80.7	99.5	84.7
60	72.3	80.7	72.7	75.4	81.0	74.6
61	82.5	88.7	82.3	--	--	--
62	71.6	81.6	74.6	88.9	99.8	92.7
63	81.2	89.9	79.9	63.2	73.0	64.1
64	74.3	97.6	79.0	75.0	92.9	83.5
65	73.8	87.7	79.5	80.5	91.3	83.8
66	80.0	83.3	86.8	82.5	84.8	85.0
67	95.4	104.4	101.0	72.9	91.4	84.2

Missing data points are due to subject withdrawal and equipment error.

Table C.8. Raw data for 5 minute average mean arterial pressure (mmHg) as measured by the finometer for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	97.2	116.6	109.5	89.9	106.7	97.6
2	90.1	104.5	90.7	97.2	113.6	99.3
3	98.5	110.7	98.6	96.4	110.4	94.1
4	102.2	118.5	105.4	100.0	115.5	100.1
5	93.5	97.5	88.2	99.8	104.1	113.9
6	97.1	103.6	98.3	81.0	88.1	83.7
7	91.6	97.8	97.3	87.6	92.3	92.2
8	89.1	98.3	81.8	90.7	106.0	89.3
9	91.8	104.8	93.6	79.3	103.6	87.4
10	97.4	109.1	90.4	88.7	95.8	94.0
11	76.3	93.1	77.7	86.5	103.1	82.2
12	96.1	115.2	99.2	87.4	105.9	92.1
13	81.0	97.3	87.5	78.7	95.0	85.2
14	101.1	105.6	100.8	86.4	104.1	--
15	82.6	91.1	81.0	90.7	98.3	90.5
16	86.9	103.3	93.0	87.1	97.3	89.6
17	82.0	93.7	88.8	84.9	102.5	93.6
18	99.6	116.4	104.5	83.8	94.1	83.6
19	95.3	105.2	95.5	81.4	93.8	84.9
20	88.6	98.9	91.2	87.7	94.7	91.1
21	89.2	94.6	86.3	92.7	94.5	92.0
22	87.5	92.6	83.7	81.8	84.3	75.6
23	87.7	99.1	94.9	83.8	87.5	87.4
24	85.8	90.2	90.9	84.5	91.0	96.0
25	90.1	99.2	96.6	88.9	95.9	93.1
26	89.1	103.6	94.3	81.0	93.9	84.9
27	91.1	95.7	93.5	84.9	94.7	94.0
28	89.1	95.0	91.1	80.2	88.3	84.0
29	101.2	116.2	105.7	85.0	97.0	87.5
30	82.2	99.4	80.6	77.7	93.5	83.4
31	95.3	98.1	99.5	91.3	96.8	94.1
32	94.8	100.3	95.9	97.4	105.8	100.0
33	90.9	98.9	91.7	83.9	95.3	85.7
34	90.4	104.5	97.6	90.4	102.9	96.9
35	92.7	101.0	96.7	76.2	86.4	84.0
36	102.0	116.0	110.6	89.7	99.8	92.5
37	89.4	108.0	90.3	80.6	92.7	81.7
38	84.4	95.5	96.3	78.1	87.6	85.0

Missing data points are due to subject withdrawal and equipment error.

Table C.9. Raw data for 5 minute average mean arterial pressure (mmHg) as measured by the finometer for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	90.4	101.0	93.1	97.8	105.9	100.9
40	97.9	110.3	100.3	94.7	110.4	102.3
41	100.6	111.9	100.6	86.2	92.2	86.2
42	97.5	115.4	101.1	90.2	106.8	88.0
43	86.7	--	92.4	--	--	--
44	90.0	114.0	93.8	91.0	118.5	90.4
45	90.1	111.3	93.2	80.5	105.4	85.4
46	90.1	102.4	93.4	91.1	97.2	95.1
47	83.8	89.3	85.9	82.8	87.7	80.8
48	79.5	90.4	81.3	81.7	93.1	86.1
49	80.8	91.6	82.3	87.4	95.9	87.9
50	102.3	111.9	107.5	94.9	106.2	101.2
51	85.5	101.4	89.2	77.8	88.9	92.4
52	88.6	101.9	88.7	82.1	96.5	85.1
53	114.2	139.0	119.0	102.7	121.9	107.4
54	81.1	102.6	96.0	82.7	96.2	90.4
55	90.4	103.4	90.5	83.1	88.1	83.3
56	96.7	106.0	95.0	97.3	102.1	100.7
57	104.4	114.9	107.0	95.4	105.7	100.6
58	95.5	101.2	98.2	98.6	105.0	103.3
59	120.4	146.7	119.4	108.5	135.8	115.4
60	91.4	103.4	93.9	92.7	101.0	94.4
61	101.5	111.6	102.9	--	--	--
62	93.9	106.1	97.6	109.8	124.7	116.5
63	100.9	111.8	100.9	82.2	94.4	83.4
64	96.9	127.4	103.6	95.1	119.1	106.3
65	94.5	110.7	102.0	102.1	115.0	108.1
66	98.0	103.4	106.9	100.8	105.9	104.5
67	110.9	122.7	118.4	89.2	111.7	105.1

Missing data points are due to subject withdrawal and equipment error.

Table C.10. Raw data for 5 minute average heart rate (beats per minute) as measured by the finometer for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	67.7	94.9	75.5	66.9	93.5	73.1
2	64.5	75.7	65.1	63.0	75.2	61.7
3	74.6	126.2	79.7	77.7	115.5	71.4
4	71.3	112.1	67.4	66.1	109.7	58.8
5	65.7	98.1	65.1	78.4	109.2	73.9
6	89.1	96.3	86.1	87.3	94.1	87.9
7	62.0	70.6	60.3	61.0	70.0	56.5
8	60.6	69.1	60.9	62.1	75.3	58.9
9	65.3	83.8	64.9	60.8	76.9	64.6
10	57.1	70.6	56.1	67.5	78.8	65.8
11	51.1	73.8	50.4	63.9	83.8	57.1
12	58.0	91.2	58.1	55.8	76.1	56.6
13	49.6	70.0	51.3	52.9	71.3	52.5
14	78.6	102.4	74.5	77.8	107.3	--
15	73.4	100.5	71.9	67.1	83.2	61.7
16	64.5	80.2	64.5	63.6	75.2	63.9
17	75.6	91.7	74.0	73.2	97.1	68.6
18	80.4	103.3	76.5	82.8	99.0	77.0
19	63.6	72.8	65.5	62.0	68.5	64.9
20	64.1	80.6	63.3	63.3	81.3	58.9
21	68.8	73.5	69.5	72.3	76.5	68.7
22	61.6	76.4	59.2	70.4	83.6	69.9
23	48.8	74.8	49.5	59.2	86.1	56.7
24	47.7	72.9	48.6	49.4	78.3	48.5
25	58.4	76.3	59.2	70.7	91.2	70.7
26	66.7	91.8	67.5	60.0	78.4	62.2
27	62.0	92.6	61.7	48.0	87.7	57.6
28	52.9	69.9	53.5	56.6	76.5	61.6
29	58.0	74.3	57.8	62.4	81.0	61.8
30	58.2	75.7	57.4	62.0	77.2	65.8
31	65.2	73.7	64.7	64.5	73.1	65.2
32	57.4	70.3	61.2	56.2	63.4	57.3
33	60.8	68.0	61.6	63.4	69.0	62.6
34	64.0	88.1	67.0	58.1	77.7	64.1
35	60.2	62.0	59.7	69.5	68.2	66.2
36	48.3	56.5	49.4	50.6	54.6	51.6
37	65.4	84.0	63.9	72.4	92.9	66.3
38	82.6	93.2	84.2	84.0	94.4	84.9

Missing data points are due to subject withdrawal and equipment error.

Table C.11. Raw data for 5 minute average heart rate (beats per minute) as measured by the finometer for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	51.2	64.8	53.4	56.3	66.4	57.5
40	71.0	87.9	67.7	56.9	83.4	57.2
41	72.0	83.7	76.0	71.2	80.8	74.1
42	67.4	84.3	65.0	63.0	74.5	60.0
43	49.6	73.6	54.0	--	--	--
44	71.2	128.9	80.9	89.3	132.0	92.9
45	80.3	114.2	83.2	67.0	96.2	76.3
46	62.0	73.8	64.6	60.4	71.4	62.9
47	71.9	92.7	78.8	73.0	91.4	76.7
48	52.9	70.6	58.0	58.0	73.9	62.0
49	77.4	88.9	77.4	63.0	74.6	59.4
50	69.1	86.6	70.0	57.2	71.3	59.9
51	79.1	86.2	78.6	74.4	82.0	78.3
52	63.5	76.1	60.9	67.1	78.6	66.0
53	67.1	96.6	65.8	70.9	89.5	67.8
54	59.0	72.3	61.7	57.8	77.8	66.2
55	69.8	80.1	74.7	69.8	71.9	69.0
56	62.9	65.2	62.9	64.1	65.2	66.0
57	81.1	100.2	79.1	72.5	89.7	72.4
58	68.1	79.6	65.9	70.9	75.1	65.6
59	76.8	92.7	77.6	69.7	95.9	73.1
60	61.2	82.2	65.1	50.1	73.1	54.0
61	69.0	80.6	67.8	--	--	--
62	65.1	79.5	64.0	78.7	92.1	76.3
63	72.8	92.2	77.2	66.7	86.0	70.9
64	69.2	91.2	72.4	72.6	95.6	75.9
65	66.8	88.4	67.9	65.8	88.5	65.4
66	55.4	78.5	59.3	52.8	73.5	50.6
67	63.4	88.2	72.3	61.8	88.9	68.0

Missing data points are due to subject withdrawal and equipment error.

