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Essential Fatty Acids, Stress Reactivity and Coping

Submitted in January 2001, for completion of PhD in Psychology

by Lorraine A. Paterson.



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Abstract

Essential Fatty Acids (EFAs) are believed to reduce cardiovascular and psychological stress responses. This study aimed to investigate the effects of omega-3, ethyl-eicosapentaenoic-acid (EPA) on cardiovascular and psychological responses to stress both within the laboratory and during everyday activity. Twenty-seven young, healthy, non-smoking males participated in a double blind, placebo-controlled 12-week study. Subjects were randomly allocated to either the EPA or Placebo group, who received vitamin E. Each subject was individually assessed at the beginning and the end of the supplementation period. Subjects provided information on their dietary and exercise habits and completed anxiety, depression and coping questionnaires. Furthermore, they were required to wear an ambulatory blood pressure monitor for one day and attend the laboratory for one morning, where their heart rate and blood pressure were monitored during a series of stress-provoking tasks and a period of cycling. Pre- and post-supplementation blood samples were drawn from each subject. At 4-week intervals the subjects completed and returned anxiety and depression scales. Results indicated that ethyl-EPA supplementation doubled levels of EPA in red blood cells, and caused a compensatory reduction in competing EFAs, whereby a static overall level of fatty acid was maintained. No significant effects of EPA supplementation were found on cardiovascular or psychological responses to stress, either in the laboratory or during ambulation. Although no effects were found, it is possible that this was due to the young, healthy student status of the subjects. Therefore future research should investigate the effects of EPA on cardiovascular and psychological responses to stress, concentrating on populations with high stress levels, depleted EPA levels, and the 35-49 age range where cardiovascular disorders begin to emerge.

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1 Introduction

1.1 Health, Stress and Coronary Heart Disease

In Western society more people suffer from, and die of cardiovascular related diseases than any other disorders, including cancers (American Heart Association, 1991, Higgens & Luepker, 1988, National Heart, Lung and Blood Institute, 1994). The major causes of heart disease, such as smoking, over-eating, lack of exercise, high alcohol consumption, high fat diets and stress, are self-inflicted. In modern, affluent society there has been a radical change in our diets where we now eat only minimal amounts of fish in comparison to the high-fish diets of our foregoing generations. Today's normal Western diet is low in the healthy, unsaturated fats, such as fish oils and high in the unhealthy saturated fats, such as those found in snacks, fast foods, convenience foods and sweets. With our high-speed, high-fat, high-stress life styles our bodies and minds are being placed under more and more strain.

1.2 Essential Fatty Acids

Essential fatty acids (EFAs) are nutrients that are required by the human body. The fats are found in small concentrations mostly in fish oils and vegetable oils. Researchers have found that EFAs are implicated in, and have varied beneficial effects on many health issues. These health benefits range from the reduction of skin inflammation, the easing of diabetic complaints, to the reduction of pain in reproductive disorders (Horrobin, 1992a, Peet & Edwards, 1997). EFAs are also beneficial to the inhibition of cancer growth, the improvement of viral infections, the reduction of symptoms in inflammatory and auto-immune disorders, the attenuation of alcohol withdrawal symptoms and the improved prognosis of various forms of coronary heart disease (Horrobin, 1992a).

1.2.1 Essential Fatty Acids Explained

Essential Fatty Acids (EFAs) are nutrients that, like vitamins, cannot be manufactured within the body and therefore must be provided in our diet, in particular from oily fish. EFAs can be found within the body as unesterified fatty acids or as components of triacylglycerols, cholesterol esters and phospholipids. The biochemical roles and factors that influence EFA transfer are, however, little understood. What is apparent is the structural requirement that all cell membranes within the body have EFAs as a component, and also that EFAs behave as precursors of vital metabolites such as leukotrienes, prostaglandins, hydroxy and hydro-peroxy-fatty acids (Horrobin, 1992a).

