The modification of heart rate variability in normal, overweight and type 2 diabetic individuals.

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"What is this life if full of care;

We have no time to stand and stare."

From the poem Leisure by W H Davies

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List of Abbreviations

ω3	Omega 3
(D)BP	Diastolic Blood Pressure
(S)BP	Systolic Blood Pressure
ANS	Autonomic Nervous System
BMI	Body Mass Index
CAN	Cardiac Autonomic Neuropathy
CSIRO	Commonwealth Scientific and industrial Research Organisation
CVD	Cardiovascular Disease
DAN	Diabetic Autonomic Neuropathy
DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
ECG	Electrocardiogram
EPA	Eicosapentaenoic Acid
HDL	High Density Lipoprotein
HF(P)	High Frequency Power
HOMA	Homeostasis Model Assessment Index
HR	Heart Rate
HRV	Heart Rate Variability
LF(P)	Low Frequency Power
HUT	Head Up Tilt
IPAQ	International Physical Activity Questionnaire
LBNP	Lower Body Negative Pressure
LDL	Low Density Lipoprotein

LF/HF	Low Frequency to High Frequency ratio
n-3 PUFA	Omega 3 Polyunsaturated Fatty Acid
NO	Nitric oxide
PNS	Parasympathetic Nervous System
PSD	Power Spectral Density
RMSSD	Square Root of the Mean Squared Differences of Successive Normal to Normal Intervals
R-R	Normal to Normal
RSA	Respiratory Sinus Arrhythmia
SDANN	Standard Deviation of the Average Normal to Normal Interval
SDNN	Standard Deviation of the Normal to Normal Interval
SEM	Standard Error of the Mean
SNS	Sympathetic Nervous System
T2D	Type 2 Diabetes Miletus
TAG	Triglycerides
ULF	Ultra Low Frequency
VLF	Very Low Frequency

Summary

The aim of this thesis was to improve our understanding of the effects that dietary therapies have on improving cardiac autonomic activity in healthy and diabetic people, particularly the effects of omega 3 polyunsaturated fatty acids (PUFA) on healthy people. Research conducted to date suggests that diet has specific effects on cardiac autonomic activity; however, much of this research has ignored the underlying influence of specific therapies and weight loss. In this thesis, heart rate variability (HRV) is used to assess cardiac autonomic activity. Cardiac autonomic activity is chiefly responsible for the beat to beat control of heart rate and has been implicated in sudden cardiac death and prognosis of an adverse cardiovacular event following myocardial infarct.

The first experiment was designed to systematically examine the dose-response changes in cardiac ANS activity and vascular compliance after supplementation with omega 3 polyunsaturated fatty acids. In sixty seven overweight middle aged volunteers, HRV, cardiac sympathetic activity (assessed via low frequency component of HRV), parasympathetic activity (assessed by the high frequency component of HRV), Low Frequency/High Frequency (LF/HF) ratio (representing the balance of sympathetic/parasympathetic nervous activity on heart rate), heart rate (HR), arterial compliance, systolic and diastolic blood pressure were assessed during rest. All variables showed the greatest change in the highest dose group. Arterial compliance and the LF/HF ratio changed in a dose-dependent manner with the omega 3 PUFAs. These results suggest that the observed relationships between fish oil dose and changes in arterial compliance and LF/HF suggest that regular fish

oil supplementation can improve the regulation of HR, HRV and consequently blood pressure by increasing parasympathetic regulation of cardiac autonomic tone in a dose-dependent manner

In the second experiment twenty healthy, young male subjects were subjected to graded lower body negative pressure (LBNP) before and after a 6 week dietary supplement intervention of omega 3 PUFAs. Both periods of LBNP were immediately followed by venepuncture to assess lipid and omega 3 content of the blood cells. After the intervention of omega 3 PUFAs an improvement in cardiac autonomic activity (HRV frequency measures) together with a reduction in HR demonstrated that cardiac autonomic activity was improved during rest. Graded LBNP significantly reduced overall HRV and increased the LF/HF ratio of the frequency domain. After the 6 week intervention of omega 3 PUFAs, the autonomic control of heart rate was improved at the highest level of LBNP. Omega 3 PUFAs were significantly increased in the treatment group. In conclusion, the changes in HR and HRV measures during orthostatic stress demonstrated a cardiovascular response likely to be caused by increasing parasympathetic regulation of cardiac autonomic tone in young active males. These mutual changes may reduce CVD risk from an early age and provide further justification for increased intakes of fish oil.

In the third experiment forty nine type 2 diabetic middle aged subjects undertook a 16 week dietary weight loss intervention. Before and after the trial, HRV measures were recorded for 10 minutes while the patients were supine and at rest for 10 minutes followed by venepuncture for metabolic and lipids markers. HRV frequency

and time domain data indicated that weight loss produced an improvement in cardiac autonomic activity and the mean level of cardiac PNS activity (assessed via the root mean square of the successive differences in R-R intervals, RMSSD) during rest. The observed changes in cardiac ANS activity were attributed to weight loss only, despite similar reductions in several metabolic and cardiovascular blood markers. The results of this study suggest that a calorically restricted diet has favourable effects on cardiac ANS activity and implicate weight loss as a mediator of these effects.

The results of this thesis indicate that dietary intervention in people with and without disease, particularly type 2 diabetes, may specifically influence cardiac autonomic activity, which may improve cardiovascular health outcomes. Moreover, the observed effects of diet on cardiac autonomic activity support the notion that weight loss and omega 3 PUFAs have positive cardiovascular health outcomes. The results of the thesis demonstrate that in order to comprehensively understand the effects of dietary therapeutics on cardiac autonomic activity, it is essential that concomitant changes in HRV are considered.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Nicholas Sjoberg and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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List of Publications

Sjoberg NJ, Milte CM, Buckley JD, Howe PR, Coates AM, Saint DA. (2010) Dose-dependent increases in heart rate variability and arterial compliance in overweight and obese adults with DHA-rich fish oil supplementation. Br J Nutr;103:243-8.

Sjoberg N, Brinkworth GD, Wycherley TP, Noakes M, Saint DA. Moderate weight loss improves heart rate variability in overweight and obese adults with type 2 diabetes. J Appl Physiol 2011;110:1060-4.

Sjoberg, NJ; Saint, DA. DHA rich fish oils restricts decreases heart rate variability during orthostatic stress in active young males. [Submitted]

Abstracts Published:

Dose-response effect of DHA rich fish oil on resting heart rate and heart rate variability N Sjoberg, C Milte, A Coates, J Buckley, PRC Howe, DA Saint
Asia Pac J Clin Nutr 2007;16 (Suppl 3): S118

Heart Rate Variability but not Corrected QT Interval has a Dose–Response Relationship with Dietary DHA Rich Fish Oil Supplementation in Overweight Humans Nicholas Sjoberg, Catherine Milte, Alison Coates, Jon Buckley, Peter Howe and David Saint Heart, Lung and Circulation, Volume 17, Supplement 3, 2008, Page S234

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North American Association for the Study of Obesity's 27th Annual Scientific Meeting of The Obesity Society, Washington D.C., USA, 2009. Presentation of: **Heart rate variability increases with weight loss in overweight and obese adults with type 2 diabetes.** Authors: Sjoberg, N; Wycherley, TP; Brinkworth, GD; Noakes, M; Saint, DA

Acknowledgements

First and foremost I would like to thank my supervisor Associate Professor David Saint for his support and mentorship, not just throughout the duration of my PhD but also in during my honours program.

I would also like to thank the team at the NPRC, particularly, Professor Peter Howe, Associate Professor Jon Buckley, Dr Alison Coates, and Miss Catherine Milte for providing me the opportunity to carry out my experiments with them. It has been an honour to be able to work with such a professional and dedicated team.

My appreciation also goes out to the team at the CSIRO, especially, Dr Grant Brinkworth, Dr Manny Noakes, and Mr Tom Wycherley for allowing me to work with the type 2 diabetic patients during their busy time. It has been great working with such a integral and enthusiastic group of people.

Throughout the duration of my PhD I have been in residence as a Residential Tutor at Lincoln College, Adelaide which is affiliated with the University of Adelaide. I would like to thank everyone there for teaching me the value of integrity, honour and sacrifice. I hope the friendships I have made there will be life-long.

Finally, I would like to thank my family who have tolerated me very well during the making of this thesis.

Chapter 1

Heart rate variability and other assessments of cardiac autonomic activity

Chapter one begins with a general discussion about the value of using heart rate variability to assess autonomic nervous system activity. The importance of the different measures within heart rate variability are discussed in detail and their importance outlined. This is followed by discussion of invasive techniques which were not used in this thesis due to the discomfort to the participants. The heart rate variability measures make up the bulk of the experimental work in the following chapters.

HRV has been accepted over the past decade by the scientific community as a measure of cardiac autonomic activity (1, 2). This includes the use of HRV in assessing cardiac autonomic activity in many recent studies involving non-alterable and alterable factors of cardiac autonomic activity (discussed in chapter 2) which include age, gender, genetics, smoking, blood pressure, circadian rhythm, diet and lifestyle (3-9). However controversy still surrounds its use in respiratory sinus arrythimia (RSA) and as a biomarker of disease. The long held discussion around assessment of HRV and RSA appears to be mostly methodological with differing views on how to best to calculate RSA using HRV (10, 11). More important is the conjecture over the usefulness of HRV as a biomarker (12). HRV and been implicated in numerous disorders and diseases which include cardiovascular diseases, pulmonary disease and arterial hypertension (13-17). The current shortcoming of HRV is the need for indexing of measurements, that is to say that there is no currently agreed 'healthy' HRV for individuals, for which large epidemiological studies are needed. As it stands, increases in HRV in many disease states are seen as positive particularly when this increase heads towards the same HRV measures as non disease groups (2, 4, 6).

Heart rate variability and cardiac autonomic dysfunction.

Cardiovascular disease (CVD) and type 2 diabetes are among the most prominent ailments in the western world and preventative measures such as exercise training and nutrition are proving to be paramount in lowering its incidence(18, 19). ECG based heart rate variability (HRV) measurements have become a vital method of assessing cardiac autonomic regulation. There is a large amount of clinical evidence showing that reduced HRV is a predictor of death in patients after myocardial infarction and with heart failure. Diabetic autonomic neuropathy (DAN) is a common and serious complication of diabetes. Cardiovascular autonomic neuropathy (CAN) is the most investigated and clinically significant form of DAN. Published data shows that CAN is strongly linked with an increased risk of myocardial ischaemia, serious arrhythmias, exercise intolerance and mortality. Measurement of HRV is a readily available technique and consists of a remarkable index of cardiac autonomic dysfunction(20) which serves to establish diagnosis of autonomic dysfunction. The decreases in parameters of HRV seem not only to carry negative prognostic value in patients with diabetes but also to precede the clinical expression of autonomic neuropathy.

While it is known that dieting and its associated weight loss is beneficial to the cardiovascular system the exact mechanisms remain unclear. We aim to investigated whether omega 3 PUFAs or calorically restricted diet have any potential cardiovascular benefits in the healthy and type 2 diabetic population as assessed by HRV.

Cardiac Control and HRV

Heart rate variability refers to the beat-to-beat (R-R) alterations in heart rate thought to reflect changes in autonomic nervous system activity. In healthy individuals during rest, the electrocardiogram (ECG) displays periodic variations in R-R intervals. One example of these rhythmic variations is cardio-acceleration during inspiration and cardio-deceleration during expiration, called respiratory sinus arrhythmia (RSA) which is an indicator of parasympathetic activity(21). There are two frequency components of HRV (Figure 1), high frequency (HF, 0.15-0.4 Hz) and low frequency (LF, 0.04-0.15 Hz)(1). The HF component has been considered to reflect efferent parasympathetic activity (PNS, predominant at rest), whereas the LF component is thought to reflect sympathetic (SNS) and PNS interactions controlled largely by baroreceptor activity. The latter factor seems to rely on more sophisticated mechanisms. Manoeuvres enhancing the sympathetic drive or pathological conditions associated with sympathetic hyperactivity lead to a marked relative increase in the LF component(22). Some discrepancies in its relationship with sympathetic tone are due to the observation that both LF and HF are reduced after atropine (23). However, the hypothesis that LF can be influenced by the vagus is valid only if LF is evaluated in absolute and not in relative terms. Thus it is more appropriate to consider the relationship between LF and HF (LF/HF ratio) in terms of sympatho-vagal balance, rather than consider them independently as separate indices of sympathetic and vagal activity. In fact, several studies (24-28) have confirmed that the LF/HF ratio offers a suitable indication of the autonomic vagal-sympathetic balance in heart control. Previous studies have established that an unbalanced sympathetic/parasympathetic tone, with a predominance of sympathetic activity, is associated with an increased cardiovascular mortality in type II diabetic

patients (29-32). Such unbalanced sympathetic/ parasympathetic tone can be responsible for several cases of sudden death (29-32) in diabetic patients, despite the absence of documented pre-existing heart disease(31).

Time Domain

Heart rate variability may be calculated by several methods, the simplest of which are the time domain measures. These methods are determined by using the intervals between successive normal complexes. In a ECG recording, each QRS complex is detected, and the R-R intervals or the instantaneous heart rate is determined. Time domain variables that can be calculated include the mean NN (the same as R-R interval) interval, the difference between the longest and shortest NN interval, the median NN interval and so on. Other measurements that can be shown in this method include variations in instantaneous heart rate secondary to respiration (RSA), tilt and Valsalva manoeuvre.

A general time-domain measure is the standard deviation of all normal R-R intervals (SDNN). SDNN reflects all of the cyclic mechanisms responsible for variability during the time of recording and has been proposed to reflect PNS activity or a mixture of SNS and PNS activity (22, 33). In addition to being an imprecise marker, SDNN can be argued as inaccurate on the fact that it is directly reliant on the length of time being analysed. SDNN increases in accuracy with length of recording and as such can't be reliably compared with records of a shorter period. A comparable method which controls for the length of recording is the standard deviation of the average of normal R-R intervals (SDANN). This measure may have prognostic value in determining the probability of recovery following acute myocardial infarction (34).

Although, the level to which SDANN reflects cardiac SNS or cardiac PNS activity has not been comprehensively examined.

A different and more suitable measure of cardiac PNS activity is the root mean square of the successive differences in normal R-R intervals, measured in milliseconds (1). Similar to RSA, RMSSD is a measurement of the fast HR changes that are mediated by the modulation of vagal activity. While RSA forms part of a frequency measure of HRV, RMSSD is a time domain measure and is therefore less sensitive to the confounding effects of respiration.
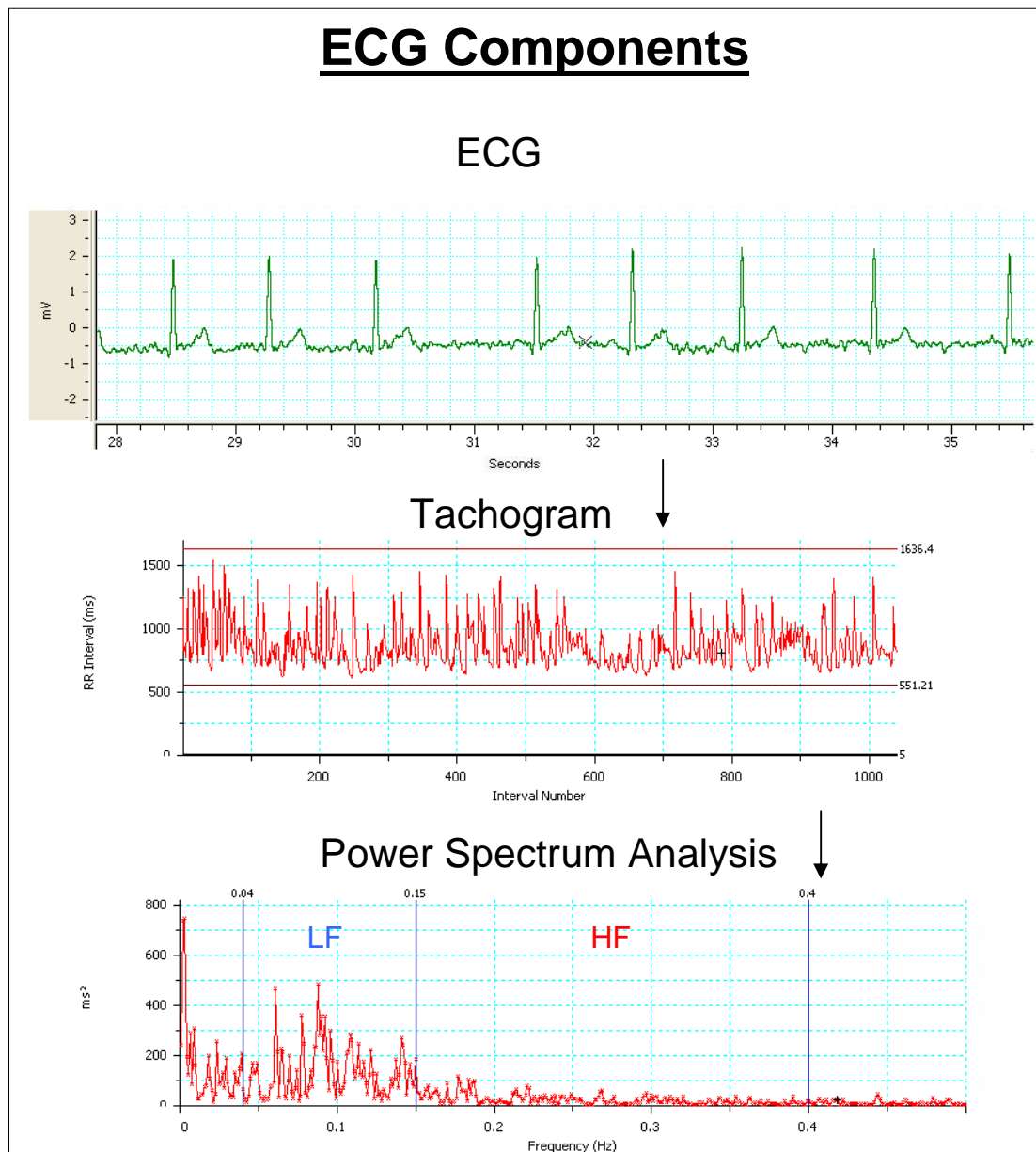
Figure 1.