Table C.12. Raw data for 5 minute average MSNA (burst per minute) for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	8.6	12.4	6.4	8.2	18.0	15.4
2	5.2	--	--	13.6	20.5	19.8
3	2.6	--	--	12.6	17.4	17.2
4	16.6	25.2	27.8	11.6	14.8	15.4
5	--	--	--	--	--	--
6	6.2	--	--	--	--	--
7	13.8	18.2	20.2	--	--	--
8	7.2	11.4	17.0	3.8	10.6	--
9	20.0	--	--	20.2	24.2	28.2
10	10.4	10.8	18.0	4.2	5.4	4.6
11	6.6	12.6	11.6	2.0	--	--
12	12.7	--	--	12.4	16.0	15.8
13	1.0	19.4	--	6.4	12.4	4.4
14	8.8	13.2	10.8	3.6	10.8	--
15	13.4	18.4	24.6	16.3	--	--
16	10.8	7.0	8.2	7.6	12.2	9.0
17	7.6	--	--	7.2	9.8	9.0
18	8.2	--	--	5.0	--	--
19	10.8	--	--	12.2	19.8	10.2
20	6.2	24.2	7.8	4.8	14.0	6.4
21	15.6	21.2	19.2	9.4	12.8	15.2
22	9.2	21.0	16.4	28.4	42.6	32.6
23	4.0	5.8	3.2	7.8	11.0	12.6
24	--	--	--	4.6	9.0	8.8
25	6.6	--	10.2	8.0	--	--
26	--	--	--	12.4	18.6	26.4
27	8.8	11.8	13.2	5.6	11.2	12.0
28	16.0	17.0	28.0	9.2	15.2	14.8
29	10.6	--	--	6.4	--	--
30	4.0	14.0	9.2	14.0	28.0	17.2
31	9.0	10.0	9.8	16.0	22.0	15.0
32	10.3	--	--	3.7	6.2	6.0
33	29.4	37.2	29.6	13.0	23.2	23.4
34	8.2	--	--	8.8	--	--
35	22.4	28.8	20.4	38.4	34.7	--
36	10.6	19.8	16.8	18.2	29.0	--
37	6.8	16.6	21.2	9.6	19.6	10.2
38	24.6	26.8	31.0	20.6	30.4	29.0

Missing MSNA data are due to either failure to locate the nerve or loss of nerve during recording

Table C.13. Raw data for 5 minute average MSNA (burst per minute) for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	7.0	--	--	19.2	20.4	22.2
40	27.0	31.7	--	13.0	19.4	24.8
41	25.2	29.2	28.8	11.0	18.4	11.4
42	--	--	--	3.0	--	--
43	3.4	--	--	4.8	7.0	11.7
44	8.2	19.0	23.4	3.4	10.0	6.4
45	3.8	9.6	6.2	3.0	--	--
46	2.8	6.2	6.0	--	--	--
47	7.2	--	--	10.2	13.2	12.8
48	18.0	--	--	27.0	22.8	32.0
49	7.0	--	--	--	--	--
50	10.8	12.6	15.2	14.2	14.4	20.4
51	17.0	11.8	17.8	7.0	--	--
52	6.4	20.3	--	13.2	16.0	14.6
53	21.4	--	--	18.0	--	--
54	4.8	13.6	10.0	8.8	--	--
55	26.8	28.6	38.2	18.4	21.0	--
56	9.3	--	15.2	4.2	9.6	4.6
57	2.6	3.6	7.0	7.4	2.6	8.2
58	13.2	--	--	19.2	27.2	15.6
59	9.4	--	--	--	--	--
60	9.6	18.2	15.6	10.0	7.0	14.0
61	9.6	13.2	15.0	--	--	--
62	17.0	34.3	30.0	--	--	--
63	14.0	15.3	21.0	6.2	8.4	6.6
64	16.4	27.0	27.2	--	--	--
65	19.0	30.8	27.2	--	--	--
66	10.8	--	--	3.8	--	--
67	7.4	--	15.6	7.8	11.2	6.0

Missing MSNA data are due to either failure to locate the nerve or loss of nerve during recording

Table C.14. Raw data for 5 minute average MSNA (burst per 100 heart beats) for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	12.7	13.2	8.4	12.3	19.4	21.2
2	8.1	--	--	21.7	27.2	32.3
3	3.5	--	--	17.0	15.0	24.8
4	23.4	22.8	41.6	17.7	13.6	26.2
5	--	--	--	--	--	--
6	7.0	--	--	--	--	--
7	22.5	26.0	33.8	--	--	--
8	12.0	16.6	28.7	6.2	14.6	--
9	30.8	--	--	33.6	31.7	43.8
10	18.4	15.4	32.1	6.3	6.9	7.0
11	12.9	17.9	23.1	3.1	--	--
12	22.0	--	--	22.2	21.1	28.1
13	2.0	28.1	--	12.2	17.5	8.5
14	11.3	13.2	14.6	4.6	10.3	--
15	18.5	18.3	34.9	24.7	--	--
16	16.8	9.0	12.9	12.0	16.4	14.1
17	10.2	--	--	10.0	10.2	13.4
18	11.1	--	--	6.1	--	--
19	17.2	--	--	19.8	29.0	15.9
20	9.7	30.2	12.4	7.8	17.3	11.0
21	23.1	29.1	28.3	13.4	17.0	22.4
22	15.0	27.7	28.0	40.6	51.3	46.8
23	8.3	7.8	6.5	13.3	12.9	22.4
24	--	--	--	9.3	11.8	18.0
25	11.5	--	17.3	11.4	--	--
26	--	--	--	20.7	24.1	42.5
27	14.2	12.8	21.5	11.8	12.8	21.2
28	30.4	24.4	52.8	16.4	19.9	24.2
29	18.4	--	--	10.3	--	--
30	7.0	18.6	16.1	22.7	36.4	26.4
31	14.3	13.7	15.5	24.9	30.1	23.2
32	18.0	--	--	6.7	9.8	10.5
33	48.6	54.6	48.2	20.6	33.9	37.4
34	12.9	--	--	15.7	--	--
35	37.3	46.8	34.2	55.2	50.7	--
36	22.0	35.1	34.1	36.2	51.8	--
37	10.4	20.1	33.3	13.5	21.7	15.5
38	29.8	28.6	36.9	24.5	32.3	34.3

Missing MSNA data are due to either failure to locate the nerve or loss of nerve during recording

Table C.15. Raw data for 5 minute average MSNA (burst per 100 heart beats) for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	13.7	--	--	34.2	30.9	38.7
40	38.7	36.8	--	23.2	23.4	43.4
41	35.0	35.1	38.0	15.5	22.9	15.4
42	--	--	--	4.9	--	--
43	7.0	--	--	9.0	8.7	18.8
44	11.7	14.2	29.1	3.8	7.8	7.0
45	4.8	8.4	7.4	4.5	--	--
46	4.6	8.4	9.4	--	--	--
47	10.5	--	--	14.1	14.5	16.9
48	34.2	--	--	47.0	31.2	51.8
49	9.1	--	--	--	--	--
50	15.7	14.5	21.8	25.0	20.3	34.2
51	21.7	13.8	22.7	9.4	--	--
52	10.1	26.1	--	19.8	20.6	22.3
53	32.1	--	--	26.0	--	--
54	8.2	19.1	16.4	15.4	--	--
55	38.6	35.7	51.2	26.5	29.0	--
56	14.6	--	24.2	6.6	14.9	7.1
57	3.2	3.5	8.9	10.3	2.8	11.4
58	19.5	--	--	27.4	36.6	24.1
59	12.3	--	--	--	--	--
60	16.1	22.7	24.2	20.5	9.6	27.3
61	14.0	16.6	22.2	--	--	--
62	26.5	44.0	47.2	--	--	--
63	19.3	16.5	27.3	9.5	9.9	9.3
64	23.9	30.2	38.0	--	--	--
65	28.6	35.1	40.2	--	--	--
66	19.8	--	--	7.4	--	--
67	11.8	--	21.8	12.7	12.3	8.9

Missing MSNA data are due to either failure to locate the nerve or loss of nerve during recording