Biochemistry

The biochemistry of EFAs has been reviewed extensively by Horrobin (1992a). Structurally, EFAs have at least two double bonds in a carbon chain. The group of EFA, whether omega-3 (n-3) or omega-6 (n-6), is "defined by the position of the first double bond in the molecule starting from the carbon atom at the methyl end of the chain" (Horrobin, 1992a). As an example DHA (docosahexaenoic acid) is written as 22:6n-3, where the number of carbon atoms in the chain is defined by the first number (22) and the number of double bonds is defined by the number after the colon (6). LA (linoleic acid, 18:2n-6) and ALA (alpha-linolenic acid, 18:3n-3) are the parent compounds of the two groups n-6 and n-3 respectively. LA and ALA are metabolised by a series of alternating elongations and desaturations, in which further double bonds and two carbon atoms are introduced at each stage. Omega-6 and omega-3 EFAs compete with each other in this metabolic pathway. Predominantly n-3 EFAs are more effective at displacing the n-6 and are therefore preferentially metabolised.

An EFA can be described as a substance that is both present in food and can reverse the deficiencies caused by a lack of other EFAs in their class, from the diet. In other words, an EFA is a nutrient that is capable of replenishing a deficit of its own class of EFA (omega-3 vs. omega-6), and can also reverse the effects of that deficit. For example GLA (gamma-linolenic acid, 18:3n-6) can reverse n-6 EFA deficiency and can be found in oats, barley and human milk. It is therefore an n-6 EFA. Omega-3 fatty acids,

such as eicosapentaenoic acid (20:5n-3) can replace a deficit of other n-3s and reverse the effects of the deficiency. Omega-3s are predominately found in fish oils, such as mackerel, salmon, tuna fish, as well as seeds, nuts and some vegetables and the oils derived from them. It should be noted that omega-6s have the additional benefit of being capable of reversing omega-3 deficiencies as well as their own class deficiencies. People deficient in fatty acid concentration often show physical symptoms of increased thirst and fluid consumption, frequent urination, dry skin and dry hair, brittle nails, dandruff and follicular keratosis (Stevens et al., 1995).

Functions and Impact

Omega-6 EFAs have four known roles, with the modulation of membrane structure perhaps being the most important. The concentration of EFA and the number of double bonds in the molecule influence the flexibility and fluidity of all membranes in the body. However, although n-3 EFAs have at least the same number of double bonds as n-6 EFAs, they are unable to reverse the features of n-6 deficiency.

A second role of n-6 EFAs is the formation of short-lived, local regulating, biologically active molecules such as leukotrienes and prostaglandins. A third role is that of regulation of the water impermeability of the skin and conceivably that of other membranes such as the blood-brain barrier. Lastly n-6 EFAs are known to regulate cholesterol transport and synthesis, hence an abnormal EFA metabolism or deficiency could be detrimental to every cell and organ of the body (Horrobin, 1992a).

Omega-6 EFA concentrations can be affected by many factors: high cell division in the presence of cancer or inflammation; increased oxidation of EFAs for energy; high intake of non-EFAs such as trans and positional non-EFA isomers; interference with LA metabolism, as occurs in diabetes, and zinc and calcium deficiencies; and gender. Males appear to require a higher intake of EFAs than women do for normal functioning, therefore a deficit of fatty acid would be expected to have a greater impact on males than females (Pudelkewicz, Seufert, & Holman, 1968).