This figure shows the conversion of the ECG to a power spectrum. The ECG recording is used to establish the series of beat-to-beat intervals. These beat-to-beat intervals have then been plotted on a tachogram in milliseconds vs the interval number. The tachogram has then been converted into a power spectrum by fast Fourier transformation and divided into its corresponding frequencies, including LF and HF.

Power Spectrum

Several spectral methods(1) for the analysis of the variations in heart rate are available to be used in the analysis of HRV. Power spectral density (PSD) provides the essential information of how power (or in other terms, variance) allocates itself as a function of frequency. In any of the methods used, only an estimation of the actual PSD of the signal can be gathered by suitable mathematical algorithms.

Nonparametric and parametric methods can generally be utilised for the calculation of PSD. In many cases, both methods show comparable results. The advantages of the nonparametric methods are the simplicity of the algorithm used (fast fourier transform) and the high processing speed. On the other hand, the advantages of parametric methods are smoother spectral components that can be determined independent of preselected frequency bands, easy post-processing of the spectrum with an auto-calculation of LF and HF power bands. This can be done with simple detection of the central frequency of each component, and precise assessment of PSD even on a small amount of samples on which the signal is supposed to preserve lack of change. The disadvantage of parametric methods is the requirement of confirmation of the suitability of the selected model and of its complexity.

Within the PSD there are three main spectral components which can be calculated from short-term recording of 2 to 5 minutes(28, 35-38): LF and HF components as discussed previously and very low frequency (VLF). The power distribution and the central frequency of LF and HF vary as they relate to changes in autonomic fluctuations of heart rate (28, 37, 39). The physiological mechanism controlling the VLF component is much less defined, and a precise physiological mechanism attributable to these heart rate changes is unknown. VLF requires longer recordings

to be effective as it assesses individual frequency changes with cycle lengths of between 25-250 seconds (i.e. up to more than 4 minutes) in duration. To obtain reasonable estimates of power from a Fourier transform, about 10 "wavelengths" of data are needed, meaning that estimates of VLF need over 40 minutes of recording (in practice, more is needed because of loss of accuracy near the beginning and end sections of the record). Shorter recording periods than this suffer unacceptable degradation of accuracy. Therefore, VLF evaluated from short-term recordings is a dubious measure and should not be used when the PSD of short ECG recordings is assessed. Ultra low frequency is another measure which is occasionally taken during HRV recordings. However, as for VLF, ultra low frequency requires much longer recordings (approx. 24hrs) and the physiological mechanisms behind it are not well understood.

The use of VLF, LF, and HF components is typically made in absolute values of power (milliseconds squared). LF and HF may also be calculated in normalized units,(28, 37) which correspond to the relative value of each component in proportion to the total power minus the VLF component. The use of LF and HF in normalized units highlights the controlled and balanced behaviour of the SNS and PNS. Furthermore, the normalisation tends to reduce the effect of the changes in total power on the values of LF and HF power components.

Controversy around low frequency power.

Spectral analysis of HRV shows not only a HF peak at 0.15 to 0.40 Hz, but also a low frequency peak located at about 0.10 Hz. These slower heart rate periods have been linked with efferent SNS activity in the baroreflex control of blood pressure(40).

However, the value of the LF component of the and its relation to the power spectrum in cardiac autonomic balance is controversial.

Many studies have established that sympathetic activity is a component of LF of the cardiac power spectrum. These experiments changedSNS activity pharmacologically (37, 41, 42), by changing posture (37, 41, 43) and by inducing mental stress (40, 44). With regards to these experiments, the LF component has been used as an index of cardiac SNS activity by researchers investigating the cardiac effects of diet (45-47) and weight loss in general (48-50). The LF to HF ratio of the cardiac power spectrum has been considered an indicator of sympatho-vagal balance(1, 35, 51-53).

A major body of research has established that the LF component of the cardiac PSD is not a precise indicator of cardiac SNS activity. This is confirmed by the observation that SNS spinal anaesthesia does not change the LF component of the cardiac PSD in the supine posture (54, 55). Another significant study conducted by Jokkel showed that short-term beta-adrenergic blockade resulted in decreases in the power in the LF component of the cardiac PSD (56). This emerging data shows that the LF component of the cardiac PSD holds both sympathetic and vagal properties. In agreement with this idea, the LF component has varied in response to interventions known to change vagal activity, including pharmacological interventions (35, 41, 42, 44). Consequently, many researchers now refer to the LF component of the cardiac PSD as an index of sympathovagal balance (33, 35, 44, 57).

While the LF component is usually taken as an index of sympathovagal

balance, this explanation remains problematic. This is because it cannot be inferred whether alterations of the LF component of the PSD are the consequence of reciprocal changes in the SNS and the PNS, independent changes in the SNS or the PNS, or exchanges between the SNS and the PNS.

Indirect versus direct measurements of autonomic activity

In this thesis cardiac ANS activity was assessed using a mathematical analysis of HRV, specifically using the ECG. This method is beneficial because it is non-invasive, does not influence the resting state of the body and allows the concurrent evaluation of the activity of both branches of the ANS. Additionally, ECGs can be collected over short or long periods of time. A multitude of mathematical analyses have been created in order to separate the SNS and PNS involvement with the ECG. The following discussion establishes some of the more accepted types of analyses accessible and their advantages and disadvantages. There are three common comments concerning the non-invasive assessment of cardiac ANS activity.

First, to make sure that non-invasive methods of cardiac ANS activity are meaningfully analysed, it should simultaneously consider associated practical information, such as heart rate (1). The reason being that, in order for a measured change in the activity of either the SNS or the PNS to be regarded as meaningful, it should be reproduced in changes in cardiac function, rather than being compensated for by a mutual change in the opposite ANS branch or another system of regulation.

Secondly, most non-invasive measurements of cardiac ANS activity presume that instances such as the central respiratory generator, baroreflex and chemoreceptor and pulmonary stretch receptor afferents remain constant(58). These influences can be altered by cognitive and behavioural changes coming from brain sites such as the hypothalamus and amygdala. Consequently, non-invasive measures of cardiac ANS activity should be used while the use of higher brain sites is relatively constant, such as during a rested supine position.

The third point to be considered when utilising non-invasive measures of cardiac ANS activity is that cardiac ANS activity changes in response to many influences which include neurotransmitter release, nerve activity and tissue responsiveness. While non-invasive measurements give important information concerning the scale of cardiac ANS changes, from these measures one cannot deduce the exact cause of change.

The main aim of this thesis was to investigate the effects of specific dietary interventions on cardiac SNS and cardiac PNS activity. This could have preferably been attained by directly assessing PNS and SNS activity. Many factors stopped such an measurement from being possible. First, the direct measurement of post-ganglionic sympathetic activity is attained using a technique called microneurography, which utilises the insertion of a small tungsten electrode into a superficial nerve(59). Microneurography is invasive, painful, cannot be utilised over a extended time period, and is susceptible to error by movement. Secondly, microneurography is used to assess SNS activity innervating the vaso-constricting muscle in the skeletal muscles and not cardiac muscle (60-62). The last reason that

SNS and PNS activity of the heart could not be directly measured in this thesis is that a method allowing the direct assessment of post-ganglionic PNS activity in humans has not yet been developed (63).

Other methods of assessing cardiac ANS activity include the extraction and measurement of plasma catecholamines, or NA spill-over in the plasma from the sympathetic nerve terminals (57). The evaluation of NA spill-over uses the injection of a NA tracer into the blood stream, and then waits for a equilibrium to be attained and then taking coronary sinus venous plasma. NA spill over was unsuitable to be used in this thesis because it can only be assessed for short time periods and is not specifically reflective of cardiac SNS activity. Moreover, the evaluation of catecholamines doesn't provide evidence on the activity of the PNS(57).

ANS activity can also be determined via the use of ANS blockades. This technique is based on the idea that a selective blockade of one branch of the ANS gives evidence on the activity of both branches. Pharmacological blockades were not used in the experiments contained in this thesis because they cannot be applied for extended time periods without harming the individual(57). Moreover, the use of these blockades doesn't allow the concurrent assessment of the activity of both branches of the ANS, and can show inconsistent results because of connections between the ANS branches, indirect or reflexive alterations in the unblocked branch, or the non-selective behaviour of the blocker agents(40, 44, 64).

To sum up, ANS activity can be determined using methods which include

microneurography, the evaluation of plasma catecholamines and pharmacological blockades. These methods are useful in that they provide direct evidence regarding the ANS. Nevertheless, as these methods are invasive and could cause pain to the participants in these studies they were not utilised.

Chapter 2

Factors influencing cardiac autonomic activity

The following chapter demonstrates the significance and the way by which particular non-modifiable and modifiable factors may influence cardiovascular regulation. This is not an exhaustive list and rather means to present some important examples. The large background on the effects of age on HRV and HR set the scene for the following factors that can alter HRV. This is to demonstrate that the other factors can act to simply accelerate the negative impact of age on the cardiovascular system.

<u>Non-alterable factors of HRV</u>

Age

Several cross-sectional studies have shown that HRV, measured using time and frequency domain measures, is higher in young people than in middle-aged or elderly healthy subjects (33, 34). Studies in small groups of selected subjects have suggested that non-linear HR dynamics may also have age-dependent features(65-67). Studies investigating both time and spectral domain indices of HRV have shown that HRV decreases with increasing age (68-70). This has been attributed to a decline in parasympathetic activity and also possibly to a decline in sympathetic activity (69)

Age has a strong influence on short term HRV, and hence should be considered in the analysis of HRV data comparing diseased and normal populations (69, 71-73). With increasing age, there is a decrease in RSA, which is related to the high frequency components of HRV (10, 68). There is nearly a linear decrease of RSA from 20 to 80 years old. Shannon et al. and Schwartz et al. found that HF declined linearly in supine posture in subjects of 9 to 28 years(71, 72). Yeragani et al. found on short term HRV using orthostatic challenge in the age range of 4 to 43 years

demonstrated significant negative correlations between age and supine LF and HF, and standing HF (73).

As HRV and heart rate are so closely interrelated it is likely that the mechanisms governing heart rate also affect HRV (1, 74, 75). There has been a defined physiological related effect of age on the normal control of heart rate. First, a physiological age-related modification in left ventricular diastolic function is a predisposing factor in the progression of diastolic heart failure, which is prevalent in elderly patients, with up to 50% of all heart failure patients in this age range(76, 77).

Secondly, the occurrence of enhanced pulse wave velocity together with prolonged ejection time contributes to summation of antegrade and retrograde arterial waves, which may lead to an increase of systolic blood pressure and pulse pressure in older subjects.(78, 79) This has clear implications as a strong mechanism favouring the beginning and/or progression of vascular damage and higher risk of adverse physiological or clinical outcomes, including extreme cardiac workload and oxygen demand, left ventricular hypertrophy, advanced arterial stiffening itself, vascular events, and impaired renal function.(80-82)

Third, impaired endothelial function in aging coronary vessels is an additional element that causes advanced age to be listed among coronary risk factors (83). Similarly, there is substantial evidence that increased carotid intima or media thickness predicts incidences of cardiovascular events(84, 85)

Fourth, slowed arterial baroreceptor-mediated blood pressure reactions in the elderly may weaken moment-to-moment adjustments of sympathetic nerve activity and peripheral vascular resistance, with increased tendency of elderly subjects to

postural or postprandial hypotension as well as excessive blood pressure peaks(86-88). Even without such clinically significant occurrences, the age-related changes in neural cardiovascular control are likely to be responsible for the higher spontaneous blood pressure variability (with associated reduction of heart rate variability) characteristic of middle-aged or elderly subjects(89-91).

Fifth, there is a persuasive argument from studies linking decreased baroreceptor control of heart rate to the risk of life-threatening arrhythmias in cardiac patients and foresee that the adverse potential of this change may also worsen the aging condition(92-97). In many respects, habitual exercise is well known to show an antiarrhythmic effect and that in elderly populations it was shown to counter several age-related alterations (82), including impairment of the arterial baroreflex (90).

With an increase in age there is a lowering in maximal heart rate and HRV in ostensibly healthy subjects and this has been consistently reported (98). There are several reasons for this.

Heart rate has been demonstrated by many studies to gradually decrease with aging (98-102). These changes have been shown to be related when the effects of multiple confounders (some of which are mentioned in this chapter) were taken away by multivariate analysis in data coming from a cardiovascular survey (100). However, some evidence has established a positive relationship between heart rate and increasing age (103, 104). This has been establish by large prospective population studies (103-105). Some other reports have shown that heart rate is reasonably stable throughout adulthood (106, 107).

Maximum heart rate is lowered with age independent of additional factors such as sex and habitual level of physical activity (108, 109). The lowered maximal heart rate shown with age signifies the main determinant of the gradual decrease in aerobic exercise capacity primarily by way of a decline in maximal cardiac output (108, 109). While the mechanisms concerned in the decrease of maximal heart rate with age in ostensibly healthy subjects is still a controversy, new data proposes that such change is mainly shown by a reduction in both intrinsic heart rate and chronotropic beta-adrenergic sensitivity (110). In short, increasing age seems to alter autonomic control of heart rate, this is supported by other studies using a variety of measures of short and long-term HRV (111, 112).

Gender

Gender also a determinant of HRV. There is a large amount of data concerning the effects of gender on HRV, however most have been determined incidentally in experiments that focused in other areas (34, 113-118). Currently the available evidence is controversial, with some showing a higher HRV for female than for male participants (113, 115, 117) and others reporting the opposite (34, 114, 116, 118). Therefore, genders relationship to HRV requires clarification. Also, because HRV is correlated with HR in healthy subjects(116), and HR is determined by gender (113, 116), the effects of aging and gender on HR also need to be elucidated. The scope of this thesis was to determine the effects of dietary supplementation on humans and while gender was taken into consideration, much larger population studies would be required to determine the HR and HRV differences between genders.

Genetic determinants

There is a significant genetic factor to the variation in heart rate. However, the molecular mechanisms which form the basis for the control of heart rate variability (HRV) remain unknown(119). New evidence from experiments as part of the Framingham Heart Study(120) and other groups(121) show that there is a genetic factor that accounts for a significant amount of the HRV. HRV phenotypes(120) have been established to be heritable, with heritability estimates similar to traditional cardiovascular risk factors such as high-density lipoprotein cholesterol, triglycerides(122), and complex qualities like hypertension(123). Further studies are warranted to identify genes in these regions that may influence autonomic tone. Acknowledgment of the genetic factors of HRV may give added insights into the pathophysiology of the autonomic nervous system and present evidence to its modulation. Genetics were not considered in this thesis due to the difficulty of genetic screening which would require clear evidence on which genes were responsible for HRV and the magnitude for which these genes alter HRV.

Alterable factors of HRV

Smoking

Short and long term effects of smoking on heart rate variability and on baroreflex responses have been well described in the literature. Most of these experiments have shown that cigarette smoking seriously disturbs the cardiovascular physiology. Short term effects of smoking comprise of a heightened heart rate and blood pressure (124-128). The chronotropic and pressor effects of smoking are partly as a

result of peripheral adrenergic receptors which have resulted in increased concentrations of plasma catecholamines(124, 125). It has also been reported that smoking a filtered cigarette markedly decreased, in healthy subjects, sympathetic nervous activity(129). Such decreases in sympathetic nervous activity, which was inversely associated to the pressor response, were likely to be secondary to an increase baroreflex activity. This has been shown in multiple experiments in which cigarette smoking decreases sympathetic nervous activity in healthy subjects whose baroreflex responses were slowed by nitroprusside(130) and in regular smokers with coronary artery disease and impaired baroreflex function (131).