Table C.16. Raw data for 5 minute average total MSNA (arbitrary units) for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	4665.4	12323.9	4687.6	3820.1	11286.9	7339.5
2	2704.6	--	--	6662.3	18142.9	12903.2
3	2265.5	--	--	6584.6	6934.1	5706.1
4	6730.0	24173.5	19916.7	5967.3	11408.5	7038.5
5	--	--	--	--	--	--
6	3065.7	--	--	--	--	--
7	5554.5	7736.9	7285.6	--	--	--
8	3667.7	7267.5	7422.4	224.6	615.8	--
9	9606.9	--	--	10124.0	12093.9	10585.7
10	5046.7	9533.7	8090.3	2241.0	--	2193.5
11	3346.8	12917.3	9870.0	935.8	--	--
12	4938.3	--	--	5586.5	6831.5	6436.7
13	800.5	24062.9	--	3496.2	11280.5	3698.6
14	4711.7	14853.2	9564.4	1152.1	6194.4	--
15	5748.1	10092.1	12825.4	7294.1	--	--
16	4574.9	4998.9	2618.6	3289.1	7299.5	3498.3
17	2571.1	--	--	2546.9	7136.8	3388.1
18	3098.4	--	--	1817.0	--	--
19	4260.0	--	--	5683.6	14405.7	4319.5
20	3143.7	16129.0	2970.2	2640.1	9107.4	3399.0
21	7878.0	8252.9	6569.8	5130.1	10731.5	6840.0
22	2350.2	7873.3	3258.2	14351.3	28460.9	17619.9
23	1921.1	4301.4	1921.2	3973.7	9161.5	7003.4
24	--	--	--	1963.6	7147.8	3867.8
25	2973.5	--	4466.0	3614.3	--	--
26	--	--	--	6025.5	6699.9	8232.9
27	4209.9	6218.5	4504.8	3132.7	4552.0	3263.5
28	6464.1	13705.9	11897.5	4365.2	6656.8	5332.4
29	6906.8	--	--	4188.6	--	--
30	1286.6	8854.8	3526.3	8400.2	26312.9	9876.6
31	5684.6	5717.6	6768.9	5462.7	8503.3	5482.3
32	5504.7	--	--	1591.1	3297.7	3265.0
33	--	--	--	5099.0	12838.4	14463.3
34	3072.2	--	--	3417.9	--	--
35	10041.5	10316.6	5727.2	16248.8	12310.7	--
36	2843.0	7800.8	3868.1	5269.1	8711.6	--
37	3140.3	18631.6	12948.5	4424.3	28032.8	6547.7
38	12032.9	15429.1	17749.6	7990.9	--	--

Missing MSNA data are due to either failure to locate the nerve or loss of nerve during recording

Table C.17. Raw data for 5 minute average total MSNA (arbitrary units) for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	3919.4	--	--	5162.6	11610.4	5027.6
40	15174.3	25679.3	--	6096.6	10364.8	12806.8
41	9522.4	26003.6	9210.4	6195.4	15282.2	5933.5
42	--	--	--	1665.6	--	--
43	2048.1	--	--	2345.6	4604.0	3760.4
44	--	--	--	351.2	13771.8	3820.4
45	2427.1	7951.7	2560.0	1884.8	--	--
46	1349.4	4434.6	4417.1	--	--	--
47	2875.2	--	--	6176.8	15374.2	9785.8
48	9547.2	--	--	14516.6	15627.3	16769.1
49	3173.9	--	--	--	--	--
50	4089.3	14199.4	6910.5	6542.0	4607.5	6222.0
51	8623.5	9246.8	9207.2	3472.3	--	--
52	4365.1	13956.6	--	--	--	--
53	10038.6	--	--	9243.8	--	--
54	2691.8	10415.2	5714.0	3574.1	--	--
55	11721.3	20335.4	26744.4	6887.0	8873.3	--
56	3117.0	--	6244.8	1545.2	3976.4	1160.3
57	1017.4	--	--	2139.2	695.2	1795.5
58	6540.3	--	--	9107.3	--	--
59	2077.1	--	--	--	--	--
60	5505.9	10479.3	8407.0	3909.1	2175.1	3979.0
61	5691.5	11318.9	9139.0	--	--	--
62	7047.0	13083.2	15772.5	--	--	--
63	8480.7	19125.3	18473.9	2315.6	4114.8	3247.8
64	8304.4	39597.7	23942.1	--	--	--
65	9208.9	24776.4	14069.5	--	--	--
66	5690.6	--	--	2259.4	--	--
67	3395.2	--	10171.7	4884.4	7990.8	2819.0

Missing MSNA data are due to either failure to locate the nerve or loss of nerve during recording

Table C.18. Raw data for 5 minute average forearm blood flow for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	5.80	9.77	6.78	5.21	7.64	7.53
2	3.31	4.26	3.53	2.76	4.47	2.88
3	4.10	10.50	4.03	3.84	6.43	2.89
4	3.55	9.23	3.35	5.21	14.98	3.94
5	2.84	8.17	3.25	5.66	9.03	4.42
6	2.94	3.59	2.69	3.61	2.90	2.74
7	2.07	4.49	2.04	3.10	4.23	2.65
8	2.18	3.10	1.72	1.98	3.03	1.52
9	2.36	3.75	2.50	2.31	4.17	2.39
10	1.45	1.86	1.36	1.92	2.64	1.82
11	2.04	6.81	2.00	3.48	7.43	2.60
12	2.54	5.99	2.44	2.32	5.52	1.77
13	1.16	--	1.29	1.80	--	1.46
14	2.72	4.04	2.56	3.01	5.73	2.88
15	1.70	5.08	1.84	--	--	--
16	1.15	2.47	1.34	1.37	2.18	1.28
17	3.91	5.13	3.23	3.52	6.69	2.74
18	1.81	2.54	1.55	1.04	2.73	1.40
19	2.49	4.72	3.41	2.07	4.21	2.23
20	1.99	6.27	1.84	--	--	--
21	2.23	4.09	2.80	3.06	4.82	3.28
22	2.61	3.35	2.25	2.12	2.27	1.57
23	1.79	4.16	1.68	1.41	3.33	1.31
24	1.73	3.57	1.80	2.08	4.10	1.94
25	1.36	3.13	1.26	1.22	--	1.21
26	1.94	3.63	1.54	2.01	2.41	2.04
27	2.25	6.00	1.93	1.75	6.91	1.89
28	1.62	3.57	2.62	3.55	4.90	4.50
29	2.44	3.82	2.39	3.09	3.81	3.25
30	1.75	3.99	1.54	2.34	4.05	3.12
31	0.96	1.30	1.28	1.22	1.48	1.28
32	1.41	2.11	1.44	1.19	1.55	1.27
33	3.12	3.22	3.32	3.48	5.06	3.32
34	2.92	6.64	2.90	2.03	5.60	2.56
35	1.02	--	1.16	0.93	--	1.05
36	1.20	--	1.45	3.03	--	3.35
37	1.98	5.45	1.83	3.67	7.55	2.91
38	3.16	5.25	3.98	2.61	3.69	2.39

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.19. Raw data for 5 minute average forearm blood flow for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	2.49	4.68	2.53	3.34	5.31	3.24
40	2.69	3.54	2.47	2.55	--	2.24
41	2.25	3.24	2.65	2.27	3.30	3.27
42	4.21	6.91	3.96	3.46	5.57	3.17
43	1.28	3.09	1.29	--	--	--
44	5.34	10.05	6.17	9.61	11.41	9.11
45	3.72	8.37	3.66	3.08	7.01	3.28
46	2.65	3.74	3.38	2.08	3.26	2.36
47	2.40	5.48	2.85	4.09	4.66	3.50
48	3.61	4.15	3.54	3.21	5.30	3.79
49	4.38	7.50	4.00	3.48	4.13	2.79
50	3.43	4.36	3.90	2.07	3.49	2.52
51	5.93	5.69	5.79	3.49	2.91	3.58
52	4.15	5.22	3.19	6.55	6.35	5.21
53	2.23	6.06	2.09	2.52	3.71	1.37
54	3.77	4.73	3.32	2.60	3.83	2.72
55	2.52	2.65	2.35	2.19	2.61	2.20
56	3.76	3.87	3.88	3.63	3.81	4.07
57	4.03	8.85	3.38	3.61	8.61	3.48
58	4.16	8.80	3.44	4.41	5.56	3.05
59	2.81	--	3.08	1.38	--	1.69
60	3.92	6.36	3.93	1.61	4.26	2.02
61	2.35	3.96	2.38	--	--	--
62	2.59	5.23	2.67	5.91	8.77	5.84
63	3.42	6.89	4.30	4.84	6.51	4.54
64	3.81	5.17	3.43	4.25	4.57	2.95
65	2.15	5.54	2.21	2.27	3.59	2.33
66	6.40	--	6.17	2.81	--	2.45
67	2.64	4.65	2.05	1.58	--	1.58

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.20. Raw data for 5 minute average forearm vascular resistance for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	16.99	12.19	16.32	17.26	14.16	13.11
2	27.35	25.61	25.75	35.37	26.01	34.85
3	24.21	11.11	24.96	28.34	20.50	33.82
4	28.98	12.93	31.64	19.88	7.94	25.62
5	33.66	12.66	28.28	18.62	11.96	26.12
6	33.22	29.51	36.66	22.46	30.71	30.75
7	45.99	22.12	48.87	28.95	21.94	35.43
8	42.23	32.29	48.65	45.92	36.93	60.39
9	38.97	33.78	37.71	34.42	26.74	36.69
10	67.21	59.29	66.82	46.16	36.49	52.20
11	37.69	14.06	39.22	24.90	16.92	32.12
12	37.93	19.73	40.66	37.83	19.51	52.49
13	70.74	--	68.13	43.69	--	60.14
14	37.29	28.47	39.62	28.74	18.46	--
15	49.64	18.70	44.07	--	--	--
16	76.10	44.11	70.75	63.97	45.05	70.44
17	21.18	18.57	27.87	24.72	16.68	34.65
18	55.04	46.71	67.59	80.54	37.58	59.70
19	38.59	22.47	28.19	39.50	23.53	38.68
20	44.76	20.22	50.66	--	--	--
21	40.18	23.50	31.57	30.95	21.07	28.57
22	33.84	28.18	37.46	39.91	39.30	51.99
23	48.97	25.98	56.82	60.20	29.39	71.06
24	49.85	28.39	51.41	41.88	22.68	50.51
25	66.44	31.94	77.24	73.03	--	77.61
26	46.85	31.43	62.82	40.55	40.56	41.73
27	41.18	16.75	49.94	50.96	13.96	52.00
28	55.22	26.59	35.73	22.82	18.09	19.19
29	41.67	30.99	44.41	27.60	25.88	26.90
30	47.10	26.13	53.49	33.59	24.23	28.09
31	101.3	76.79	79.25	76.21	66.75	76.13
32	66.52	47.95	67.41	81.65	68.59	79.19
33	29.26	31.63	28.62	24.14	20.83	26.00
34	30.98	16.72	33.73	45.46	18.65	38.25
35	92.95	--	84.29	83.08	--	80.69
36	84.72	--	76.94	29.62	--	27.71
37	46.01	29.68	50.34	22.55	17.36	28.14
38	27.07	18.67	24.83	30.16	24.45	35.57