1.3 EFAs and Psychological Functioning

1.3.1 Learning and Developmental Problems

It is known that infants require essential fatty acids for normal growth and development of the brain, eyes, nervous system and all other tissues (Ballabriga, 1994, Birch, Garfield, Hoffman, Uauy, & Birch, 2000, Burr & Bur, 1930, Neuringer & Connor, 1985, Wainwright, 1992). Studies have shown that development of pre-term and low birth-weight infants benefit from n-3 fatty acid supplemented formulas. In particular, n-3 fatty acid supplementation helps the development of the brain in these populations (Carlson et al., 1991, Clark, Madrises, Neumann, & Gibson, 1992, Innis, Foote, MacKinnon, & King, 1990, Makrides, Neumann, Simmer, Pater, & Gibson, 1995, Neuringer, Anderson, & Connor, 1988). There is also evidence that omega-3 supplementation can benefit those with behavioural, learning, cognitive or attentional disorders. For example, Stevens et al. (Stevens, Zentall, Abate, Kuczek, & Burgess, 1996, Stevens & Bugress, 1999) found that young boys with behaviour and learning problems had deficiency of omega-3 fatty acids in comparison to normally behaved boys. The boys deficient in omega-3 showed learning and health problems together with, sleeping problems and temper tantrums. Furthermore Stevens et al. (1995), found that boys diagnosed with attention-deficit hyperactivity disorder had significantly lower blood concentrations of fatty acids than a control group.

It has also been suggested that neuro-developmental disorders, such as dyslexia and dyspraxia are related to omega-3 deficiency (Puri & Richardson, 1999, Richardson et al., 1999). Dark adaptation has even been found to improve in dyslexics following EPA supplementation (Stordy, 1995). Furthermore, Stordy has posited a model of dietary management, rich in omega oils, for dyslexic and dyspraxic sufferers (Stordy, 1999). It is not only the development of psychological functions that are impacted by EFAs, but also their decline with ageing. Yehuda et al. (Yehuda, Rabinovitz, Carasso, & Mostofsky, 2000) found that Alzheimer's disease (AD) could be linked to the depletion of fatty acids within the body. Yehuda proposed that intense stress produces cortisol damage, that leads to the cognitive decline witnessed in Alzheimer's. Thus, he

postulated that a fatty acid mixture could modulate the stress effects on AD. In support, of this notion, Gattaz et al. (Gattaz, Maras, Cairns, Levy, & Forstl, 1995, Gattaz et al., 1999), demonstrated that reduced metabolism and activity of fatty acids is evident in the brains of Alzheimer's sufferers.

1.3.2 Schizophrenia

The importance of omega oils in development and learning has led to investigation into their effects and implications in other psychological disorders, such as schizophrenia (Horrobin, Glen, & Hudson, 1995). There is a wealth of research that relates schizophrenia to depleted levels of fatty acids (Gattaz, Kolliisch, Thuren, Virtanen, & Kinnunen, 1987, Gattaz, Hubner, Nevalainen, Thuren, & Kinnunen, 1990, Horrobin, 1998a, Mellor, Laugharne, & Peet, 1995, Vaddadi et al., 1996, Yao, Leonard, & Reddy, 2000). The development of schizophrenia has been linked to the early depletion and inefficient metabolism of omega-3 oils and the associated developmental deficiencies (Crawford et al., 1997, Horrobin, Glen, & Vaddadi, 1994, Horrobin, 1998a, Horrobin, 1992b, Rifkin, Lewis, Jones, Toone, & Murray, 1994, Ross, Hudson, Erlich, Walsh, & Kish, 1997). Further to this research studies have found that the prevalence of schizophrenia is related to the variation in the intake of dietary fat (Christensen & Christensen, 1988). The Christensens verified that 98% of the variation in the development of schizophrenia could be explained by variations of fat intake. Undeveloped countries where the diet was high in fibre and low in fat (predominately derived from vegetables and seafood) had a less severe course of the disease than developed countries whose diets consisted of fat, from land animals and birds. Not only can schizophrenia be linked to high fat diets, but it has also become more prevalent since the decline of healthier dietary habits (Christensen & Christensen, 1988, Horrobin, 1998b).