Some experiments have proposed that smoking damages the baroreflex input into the variation of heart rate (129, 132) when the baroreceptor activity comes from instantaneous blood pressure modulation and not when reflex changes in heart rate depend on laboratory stimuli such as carotid mechanical stimulation by a neck suction device (133). Decreases in baroreflex sensitivity has been found to a reduce HRV(132), this has been established more accurately in an additional experiment in which 24 hour ECG monitoring (129) with Portapres was performed in daily life in smoking individuals. These experiments have shown that smoking impairs baroreflex responses over the long-term. Indeed, smokers showed considerably elevated heart rate and blood pressures compared to non-smokers. This was established in a large population study measuring the determinants of resting heart rate involving more than 5000 men and 4000 women (134). In males, cigarette smoking was the second largest factor affecting resting heart rate after blood pressure; such a correlation was smaller in females.

Since smoking has profound effects on HRV, it was used as an exclusion criterion (124, 127, 129-132, 135, 136).

Blood pressure

While the cause of the majority of hypertension cases is uncertain, a poor regulation of the autonomic nervous system has been suggested in its development. HRV has grown as a useful, non-invasive means to quantitatively examine cardiac autonomic dysregulation in hypertension. Experiments have shown lowered HRV among hypertensives (137-148) and that the correlation with blood pressure and HRV is present across a broad array of blood pressures(148, 149). The Framingham heart study has found data in its cohort and in a subset of the Atherosclerosis Risk in Communities cohort that suggests that people with lowered HRV have a higher risk of developing hypertension, while the outcomes are conflicting across different measures of HRV(137, 138).It is not yet known to what magnitude hypertensives and normotensives experience similar declines in HRV. Therefore, while the autonomic nervous system is implicated in the regulation of blood pressure, the temporal relationship between hypertension and HRV is unclear.

Heart rate has also consistently been implicated with blood pressure in both epidemiologic and pathophysiologic experiments (99-101, 134, 150-153). In the population, the association linking heart rate and blood pressure has been long-established over the entire range of blood pressure values and has been accounted for at any age (100, 134, 151, 153).

Because of the strong effect that blood pressure exerts on both HR and HRV people that were hypertensive or hypotensive were excluded from the experiments within this thesis.

Influence of circadian cycle

The way in which circadian rhythm influences the heart has been shown by neuroanatomical and physiological evidence. Research performed on animals shows how the suprachiasmatic nucleus works to control the heart. Particularly, in rats, lesions of the suprachiasmatic nucleus eliminate the 24 hour rhythmicity in blood pressure, heart rate, food intake and motor control (154-156). The effects of suprachiasmatic nucleus -lesions on these cardiac dependent factors are not a result of the changes in motor activity. This was established in a study of rats with suprachiasmatic nucleus lesions that determined that there was no 24 hour rhythm in blood pressure and heart rate in data collected only throughout periods of temporary voluntary inactivity (157).

Circadian rhythms are made by transcriptional and translational feedback loops connecting numerous clock genes (158) and these genes have been shown in heart tissue (159). However, the way the circadian pacemaker intervenes with the control of heart rate is not yet known. As the circadian variation in HR is usually not present after heart transplantation, it seems probable that synaptic activities are mainly responsible (160, 161). Therefore, a multi-synaptic autonomic association from suprachiasmatic nucleus neurones to the heart has been suggested (157, 162). Additionally, the suprachiasmatic nucleus has many projections into the

paraventricular nucleus of the hypothalamus, a higher brain centre that is highly implicated in the regulation of cardiac activity (163, 164).

It has been shown that some of the hormones that may have a secondary function in the circadian regulation of cardiac activity comprise of the renin angiotensin system, predominantly angiotensin II (165), atrial natriuretic peptide (166), insulin (167, 168) and cortisol (169). Lately, researchers have started to develop an interest in the significance of melatonin in the circadian control of the heart. Melatonin is secreted in a circadian rhythm and it has been hypothesised that it has a vital role in the communication linking the pacemaker and secondary physiological systems. Furthermore, exogenous melatonin lowers blood pressure in healthy and hypertensive rats (170, 171), and potentiates dose dependent vasoconstriction of isolated coronary arteries (172).

From the above evidence it was decided in this thesis that when participants were to return for their final test they would be done at the same time in the morning. It was also a requirement of each volunteer to remain awake throughout the ECG recording. Therefore, the effect of the circadian rhythm carries through the experiment and can be considered the same for both the baseline and final recordings.

Excess body weight

There is currently a large body of evidence linking obesity with reduced HRV, which confirms negative changes in cardiac regulation by the ANS (173-176). Weight loss undoes this alteration and ANS dysfunction is considered to play a part in obesity

related cardiac pathology. However despite this knowledge few studies have examined the influence of weight loss on cardiac autonomic control

Despite the comparatively reliable conclusions of increased occurrence of cardiovascular disease in obesity, the cause for these relations remain unclear. Several contributing factors have been suggested as reasons for this association, such as insulin resistance, hypertension, and lowered high density lipoprotein. Conversely, it has also been hypothesised that a decrease in autonomic function may be the mechanism for the increase in frequency of cardiovascular disease in obesity (173-179). However, these experiments have not focussed on the autonomic activity of the heart itself. HRV measures the effect of autonomic function on the heart alone. It is vital to highlight the effect of obesity on HRV; lowered HRV considerably increases cardiovascular mortality (29, 180-183). Since obesity is connected to increased morbidity and mortality in cardiovascular diseases it is prudent to determine whether excess body weight has a effect on HRV which may be prognostic of cardiovascular outcomes (184-186).

The studies involving overweight and obese subjects contained in this thesis were performed on the assertion that obese people have a different HRV response at rest and to applied stimulus. As obesity is known to lower HRV, it is likely that responses to orthostatic challenges will also be lowered as compared with normal weight population. To test this hypothesis, HRV was measured at rest and in response to such stimuli as LBNP and postural changes.

Exercise and lifestyle

Recently, both time and frequency domain indices of HRV have gained increasing interest in sports and training sciences(187). In these fields, HRV is currently used for the non-invasive evaluation of autonomic changes linked with short term and long term endurance exercise training in both leisure sports activity and high performance training. Additionally, HRV is being studied as a diagnostic marker of overreaching and overtraining(188-191). A large body of evidence shows that, in healthy subjects and cardiovascular patients of all ages (up to an age of 70 years), regular aerobic training usually results in a significant improvement of overall HRV(20, 192, 193). These changes, which are accompanied by significant reductions in heart rates both at rest and during submaximal exercise, reflect an increase in autonomic efferent activity and a shift in favour of enhanced vagal modulation of the cardiac rhythm. Consistent aerobic training to a moderate degree and intensity over a minimum 3 months seems to be essential to ensure these effects, which might be associated with a prognostic benefit concerning overall mortality(187, 194). At present, available data does not allow for final conclusions with respect to the usefulness of traditional HRV indices in assessing an individual's exercise performance and monitoring training load. The ambiguous results published so far are due to several factors including insufficient study size and design, and different HRV methods. Large-sized and well planned studies are necessary for clarification.

On the other hand, the effects of habitual aerobic exercise have been demonstrated to decrease factors linked with atherogenesis. Positive lipoprotein results have been suggested as the cause. Cardioprotective modifications in haemodynamic factors

have been shown, by altering sympathetic activity and a associated change in parasympathetic outflow (195, 196). Other studies have established a reduction in arterial compliance among smokers and sedentary people when compared to physically trained ones (197, 198). Additionally, it has demonstrated a change in respiratory sinus arrhythmia following an augmentation in aerobic capacity (135, 199).

There is no clear understanding of the relative benefits of aerobic and resistance exercise training for the treatment of poor HRV in type 2 diabetes, and there is confusion surrounding what exercise prescription will be most beneficial for improving HRV in this patient group. For the purposes of this thesis we turned our attention to the effects of habitual exercise and lifestyle on spectral analysis of HRV.

Diet supplementation (emphasis on ω3 polyunsaturated fatty acids)

The importance of the type, as opposed to quantity, of fat in the diet is increasingly emphasized by nutritionists and health authorities (200-205) who recommend that we eat more polyunsaturated fatty acids, particularly the ω3, EPA and DHA, in seafood. The extensive health benefits of ω3 are well documented including improved cardiovascular health, a reduced risk of sudden cardiac death and reduced inflammation (206-212). Despite the evidence that increased consumption of ω3 can improve health, there is little information on intakes needed to attain a given level of risk reduction. There has never been a controlled study to ascertain the minimum ω3 intake required for a specified health benefit. Recent estimates of ω3 intakes in Australia suggest that most Australians consume less than 0.2 g/day (7,8). This

means that the potential benefits of dietary ω3 are not accessed by the vast majority of Australians and evidence suggests that identifiable risk groups (individuals with high blood fats, high blood pressure or obesity) may be suffering due to a lack of protective effects that ω3 afford. The latest recommendation of ω3 intake in Australia is DHA + EPA + DPA: 160 mg/day for men and 90 mg/day for women, however a recent report written by Colquhoun et al. has suggested that to reduce chronic disease a dose of 610 mg/day for men and 430 mg/day for women OF DHA + EPA + DPA is required (213). This effect on chronic disease has been suggested by some groups to be from a reduction of cardiac arrythmias, coronary heart disease, and cardiac arrest (214-219). This reduction in cardiovascular risk by ω3 intake may be able to be assessed by HRV (45, 46, 220)

By establishing a dose-response relationship between changes in erythrocyte ω3 levels and changes in cardiovascular and anti-inflammatory risk factors, the incorporation of ω3 can be used as an index of the protective benefit that can be obtained by consuming supplements or foods containing ω3. Increased dietary intakes of EPA, DPA and DHA will result in increased levels of the respective fatty acids in erythrocytes. The integration of DHA and EPA into the diet has proven to be efficacious in raising both DHA and EPA in human tissue, with DHA being more readily absorbed (221). However, all will contribute to elevation of DHA. As the cardiovascular health benefits of ω3 are predominantly associated with DHA, our aim is to establish a DHA Index.

There is a large body of evidence linking ω3 and cardiovascular risk, however the role the HRV plays remains to seen (173, 174, 176, 179, 222, 223). Large

prospective studies have found that ω3 reduce heart rate but the mechanisms for this remain unknown(224, 225). Thus one of the primary aims of this thesis is to evaluate whether a dietary supplementation with ω3 fatty acids improves cardiovascular markers and whether the level of improvement is related to the levels of DHA incorporated into erythrocyte membranes

Disease state

It has been demonstrated that HRV is affected by not only ageing, but also by other additional factors, such as ischaemic heart disease (29, 226, 227), exercise capacity (228) and glucose intolerance (229). The purpose of one of the experiments in the thesis was to study the changes in HRV in a population of patients with type 2 Diabetes mellitus (T2D). Although several diseases affect autonomic activity, our interest in T2D is principally formed on the association of T2D and HRV which will be discussed in detail in the following paragraphs.

Diabetes, Autonomic Control and Cardiac Risk

Diabetic autonomic neuropathy is perhaps the least understood problem of diabetes, having a profound effect on survival and quality of life (230). It has been established that metabolic diseases complications (eg. CVD, neuropathy, nephropathy and retinopathy) with diabetes cause extensive damage to peripheral nerves and small vessels. Indeed, a commonly overlooked problem involved with diabetes is cardiac autonomic neuropathy (CAN) (231). CAN occurs from injury to the autonomic nerve

fibres that supply the heart and blood vessels and it creates defective heart rate control and vascular dynamics (232). Lowered heart rate variation is the initial marker of CAN (233). The association between T2D and CVD is well established (234). It has been suggested that better defensive actions, comparable to those for recognised CVD, should be used in people with diabetes. These modifications are supported by evidence demonstrating that CVD happens at a considerably increased rate in patients with diabetes than in the general populace (235-237). Particularly, the Nurse's Healthy Study has given data of higher risk of CVD events prior to diagnosis of diabetes, with risk further increasing following diabetes diagnosis (237). The authors of this study also established that cardiovascular risk levels remained high after modifying for obesity and history in the family of diabetes. When compared with non-diabetic individuals, diabetes patients hold a significantly higher risk not only of developing and sustaining cardiovascular events, but also of worse outcomes connected with CVD.

Numerous mechanisms have been hypothesised as contributing to ANS imbalance and, amongst these, hyperinsulinaemia/insulin resistance appear to have a critical function. There is a large body of evidence that shows that acute physiological and pharmacological (euglycaemic clamp) increments in plasma insulin concentration promotes SNS activity, as established by recordings of venous plasma catecholamine concentration (238), plasma NE spill over (239) or direct microneurographic recordings of sympathetic nerve action potentials aimed at the skeletal muscle vasculature (240). Particularly, a short-term infusion of insulin and chronic hyperinsulinaemia stimulates a resetting in the balance of cardiac autonomic activity, mainly accompanied by a increase in sympathetic activity (241-243). This

evidence strengthens the theory that hyperinsulinaemia and insulin resistance is connected directly in the pathogenesis of cardiovascular mortality linked with T2D (241) throughout continuous over-activation of cardiac sympathetic nervous activity.

Insulin has a dual mechanism: a peripheral action and a central neural action. Insulin traverses the blood brain barrier (244-246), and insulin receptors have been located in numerous discrete sections of the central nervous system, for example the median hypothalamus (245) suggesting a central effect. The level of cardiac SNS activity caused by the peripheral effects of insulin, are mediated by non-esterified fatty acids (NEFAs) and the NO (nitric oxide)/ l-arginine pathway (247). Insulin resistant conditions are typified by change in both of these mechanisms. Current data points to the fact that these two regulatory systems act together directly and that a failing in NO synthesis and rise in plasma NEFAs may have a significant role in relation to SNS action (Figure 2).
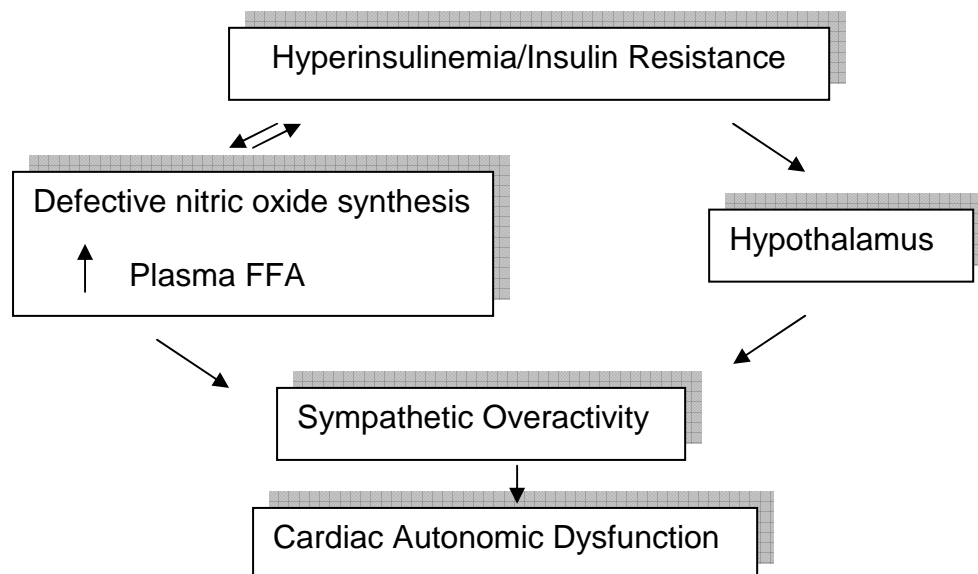
Figure 2: Effect of type 2 diabetes on the cardiac autonomic system.

Interventions for CAN

Appropriate recognition of autonomic dysfunction in diabetic patients may accelerate prophylaxis. Additionally, better nutrition and reduced alcohol and smoking habits are alternatives available to people with T2D who have known cardiac autonomic dysfunction (248). Identification that is made early for CAN allows for appropriate interventions with the anti-oxidant α-lipoic acid, which appears to reverse the progression of neuropathy in a few studies (249, 250). Additional antioxidants, like vitamin E, have been shown to improve oxidative stress in people with T2D, and this effect appears to be connected with a reduction in plasma catecholamine concentration and SNS activity (250, 251). The consumption of vitamin E for a long period improves the cardiac sympathetic balance in T2D patients (251).

Influence of posture

HUT and Cardiovascular Response

Head up tilt (HUT) elicits cardiovascular responses due to gravity and posture, both of which upon upright tilt, cause pooling of the blood into the periphery, particularly the femoral and adjoining veins. The body's ability to return this blood to normal circulation is known as orthostatic tolerance. Head-up tilt testing has become a widely accepted tool in the clinical evaluation of patients presenting with autonomic dysfunction. Currently, there is considerable agreement that tilt table testing is an efficient technique for providing direct diagnostic evidence indicating susceptibility to vasovagal syncope. The most common tilts vary between 60^0 and 80^0 from the supine position with 70^0 being the most common (252-254).