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.21. Raw data for 5 minute average forearm vascular resistance for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	36.57	24.07	37.17	29.42	22.40	31.40
40	37.92	34.36	43.98	38.66	--	46.75
41	44.86	36.57	38.45	38.68	33.66	26.86
42	23.34	18.45	25.87	26.86	20.78	27.95
43	73.32	--	73.27	--	--	--
44	16.87	11.32	15.34	9.56	10.51	9.98
45	24.49	15.15	26.01	26.22	16.09	26.68
46	34.72	27.73	30.63	44.27	33.42	42.21
47	35.11	18.09	30.41	20.48	20.87	23.90
48	23.21	22.32	23.02	25.64	17.90	22.78
49	18.51	12.92	20.60	25.10	24.69	31.64
50	29.98	26.22	28.01	46.78	31.18	41.47
51	14.47	17.72	15.44	22.40	31.91	26.64
52	21.38	20.81	27.85	12.56	15.58	16.46
53	51.64	25.84	59.20	48.41	34.64	79.67
54	21.69	25.80	29.42	32.03	27.46	33.84
55	36.14	39.17	38.59	38.44	34.49	38.64
56	25.92	28.33	24.85	26.87	27.62	24.94
57	26.20	15.26	32.13	26.57	14.41	29.01
58	23.08	12.92	28.75	22.56	22.66	34.14
59	43.04	--	39.12	83.39	--	70.34
60	23.48	16.72	24.02	58.51	25.34	51.22
61	43.93	32.30	44.13	--	--	--
62	37.23	20.77	36.85	18.74	14.50	20.19
63	29.58	16.35	23.69	16.99	14.70	18.58
64	25.94	27.69	30.33	22.79	28.92	36.33
65	44.02	23.48	46.28	45.32	35.84	46.45
66	15.40	--	17.34	36.66	--	44.11
67	44.32	31.42	57.95	56.63	--	67.97

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.22. Raw data for 5 minute average forearm vascular conductance for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	0.060	0.084	0.062	0.058	0.072	0.077
2	0.037	0.041	0.039	0.028	0.040	0.029
3	0.042	0.096	0.041	0.040	0.059	0.031
4	0.035	0.078	0.032	0.052	0.130	0.039
5	0.030	0.084	0.037	0.057	0.087	0.039
6	0.030	0.035	0.027	0.045	0.033	0.033
7	0.023	0.046	0.021	0.036	0.046	0.029
8	0.024	0.032	0.021	0.022	0.029	0.017
9	0.026	0.037	0.027	0.029	0.041	0.027
10	0.015	0.017	0.015	0.022	0.028	0.019
11	0.027	0.073	0.026	0.040	0.072	0.032
12	0.026	0.052	0.025	0.027	0.052	0.019
13	0.014	--	0.015	0.023	--	0.017
14	0.027	0.038	0.025	0.035	0.055	--
15	0.021	0.056	0.023	--	--	--
16	0.013	0.024	0.014	0.016	0.022	0.014
17	0.048	0.054	0.036	0.042	0.064	0.029
18	0.018	0.022	0.015	0.012	0.029	0.017
19	0.026	0.045	0.036	0.025	0.045	0.026
20	0.022	0.064	0.020	--	--	--
21	0.025	0.043	0.032	0.033	0.051	0.036
22	0.030	0.036	0.027	0.026	0.027	0.021
23	0.020	0.042	0.018	0.017	0.039	0.015
24	0.020	0.039	0.020	0.025	0.045	0.020
25	0.015	0.031	0.013	0.014	--	0.013
26	0.022	0.035	0.016	0.025	0.026	0.024
27	0.025	0.063	0.021	0.021	0.073	0.020
28	0.018	0.038	0.029	0.044	0.055	0.053
29	0.024	0.032	0.023	0.036	0.039	0.037
30	0.021	0.040	0.019	0.030	0.044	0.037
31	0.010	0.013	0.013	0.013	0.015	0.014
32	0.015	0.021	0.015	0.012	0.015	0.013
33	0.034	0.033	0.036	0.041	0.054	0.039
34	0.032	0.064	0.030	0.023	0.055	0.026
35	0.011	--	0.012	0.012	--	0.012
36	0.012	--	0.013	0.034	--	0.036
37	0.022	0.052	0.020	0.045	0.082	0.036
38	0.038	0.055	0.041	0.033	0.042	0.028

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.23. Raw data for 5 minute average forearm vascular conductance for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	0.028	0.047	0.027	0.034	0.051	0.032
40	0.028	0.032	0.025	0.027	--	0.022
41	0.022	0.029	0.026	0.026	0.036	0.038
42	0.043	0.061	0.039	0.038	0.053	0.036
43	0.014	--	0.014	--	--	--
44	0.059	0.089	0.066	0.105	0.096	0.101
45	0.041	0.076	0.039	0.038	0.066	0.038
46	0.029	0.037	0.036	0.023	0.033	0.025
47	0.029	0.055	0.033	0.049	0.052	0.043
48	0.045	0.045	0.044	0.039	0.057	0.044
49	0.054	0.082	0.049	0.040	0.043	0.032
50	0.034	0.039	0.036	0.022	0.033	0.025
51	0.069	0.057	0.065	0.045	0.032	0.039
52	0.047	0.052	0.036	0.080	0.066	0.061
53	0.019	0.042	0.018	0.025	0.030	0.013
54	0.047	0.048	0.035	0.031	0.040	0.030
55	0.028	0.026	0.026	0.026	0.030	0.026
56	0.039	0.037	0.041	0.037	0.037	0.040
57	0.039	0.078	0.032	0.038	0.082	0.035
58	0.044	0.088	0.035	0.045	0.054	0.030
59	0.023	--	0.026	0.013	--	0.015
60	0.043	0.062	0.042	0.017	0.042	0.021
61	0.023	0.036	0.023	--	--	--
62	0.028	0.049	0.027	0.054	0.070	0.050
63	0.034	0.061	0.043	0.059	0.069	0.055
64	0.039	0.041	0.033	0.045	0.039	0.028
65	0.023	0.051	0.022	0.022	0.032	0.022
66	0.065	--	0.058	0.028	--	0.024
67	0.024	0.038	0.017	0.018	--	0.015

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.24. Raw data for 5 minute average calf blood flow for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	3.18	4.34	--	2.59	3.15	2.89
2	1.74	2.03	1.98	2.16	2.32	2.21
3	3.58	4.50	3.50	2.21	2.79	1.89
4	3.94	7.66	4.59	3.29	6.48	2.71
5	2.18	4.71	2.00	3.14	4.25	2.47
6	3.91	3.76	3.53	3.51	3.73	3.67
7	2.81	3.62	2.59	2.12	2.00	1.55
8	1.75	2.36	1.65	2.04	2.92	2.08
9	1.70	2.74	1.87	1.89	2.57	1.79
10	1.57	1.85	1.33	--	--	--
11	1.21	1.20	1.12	2.26	2.37	2.22
12	1.53	2.79	1.35	3.39	4.61	3.19
13	0.98	1.04	1.00	1.60	1.59	1.51
14	2.25	3.99	2.36	2.54	4.22	2.51
15	0.93	1.90	0.87	--	--	--
16	1.43	2.34	1.66	1.72	2.06	1.55
17	2.14	3.06	1.92	3.44	5.55	3.18
18	1.26	1.83	1.23	0.95	1.44	0.96
19	2.49	2.81	2.89	2.70	2.70	2.89
20	1.79	1.98	2.12	--	--	--
21	1.94	2.37	2.07	1.87	2.12	1.91
22	1.52	1.32	1.58	1.79	1.79	1.80
23	1.66	3.83	1.73	1.37	3.25	1.47
24	1.84	3.10	1.92	2.00	2.69	1.91
25	1.51	1.93	1.29	--	--	--
26	1.40	2.44	1.23	--	--	--
27	--	--	--	--	--	--
28	3.02	3.46	2.26	2.14	2.24	2.03
29	1.80	2.41	1.76	1.50	2.22	1.58
30	2.38	2.77	1.42	2.01	3.00	1.96
31	0.96	1.30	1.28	1.62	1.53	1.69
32	1.50	1.72	1.41	1.60	1.58	1.36
33	1.60	1.72	1.56	3.01	3.37	2.59
34	1.70	4.11	2.32	1.60	2.98	1.98
35	1.94	--	1.84	2.42	2.27	2.47
36	1.67	1.53	1.57	1.71	1.53	1.62
37	2.08	1.80	1.50	2.45	2.40	2.21
38	2.65	2.79	2.62	4.01	3.39	4.15