Multiple studies have investigated the effects of omega oil supplementation on schizophrenic symptoms. For example, Peet et al. (Peet, DeLaugharne, & Mellor, 1997a) provided a small group of sufferers with either EPA (eicosapentaenoic acid) or DHA. They found sufferers positive symptoms were reduced by a clinically defined level of intensity, after EPA supplementation. Puri et al., (2000) found that after 8-

weeks supplementation with EPA both the positive and negative symptoms of schizophrenia had reduced in sufferers, with optimal effects occurring after 12-weeks supplementation. Several studies have found results supporting the two examples above (Mellor & Peet, 1998, Mellor et al., 1995, Mellor, Laugharne, & Peet, 1996, Mahadik & Evans, 1997, Peet & Mellor, 1998, Puri et al., 2000). Further studies have shown that the reduction of schizophrenic symptoms by EPA have been maintained over time (Puri, 1998, Puri et al., 2000). Evidence of the beneficial effect of EPA is so strong that niacin skin tests¹ are being developed to diagnose psychiatric patients susceptible to omega-depleted schizophrenia (Mahadik, Shendarkar, Scheffer, Mukherjee, & Correnti, 1996b, Mahadik et al., 1996a, Ward, Sutherland, Glen, & Glen, 1998).

1.3.3 Depression

How we feel emotionally has a large impact upon our physical and mental health. Clinical depression is on the increase along with the prescription of anti-depressant drugs to combat it. However researchers have found a link between depleted omega-3 levels and depression (Hibbeln & Salem, 1995, Hibbeln, Umhau, George, & Salem, 1997, Maes & Smith, 1998) that may lessen the need for psycho-tropic drugs and their known side effects, such as increased risk of myocardial infarction (Pratt et al., 1996). Hibbeln and Salem (1996), have posited that omega-3 acids are protective against depression, and if omega-3 can be increased in the diet then the incidence of major depression would be lower than in cultures where omega-3 was not an important element of the diet. A number of dietary and epidemiology studies have positively supported this prediction. High rates of depression have been noted in the USA where fish consumption, hence omega-3 acid intake is low (Blazer & Williams, 1980, Jonnalagada, Egan, & Heimbach, 1995, O'Hara, Kohout, & Wallace, 1985, Krause & Liang, 1992, Zung, 1967). Meanwhile in Japan where fish consumption is high, resulting in large intakes of omega-3 acids, levels of depression and the associated symptomology are low (Hasegawa, 1985, Sarai, 1979). Although considerable research tells us that other social and cultural factors influence the depression statistics, such as stress, the correlational data from these studies are strong. Recent clinical studies have

¹ When administered niacin produces a skin flushing in normal individuals whereas flushing is greatly reduced in schizophrenic patients.

found evidence to support the link between lack of n-3 intake and depression (Hibbeln et al., 1998, Maes et al., 1996, Peet, Murphy, Shay, & Horrobin, 1998, Peet & Mellor, 1998). Furthermore dietary supplementation with n-3 has revealed a lessening of depressive symptoms and actual remission periods in bipolar depressives (Hamazaki et al., 1996, Stoll et al., 1998).

1.3.4 Summary

There is strong research evidence that essential fatty acids not only play a large part in physiological health and development but also in psychological development and well being. Depleted levels of fatty acids have been linked with learning, cognitive, behavioural and attention development disorders as well as being associated with cognitive decline such as Alzheimer's disease. There is also strong evidence that psychological disorders such as schizophrenia and depression are more prevalent when omega-3 intake is low and that these disorders show an improvement after omega-3 supplementation.

The influence of omega-3 levels on depression has strong implications for the impact of depression on stress responses and cardiovascular functioning (Peet & Edwards, 1997, Pratt et al., 1996) as well as other health conditions such as chronic back pain, rheumatoid arthritis and asthma (Herbert & Cohen, 1993). Carney et al. (1988) found that depression was related to heart rate and heart rate variability in coronary heart patients (Carney et al., 1988). In support of these findings, Krittayaphong et al. (1997) demonstrated that depressive state negatively related to heart rate variability in coronary patients (Krittayaphong et al., 1997). Furthermore, depressive state has been identified as an indicator of future heart rate and blood pressure levels (Thornton & Hallas, 1999). Depression is now considered as a risk factor for coronary heart disease (Carney, Freedland, Rich, & Jaffe, 1995).