LBNP and Cardiovascular Reflex

Unlike HUT, lower body negative pressure (LBNP) can be applied in a graded manner to elicit cardiovascular reflexes without a changing posture, and without gravitational control of the main blood volume into the periphery. This process creates a controlled, non-haemorrhagic hypovolemia. It may be used to make a distinction between the cardiovascular reflex effects induced by the deactivation of the low pressure cardiovascular receptors (LBNP < 30mmHg)(255), and those produced by the deactivation of the arterial baroreceptors (LBNP > 30 mmHg)(256). The low LBNP forces result in an increase in the limb vascular resistance without a change in heart rate and blood pressure(257), while the latter produces a marked increase in heart rate (tachycardia), an increase in the diastolic BP with a fall in the systolic BP(258, 259). Cardiovascular reflex effects of -40 mmHg are similar to those produced by a change of posture from supine to standing. It is apparent that vaso-vagal syncope is more frequent when LBNP exceeds -60 mmHg even in normal healthy subjects(260). The LBNP test is valuable in the evaluation of apparently healthy individuals with poor orthostatic tolerance(261), in the evaluation of the effects of physiological and pharmacological interventions on cardiovascular reflexes(262), and in the evaluation of patients of autonomic neuropathies(263). LBNP is also a way of inducing safe, well controlled hypotension in order to study the genesis of this phenomenon.

Chapter 3

General experimental information

Aims and Hypotheses

In chapters one and two the current understanding of multiple intrinsic and environmental factors and the influence that these have on HRV representing cardiac ANS activity was described in detail. The aim of this thesis was to gain extra knowledge on this subject. In chapter two it was noted that a number of occurrences, particularly poor diet, lack of exercise and disease state increase the incidence of CVD. A better understanding of the consequences of weight loss and improved diet for cardiac ANS activity may have community health benefits, particularly for T2D patients, a subgroup of the population who develop CVD particularly frequently.

The first experiment of this thesis was designed to examine the dose dependant effect DHA rich fish oils had on cardiac ANS activity as assessed by HRV measures and large arterial compliance which may suggest a baroreflex involvement in middle aged overweight or obese but otherwise healthy volunteers. HRV and arterial compliance was expected to increase gradually with increasing does of DHA rich fish oil. In addition to this we also expected to see heart rate decrease in dose dependant manner with an increasing dose of the fish oil.

Using the data from the first experiment the second experiment examined the effect that high doses of DHA rich fish oil would have on young active and sedentary individuals. It was hypothesised that the high dose DHA rich fish oil would improve the HRV measures in the young individuals. LBNP and posture change was also utilised in this experiment and it was hypothesised that HRV would decrease with high negative pressure and in a standing posture. After the trial of high dose DHA

rich fish it was expected that the HRV results of participants during increased LBNP or posture change would be lower, which would exhibit a cardioprotective effect.

In the third experiment it was examined what effects calorically restricted diet and weight loss would have on HRV measures in T2D patients. As mentioned in chapter two T2D patients show a decreased level of control of heart rate and an increased level of CVD. It was hypothesised that HRV measures could be improved by weight loss in these overweight and obese T2D patients. Metabolic markers were also taken as part of this experiment and it was expected that they would also improve with weight loss.

When taken together it was expected that HRV measures would improve with the treatments given for the young, middle aged overweight and obese, and middle aged T2D patients in each of their experiments.

General methods

Ethical considerations

All experiments contained within this thesis were approved by The University of Adelaide Human Research Ethics Committee and/or the University of South Australia/CSIRO Human Research Ethics Committee. All participants gave written informed consent before partaking the trials (Appendix 1) and the experimental procedures conformed to the Declaration of Helsinki.

Subject selection criteria

Potential subjects responded to advertisements on University notice boards. They attended an interview during which the experimental protocol was explained and they received a Subject Information Sheet (example: Appendix 2). If interested in participating in the experiment, subjects completed a diet and lifestyle questionnaire (Appendices 3 and 4). The T2D patients were contacted via the CSIRO database for volunteers and asked if they would like to attend an information session on the experiment followed by a sign up session.

The participants in each experiment were chosen on specific exclusion and inclusion criteria which has been included in the methods section of each experiment. All subjects had no personal or parental history of cardiovascular or respiratory disease and were non-smokers. They did not frequently consume alcohol, or large caffeine doses and were not taking any medication except in experiment three involving T2D where all medications had to be stated, and that all the females were taking an oral contraceptives. Subjects were instructed not to change any of their habitual exercise or dieting habits except for those required with the experiment. They were not experiencing any major life stresses for a few days before, during, or after the study.

General design details

Experiments were held at the Nutritional Physiology Research Centre in the University of South Australia for experiment one, within the discipline of physiology at the University of Adelaide for experiment two, and at the CSIRO food and nutritional sciences building, Adelaide for experiment three. These establishments were

temperature controlled by means of an air-conditioner and the room used in the experiment lit by standard fluorescent lighting. Each room had a standard clinical bed from which the ECG recordings were measured. Volunteers abstained from caffeine, alcohol and other stimulants for twenty four hours before and during each of the experimental procedures.

General equipment and assessment

Supplementation and diet

Participants were required to maintain the proper supplementation with omega 3 PUFAs for experiments one and two. This require them to take 6 tablets on a daily basis. It was asked that subjects space the tablets out so that they would be consumed over the spread of the day (ie. two at breakfast, 2 at lunch and 2 at dinner). These instructions were given on the container either the placebo or omega 3 PUFAs were given in. Subjects were excluded if they had been taking omega 3 PUFAs eight weeks prior to the commencement of the trials. With experiment 3, participants were provided with the meals needed for the calorically restricted diet and instructed not to deviate from that diet. Compliance to diet was checked on a biweekly basis by nutritionists and if the participants did not comply to the diet they were excluded from analysis.

Collection of Anthropometric Data and Metabolic Markers

Height, weight, HR, arterial compliance and blood pressure were measured at baseline and after 12 weeks after an overnight (10 – 12 h) fast and blood was collected by venepuncture in all experiments.   Weight was measured using calibrated weight scales (Mercury, AMZ 14, Tokyo, Japan) to the nearest 0.05 kg, with subjects wearing light clothing and no footwear. Waist circumference was measured to the nearest millimetre using a standard tape measure 3 cm above the iliac crest. The average of 2 measures was recorded as the measured value (cm). Systolic and diastolic blood pressure was measured by an automatic sphygmomanometer (Dinamap™, 845XT/XT-IEC, Tampa, Florida, USA) with participants in a seated position after having rested for 5 min. Three readings were taken, each separated by 2 min, with the average score recorded as the measured value. Arterial compliance had comparable results with published studies (279).

Venous blood samples were taken into vacutainer tubes containing either no additives or sodium fluoride/ EDTA (1g/L) and the serum/plasma isolated by centrifugation for 10 min at 2000 g, ($5^{o}C$) (Beckman GS-6R Centrifuge CA) and stored at $-80^{o}C$ until study completion. Serum lipids (total cholesterol, HDL cholesterol and triglycerides) and plasma glucose were measured on a Hitachi 902 autoanalyzer (Roche Diagnostics, Indianapolis, IN) using commercial enzymatic kits (Roche Diagnostics, Basel, Switzerland). The Friedewald equation was used to calculate LDL-C levels (264). HbA1c was measured using high-performance liquid chromatography at a commercial laboratory (IMVS, Adelaide, Australia). Insulin was measured in duplicate using Mercodia Insulin ELISA (Mercodia AB, Uppsala, Sweden). The Computerized Homeostatic Model Assessment 2 (HOMA2-IR) were

used as a surrogate measure of insulin resistance based on fasting glucose and insulin concentrations (265).

Heart Rate Variability Measures

All measures were taken according to the standards and measurements set down by the European task force on HRV (1). These measures were calculated as mentioned in chapter one. Briefly the measures taken in all experiments were; LFP, HFP, LF/HF ratio, SDNN, RMSSD, SDANN and heart rate. These were all derived from ECG recordings. Results of the HRV measurements were compared with the standards of the european task force on HRV (1).

The Electrocardiogram

ECG recordings were taken while subjects were supine for 5 - 20 mins and recorded digitally using a biological amplifier (Bio Amp Model ML132, ADInstruments, Bella Vista, NSW, Australia) connected to a data acquisition system (Powerlab Model ML880, ADInstruments, Bella Vista, Australia). ECG data were analysed offline by an assessor who was blinded to the treatments using the HRV Module 1.01 for Chart 5 (ADInstruments, Bella Vista, Australia). Poincare analysis was utilised to ensure correct distribution of the beat to beat differences included in the analysis.

Chapter 4.

Dose-dependent increases in heart rate variability and arterial compliance in overweight and obese adults with DHA-rich fish oil supplementation

Contextual Statement

This chapter is a published study utilising DHA rich fish oil supplementation in over weight and obese subjects. The main outcomes of this study were increases in HRV and large arterial compliance. Statistical significance was reached and showed that there was a dose response relationship up to 6 grams of the DHA rich fish oil. However the correlations although present were weak. This was the first experiment undertaken of three and proved that DHA rich fish oil could increase HRV. At this stage it should also be noted that power calculations were performed retro spectively following patients drop out and found that there was 60% power, to detect significant treatment effects of HRV measures at P=0.05. Moreover, confidence in these outcomes is strengthened by the accompanying observations of significant dose-relationships (linear regression) and inter-relationships (LAC changes correlate with LF:HF changes).

Post publication it was found that in the discussion in this paper attributed the effect of DHA as being similar to EPA. Indeed this may be the case but the one particular reference (Yokoyama et al.) was misquoted and its effects are discussed as being due to EPA, not DHA.

Dose-dependent increases in heart rate variability and arterial compliance in overweight and obese adults with DHA-rich fish oil supplementation

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STATEMENT OF AUTHORSHIP

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British Journal of Nutrition;103:243-8. 2010.

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ABSTRACT

Heart rate variability and large arterial compliance can be improved using fish oils.
DHA, a component of fish oil, has cardiovascular health benefits but its effect on
heart rate variability and arterial compliance is yet to be quantified. 67 overweight or
obese adults (36 male, 31 female; 53 (2) yr; BMI 31.7 (1.1) kg/m2) were randomly
allocated to consume either 6 g/day sunola oil (control; n = 17), fish oil (260mg DHA
+ 60mg EPA per gram) at doses of 2 g/day (n = 16), 4 g/day (n = 17) or 6 g/day (n =
17). Blood pressure, heart rate, and compliance of large and small arteries were
measured while supine at baseline and after 12 weeks in all participants and heart
rate variability was assessed in a sub-group of 46 participants. There was no effect
of fish oil on blood pressure, small artery compliance or heart rate. However, the low
frequency:high frequency ratio of heart rate variability decreased with increasing
doses of fish oil (r= -0.34, P=0.02) while large artery compliance increased (r=0.34,
P=0.006). Moreover, the changes in these biomarkers were significantly correlated
(r=-0.31 P=0.04) and may reflect fish oil induced improvements in arterial function
and cardiac autonomic regulation.

INTRODUCTION

Elevated heart rate (HR) is a risk factor for cardiovascular death, particularly sudden death(104) while impaired heart rate variability (HRV) is an indicator of mortality risk both in patients suffering from heart disease(266) and in the general population(267). Arterial compliance is also an independent risk factor for CVD(268) and may contribute to cardiovascular risk by contributing to a reduction in HRV as a result of baroreceptors in the walls of less compliant arteries being less able to respond to changes in blood pressure and therefore provide less sensitive regulation of HRV.

HRV refers to the beat-to-beat alterations in HR thought to reflect changes in autonomic nervous system activity. In healthy individuals during rest, the electrocardiogram (ECG) displays periodic variation in R-R intervals. There are two important frequency components of HRV, high frequency (HF, 0.15-0.4 Hz) and low frequency (LF, 0.04-0.15 Hz). The HF component has been shown to reflect efferent parasympathetic activity (predominant at rest), whereas the LF component reflects sympathetic and parasympathetic interactions as well as baroreceptor activity. The LF:HF of HRV therefore reflects the balance between the sympathetic and parasympathetic nervous activity known as sympathovagal balance. Depressed HRV has been identified as a cardiovascular risk factor and increases the mortality risk amongst patients with and without heart disease(266, 267).

HRV and arterial compliance are known to be attenuated in people with elevated levels of TAG(269, 270). High TAG are also strongly associated with obesity and have been evaluated as a significant risk factor for CVD(271, 272). Obesity is an independent cardiovascular risk factor; however the mechanism underlying this

association remains unclear. Several causes for the relationship between obesity and CVD have been suggested, including that a reduction in HRV or impaired arterial compliance might be the means for the increased cardiovascular risk(173, 174, 176, 179, 222, 223). It remains unclear whether arterial compliance or HRV measures in obese people with elevated blood TAG are affected by long-chain omega-3 (LC n-3) PUFA in a dose dependant manner.

Supplementation of the diet with fish oil containing LC n-3 PUFA has previously been shown to reduce TAG and HR(273, 274) and improve HRV(45, 275, 276). Epidemiological evidence also suggests that HRV is improved in populations that have a higher intake of LC n-3 PUFA over a prolonged period(277). The effects of LC n-3 PUFA on HR and HRV are likely to be attributable to increased parasympathetic activation(276). We hypothesise that the latter could result from fish oil mediated improvements in arterial compliance thereby increasing baroreceptor sensitivity.

While the effects of LC n-3 PUFA on HR and HRV could reduce the risk of cardiovascular events, it is unclear what dose is needed to achieve these benefits. The purpose of this study was to investigate the dose-response effects of LC n-3 PUFA on HR and HRV in order to better understand what dose is required to achieve benefit and to determine whether some of the effect of LC n-3 PUFA on HR and HRV might be mediated by improvements in arterial compliance.

MATERIALS AND METHODS

A randomized, double-blind, placebo-controlled, parallel dose–response

supplementation trial of 12 weeks duration was undertaken. This study was

conducted according to the guidelines laid down in the Declaration of Helsinki and all

procedures involving human subjects were approved by the Human Research Ethics

Committees of the University of Adelaide and the University of South Australia

(Adelaide, Australia). Written informed consent was obtained from all subjects prior

to commencement.

Participants

Seventy five overweight adults (38 male, 37 female; 53 ± 2 yr; BMI =31.7 (1.1)

kg/m2) were recruited for the study. Participants taking lipid-lowering, blood-thinning

or antihypertensive medication, fish oil supplements or consuming more than one

serving of fish per week were excluded. Participants were all non smokers and were

instructed to maintain their habitual exercise levels.

Study design

Participants were block-matched into four groups which were stratified according to

fasting serum TAG concentration. The groups were then randomized to consume six

1 g oil capsules/d comprising either 0 (n=17), 2 (n=16), 4 (n=17) or 6 (n=17) x 1 g

capsules of DHA-rich fish oil (NuMega Ingredients, Victoria, Australia) with the

balance of the capsules made up of 1 g Sunola oil (contents are specified in **Table 1**)

capsules (NuMega Ingredients). The 2, 4 and 6 g/d doses of fish oil provided 0·52,

1·04 and 1·56 g DHA/d, respectively. Height and weight, HR, arterial compliance and

blood pressure were measured at baseline and after 12 weeks after an overnight (10

– 12 h) fast and blood was collected by venepuncture. Forty nine participants who were willing to undertake an ECG were measured for HRV.

Assessment of erythrocyte fatty acid profiles

The relative proportions of n-3 PUFA in erythrocytes were determined as described previously and these assessments with blood lipids been have reported elsewhere(278).

Resting heart rate, arterial compliance and blood pressure

Assessments of compliance in large (LAC) and small (SAC) arteries were obtained using the HDI/Pulsewave CR-2000 Cardiovascular Profiling System (Hypertension Diagnostic Inc, Eagan, Minnesota) following 10 minutes of rest in the supine position. An appropriate blood pressure cuff was placed around the subject's left upper arm, and a rigid plastic wrist support was placed on the subject's right wrist to minimize wrist movement and to stabilize the radial artery during the measurement. An arterial pulsewave sensor was placed on the skin directly over the radial artery at the point of the strongest pulse. The non-invasive acoustic sensor was adjusted to the highest relative signal strength, and the compliance measures were obtained during 30 seconds of blood pressure waveform collection. This device measures the decay in diastolic pressure in the large arteries and the decay in the reflective waves of the small arteries. Blood pressure and HR were measurements were also recorded at the same time. Three consecutive measures were collected and the average recorded. This non-invasive approach is repeatable and reliable both during long-term and short-term observations(279).