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.25. Raw data for 5 minute average calf blood flow for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	2.78	4.01	2.87	3.42	4.11	3.57
40	2.41	2.19	2.06	2.69	2.98	2.74
41	2.02	2.74	2.04	1.80	2.35	1.84
42	3.21	5.34	3.27	4.80	5.18	3.61
43	--	--	--	--	--	--
44	2.20	3.78	2.41	3.57	5.79	3.86
45	3.88	5.80	3.93	3.93	5.64	4.63
46	1.84	2.28	2.28	1.66	2.20	1.70
47	3.04	3.51	3.38	4.43	3.40	3.45
48	1.64	2.08	1.65	--	--	--
49	--	--	--	2.84	3.29	2.87
50	1.74	2.91	1.51	1.36	1.59	1.37
51	2.05	2.61	1.90	2.60	2.88	3.11
52	3.60	3.50	3.11	3.51	3.84	2.86
53	3.22	4.84	2.89	4.26	5.06	2.94
54	2.42	2.32	2.36	3.52	3.89	3.92
55	2.03	2.26	2.02	1.89	2.38	1.91
56	3.17	3.67	3.23	4.12	4.15	4.31
57	3.88	6.40	3.39	2.76	5.02	2.70
58	3.86	2.47	2.49	1.76	1.46	1.29
59	2.73	4.58	3.03	2.01	--	2.09
60	2.00	2.80	1.91	1.69	2.13	1.93
61	1.62	2.46	1.50	--	--	--
62	1.76	1.73	1.46	1.53	2.16	1.51
63	2.42	3.10	2.28	2.71	3.49	3.33
64	2.68	2.94	2.30	3.82	3.25	3.09
65	2.09	2.61	3.34	2.19	2.57	2.25
66	2.28	--	2.43	2.23	--	2.04
67	3.04	3.93	4.28	1.56	2.16	1.54

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.26. Raw data for 5 minute average calf vascular resistance for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	31.03	32.37	--	34.72	34.50	34.12
2	52.15	52.32	46.31	45.16	49.29	45.16
3	27.66	26.09	30.37	45.53	43.42	50.33
4	25.97	15.63	22.99	30.60	19.83	37.16
5	43.80	26.03	44.69	33.23	27.30	47.28
6	25.62	27.67	27.99	23.18	23.85	22.87
7	33.03	27.82	38.70	43.67	47.65	61.59
8	50.95	42.00	50.41	44.52	37.16	43.35
9	54.15	44.34	50.76	42.40	40.61	48.78
10	62.13	60.09	68.11	--	--	--
11	63.18	82.52	70.01	38.46	47.92	37.19
12	62.83	42.31	74.26	25.87	23.10	29.04
13	83.32	99.40	88.95	49.52	61.58	57.09
14	44.99	29.51	45.17	34.29	25.52	--
15	93.09	54.98	94.13	--	--	--
16	61.02	46.00	57.15	50.72	48.15	58.50
17	38.43	33.04	47.44	24.89	18.79	29.65
18	78.96	63.94	87.09	88.47	67.29	89.65
19	38.37	38.26	33.28	30.27	34.80	29.64
20	49.94	51.17	43.25	--	--	--
21	46.00	41.07	41.78	49.82	44.49	48.70
22	58.77	72.71	56.56	46.09	47.67	42.22
23	53.14	30.72	55.10	63.15	28.22	60.96
24	46.66	32.05	47.79	42.39	34.43	50.66
25	59.57	52.17	75.16	--	--	--
26	65.85	43.86	78.13	--	--	--
27	--	--	--	--	--	--
28	29.54	27.59	41.58	37.55	40.40	41.80
29	56.41	48.90	61.41	56.85	43.87	55.94
30	34.71	36.27	58.14	38.69	31.49	42.68
31	101.3	73.15	79.25	56.94	64.48	57.25
32	64.73	58.56	68.61	61.28	67.34	73.66
33	57.38	57.52	59.33	27.94	28.34	33.13
34	54.10	28.00	42.52	57.12	36.60	49.68
35	47.81	--	54.44	31.51	38.10	34.06
36	61.15	77.61	70.68	52.42	65.43	57.35
37	43.44	61.54	62.21	33.24	40.01	37.50
38	31.89	35.47	37.03	19.48	26.01	20.76

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.27. Raw data 5 minute average for calf vascular resistance for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	32.54	28.64	32.48	28.65	25.89	28.30
40	41.44	52.62	49.10	35.87	38.69	37.58
41	49.91	42.67	49.57	47.92	40.91	46.85
42	30.77	21.98	31.12	18.93	21.94	24.77
43	--	--	--	--	--	--
44	41.22	33.78	39.25	25.82	21.97	23.74
45	23.27	21.87	23.85	20.58	19.81	18.51
46	49.26	45.69	41.68	55.08	45.17	56.74
47	27.68	32.66	25.62	18.95	26.52	23.41
48	49.30	43.84	49.50	--	--	--
49	--	--	--	30.86	30.26	30.73
50	58.92	46.26	71.28	69.95	67.33	73.96
51	41.84	39.78	47.12	30.06	31.36	29.72
52	24.72	31.31	28.62	23.56	25.21	29.92
53	35.64	31.86	41.65	24.69	26.16	36.61
54	33.86	45.34	41.02	23.80	24.90	23.14
55	44.70	46.05	45.36	43.97	37.36	44.24
56	30.71	29.08	29.55	23.67	24.66	23.45
57	26.98	19.43	31.75	34.72	22.79	37.72
58	25.22	46.19	39.48	57.89	73.91	81.72
59	44.67	34.02	39.46	54.34	--	56.40
60	46.41	38.95	49.71	55.21	49.03	49.36
61	63.84	50.32	69.44	--	--	--
62	54.35	62.30	68.44	71.91	58.02	78.05
63	41.81	36.48	44.80	30.38	27.19	25.08
64	37.40	47.50	45.54	25.11	39.20	35.25
65	45.78	42.50	33.21	46.77	45.28	48.40
66	43.04	--	44.28	45.41	--	52.29
67	36.64	33.86	27.76	57.25	54.62	68.36

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.28. Raw data for 5 minute average calf vascular conductance for normotensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
1	0.033	0.037	--	0.029	0.030	0.030
2	0.019	0.020	0.022	0.022	0.020	0.022
3	0.036	0.041	0.035	0.023	0.025	0.020
4	0.039	0.065	0.044	0.033	0.057	0.027
5	0.023	0.048	0.023	0.031	0.041	0.022
6	0.040	0.036	0.036	0.043	0.042	0.044
7	0.031	0.037	0.027	0.024	0.022	0.017
8	0.020	0.024	0.020	0.022	0.028	0.023
9	0.019	0.027	0.020	0.024	0.025	0.021
10	0.016	0.017	0.015	--	--	--
11	0.016	0.013	0.014	0.026	0.023	0.027
12	0.016	0.024	0.014	0.039	0.044	0.035
13	0.012	0.011	0.011	0.020	0.017	0.018
14	0.022	0.038	0.024	0.029	0.041	--
15	0.011	0.021	0.011	--	--	--
16	0.016	0.023	0.018	0.020	0.021	0.017
17	0.026	0.033	0.022	0.041	0.054	0.034
18	0.013	0.016	0.012	0.011	0.015	0.012
19	0.026	0.027	0.030	0.033	0.029	0.034
20	0.020	0.020	0.023	--	--	--
21	0.022	0.025	0.024	0.020	0.023	0.021
22	0.017	0.014	0.019	0.022	0.021	0.024
23	0.019	0.039	0.018	0.016	0.037	0.017
24	0.021	0.034	0.021	0.024	0.030	0.020
25	0.017	0.019	0.013	--	--	--
26	0.016	0.024	0.013	--	--	--
27	--	--	--	--	--	--
28	0.034	0.036	0.025	0.027	0.025	0.024
29	0.018	0.021	0.017	0.018	0.023	0.018
30	0.029	0.028	0.018	0.026	0.032	0.023
31	0.010	0.014	0.013	0.018	0.016	0.018
32	0.015	0.017	0.015	0.016	0.015	0.014
33	0.018	0.017	0.017	0.036	0.035	0.030
34	0.019	0.040	0.024	0.018	0.029	0.020
35	0.021	--	0.019	0.032	0.026	0.029
36	0.016	0.013	0.014	0.019	0.015	0.017
37	0.023	0.017	0.017	0.030	0.026	0.027
38	0.031	0.029	0.027	0.051	0.039	0.049

Missing blood flow data are due to movement artifact or equipment failure during recording

Table C.29. Raw data for 5 minute average calf vascular conductance for prehypertensive group in study 2

Subject	Pre supplementation			Post supplementation		
	Base	Mental Stress	Recovery	Base	Mental Stress	Recovery
39	0.031	0.040	0.031	0.035	0.039	0.035
40	0.025	0.020	0.021	0.028	0.027	0.027
41	0.020	0.024	0.020	0.021	0.025	0.021
42	0.033	0.046	0.032	0.053	0.049	0.041
43	--	--	--	--	--	--
44	0.024	0.033	0.026	0.039	0.049	0.043
45	0.043	0.053	0.042	0.049	0.054	0.054
46	0.020	0.022	0.024	0.018	0.023	0.018
47	0.036	0.031	0.039	0.054	0.038	0.043
48	0.021	0.023	0.020	--	--	--
49	--	--	--	0.032	0.035	0.033
50	0.017	0.026	0.014	0.014	0.015	0.014
51	0.024	0.025	0.021	0.033	0.032	0.034
52	0.041	0.035	0.035	0.043	0.040	0.034
53	0.028	0.035	0.024	0.042	0.042	0.027
54	0.030	0.023	0.025	0.043	0.041	0.043
55	0.022	0.022	0.022	0.023	0.027	0.023
56	0.033	0.035	0.034	0.042	0.041	0.043
57	0.037	0.056	0.032	0.029	0.048	0.027
58	0.041	0.024	0.025	0.018	0.014	0.012
59	0.023	0.031	0.025	0.018	--	0.018
60	0.022	0.027	0.020	0.018	0.021	0.020
61	0.016	0.022	0.015	--	--	--
62	0.019	0.016	0.015	0.014	0.017	0.013
63	0.024	0.028	0.023	0.033	0.037	0.040
64	0.028	0.023	0.022	0.040	0.028	0.029
65	0.022	0.024	0.033	0.021	0.022	0.021
66	0.023	--	0.023	0.022	--	0.020
67	0.027	0.032	0.036	0.018	0.020	0.015

Missing blood flow data are due to movement artifact or equipment failure during recording

Appendix D

Appendix D-1. Repeated Measures ANOVA in normotensive group, 2 Time (pre/post) vs. 2 Drug (Fish Oil/Placebo) resting values presented; two-tailed significance

Variable	Time	Time x Drug Interaction	N (fish oil/placebo)
SAP (mmHg)	.194	.169	19/19
DAP (mmHg)	.279	.693	19/19
MAP (mmHg)	.778	.420	19/19
HR (beats/min)	.464	.427	19/19
MSNA (bursts/min)	.729	.160	16/17
MSNA (bursts/100hb)	.864	.340	16/17
FBF (mL/100mL/min)	.045	.814	18/18
FVR (mmHg/mL/100mL/min)	.020	.681	18/18
FVC (mL/100mL/min/mmHg)	.005	.947	18/18
CBF (mL/100mL/min)	.138	.701	16/16
CVR (mmHg/mL/100mL/min)	.006	.555	16/16
CVC (mL/100mL/min/mmHg)	.012	.926	16/16

Appendix D-2. Repeated Measures ANOVA in prehypertensive group, 2 Time (pre/post) vs. 2 Drug (Fish Oil/Placebo) resting values presented; two-tailed significance

Variable	Time	Time x Drug Interaction	N (fish oil/placebo)
SAP (mmHg)	.042	.585	15/14
DAP (mmHg)	.522	.931	15/14
MAP (mmHg)	.221	.794	15/14
HR (beats/min)	.264	.526	15/14
MSNA (bursts/min)	.288	.381	12/10
MSNA (bursts/100hb)	.448	.388	12/10
FBF (mL/100mL/min)	.797	.374	14/13
FVR (mmHg/mL/100mL/min)	.207	.752	14/13
FVC (mL/100mL/min/mmHg)	.961	.545	14/13
CBF (mL/100mL/min)	.481	.822	14/11
CVR (mmHg/mL/100mL/min)	.923	.566	14/11
CVC (mL/100mL/min/mmHg)	.155	.686	14/11

Table D.3. Post-hoc paired t-test of **resting SAP (mmHg)** prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	-1.76	5.42	-4.76	1.24	.229	15

Table D.4. Post-hoc paired t-test of **resting SAP (mmHg)** prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	-2.99	5.56	-6.78	.795	.112	14

Table D.5. Post-hoc paired t-test of **resting FBF (mL/100mL/min)** for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	.343	.998	-.153	.840	.082	18

Table D.6. Post-hoc paired t-test of **resting FBF (mL/100mL/min)** for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	.273	.763	-.107	.652	.074	18

Table D.7. Post-hoc paired t-test of **resting FVR (mmHg/mL/100mL/min)** for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	-5.25	-17.68	-14.04	3.54	.112	18

Table D.8. Post-hoc paired t-test of **resting FVR (mmHg/mL/100mL/min)** for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	-7.41	-13.20	-13.97	-.843	.015	18

Table D.9. Post-hoc paired t-test of **resting FVC (mL/100mL/min/mmHg)** for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	.005	.0097	.0002	.0098	.021	18

Table D.10. Post-hoc paired t-test of **resting FVC (mL/100mL/min/mmHg)** for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	.048	.0100	-.0002	.0098	.029	18

Table D.11. Post-hoc paired t-test of **resting CVR (mmHg/mL/100mL/min)** for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	-9.12	-15.67	-17.47	-.772	.16	.017

Table D.12. Post-hoc paired t-test of **resting CVR (mmHg/mL/100mL/min)** for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	-6.03	-13.48	-13.22	1.15	.16	.047

Table D.13. Post-hoc paired t-test of **resting CVC (mL/100mL/min/mmHg)** for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	.0040	.0088	-.00073	.0087	16	.046

Table D.14. Post-hoc paired t-test of **resting CVC (mL/100mL/min/mmHg)** for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Resting vs. Post Resting	.0037	.0074	-.00023	.0077	16	.032

Table D.15. Normotensive repeated measures ANOVA of changes in 5 minute average Δ SAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.383
Time x Drug	.267
Condition	.000
Condition x Drug	.050
Time x Condition	.383
Time x Condition x Drug	.267

Table D.16. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ SAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.422
Time x Drug	.276
Condition	.000
Condition x Drug	.495
Time x Condition	.422
Time x Condition x Drug	.276

Table D.17. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ SAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.249
Time x Drug	.484
Condition	.347
Condition x Drug	.145
Time x Condition	.249
Time x Condition x Drug	.484

Table D.18. Post-hoc paired t-test of condition interaction \square **SAP (mmHg)** responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	12.20	8.07	8.31	16.10	.000	19
Post Base vs. Post MS	12.45	7.83	8.68	16.22	.000	19

Table D.19. Post-hoc paired t-test of condition interaction \square **SAP (mmHg)** responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	9.32	5.00	6.91	11.72	.000	19
Post Base vs. Post MS	8.37	6.92	5.04	11.71	.000	19

Table D.20. Post-hoc paired t-test of condition interaction \square **SAP (mmHg)** responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	14.57	10.10	9.00	20.16	.000	15
Post Base vs. Post MS	13.58	9.93	7.85	19.31	.000	14

Table D.21. Post-hoc paired t-test of condition interaction \square **SAP (mmHg)** responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	13.83	9.01	8.39	19.28	.000	13
Post Base vs. Post MS	14.42	13.7	6.14	22.70	.003	13

Table D.22. Normotensive repeated measures ANOVA of changes in 5 minute average Δ DAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.254
Time x Drug	.101
Condition	.000
Condition x Drug	.019
Time x Condition	.254
Time x Condition x Drug	.101

Table D.23. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ DAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.189
Time x Drug	.491
Condition	.000
Condition x Drug	.428
Time x Condition	.189
Time x Condition x Drug	.491

Table D.24. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ DAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.262
Time x Drug	.173
Condition	.215
Condition x Drug	.175
Time x Condition	.262
Time x Condition x Drug	.173

Table D.25. Post-hoc paired t-test of condition interaction \square **DAP (mmHg)** responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	9.66	3.75	7.86	11.47	.000	19
Post Base vs. Post MS	10.70	3.27	9.12	12.28	.000	19

Table D.26. Post-hoc paired t-test of condition interaction \square **DAP (mmHg)** responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	8.15	3.37	6.53	9.78	.000	19
Post Base vs. Post MS	7.82	3.73	6.03	9.62	.000	19

Table D.27. Post-hoc paired t-test of condition interaction \square **DAP (mmHg)** responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	10.49	5.50	7.32	13.67	.000	15
Post Base vs. Post MS	9.89	4.89	7.07	12.71	.000	14

Table D.28. Post-hoc paired t-test of condition interaction \square **DAP (mmHg)** responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	10.87	4.96	7.87	13.86	.000	13
Post Base vs. Post MS	10.20	5.96	6.59	13.80	.000	13

Table D.29. Normotensive repeated measures ANOVA of changes in 5 minute average Δ MAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.483
Time x Drug	.150
Condition	.000
Condition x Drug	.026
Time x Condition	.483
Time x Condition x Drug	.150

Table D.30. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ MAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.079
Time x Drug	.194
Condition	.000
Condition x Drug	.382
Time x Condition	.079
Time x Condition x Drug	.194

Table D.31. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ MAP (mmHg) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.204
Time x Drug	.410
Condition	.397
Condition x Drug	.064
Time x Condition	.204
Time x Condition x Drug	.410

Table D.32. Post-hoc paired t-test of condition interaction \square **MAP (mmHg)** responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	12.03	5.42	9.42	14.64	.000	19
Post Base vs. Post MS	12.82	4.90	10.46	15.18	.000	19

Table D.33. Post-hoc paired t-test of condition interaction \square **MAP (mmHg)** responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	9.99	4.26	7.94	12.04	.000	19
Post Base vs. Post MS	9.26	4.78	6.95	11.57	.000	19