Heart rate variability

Electrocardiogram (ECG) recordings were taken supine for 20 min and recorded digitally using a biological amplifier (Bio Amp Model ML132, ADInstruments, Bella Vista, NSW, Australia) linked to a data acquisition system (Powerlab Model ML880, ADInstruments, Bella Vista, Australia). ECG data were analysed off-line by an assessor who was blinded to the treatments using the HRV Module 1.01 for Chart 5 (ADInstruments, Bella Vista, Australia). Frequency domain parameters of HRV were derived using power spectrum analysis (fast Fourier transforms) with high frequency power (HFP, defined as 0.15-0.40 Hz) and low frequency power (LFP, defined as 0.04-0.15 Hz) expressed in normalised units adjusting for changes in total power. Poincare plots were used to determine the normal distribution of HRV. All ECGs were recorded according to the standards of measurements, physiological interpretation and clinical use guidelines for assessment of HRV(280).

Statistics

Statistical analysis was performed using SPSS for Windows 6.0 (SPSS, Chicago, IL, USA). Baseline characteristics between the treatment groups were compared using one-way ANOVA. A logarithmic scale was applied to the LF:HF data to ensure normal distribution for statistical analysis. The effects of the oil treatments on the dependent measures over time were analysed using random effects mixed models, with oil treatment (dose of DHA-rich fish oil or control treatment) and time being the factors in the analysis, with time being the repeated measurement. For significant interactions, Bonferroni post hoc pairwise comparisons were performed to indentify differences between means. Relationships between oil doses and changes in the dependent measures by Week 12 were determined by linear regression. Changes

were compared against baseline values to detect any regression to the mean. If the latter was found to be significant, a General Linear Model was used to assess treatment related effects with baseline as a covariate. Statistical significance was set at $P < 0.05$. All data are shown as mean (SEM).

RESULTS

Study Population

Seventy Five participants were recruited for the study. A sub-group of 53 participants agreed to undergo HRV assessment. Eight participants withdrew from the trial due to time constraints or other factors unrelated to the study. Of these, four were in the group who underwent HRV assessment. One of the participants in the HRV assessment suffered an anxiety attack the day before testing at Week 12 and did not complete this assessment. Additionally data from two participants who underwent HRV assessment were excluded as statistical outliers (LF:HF for HRV was >3 standard deviations from the mean). Thus at the end of the 12 week intervention there was complete data on 67 participants for LAC, blood pressure and HR and on 46 participants for HRV.

Baseline (week 0) characteristics are shown for all participants in **Table 2** and for the subgroup of participants who underwent HRV assessments in **Table 3**. No difference in any parameter was noted between treatment groups at baseline and there was no significant effect of gender. Baseline and week 12 measurements of LC n-3 PUFA content in erythrocytes have already been published elsewhere(278).

Arterial Compliance and blood pressure

There was a significant time x dose (P=0.027) effect for LAC, and post hoc tests revealed that, compared with placebo, the 6g dose elicited a significant (P<0.001) improvement from weeks 0 to 12. Changes in LAC were correlated with dose of fish oil (r=0.34 P=0.006) but there was no relationship with SAC. Changes in LAC were also correlated with changes in erythrocyte DHA content (P=0.02 r=0.29) but not with

changes in erythrocyte EPA (P=0.077 r=0.22). Although there were no significant differences between the groups at baseline, regression analysis showed that the change in LAC correlated with baseline LAC (r=-0.41, P<0.001), raising the possibility that the result may have been influenced by regression to the mean. However, analysis by a General Linear Model comparing treatment groups to placebo showed the following levels of significance for effects of treatment: 2 g/day, P = 0.408; 4 g/day, P = 0.784; 6 g/day, P = 0.004. Hence there was a highly significant treatment effect at the highest dose, as well as a significant correlation with dose, as noted above.

Heart Rate

There was no significant effect in time (P=0.74) or dose (P=0.70) with HR. There was no correlation between change in HR and change in erythrocyte DHA (P=0.34 r=-0.12) or EPA (r= -0.022 P=0.86) content. However, changes in HR over 12 weeks correlated significantly with the corresponding changes in LAC (r=-0.48 P<0.001).

Heart Rate Variability

The individual LF and HF components of HRV showed no dose x time effect or correlation with changes in erythrocyte DHA or EPA content or fish oil dose. However, there was a significant dose x time (P=0.022) effect for the LF:HF; post hoc tests revealed significant differences from weeks 0-12 for the 4g (P=0.0049) and 6g (P=0.0015) doses versus placebo. The LF:HF for HRV decreased with increasing dose of fish oil (r=-0.34 P=0.023). There was no correlation of change in LF:HF with change in erythrocyte DHA content (r=0.23 P=0.13) but there was a strong correlation with change in erythrocyte EPA content (r=0.47 P<0.001). Changes in the

LF:HF were inversely related to the corresponding changes in both LAC (r=-0.31

P=0.04) and MAP (r=0.11 P=0.024).

DISCUSSION

This study demonstrates that dietary supplementation with DHA-rich fish oil over a 12 week period can produce dose-related improvements in both LAC and HRV. Even though these were secondary outcome measures in a broader-based study of dose-related cardiovascular benefits of DHA-rich fish oil(21), retrospective assessments indicate that there was 90% and 60% power, respectively, to detect significant treatment effects in LAC and LF:HF at P=0.05. Moreover, confidence in these outcomes is strengthened by the accompanying observations of significant dose-relationships (linear regression) and inter-relationships (LAC changes correlate with LF:HF changes). Impaired arterial compliance and HRV are independent risk factors for CVD (104, 266, 267, 281) ; hence the dose-related increases in both of these parameters suggest that increased intakes of DHA-rich fish oil are likely to be associated with dose-related reductions in CVD.

Raised HR per se is a risk factor for CVD(274). A recent study utilising the HR lowering drug ivabradine found that reducing HR below 70 bpm reduced the incidence of CHD(282). There is strong evidence that regular consumption of LC n-3 PUFA can also reduce HR. In a meta analysis of 32 trials, Mozaffarian et al(274) found that fish oil consumption for greater than 12 weeks reduced HR, particularly with groups that had a resting HR equal to or above 69 bpm. However, in our current study we did not find a significant reduction in resting HR, perhaps because resting HR in our study was below 69 bpm for each group. Nevertheless, the dose-related changes in LAC were significantly correlated with changes in HR.

The dose-related increase in LAC may have facilitated the improvement in HRV by increasing baroreflex sensitivity, a possibility which is supported by the observed correlations between changes in LAC and changes in both HR and LF:HF. The responsiveness of stretch sensitive afferent baroreceptors within the arterial wall would be facilitated by an increase in LAC, resulting in heightened baroreceptor sensitivity and afferent input leading to improved autonomic regulation of HRV. We also found that increasing HRV correlated with a decreasing MAP, again suggesting enhanced baroreflex activity.

The increase in LAC was related predominantly to DHA incorporation in erythrocytes, which is consistent with previous studies(283-285).On the other hand, the reduction in LF:HF with increasing dose of fish oil, which indicates an increasing shift toward parasympathetic regulation, appeared to be mediated predominantly by EPA. EPA has been associated with a lower incidence of death from coronary heart disease and arrhythmias(286) and it is known that, compared to DHA, EPA is more readily incorporated into human atrial tissue(221). In animal models, dietary fish oils have been shown to confer resistance to atrial fibrillation(287).The incorporation of EPA into atrial tissue is thought to be antiarrhythmic by virtue of EPA's ability to displace arachidonic acid, which is known to have pro-arrhythmic properties(288, 289). Thus increased consumption of EPA and DHA may possibly improve HRV by both local and baroreflex modulation of sinoatrial function.

In conclusion, the observed relationships between fish oil dose and changes in LAC and LF:HF suggest that regular fish oil supplementation can improve the regulation of HR, HRV and consequently blood pressure by increasing parasympathetic

regulation of cardiac autonomic tone in a dose-dependent manner. These combined

benefits may be expected to reduce CVD risk and provide further justification for

increased intakes of fish oil.

Acknowledgements

Table 1. Composition of fatty acids in 1000mg fish oil and placebo (Sunola oil) capsules

Fatty Acid	Fish Oil (mg)	Placebo (mg)
14:0	30	-
14:1	2	-
15:0	10	-
15:1	1	-
16:0	204	38
16:1trans	5	-
16:1n5	6	-
16:1n7	36	1
16:1n9	3	-
16:2n4	1	-
16:3n3	9	-
17:0	12	-
17:1	8	1
18:0	58	35
18:1n7	21	-
18:1n9	134	837
18:2n6	14	63
18:3n3	6	4
18:3n6	2	-
18:4n3	3	-
20:0	7	3
20:1n11	12	3
20:1n9	1	-
20:2n6	3	-
20:3n6	2	-
20:4n3	2	-
20:4n6	18	-
20:5n3 (EPA)	56	-
22:0	2	-
22:1n11	4	-
22:1n9	3	-
22:4n6	2	-
22:5n3	10	-
22:5n6	16	-
24:0	2	-
24:1	4	2
22:6n3 (DHA)	262	-
Minor Fatty Acids	29	13

Table 2. Dose related effects of fish oil supplementation for 12 weeks in all study participants

	0g/day (n 10M/7F)				2g/day (n 7M/9F)				4g/day (n 9M/8F)				6g/day (n 10M/7F)			
	Baseline		Week 12		Baseline		Week 12		Baseline		Week 12		Baseline		Week 12	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age (years)	52.6	2.5			53.4	2.2			54.0	2.1			54.0	1.6		
BMI (kg/m2)	30.8	0.8	30.9	0.9	32.4	1.1	32.5	1.1	31.5	1.2	31.4	1.2	32.2	1.3	32.2	1.3
SBP (mmHg)	129.4	2.7	127.6	2.6	124	1.9	124.2	2.6	123.1	2.5	121.8	2.3	137.6	4.3	134.3	4.3
DBP (mmHg)	75.6	2.1	73.3	2.5	72.0	1.8	73.0	2.0	70.1	2.3	69.1	2.0	79.2	2.4	78.5	2.4
MAP (mmHg)	94.0	2.1	92.4	2.3	90.2	1.6	91.0	2.0	88.3	2.1	87.1	2.2	99.8	3.5	98.0	3.2
HR (bpm)	59.5	1.4	60.2	2.1	61.1	1.6	61.7	1.5	59.5	1.3	58.6	1.4	61.4	1.6	60.2	2.1
LAC (ml/mmHg x 10)*	17.1	1.0	16.5	0.86	17.6	0.7	17.8	0.66	15.3	1.1	15.8	1.41	14.6	0.78	16.7†	0.92
SAC (ml/mmHg x 100)	8.8	0.97	9.1	0.9	8.9	0.81	8.2	0.75	7.67	0.72	8.8	0.9	7.5	0.89	7.0	0.8

Volunteers (n=67) consumed 0, 2, 4 or 6 g/day of fish oil. BMI, body mass index, SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, Mean Arterial Pressure; HR, heart rate; LAC, large artery compliance; SAC, small artery compliance. SEM = Standard Error of the Mean. * There is a significant dose x time (P<0.05) effect of LAC. † Mean week 12 values significantly different from mean baseline value using Bonferroni post hoc pairwise comparisons (P<0.05).

Table 3. Dose related effects of fish oil supplementation for 12 weeks in the HRV sub-group[‡]

| | 0g/day (n 8M/6F) | | | | 2g/day (n 5M/6F) | | | | 4g/day (n 5M/6F) | | | | 6g/day (5M/5F) | | | |
| | Baseline | | Week 12 | | Baseline | | Week 12 | | Baseline | | Week 12 | | Baseline | | Week 12 | |
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age (years)	51.4	2.9			52.2	2.7			53.9	2.9			54.3	2.1		
BMI (kg/m^2)	30.0	0.8	30.1	0.8	32.3	1.6	32.0	1.5	31.4	1.8	31.2	1.8	34.2	1.7	34.2	1.8
SBP (mmHg)	129.1	3.3	126	3.0	124.2	2.1	124.1	3.4	124.8	3.7	122	3.4	142.2	6.5	138.6	6.2
DBP (mmHg)	74.7	2.3	71.6	2.8	73.4	1.9	74.2	2.6	71.0	3.2	69.1	2.4	81.4	3.5	80.8	3.5
MAP (mmHg)	92.9	2.4	90.6	2.4	90.3	1.9	92.0	2.6	88.8	3.1	87.2	3.2	104.4	5.1	101.7	4.7
HR (bpm)	62.9	1.9	60.8	1.7	66.0	2.2	62.0	1.9	62.3	1.7	58.2	2.1	64.6	2.7	57	2.7
LAC (ml/mmHg x 10)	17.4	1.1	16.6	0.9	17.2	0.7	17.0	0.5	14.6	1.6	15.0	1.7	14.7	1.0	17.6[†]	1.2
SAC (ml/mmHg x 100)	8.8	0.9	9.7	1.2	9.3	0.9	8.9	0.9	7.2	0.86	9.1	1.1	6.4	1.0	6.2	1.0
LF (nu)	74.7	3.1	71.6	4.1	71.8	3.2	72.6	3.6	70.5	3.6	62.3	5.4	79.1	1.9	70.4[†]	2.8
HF (nu)	25.3	3.1	28.4	4.1	28.2	3.2	27.4	3.6	29.5	3.6	37.7	5.4	20.9	1.9	29.6[†]	2.8
LF:HF (ratio)*	4.0	0.73	3.8	0.76	3.0	0.41	3.5	0.8	2.9	0.44	2.2[†]	0.39	4.3	0.66	2.8[†]	0.48

[‡]Forty six of the 67 volunteers undertook assessments of HRV (see methods). Participants consumed 0, 2, 4 or 6 g/day of fish oil. SEM = Standard Error of the Mean. BMI, body mass index, SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, Mean Arterial Pressure; HR, heart rate; LAC, large artery compliance; SAC, small artery compliance; LF, low frequency; HF, high frequency. * There is a significant dose x time (P<0.05) effect of LF:HF. [†] Mean week 12 values significantly different from mean baseline value using Bonferroni post hoc pairwise comparisons (P<0.05).

Chapter 5

DHA rich fish oil increases heart rate variability during orthostatic stress in active young males.

Contextual Statement

This chapter is a written manuscript for submission which studied HRV in young active and sedentary males that had undergone DHA rich fish oil supplementation who were subjected to orthostatic tolerance tests. The main outcomes of this study were the increases of HRV in active male subjects taking DHA rich fish oil. This study looked at the relationship between fish oil intake and HRV in ostensibly healthy individuals. It was expected that the two treatment groups would have a change in HRV in accordance to the experiment in chapter 4, however only active young individuals consuming 6 grams of DHA rich fish oil over 6 weeks increased their HRV.

DHA rich fish oil increases heart rate variability during orthostatic stress in active young males.

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STATEMENT OF AUTHORSHIP

DHA rich fish oil increases heart rate variability during orthostatic stress in active young males.

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Performed analysis on all samples, conceptualised and designed study, interpreted data, wrote manuscript and acts as corresponding author
I hereby certify that the statement of contribution is accurate
Signed

……………………………………………………………….Date…………

SAINT, D.A.
Supervised development of work, helped in data interpretation and manuscript evaluation
I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the manuscript in the thesis
Signed

………………………………………………………………….Date…………

Abstract

Heart rate variability (HRV) has been established to measure cardiac autonomic activity which has been implicated in changes in adverse cardiovascular events. Changes in the levels of exercise and diet have been shown to change HRV individually. Orthostatic challenge has been established to lower HRV in individuals but it has not been shown whether changes in diet or lifestyle itself can protect against these HRV changes. This study aimed to determine whether a habitual level of exercise (sedentary or active lifestyles) with dietary intervention of omega 3s protected against lowering HRV during orthostatic challenge. HRV measures are lower in sedentary and than active people , with the most important component implicated being the parasympathetic nervous system. Twenty active and sedentary (n=20, 24 ± 1.5 yrs, BMI = 23.8 ± 1.5) males were recruited for the trial. Participants were divided into two groups having a sedentary or an active lifestyle. These were further divided into four equal groups with each group receiving either placebo or docosahexaenoic acid rich fish oil. Heart rate and HRV recordings were taken during orthostatic stress elicited by lower body negative pressure or postural change. The active lifestyle group showed improvement in square root of the mean squared differences of successive normal to normal intervals (Standing: 26.1±9.7 to 36.1±11.6ms; -40mmHg 25.9±7.6 to 33.4±9.8ms) , LF/HF ratio (Standing: 10.5±3.4 to 8.7±3.1) and heart rate (Standing: 82.7±5.5 to 74.0±4.0bpm) during orthostatic stress testing following the intervention of docosahexaenoic acid rich fish oil. The changes in heart rate and HRV measures during orthostatic stress demonstrate an improved cardiovascular condition likely be caused by

increasing parasympathetic regulation of cardiac autonomic tone in young active males.