Table D.34. Post-hoc paired t-test of condition interaction \square **MAP (mmHg)** responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	14.67	7.17	10.72	18.67	.000	15
Post Base vs. Post MS	13.09	6.25	9.48	16.71	.000	14

Table D.35. Post-hoc paired t-test of condition interaction \square **MAP (mmHg)** responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	13.52	5.70	10.08	16.97	.000	13
Post Base vs. Post MS	13.04	8.19	8.09	17.99	.000	13

Table D.36. Normotensive repeated measures ANOVA of changes in 5 minute average Δ HRR (beats/min) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.056
Time x Drug	.007
Condition	.000
Condition x Drug	.001
Time x Condition	.056
Time x Condition x Drug	.007

Table D.37. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ HRR (beats/min) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.093
Time x Drug	.189
Condition	.000
Condition x Drug	.299
Time x Condition	.093
Time x Condition x Drug	.189

Table D.38. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ HRR (beats/min) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.351
Time x Drug	.009
Condition	.026
Condition x Drug	.019
Time x Condition	.351
Time x Condition x Drug	.009

Table D.39. Post-hoc paired t-test of condition interaction \square HR (beats/min) responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	24.72	10.61	19.61	29.83	.000	19
Post Base vs. Post MS	21.61	11.27	16.17	27.04	.000	19
Pre MS vs. Post MS	-3.11	5.91	-5.96	-.263	.034	19

Table D.40. Post-hoc paired t-test of condition interaction \square HR (beats/min) responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	12.86	5.81	10.06	15.67	.000	19
Post Base vs. Post MS	13.55	7.21	10.07	17.02	.000	19
Pre MS vs. Post MS	.682	2.72	-.630	1.99	.289	19

Table D.41. Post-hoc paired t-test of condition interaction \square HR (beats/min) responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	17.52	7.26	13.50	21.54	.000	15
Post Base vs. Post MS	15.57	6.74	11.68	19.46	.000	14

Table D.42. Post-hoc paired t-test of condition interaction \square HR (beats/min) responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	19.32	12.62	12.03	26.61	.000	13
Post Base vs. Post MS	18.47	11.38	11.60	25.35	.000	13

Table D.43. Post-hoc paired t-test of condition interaction \square HR (beats/min) responses to mental stress for combined-group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	21.54	9.84	18.11	24.98	.000	34
Post Base vs. Post MS	19.05	9.95	15.52	22.58	.000	33
Pre MS vs. Post MS	-2.80	5.18	-4.63	-.959	.002	33

Table D.44. Post-hoc paired t-test of condition interaction \square HR (beats/min) responses to mental stress for combined-group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	15.60	9.71	12.16	19.05	.000	33
Post Base vs. Post MS	15.55	9.29	12.20	18.90	.000	32
Pre MS vs. Post MS	.207	4.60	-1.45	1.87	.400	32

Table D.45. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ MSNA (bursts/min) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.326
Time x Drug	.398
Condition	.010
Condition x Drug	.449
Time x Condition	.326
Time x Condition x Drug	.398

Table D.46. Post-hoc paired t-test of condition interaction Δ MSNA (bursts/min) responses to mental stress for combined-group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	5.78	6.13	1.88	9.68	.004	12
Post Base vs. Post MS	4.82	4.52	1.95	7.69	.002	12

Table D.47. Post-hoc paired t-test of condition interaction Δ MSNA (bursts/min) responses to mental stress for combined-group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	6.00	4.86	3.50	8.50	.000	17
Post Base vs. Post MS	5.89	5.00	3.32	8.46	.000	17

Table D.48. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ MSNA (bursts/100hb) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.398
Time x Drug	.364
Condition	.024
Condition x Drug	.457
Time x Condition	.398
Time x Condition x Drug	.364

Table D.49. Post-hoc paired t-test of condition interaction Δ MSNA (bursts/100hb) responses to mental stress for combined-group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	4.19	9.59	-1.91	10.28	.080	12
Post Base vs. Post MS	3.13	6.94	-1.29	7.54	.079	12

Table D.50. Post-hoc paired t-test of condition interaction Δ MSNA (bursts/100hb) responses to mental stress for combined-group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	3.99	7.05	.368	7.62	.016	17
Post Base vs. Post MS	3.98	6.03	.883	7.08	.008	17

Table D.51. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ Total MSNA (arbitrary units) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.102
Time x Drug	.041
Condition	.113
Condition x Drug	.459
Time x Condition	.102
Time x Condition x Drug	.041

Table D.52. Post-hoc paired t-test of condition interaction Δ Total MSNA (arbitrary units) responses to mental stress for combined-group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	9627.9	7219.5	4078.5	15177.3	.002	9
Post Base vs. Post MS	3830.2	3087.8	1456.7	6203.6	.003	9
Pre MS vs. Post MS	-5797.7	6576.1	-10852.6	-742.9	.015	9

Table D.53. Post-hoc paired t-test of condition interaction Δ MSNA (bursts/100hb) responses to mental stress for combined-group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	6860.4	5474.9	3699.3	10021.6	.000	14
Post Base vs. Post MS	6302.3	6589.6	1890.5	10714.2	.004	14
Pre MS vs. Post MS	-558.10	6589.6	-4362.8	3246.6	.378	14

Table D.54. Normotensive repeated measures ANOVA of changes in 5 minute average Δ FBF (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.337
Time x Drug	.497
Condition	.000
Condition x Drug	.003
Time x Condition	.337
Time x Condition x Drug	.497

Table D.55. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ FBF (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.043
Time x Drug	.491
Condition	.000
Condition x Drug	.396
Time x Condition	.043
Time x Condition x Drug	.491

Table D.56. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ FBF (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.073
Time x Drug	.478
Condition	.000
Condition x Drug	.011
Time x Condition	.073
Time x Condition x Drug	.478

Table D.57. Post-hoc paired t-test of condition interaction \square FBF (%) responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	109.1	62.65	75.67	142.44	.000	16
Post Base vs. Post MS	104.1	72.23	65.66	142.63	.000	16

Table D.58. Post-hoc paired t-test of condition interaction \square FBF (%) responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	58.86	45.64	34.54	83.18	.000	16
Post Base vs. Post MS	54.11	40.25	32.67	75.56	.000	16

Table D.59. Post-hoc paired t-test of condition interaction \square FBF (%) responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	71.76	51.86	40.43	103.1	.000	13
Post Base vs. Post MS	50.36	45.11	23.10	77.62	.002	13

Table D.60. Post-hoc paired t-test of condition interaction \square FBF (%) responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	67.56	52.38	30.08	105.03	.003	10
Post Base vs. Post MS	45.57	46.34	12.43	78.72	.013	10

Table D.61. Normotensive repeated measures ANOVA of changes in 5 minute average Δ FVR (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.336
Time x Drug	.497
Condition	.000
Condition x Drug	.003
Time x Condition	.336
Time x Condition x Drug	.497

Table D.62. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ FVR (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.081
Time x Drug	.349
Condition	.000
Condition x Drug	.351
Time x Condition	.081
Time x Condition x Drug	.349

Table D.63. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ FVR (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.024
Time x Drug	.366
Condition	.000
Condition x Drug	.127
Time x Condition	.024
Time x Condition x Drug	.366

Table D.64. Post-hoc paired t-test of condition interaction \square FVR (%) responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	-37.88	20.19	-48.64	-27.12	.000	16
Post Base vs. Post MS	-35.63	19.71	-46.13	-25.13	.000	16

Table D.65. Post-hoc paired t-test of condition interaction \square FVR (%) responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	-25.64	18.35	-35.42	15.86	.000	16
Post Base vs. Post MS	-20.08	19.38	-30.41	-9.75	.000	16

Table D.66. Post-hoc paired t-test of condition interaction \square FVR (%) responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	-18.07	24.97	-33.94	-2.21	.029	13
Post Base vs. Post MS	-12.63	28.24	-30.57	5.32	.150	13

Table D.67. Post-hoc paired t-test of condition interaction \square FVR (%) responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	-23.95	21.74	-39.14	-8.04	.007	10
Post Base vs. Post MS	-14.07	19.67	-28.14	.006	.050	10

Table D.68. Normotensive repeated measures ANOVA of changes in 5 minute average Δ FVC (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.146
Time x Drug	.326
Condition	.000
Condition x Drug	.012
Time x Condition	.146
Time x Condition x Drug	.326

Table D.69. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ FVC (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.245
Time x Drug	.413
Condition	.000
Condition x Drug	.006
Time x Condition	.244
Time x Condition x Drug	.413

Table D.70. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ FVC (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.066
Time x Drug	.407
Condition	.000
Condition x Drug	.025
Time x Condition	.066
Time x Condition x Drug	.407

Table D.71. Post-hoc paired t-test of condition interaction \square FVC (%) responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	86.02	55.06	56.68	115.37	.000	16
Post Base vs. Post MS	78.42	64.12	44.25	112.59	.000	16

Table D.72. Post-hoc paired t-test of condition interaction \square FVC (%) responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	45.13	39.66	24.00	66.26	.000	16
Post Base vs. Post MS	39.29	36.54	19.81	58.76	.001	16

Table D.73. Post-hoc paired t-test of condition interaction \square FVC (%) responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	48.94	45.30	21.57	76.31	.002	13
Post Base vs. Post MS	30.75	38.94	7.22	54.29	.015	13