Introduction

Lowered heart rate variability (HRV) is an indicator of mortality risk both in people suffering from heart disease(266) and in the general population(267). The lowering of HRV has been said to be controlled principally by the autonomic nervous system (ANS). HRV refers to the change in time between beats (R-R intervals) with regard to heart rate. In the normal populace, the electrocardiogram (ECG) shows intermittent changes in R-R intervals. There are two main frequency components of HRV, high frequency (HF, 0.15-0.4 Hz) and low frequency (LF, 0.04-0.15 Hz). The HF component has been shown to reflect efferent parasympathetic activity (predominant at rest), whereas the LF component is thought to reflect sympathetic and parasympathetic interactions as well as baroreceptor activity. The balance between sympathethic and parasympathetic nervous activity is known as sympathovagal balance and can be shown as the LF:HF ratio. Increasing HRV has been identified to lower cardiovascular risk and decreases the mortality risk amongst patients with and without heart disease (266, 267).

A major determinant of HRV is the level of habitual exercise a person undertakes (135, 290). The difference between the HRV measures of sedentary and active people appear to be because of many contributing factors, which also involve the parasympathetic nervous system (291-293). It is also well established the individuals with a sedentary lifestyle who increase their level of physical activity lower their risk of cardiovascular disease by reducing traditional metabolic factors and increasing HRV (135, 290, 292, 293). Lower body negative pressure (LBNP) is a method used to elicit a

controlled reaction from the cardiovascular system(294-296). It causes changes in heart rate due to pooling of blood in the legs which mimics orthostatic stress (296, 297). The reaction of the cardiovascular system to orthostatic stress is thought to brought about by an increase in autonomic tone, particularly sympathetic activity (294, 296, 298).

Diets containing omega-3 polyunsaturated fatty acids (n-3 PUFA) have been shown to reduce traditional cardiovascular risk markers (273, 274) and improve HRV(45, 275, 276). Evidence from large population studies have also suggested that HRV is increased in humans that consume more n-3 PUFA over a extended time(277). The mechanism of action which has been suggested to be responsible for the changes in HR and HRV with n-3 PUFA is increased parasympathetic activation (276). Whilst fish oils are known to confer benefits to the cardiovascular system it is not known which branch of the autonomic nervous system is responsible for conferring cardio-protective changes as seen with HRV. By utilising LBNP and postural change we hope to elucidate whether sympathetic or parasympathetic nervous activity is responsible for changes in HRV in sedentary and active young men after supplementation with DHA rich fish oils.

<u>Methods</u>

A randomized, double-blind, placebo-controlled, parallel dose–response supplementation trial of 6 weeks duration was undertaken. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Human Research Ethics Committees of the University of Adelaide and the University of South Australia (Adelaide, Australia). Written informed consent was obtained from all subjects prior to commencement.

Participants

Twenty active and sedentary (n=20, 24 ± 1.5 yrs, BMI = 23.8 ± 1.5) males were recruited for the trial (Figure 1). Participants taking lipid-lowering, blood-thinning or antihypertensive medication, fish oil supplements or consuming more than one serving of fish per week were excluded. Participants were also excluded from the study if they suffered from cardiovascular disease, high blood pressure (BP> 140/90), any autonomic dysfunction, or diabetes. Participants were all non smokers and were instructed to maintain their habitual exercise levels. All participants were screened by a 12 lead ECG and were excluded if the ECG showed any abnormalities.

Experimental Plan

The study was six weeks in duration, and used a placebo. The 20 subjects were randomly allocated to either a control group or a treatment group (n=5 per group). The control group consumed five 1g capsules per day of a sunola oil placebo. The treatment groups consumed five 1g capsules per day

containing DHA rich fish oil (500mg DHA, 100mg EPA, Blackmores, Sydney, Australia). The supplements for each were then allocated into lifestyle groups as follows: 5g Sunola oil (Sedentary), 5g Sunola oil (Active), 5g DHA-rich oil (Sedentary) 5g DHA-rich oil (Active).

Participants completed a diet and lifestyle (International Physical Activity Questionnaire, IPAQ) questionnaire prior to the intervention, and this was repeated twice during the study period to determine whether their diet or lifestyle had changed (other than due to the prescribed intervention). Using these forms, the level of activity was calculated as has been previously described(299).

Applying LBNP

All participants initially rested for 10 mins in the supine position after which progressive orthostatic stress was elicited by LBNP. Participants' legs were placed in a transparent semi-cylindrical chamber that was sealed at the level of the iliac crest using neoprene skirting. Pressure within the chamber was indicated by a manometer and could be lowered using a variable vacuum source. After a period of at least 15 min of quiet supine rest, the following graded orthostatic stress protocol was applied. Baseline recordings were initially made during 5 min without LBNP application. Then, graded LBNP was applied at -0, -20, and -40 mmHg. These levels were each applied for 5 min to allow for sufficient time for HRV to be recorded. The LBNP was terminated if the participant developed pre-syncope, which was defined as a drop in systolic blood pressure below 80 mmHg, accompanied by symptoms of

approaching syncope such as dizziness, light-headedness, nausea, pallor or visual disturbances(300). Blood pressure was also recorded before LBNP. Three consecutive measures were collected and the average recorded.

Assessment of erythrocyte fatty acid profiles

Blood samples were taken by venepuncture. Blood samples were taken more than half an hour after LBNP testing at baseline and week 6. The n-3 PUFA content of erythrocytes was determined by isolating, lysing and extracting the membrane lipids. Fatty acid contents were trans-methylated and the methyl esters were then assayed by gas chromatography; individual fatty acids were identified by comparison with known standards.

Assessment of metabolic markers

The blood collected from venepucture was centrifuged for 10 min at 2000 g, (5°C) (Beckman GS-6R Centrifuge CA) and plasma stored at -80°C until study completion. Serum lipids (total cholesterol, HDL cholesterol and triglycerides) and plasma glucose were measured on a Hitachi 902 autoanalyzer (Roche Diagnostics, Indianapolis, IN) using commercial enzymatic kits (Roche Diagnostics, Basel, Switzerland). The Friedewald equation was used to calculate LDL-C levels (264).

Heart rate variability

Participants lay on the LBNP bed where electrocardiogram (ECG) recordings were recorded digitally using a biological amplifier (Bio Amp Model ML132,

ADInstruments, Bella Vista, NSW, Australia) linked to a data acquisition system (Powerlab Model ML880, ADInstruments, Bella Vista, Australia). ECG data were analysed off-line by an assessor who was blinded to the treatments using the HRV Module 1.01 for Chart 5 (ADInstruments, Bella Vista, Australia). Frequency domain parameters of HRV were derived using power spectrum analysis (fast Fourier transforms) with high frequency power (HFP, defined as 0.15-0.40 Hz) and low frequency power (LFP, defined as 0.04-0.15 Hz) expressed in normalised units adjusting for changes in total power. Poincare plots were used to determine the normal distribution of HRV. All ECGs were recorded according to the standards of measurements, physiological interpretation and clinical use guidelines for assessment of HRV (1).

Statistics

Statistical analysis was performed using SPSS for Windows 15.0 (SPSS, Chicago, IL, USA). Data was assessed for normality and a logarithmic scale was applied to the frequency domain measures of HRV to ensure normal distribution for statistical analysis. The effects of the DHA rich fish oil on HRV and other outcomes parameters were determined by One-way ANOVA. Correlation analysis was used to assess relationships between variables. Statistical significance was set at $P<0.05$. All data are shown as mean (SEM).

Results

DHA (P<0.01), EPA (P<0.01) and total n3-PUFAs (P<0.01) increased in the active individuals on high DHA fish oil. The sedentary group and taking n-3 PUFAs showed no significant change in erythrocyte n-3 PUFA content (p=0.065). The two placebo controlled groups did not see changes to the erythrocyte n-3 PUFA content which can be seen in **table 1**. Each group had 5 participants from start to finish.

Each group, except the active group taking the placebo, had significant stepwise declines in the HRV measures RMSSD and stepwise increases in the LF/HF ratio to LBNP and postural change at baseline. Similarly HR showed increases to both stressors. This significance remained the same at week 6. P<0.01 for all measures. HR (r=0.74, p<0.01), LF/HF (r=0.84, p<0.01) and RMSSD (r=0.90, p<0.01) were strongly correlated with each other in LBNP and postural change.

Correlations were found between changes total n-3 PUFA (r=0.52, p=0.017), DHA (r=0.46, p=0.04) and EPA (r=0.48, p=0.03) content of the red blood cells with changes in RMMSD in the whole group (n=20) at rest. No correlations were found between n-3 PUFA content and the metabolic biomarkers. Activity level was also not correlated to blood pressure, lipids, HR or HRV measures.

By week 6 there was a significant restriction on the decrease in HRV measures taken while standing and on the application of LBNP at -20 and -40mmHg in the active/treated group only. There was also another significant

effect on HR in the standing posture. (P<0.05 for all variables). These results are shown in **table 2**.

Discussion

This study establishes that dietary supplementation with DHA-rich fish oil over a 6 week period can produce improvements in HRV in young active men. This study was based on a previous trial conducted by our group on the dose-related cardiovascular benefits of DHA-rich fish oil (301), and it was from this study that it was decided more than 2g of n-3 PUFA would be required to achieve a difference in HRV outcomes. Moreover, data from other groups suggested that changes in autonomic activity might be able to be detected under a stressed state such as orthostatic challenge. HRV is an independent risk factor for CVD (104, 266, 267, 281) ; hence the improvements in this parameter suggests that higher intakes of DHA-rich fish oil could be associated with prevention of CVD.

There was no significant rise in EPA and DHA level in sedentary individuals taking DHA-rich fish oil. This is contradictory to many studies although may be attributable to the 6 week supplementation time which is shorter than most trials and that given more time these levels would rise (45). This lack of an effect could also be dues to a lower metabolism rate and turnover of lipids into the blood cell membranes of sedentary as compared to active individuals (302-304)

 Another factor involved with cardiac risk is a high HR (274). This has been implicated in active individuals over sedentary people (195). It has been recognised that lowering HR reduced the incidence of CHD (282). There is

mounting evidence that a consistent intake of n-3 PUFA can also decrease HR. In a meta analysis of 32 trials, Mozzafarian et al (274) established that fish oil consumption for more than 6 weeks reduced HR, mainly with groups that have a resting HR equivalent to or over 69 bpm. However, in this trial it was not found the resting heart rate declined, perhaps because resting HR in our study was below 69 bpm for each group. However, after being stressed with postural change and LBNP it was found that young active individuals had attenuated heart rate response after supplementation with DHA rich fish oil. The change in total n-3 PUFA, DHA and EPA level was also linked with the change in RMSSD, a time domain measure of HRV. This is likely to have to do with the improvement in vagal tone(21, 194, 196, 225, 228, 290).

The restriction of HRV may have been facilitated by increasing baroreflex sensitivity (89, 92, 195), a option which is made more likely by the observed correlations seen in the previous experiment carried out by our group in LAC and changes in both HR and LF:HF. It is therefore likely that the changes in HRV witnessed in this trial were facilitated by increased baroreceptor sensitivity and afferent input leading to improved autonomic regulation (90, 258, 305).

The increases in RMSSD were related principally with total n-3 PUFA but also DHA and EPA incorporation in erythrocytes, as has been demonstrated in earlier studies(283-285). Conversely, the restriction of the increase in LF:HF suggests a decrease in sympathetic activity, shown to be mediated mainly by EPA. EPA has been connected with a reduced prevalence of mortality from

coronary heart disease (286) and it is acknowledged that, in comparison to EPA, DHA has the ability to be incorporated further into human atrial tissue(221). Therefore greater intake of EPA and DHA could improve HRV by both local and baroreflex modulation of sinoatrial function.

In conclusion, the changes in HR and HRV measures during orthostatic stress demonstrate an improved cardiovascular condition likely to be caused by decreasing sympathetic innervation of cardiac autonomic activity in young active males. These mutual changes may reduce CVD risk from an early age and provide further justification for increased intakes of fish oil.

Limitations

Power calculations were performed prior to the beginning of this experiment and were based on the study by Sjoberg et al.(301) with the expectation that the heart rate and HRV changes in this study would be the similar. These calculations revealed that 20 people with 5 participants per group would reveal a significant change, While the mean heart rate and HRV measure were expected, ideally, a larger number of participants would have been better to reduce the standard deviation of the groups to demonstrate a greater sensitivity of HRV changes created by omega-3 supplementation, lifestyle and orthostatic challenge.

Figure 1. Study design showing group allocations.
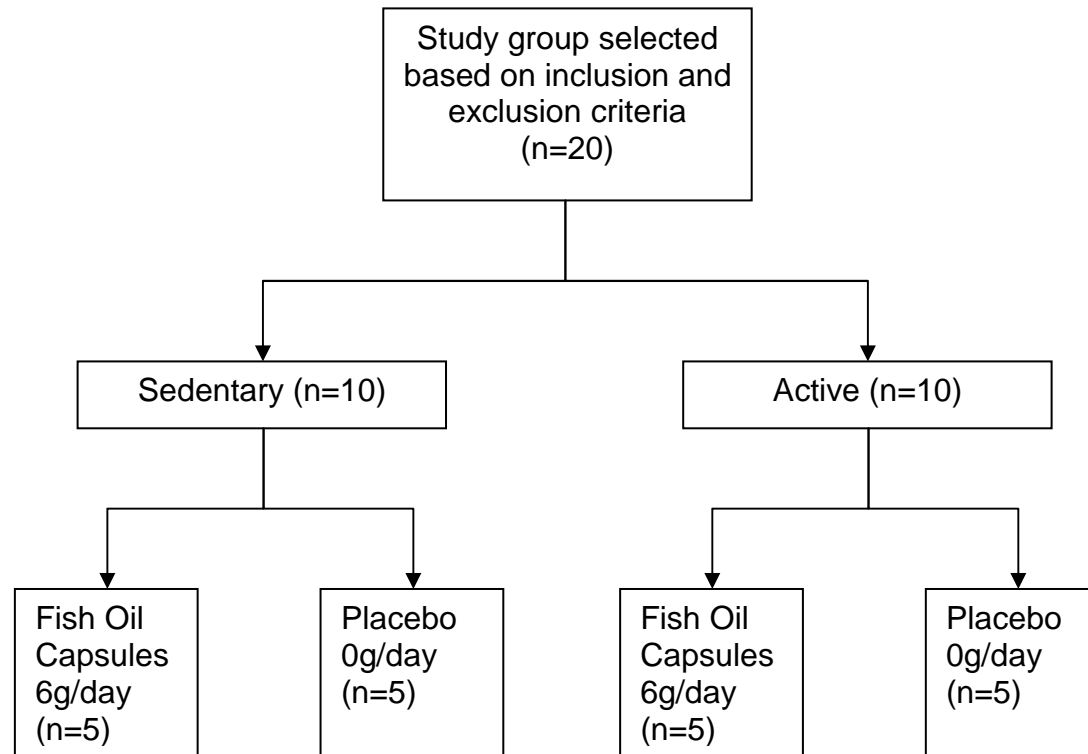
Table 1 . Baseline characteristics and changes before and after a 6 week intervention of DHA rich fish oils. (N=20)

		Active								Sedentary							
		6g/day (n=5)				0g/day (n=5)				6g/day (n=5)				0g/day (n=5)			
		Baseline		Week 6		Baseline		Week 6		Baseline		Week 6		Baseline		Week 6	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Age	(yrs)	26.8	3.3			24.6	1.8			22.8	1.2			22.4	1.3		
Weight	(kg)	77.9	3.5	77.1	3.8	75.2	5.2	74.7	4.8	85.0	7.0	86.7	6.8	71.7	6.3	71.8	6.1
BMI	(kg/m2)	23.1	1.1	22.8	0.9	23.7	1.5	23.5	1.4	26.2	2.0	26.7	2.0	22.5	1.6	22.5	1.5
SBP	(mmHg)	126.0	2.1	127.8	7.2	132.5	4.5	120.3	3.9	118.0	2.8	132.8	4.5	126.0	3.7	122.4	2.9
DBP	(mmHg)	72.5	5.6	74.0	4.7	78.5	2.4	68.0	4.4	77.7	3.0	72.0	5.0	76.3	4.5	74.3	1.9
Activity	(MET)	2817.0	856.3	3073.1	636.7	3646.8	609.7	3826.8	522.4	883.6	215.5	1064.3	276.2	931.2	198.0	1074.6	176.9
HDL	(mmol/L)	1.4	0.1	1.4	0.1	1.6	0.1	1.6	0.1	1.3	0.1	1.4	0.1	1.5	0.1	1.5	0.1
LDL	(mmol/L)	2.6	0.1	2.8	0.2	2.3	0.4	2.1	0.3	2.1	0.2	2.2	0.2	2.8	0.4	2.9	0.4
Total Cholesterol	(mmol/L)	4.4	0.1	4.6	0.2	4.3	0.4	4.0	0.3	3.9	0.2	3.9	0.2	4.7	0.5	4.7	0.5
Triglycerides	(mmol/L)	0.9	0.2	0.6	0.1	0.9	0.2	0.7	0.1	0.8	0.1	0.6	0.1	0.9	0.2	0.8	0.1
Cholesterol/HDL	ratio	3.2	0.2	3.3	0.3	2.7	0.2	2.6	0.2	2.9	0.3	2.8	0.2	3.1	0.2	3.1	0.2
EPA	(%)	0.8	0.1	2.0**	0.1	1.1	0.1	1.0	0.1	1.1	0.3	1.9	0.2	0.8	0.1	0.7	0.1
DHA	(%)	4.5	0.6	7.5**	0.4	4.8	0.5	4.7	0.5	4.2	0.6	6.0	0.4	4.4	0.2	4.4	0.2
Total n-3	(%)	8.0	0.5	12.1**	0.4	8.7	0.5	8.7	0.7	8.5	1.1	10.9	0.5	8.0	0.4	7.9	0.3
HR	(bpm)	62.7	4.5	57.6	3.2	61.6	4.5	66.7	5.6	59.9	5.4	60.7	3.7	64.9	6.2	65.4	1.7
LF:HF	ratio	1.4	0.5	1.5	0.5	2.8	1.5	3.8	2.5	1.2	0.3	1.9	0.6	2.5	1.1	1.7	0.8