Table D.74. Post-hoc paired t-test of condition interaction \square FVC (%) responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	45.38	44.09	13.84	76.92	.010	10
Post Base vs. Post MS	31.21	43.49	.102	62.32	.049	10

Table D.75. Normotensive repeated measures ANOVA of changes in 5 minute average Δ CBF (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.007
Time x Drug	.025
Condition	.000
Condition x Drug	.080
Time x Condition	.007
Time x Condition x Drug	.025

Table D.76. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ CBF (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.145
Time x Drug	.166
Condition	.000
Condition x Drug	.448
Time x Condition	.145
Time x Condition x Drug	.332

Table D.77. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ CBF (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.005
Time x Drug	.018
Condition	.000
Condition x Drug	.110
Time x Condition	.005
Time x Condition x Drug	.018

Table D.78. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	52.99	45.06	28.03	77.94	.000	15
Post Base vs. Post MS	32.87	31.65	15.34	50.39	.001	15
Pre MS vs. Post MS	-20.12	26.57	-34.83	-5.41	.005	15

Table D.79. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	25.21	33.24	6.81	43.62	.011	15
Post Base vs. Post MS	22.72	39.54	.825	44.62	.043	15
Pre MS vs. Post MS	-2.49	20.12	-13.63	8.65	.639	15

Table D.80. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	29.87	31.63	10.75	48.99	.005	13
Post Base vs. Post MS	19.12	25.25	3.86	34.38	.018	13

Table D.81. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	23.42	23.08	6.91	39.94	.011	10
Post Base vs. Post MS	22.95	23.57	6.09	39.81	.013	10

Table D.82. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for combined-group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	45.61	40.33	30.82	60.40	.000	31
Post Base vs. Post MS	26.67	28.67	15.41	37.22	.000	29
Pre MS vs. Post MS	-15.77	26.20	-25.93	-5.61	.002	29

Table D.83. Normotensive repeated measures ANOVA of changes in 5 minute average ΔCVR (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.011
Time x Drug	.218
Condition	.023
Condition x Drug	.198
Time x Condition	.011
Time x Condition x Drug	.218

Table D.84. Prehypertensive repeated measures ANOVA of changes in 5 minute average ΔCVR (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.403
Time x Drug	.390
Condition	.441
Condition x Drug	.285
Time x Condition	.403
Time x Condition x Drug	.390

Table D.85. Combined-groups repeated measures ANOVA of changes in 5 minute average ΔCVR (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.107
Time x Drug	.274
Condition	.088
Condition x Drug	.487
Time x Condition	.107
Time x Condition x Drug	.274

Table D.86. Post-hoc paired t-test of condition interaction \square **CVR** (%) responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	-15.13	25.09	-29.02	-1.23	.035	15
Post Base vs. Post MS	-6.46	20.52	-17.82	4.91	.243	15

Table D.87. Post-hoc paired t-test of condition interaction \square **CVR** (%) responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	-6.70	16.35	-15.75	2.36	.135	15
Post Base vs. Post MS	-2.28	22.38	-14.68	10.11	.699	15

Table D.88. Normotensive repeated measures ANOVA of changes in 5 minute average Δ **CVC** (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.003
Time x Drug	.036
Condition	.002
Condition x Drug	.180
Time x Condition	.003
Time x Condition x Drug	.036

Table D.89. Prehypertensive repeated measures ANOVA of changes in 5 minute average Δ **CVC** (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.243
Time x Drug	.116
Condition	.046
Condition x Drug	.428
Time x Condition	.243
Time x Condition x Drug	.116

Table D.90. Combined-groups repeated measures ANOVA of changes in 5 minute average Δ CVC (%) in Study 2, 2 time (pre/post) vs. 2 condition (base/mental stress) vs. 2 Drug (fish oil/placebo), presented as one-tailed significance.

Interaction	Base – Mental Stress
Time	.004
Time x Drug	.017
Condition	.000
Condition x Drug	.178
Time x Condition	.004
Time x Condition x Drug	.017

Table D.91. Post-hoc paired t-test of condition interaction \square CVC (%) responses to mental stress for normotensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	36.29	41.82	13.13	59.44	.005	15
Post Base vs. Post MS	16.14	27.95	.659	31.61	.042	15
Pre MS vs. Post MS	-20.15	24.61	-33.78	-6.52	.007	15

Table D.92. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for normotensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	14.55	29.22	-1.63	30.73	.074	15
Post Base vs. Post MS	10.42	37.22	-10.19	31.03	.297	15
Pre MS vs. Post MS	-4.13	20.52	-15.49	7.23	.449	15

Table D.93. Post-hoc paired t-test of condition interaction \square CVC (%) responses to mental stress for prehypertensive group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	12.10	28.84	-5.33	29.53	.156	13
Post Base vs. Post MS	3.74	23.41	-10.40	17.89	.575	13

Table D.94. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for prehypertensive group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	6.22	18.53	-7.04	19.48	.316	10
Post Base vs. Post MS	8.46	17.17	-3.82	20.74	.154	10

Table D.95. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for combined-group, fish oil.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	28.41	37.91	14.50	42.31	.000	31
Post Base vs. Post MS	10.15	25.79	.337	19.96	.043	29
Pre MS vs. Post MS	-14.68	24.19	-24.06	-5.29	.003	29

Table D.96. Post-hoc paired t-test of condition interaction \square CBF (%) responses to mental stress for combined-group, placebo.

Variable	Mean	Std. Dev.	95% Confidence Interval		Sig.	N
			Lower	Upper		
Pre Base vs. Pre MS	-11.68	23.75	2.81	20.55	.012	30
Post Base vs. Post MS	-8.52	29.59	-3.19	20.22	.147	27
Pre MS vs. Post MS	-1.58	18.95	-9.41	6.24	.680	27

Table D.97. Combined-group regression analyses of Δ MSNA (bursts/min) with Δ MAP and Resting HR following fish oil supplementation.

Variable		Δ MSNA (bursts/min)
ΔMAP (mmHg)	Pearson Correlation	.354
	Sig. (one-tailed)	.035
	N	27
Resting HR (beats/min)	Pearson Correlation	-.466
	Sig. (one-tailed)	.007
	N	27

Table D.98. Combined-group regression analyses of **ΔMSNA (bursts/min)** with **ΔMAP** and **Resting HR** following placebo supplementation.

Variable		ΔMSNA (bursts/min)
ΔMAP (mmHg)	Pearson Correlation	-.036
	Sig. (one-tailed)	.860
	N	27
Resting HR (beats/min)	Pearson Correlation	-.227
	Sig. (one-tailed)	.123
	N	28

Table D.99. Combined-group regression analyses of **ΔMSNA (bursts/100hb)** with **ΔMAP** and **Resting HR** following fish oil supplementation.

Variable		ΔMSNA (bursts/100hb)
ΔMAP (mmHg)	Pearson Correlation	.325
	Sig. (one-tailed)	.049
	N	27
Resting HR (beats/min)	Pearson Correlation	-.429
	Sig. (one-tailed)	.013
	N	27

Table D.100. Combined-group regression analyses of **ΔMSNA (bursts/100hb)** with **ΔMAP** and **Resting HR** following placebo supplementation.

Variable		ΔMSNA (bursts/100hb)
ΔMAP (mmHg)	Pearson Correlation	-.062
	Sig. (one-tailed)	.758
	N	28
Resting HR (beats/min)	Pearson Correlation	-.219
	Sig. (one-tailed)	.131
	N	28

Appendix E

Request for Permission to Reproduce Previously Published Material

(please save this file to your desktop, fill out, save again, and e-mail to permissions@the-aps.org)

Your Name: Christopher E. Schwartz E-mail: ceschwar@mtu.edu

Affiliation: Michigan Technological University

University Address (for PhD students): 1400 Townsend Dr.
Houghton, MI 49931

Description of APS material to be reproduced (check all that apply):

- Figure Partial Article Abstract
 Table Full Article Book Chapter

Other (please describe):

Are you an author of the APS material to be reproduced? Yes No

Please provide all applicable information about the APS material you wish to use:

Author(s): Christopher E. Schwartz, John J. Durocher, Jason R. Carter

Article or Chapter Title: Neurovascular responses to mental stress in prehypertensive humans

Journal or Book Title: Journal of Applied Physiology

Volume: 110 Page No(s): 76-82 Figure No(s): 1-4 Table No(s): 1-2

Year: 2011 DOI: 10.1152/jappphysiol.00912.2010

(If you are reproducing figures or tables from more than one article, please fill out and send a separate form for each citation.)

Please provide all applicable information about where the APS material will be used:

How will the APS material be used? (please select from drop-down list)

If "other," please describe:

Title of publication or meeting where APS material will be used (if used in an article or book chapter, please provide the journal name or book title as well as the article/chapter title):

Omega-3 fatty acids and neurovascular responses to mental stress in humans.

Publisher (if journal or book): ProQuest/UMI

URL (if website): _____

Date of Meeting or Publication: Summer 2011

Will readers be charged for the material: Yes No

Additional Information:

APPROVED

By *pripka* at 11:01 am, Jun 22, 2011

THE AMERICAN PHYSIOLOGICAL SOCIETY
9650 Rockville Pike, Bethesda, MD 20814-3991

Permission is granted for use of the material specified above, provided the publication is credited as the source, including the words "used with permission."