SBP, systolic blood pressure; DBP, diastolic blood pressure; MET, metabolic equivalents; EPA, eicosapentaenoic acid; DHA,

docosahexaenoic acid. ** $P<0.01$ baseline values significantly different from values at Week 6

Table 2 . Heart rate variability outcomes during LBNP and posture change before and after a 6 week intervention of DHA rich fish oils in active and sedentary young males. (N=20)

	Active								Sedentary							
	6g/day (n=5)				0g/day (n=5)				6g/day (n=5)				0g/day (n=5)			
	Baseline		Week 6		Baseline		Week 6		Baseline		Week 6		Baseline		Week 6	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
HR (bpm)																
0mmHg	62.7	4.5	57.6	3.2	59.9	5.4	60.7	3.7	61.6	4.5	66.7	5.6	64.9	6.2	65.4	1.7
20mmHg	66.6	5.0	63.2	2.8	59.8	5.0	59.5	4.7	66.6	4.3	69.1	6.4	64.2	5.2	69.3	2.9
40mmHg	76.3	5.1	68.9	2.9	67.3	4.3	64.1	5.2	77.8	6.1	76.2	7.2	71.9	6.7	78.1	3.3
Lying	61.9	3.6	56.7	1.7	58.6	5.0	57.5	3.8	62.5	3.2	65.5	4.6	62.1	3.3	66.1	1.8
Sitting	71.2	5.7	66.2	3.7	62.1	4.7	66.7	5.1	75.6	5.5	77.0	8.3	71.8	5.0	75.8	2.8
Standing	82.7	5.5	74.0*	4.0	87.8	9.1	84.8	9.9	75.4	4.7	77.2	5.1	83.9	6.7	89.4	3.3
LF:HF																
0mmHg	1.4	0.5	1.5	0.5	1.2	0.3	1.9	0.6	2.8	1.5	3.8	2.5	2.5	1.1	1.7	0.8
20mmHg	3.7	1.5	4.9	2.0	2.2	0.8	2.6	1.3	4.5	2.3	3.6	1.5	3.1	1.4	4.4	2.0
40mmHg	8.6	4.1	6.9	3.3	1.9	0.6	2.9	1.5	6.8	2.5	9.2	4.9	4.8	1.4	9.7	5.5
Lying	1.8	0.8	1.3	0.4	0.9	0.3	1.2	0.4	1.4	0.5	1.7	0.5	1.2	0.4	1.6	0.5
Sitting	5.7	2.6	4.7	1.6	2.3	1.0	3.4	1.2	3.6	1.1	4.5	1.9	5.7	1.5	4.0	0.9
Standing	10.5	3.4	8.7*	3.1	4.1	0.8	6.1	2.8	7.9	3.0	8.6	3.6	10.0	2.9	10.9	2.6
RMSSD (ms)																
0mmHg	56.3	19.1	69.8*	14.7	49.8	8.7	59.1	12.8	68.7	16.8	43.5	10.2	57.9	25.3	74.0	20.3
20mmHg	44.1	15.0	43.8	13.1	57.3	12.8	46.2	13.2	33.8	5.3	34.3	9.8	56.6	23.3	47.2	15.5
40mmHg	26.1	9.7	36.1*	11.6	40.2	6.5	42.9	13.2	26.6	5.8	23.8	6.9	46.4	20.1	26.2	5.5
Lying	59.7	18.7	73.1	16.6	58.5	8.5	60.3	11.2	66.2	17.4	47.8	6.3	72.5	13.4	58.8*	15.4
Sitting	53.0	17.6	70.1*	22.5	66.5	13.9	49.7	12.6	32.3	5.1	34.4	9.0	55.2	15.0	50.6	11.9
Standing	25.9	7.6	33.4*	9.8	36.1	8.1	32.0	11.7	18.3	4.2	20.8	5.8	31.6	7.5	26.5	3.2

* P<0.05, baseline values significantly different from values at Week 6

Chapter 6

Moderate weight loss improves heart rate variability in overweight and obese adults with type 2 diabetes

Contextual Statement

This chapter is a publish study which investigated the effects of weight loss in type 2 diabetics on HRV. This study involved utilised a calorically restricted diet to induce weight loss and the following measures were also taken insulin, insulin resistance, LDL, HDL, total cholesterol, glucose, HbA1c, BMI, heart rate and multiple HRV (LF, HF, LF/HF, SDNN, RMSSD) measures. The main aim and outcome of this study was the increase of HRV in overweight and obese type 2 diabetics.

Post publication, a review of this paper it found error in the interpretation of the ivabradine study. Indeed, in this study it was found that a sub group analysis showed that lowering heart rate below 70bpm had some beneficial outcomes on reduced coronary revascularisations and reduced infarctions, not CHD in general.

Moderate weight loss improves heart rate variability in overweight and obese adults with type 2 diabetes

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Moderate weight loss improves heart rate variability in overweight and obese adults with type 2 diabetes
Journal of Applied Physiology 2011;110:1060-4.

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Performed data collection, analysis on all samples, interpreted data and wrote manuscript.
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Chapter 7

General Discussion.

There are three main sections to this chapter. First, the experiments one, two and three are considered together and a there is general discussion of the implications of the three experiments. Second, the methodological issues arising that aren't mentioned in the papers are outlined. In the third section, the future direction of HRV research and its implications to CVD risk are examined. The discussion is based on the utilisation of HRV as an evaluative measure on the human health conditions.

Discussion combining experiment one, two and three.

The main theme throughout each experiment was that cardiovascular health and HRV are connected. While cardiovascular health and HRV decrease with age and fighting its effects using specific diets (as in experiment one and two) and weight loss (as in experiment three) have proved useful in lowering (**Figure 3**) CVD (47, 224). Whilst the specific underlying mechanisms for this remain unclear, it is certain that these changes HRV are significant and are likely to improve cardiovascular health.

Secondly carried through the experiments is a stepwise formation of disease which show signs of decaying HRV. Consequently, experiment two involving healthy active young individuals records higher HRV than seen in Experiment one involving overweight and obese participants and experiment three with T2D patients. The most consistent measure which shows this is the time domain measure RMSSD which is suggested to be a marker of vagal activity (1). For example, the RMSSD for experiment with the young volunteers return values around 60ms at rest, for the overweight and obese middle aged people

it was around 34.5ms (unpublished data) and for the T2Dpatients it was around 23.4ms before weight loss. The frequency domain data is a little more inconsistent which I will go on to explain in the next section.

This stepwise decline in HRV with age must be further analysed by experiments utilising ostensibly healthy participants in order to not only test the reliability of HRVs decline but also the variance in its recording with age. in these experiments it was found that some measurements (total power, LF and HF power) had large variances, which was improved through normalising the units. Such variances make it difficult to come to precise conclusions without the use of very large populations.

The implications of all three experiments put together are rather small, the reason being that the studies undertaken are not large epidemiological based studies needed to determine the efficacy of HRV measures. This standardisation is needed in order to be able to design experiments necessary to explore interventions that will be targeted at treat HRV itself and only then will we know if the modification of HRV can change cardiovascular or other systemic outcomes. Another possibility apart from large and expensive epidemiological studies would be a meta-analysis of the available literature based on HRV after the standardisation of measurements after the 1996 standardisation of measurements by the HRV task force (1)
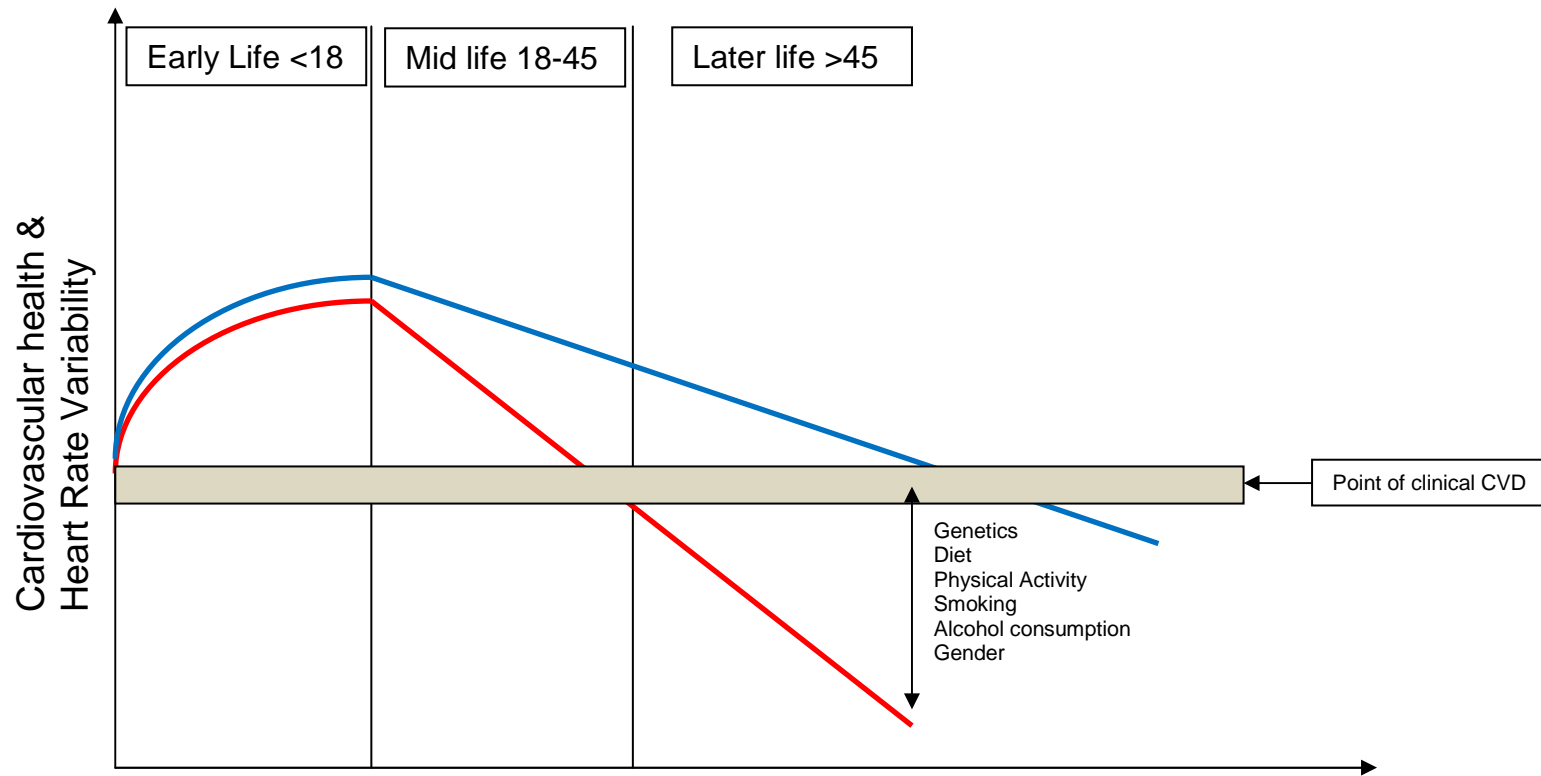
Figure 3. The effect of risk factors on cardiovascular health and HRV.

It is likely the HRV measures have a similar scale to BMI, whereby there is a level that is too high or low which shows or even contributes to poorer health outcomes. The current expectation as suggested by the literature is that increases in HRV are seen as a positive indicator of health, however this in not conclusive across all studies. As suggested above this could be because participants already have a 'healthy' level of HRV and increases in a normal level of HRV may yield little to no effects at all. In fact, the literature at this stage supports this view as the main studies are showing a positive effect are those done with a compromised cardiovascular system (through coronary heart disease inc myocardial infarction).

Frequency and time domain measures of HRV

One of the main difficulties in analysing HRV is the variety of measures that can be taken. Time domain measures are the most stable measures that can be taken showing a very good reproducibility during a 24 hour recording (116). However this reliability depreciates as the recording gets shorter. Frequency domain measures on the other hand are very good during short (5-20 minute) recordings. As mentioned in the background to this thesis there is still controversy surrounding the use of the frequency domain measures of HRV.

The overwhelming evidence points to the HF component of HRV being largely determined to be parasympathetic nervous activity. The other measures ULF, VLF and LF are much less defined. Theories around ULF point to it being dependent on circadian hormones and the sleep wake cycle. The VLF

component has been hypothesised to be associated with the renin angiotensin aldosterone pathway and thermoregulation, although these are still very speculative (1).

In contrast the LF component in HRV has had a great deal of evidence pointing to it representing either sympathetic activity, combined sympathetic/parasympathetic activity, or simply the absence of parasympathetic activity (see Chapter 2: Controversy around LF power). In the experiments covered in this thesis the participants were always rested and supine during the time that baseline HRV measures were taken. This would mean that the predominant factor on these individuals should be parasympathetic control. However numerous fluctuations were noticed in the LF measure as well as the HF measure. This would seem to be consistent with the idea that the LF measure may simply show the absence of parasympathetic activity. The vagus can increase heart rate by simply lowering its stimulus on the sino-atrial node and allowing heart rate to rise to the hearts intrinsic rate of 100bpm. To confirm this LF measures could be compared with time domain measures. An increase in SDNN or RMSSD which are simple measures of HRV with an increase in LF would not be consistent with sympathetic activity but would be consistent with vagal interaction.

Methodological issues

As with most experiments there are issues that arise during the experimental process that need to be addressed. In all of the experiments compliance with

diet or the taking of supplements was addressed. For experiment one or two if the placebo group had a significant rise in omega 3 levels they were queried about their dietary intake further and asked if they had been eating any foods containing omega 3 from a large list. On the other hand, if the participants on the omega 3 capsules, lost omega 3 from their red blood cell content they would be immediately excluded as this would imply a lack of compliance. For experiment three, weigh- ins and visits with a nutritionist were necessary for every two weeks to ensure compliance. If these T2D diabetics patients were suspected of non compliance with the prescribed diet they were excluded from the trial.

It is difficult to assess the significance of the HRV frequency domain measures without comparing it to direct measures (as in Chapter One). Methods such as muscle sympathetic nerve activity (MSNA) and microneurography which use a needle to directly assess nervous activity are well established measures of autonomic activity. Unfortunately by their very direct nature they are difficult to use for two main reasons. Firstly, the needles are very sensitive to movement and would require the recording to be taken over a long period of time to make sure there is enough viable information. Secondly participants are much less likely to be recruited if there is a direct measure involved. Nonetheless, it would be useful to have a full quantitative analysis of all measures of autonomic nervous activity

Future possibilities and implications for HRV

Standardising HRV values

A necessary area for HRV recordings to branch out into is to standardise the measures and to define precisely what constitutes a healthy HRV. At the current stage there are many measures which have a low, normal, high level attributed to them (ie, BMI, HR, blood pressure, etc.). It is in this same sense that HRV should also have a level which is too high, healthy and too low. In order to get to this stage large population experiments with longitudinal follow up are required to ascertain healthy HRV values for a variety of age and sex populations (328). Experiments from the Framingham Heart Study established the time and frequency domain measures of HRV in about 700 elderly subjects and the association of these HRV measures to all-cause mortality throughout four years of follow up (31). These experiments determined that HRV presents predictive information further than that demonstrated by traditional risk factors. Extra population HRV studies utilising the complete age range in male and female participants warrant further investigation. At the same time, it would be useful to connect the use of omega 3 PUFAs in a long term population study similar to what has been done with the DART and JELIS trials (329-331).

Risk Stratification and HRV

Measurements in the time and frequency domain of HRV considered from lengthy 24 hour and small five to fifteen minute ECG recordings have been utilised to predict time to next cardiovascular event after myocardial infarction in addition to the danger of all cause mortality and sudden cardiac death in

individuals with physical heart disease (332-334) and several other pathological conditions (334). With a multiple analysis using equipment that can calculate HRV jointly with the occurrence and intricacy of ventricular arrhythmias, QT segment variability, it ought to be possible to noticeably increase the recognition of people at risk for sudden cardiac death and arrhythmic events. Trials are required to assess the sensitivity, specificity, and prognostic value of combined recordings.

Disease manifestation and HRV

A area of research that could be productive is the use of HRV measures to discover the function of ANS changes in disease mechanisms, particularly in those circumstances in which sympathetic/parasympathetic components are considered to have a significant role..

The function that the ANS plays in essential hypertension is a significant area worthy of examination (335). The problem concerning the roles of increased SNS activity in essential hypertension may be elucidated by longitudinal studies of individuals who are originally normotensive. Essentially the question that must be asked is: Is essential hypertension a consequence of increased SNS activity with changed sensitivity of neural regulatory mechanisms?

Several secondary ANS disorders that come with major diseases such as T2D, alcoholism, and spinal cord injuries are connected with changes ANS function. In a number of these diseases, alteration in HRV may be an early symptom of the disorder and might be helpful in quantifying the speed of

disease progression and the effectiveness of therapeutic interventions such as omega 3 PUFAs and calorically restricted diet.

Conclusion

In order to develop a deep understanding of how diet, and specific dietary therapies, influence HRV, it is necessary that we distinguish how cardiac ANS activity acts in the normal healthy population. Once HRV measures have been standardised a further understanding of the important effects that aging, gender, blood pressure, disease state and various other factors have on HRV should be established. Research suggests that lowered HRV may indicate the risk of CVD. Consequently, an improved understanding of methods to increase HRV could have significant community health benefits. Particularly, further investigations in this area might be important in implementing plans to reduce the risk of CVD in people with lowered HRV.

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Appendices

THE UNIVERSITY OF ADELAIDE HUMAN RESEARCH ETHICS COMMITTEE

STANDARD CONSENT FORM
FOR PEOPLE WHO ARE PARTICIPANTS IN A RESEARCH PROJECT

1.   I, ………………………………………………………………   *(please print name)*

     consent to take part in the research project entitled:

     …………………………………………………………………………………

2.   I acknowledge that I have read the attached Information Sheet entitled:

     …………………………………………………………………………………

3.   I have had the project, so far as it affects me, fully explained to my satisfaction by the research worker. My consent is given freely.

4.   Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to me.

5.   I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.

6.   I have been informed that, while information gained during the study may be published, I will not be identified and my personal results will not be divulged.

7.   I understand that I am free to withdraw from the project at any time and that this will not affect medical advice in the management of my health, now or in the future.

8.   I am aware that I should retain a copy of this Consent Form, when completed, and the attached Information Sheet.

     ………………………………………………………………………………...
     (signature)   *(date)*

WITNESS

   I have described to   ……………………………………………….. *(name of participant)*

   the nature of the research to be carried out. In my opinion she/he understood the explanation.

   Status in Project**:** …………………………………………………………………

   Name: ……………………………………………………………………………….

   ………………………………………………………………………………...
   (signature)   *(date)*

PARTICIPANT INFORMATION SHEET

Research Project Title: **The effect of DHA rich fish oil on heart rate variability and arterial cardiovascular markers during orthostatic challenge in young sedentary and active males.**

Investigators: Dr David Saint, University of Adelaide, Discipline of Physiology, School of Molecular and Biomedical Science, phone, 8303 3931.

Mr Nicholas John Sjoberg, University of Adelaide, Discipline of Physiology, School of Molecular and Biomedical Science, 8303 3754.

Purpose of the study
The purpose of our study is to investigate the effect of DHA rich fish oil on heart rate and heart rate variability in sedentary young males. This study will allow us to determine whether DHA rich fish oil has an effect on the nervous system supplying the heart. Body Mass Index (BMI) is a determinant of health with respect to height and weight as prescribed by the national Heart Foundation and will be used to determine who is healthy in this experiment. Due to the nature of the study all participants must be under a BMI of 35, non-smoking males with no history of heart disease or objection to taking 5g of DHA rich fish oil a day. Lifestyle (ie. Sedentary, moderate or active) will be quantified using the International Physical Activity Questionnaire (IPAQ) which assesses the amount of activity you currently do over a 7 day period.

Possible benefits from the study.
To the participants involved there is no direct benefit, but this study will help us to understand the effects of DHA rich fish oils on the cardiovascular system.

Procedures that involve the participant.
Prior to the study, you will not be able to consume any stimulants such as caffeine, alcohol, or Red Bull™ for twelve hours prior to testing. No foods high in salt can be consumed on the day of testing and you are asked to refrain from eating for two hours prior to the session. Upon arrival, the study will be fully described and the investigators will ask you to read the information sheet and to sign a consent form. Initially, we will take your weight, height and blood pressure, to check whether you qualify for the experiment. Upon qualifying for the experiment, we will then place 3 surface electrodes to the upper body (right collarbone, left ribcage, left just above hip) and begin recording in the supine position after 5-10 mins rest. The recording will also be taken in a sitting and standing position for the same period of time. This will be followed by a second procedure where LBNP will be used, which will require you to lie down and place your legs into a transparent semi cylindrical chamber while a vacuum is being applied. Following these procedures you will be brought back to rest and your bloods will be taken by the qualified nurse on staff. The whole experiment will take 1 hour in total to complete. Immediately after the 6 week trial of DHA rich fish oil the above procedures will be repeated.
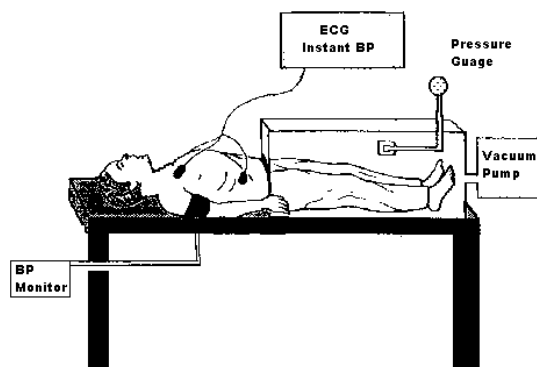

Figure 1: Sketch of LBNP setup.

Experimental Protocol

Information Sheet Consent Form, IPAQ, Diet questionnaire	HRV, Blood Pressure	6 week intervention of DHA	IPAQ, Diet questionnaire Weight, Height and Blood	HRV, Blood Pressure	Trial Completed

Approximately 1 hour for testing to take place is required from participants at an arranged time convenient to both participant and researcher. The site of experimentation is in the Physiology Teaching and Resource Centre, located on the 4th floor of Medical School South, University of Adelaide,

Possible risks and adverse effects of DHA rich fish oil.
The DHA rich fish oil used in this trial is commercially available and is made to Australian standards however known side effects exist in very high doses of omega 3 polyunsaturated fatty acids (>4g per day). These side effects are:

- Increased bleeding if overused by a patient who is also taking aspirin or warfarin.
- Hemorrhagic stroke.
- Oxidation of n−3 fatty acids forming biologically active oxidation products.
- Reduced glycemic control among diabetics.
- Suppression of immune and inflammation responses, and consequently, decreased resistance to infections and increased susceptibility to opportunistic bacteria.
- An increase in concentration of LDL cholesterol in some individuals.

This trial uses 3g of omega 3 polyunsaturated fatty acid within the 5g of fish oil per day. Each gram of fish oil provided contains 600mg of omega 3 polyunsaturated fatty acids and should only be used as directed. If any symptoms arise during the trial and persist, see your healthcare professional or use the provided contact form.

Statement of withdrawal and confidentiality
All participants' personal details will be kept confidential and only be exposed to the conductor of this experiment. Any further use or exposure of these personal details will not proceed unless participants give permission (by means of a signed consent form). All participants reserve the right to withdraw from the trial at any time without prejudice to their future treatment or involvement with the experiment group and without explanation. If you wish to speak to someone about the project please refer to the independent complaint form.

Diet and Lifestyle Questionnaire

Title: The effect of DHA rich fish oil on heart rate variability and arterial cardiovascular markers during posture change in young sedentary males.

In this questionnaire general questions are asked about your health, diet and some background information

about yourself. All information will be kept strictly confidential. If you have any concerns about the

questions in this form or have difficulty in answering any questions please do not hesitate to contact Dr.

David Saint (8303 3931)

1. Title: Dr   Mr   Mrs   Miss   Ms

2. Full name: _____

3. Today's Date: _____

4. Address:

_____

_____

5. Telephone Nos: **Home:** _____
                         Work: _____
                         Mobile: _____

6. Date of birth:_____day_____month_____year

7. Age:_____

8. DOCTOR'S INFORMATION

DOCTOR's NAME_____

Name of Practice   _____

    Address   _____

                _____ Post Code _____

    Telephone_____

Would you be happy for us to notify your doctor of your involvement in this study and if necessary contact
them directly (please tick)   **Yes**   ☐   **No**   ☐

9. PLEASE COMPLETE (IF KNOWN):

Weight:_____kg   **Height:**_____cm   **Blood Pressure:**_____**mmHg**

Has your weight changed by more than 5 kg in the last month (please tick) **Yes** ☐   **No** ☐

Are you on any weight loss or diet program (please tick)   **Yes** ☐   **No** ☐

Have you donated blood within the last 3 months   **Yes** ☐   **No** ☐

If yes, please give the date of your last donation and any other details_____

Are you currently participating in any other research study **Yes** ☐   **No** ☐

10. DO YOU HAVE A FAMILY HISTORY OF :	**Yes**	**No**	**Not Sure**
Obesity	☐	☐	☐
Heart disease	☐	☐	☐
Stroke	☐	☐	☐
High blood pressure	☐	☐	☐
Diabetes	☐	☐	☐

11. HAVE YOU HAD, OR DO YOU HAVE :	**Yes**	**No**	**Not sure**
Palpitations	☐	☐	☐
High blood pressure	☐	☐	☐
Angina	☐	☐	☐
Heart Disease	☐	☐	☐
Stroke	☐	☐	☐
High cholesterol	☐	☐	☐
Diabetes	☐	☐	☐
Renal Disease	☐	☐	☐
Peripheral Vascular Disease	☐	☐	☐
Liver Disease	☐	☐	☐
Gastrointestinal Disease	☐	☐	☐

Thyroid Disease ☐ ☐ ☐

Are you undergoing treatment for any of the above conditions?   **Yes** ☐   **No** ☐

If yes please give details:_____

_____

12. Do you have any condition that may adversely affect yourself or any other person as a result of your participation in this study? (please tick)   **Yes** ☐   **No** ☐

13. Have you ever been a smoker ?   **Yes** ☐   **No** ☐

If no, please go to Q.14

If yes, how long ago did you give up :_____

OR

If you **currently still smoke** how many cigarettes approx. would you smoke in a day:_____

How many years have you been a smoker? _____

14. Do you take any medication regularly?
- Eg: do you regularly use aspirin, lipid and or blood pressure lowering drugs, drugs for diabetes, thyroid or phosphodiesterase inhibitor medication (e.g. Viagra, Levitra) etc?

Yes ☐   No ☐

If yes, please give details: _____

_____

15. Do you regularly take vitamin, mineral, herbal or other dietary supplements?

Eg. Cod liver oil or fish oil capsules, Vitamin C etc.

Yes ☐   **No** ☐

If yes, please give details of dose and frequency of consumption:_____

_____

16. How many cups per day would you drink of the following?

Black Tea ☐   **Green Tea** ☐   **Coffee** ☐   **Decaffeinated Coffee** ☐

17. Do you have any allergies to foods, beverages or drugs?

If yes, please give details of which foods, beverages or drugs:_____

18. Would you consume more than two standard glasses of any alcoholic beverage :

Daily ☐   A few days a week ☐

Once a week ☐   Occasionally ☐

Rarely or never ☐   If yes, type of beverage:_____

19. Do you exercise?

Yes ☐   No ☐

If yes, what sort of exercise do you do each week? (please list responses)

How often do you do this type of exercise?

How long does each session last?

How would you describe your intensity of effort during exercise (light, moderate, hard)?

20. Do you eat fish regularly?

Daily ☐   A few days a week ☐

Once a week ☐   Occasionally ☐

Rarely or never ☐   If yes, type of fish and quantity (e.g 100g):_____

21. Would you be able to attend a 1 hour long visits at week 6 of testing? *

Yes ☐   No ☐

What two days per week would suit you best?

Please list:

_____

22. What time would best suit you for testing?

<u>1 hr Visit</u>
7:00-8:30 am   ☐
8:30-10:00 am   ☐
10:00-11.30 am   ☐
11.30-1:00pm   ☐

*After hours testing may also be available on request

Thank you for your co-operation. We will be selecting a study group of approximately 16 males. Please do not be

offended if you are not chosen, selection into the study is based on a set list of criteria that you may have not met for

whatever reason. We will be notifying everyone in due course, whether they have been selected or not. Those

selected will then be asked to attend The University of Adelaide, PTRC (Medical School South Building, 4th floor) on

2 occasions (over a 6 week period) to take part in the study.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ
Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation
Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an **International Physical Activity Prevalence Study** is in progress. For further information see the IPAQ website.

<u>*More Information*</u>

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at <u>www.ipaq.ki.se</u> and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1.   Do you currently have a job or do any unpaid work outside your home?

☐ **Yes**

☐ No ➡️   ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2.   During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity ➡️ ***Skip to question 4***

3.   How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____   **hours per day**
_____   **minutes per day**

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

4.   Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity   ➡️ ***Skip to question 6***

5.   How much time did you usually spend on one of those days doing **moderate** physical
       activities as part of your work?

_____   **hours per day**

_____   **minutes per day**

6.   During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time
as part of your work? Please do not count any walking you did to travel to or from work.

_____   **days per week**

☐   No job-related walking   ➡   *Skip to PART 2: TRANSPORTATION*

7.   How much time did you usually spend on one of those days **walking** as part of your
work?

_____   **hours per day**

_____   **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work,

stores, movies, and so on.

8.   During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train,
bus, car, or tram?

_____   **days per week**

☐   No traveling in a motor vehicle   ➡   *Skip to question 10*

9.   How much time did you usually spend on one of those days **traveling** in a train, bus,
       car, tram, or other kind of motor vehicle?

_____   **hours per day**

_____   **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from
work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐   No bicycling from place to place   ➡️   *Skip to question 12*

11.   How much time did you usually spend on one of those days to **bicycle** from place to place?

_____   **hours per day**

_____   **minutes per day**

12.   During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐   No walking from place to place   ➡   *Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY*

13.   How much time did you usually spend on one of those days walking *from place to place?*

_____   **hours per day**

_____   **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14.   Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐   No vigorous activity in garden or yard   ➡   *Skip to question 16*

15.   How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____   **hours per day**

_____   **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

[ ]   No moderate activity in garden or yard   ➡   *Skip to question 18*

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____   **hours per day**

_____   **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home   ➡   *Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY*

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____   **hours per day**

_____   **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time   ➡   *Skip to question 22*

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____   **hours per day**

_____   **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____   **days per week**

[ ]   No vigorous activity in leisure time   ➡   *Skip to question 24*

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____   **hours per day**
_____   **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____   **days per week**

[ ]   No moderate activity in leisure time   ➡   *Skip to PART 5: TIME SPENT SITTING*

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____   **hours per day**
_____   **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

LONG LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised October 2002.

_____   **hours per day**
_____   **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____   **hours per day**
_____   **minutes per day**

This is the end of the questionnaire, thank you for participating.

Arterial Compliance
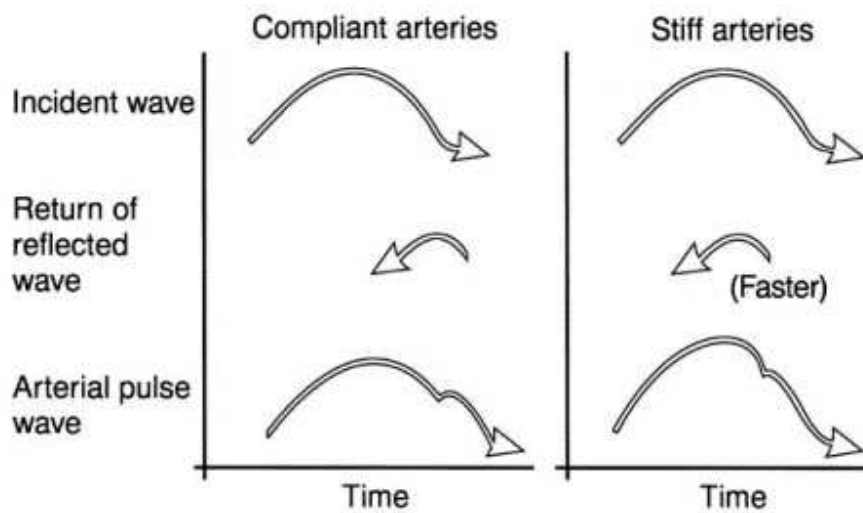
Example of arterial compliance in normal and poor compliance vessels. Adapted from London. G, M et al. Influence of arterial pulse and reflected waves on blood pressure and cardiac function (1999)