1.4 *EFA*s and Coronary Heart Disease

Elevated cholesterol and triacylglycerol levels, hypertension² and enhanced platelet aggregation are the four main accepted biological factors associated with coronary heart disease (CHD) and peripheral vascular disease (PVD). However a stronger association than any of the above can be made with inadequate levels of EFAs and high risk of coronary heart disease.

1.4.1 Inadequate EFA levels

Sinclair, as early as the 1950s, suggested that inadequate intake of n-6 EFAs may be the underlying mechanism which accounts for both CHD / PVD and the associated factors³ (Sinclair, 1958). Sinclair has since stated that an n-3 EFA supplement provided a protective effect in Eskimos, indicating that an optimal approach would combine both n-3 and n-6 EFAs (Horrobin, 1995). Population studies have shown that high risk of coronary heart disease is associated with low intake of omega-3 (McLennan, 1993, McLennan, Abeywardena, & Charnock, 1985, McLennan, Abeywardens, & Charnock, 1988). Furthermore, coronary heart disease patients show inappropriate omega-3/omega-6 ratios. It may be the fatty acids lower down the metabolic pathway (e.g. EPA and DHA) that are more directly involved in cardiovascular disease risk, than the parent compounds themselves (e.g. ALA). For example, low concentrations of both DGLA (dihomogamma-linolenic acid, 20:3n-6) and AA (arachidonic acid, 20:4n-6) in plasma and adipose tissue are also strongly indicative of coronary heart disease, rather than the parent compound. Therefore inadequate intake of the two parent compounds (LA and ALA) and, more directly, the poor metabolism may predispose an individual to a high risk of coronary heart disease, and the associated risk factors. To illustrate this point, GLA and DGLA (metabolised from the parent compound LA) can lower blood pressure in animal models of stress-related or spontaneous hypertension more effectively than LA can. There is evidence of defective desaturation of both LA and

² Abnormally high blood pressure.

³ Elevated cholesterol levels, elevated triacylglycerol levels, hypertension and enhanced platelet aggregation.

ALA in studies of human patients with hypertension. Gray (1988) summarised in Horrobin (1992a), and Venter et al., (1988) found Efamol (GLA) lowered blood pressure in humans with mild hypertension and lowered stress-related blood pressure increases more effectively than n-3 EFAs (Venter, Joubert, & Booyens, 1988). Secondly GLA and less effectively LA (parent compound), lowered cholesterol levels. Antithetically, high cholesterol levels will inhibit EFA desaturation. Additionally n-3 rather than n-6 EFAs lower triacylglycerol levels. Furthermore GLA, DGLA and EPA (eicosapentaenoic acid, 20:5n-3) effectively inhibit platelet aggregation (Leeds, Gray, & Ahmand, 1990). Horrobin suggested that both EPA and GLA, even at low doses, have synergistic effects (Horrobin, 1992). Other omega-3 supplementation studies have also shown benefits in coronary heart patients such as decreased morbidity and lessened severity of secondary heart attacks and lowered blood pressure (Christensen et al., 1997, Gustafsson, Ohrvall, Ekstrand, & Vessby, 1996, Leaf & Kang, 1996, Metha, Lopez, Lawson, Wargovich, & Williams, 1988, Mills, Prkachim, Harvey, & Ward, 1989a). Also, as previously mentioned depressive symptoms are considered a large factor in poor CHD recovery, but these depressive symptoms can be decreased with omega-3 supplementation (Carney et al., 1988, Carney et al., 1995, Krittayaphong et al., 1997, Peet & Edwards, 1997, Thornton & Hallas, 1999).

1.4.2 Summary

A currently influential hypothesis is that deficient levels of both n-3 and n-6 EFAs, combined with inadequate desaturation / metabolisation, account for both coronary heart disease and its biochemical risk factors.

1.5 EFAs and Cardiovascular Reactivity to Stress

1.5.1 Animal studies

Several studies have investigated the effects of administration and supplementation with n-3 and n-6 fatty acids on cardiovascular responses to acute and chronic stress in

animals. Eight weeks DHA supplementation in rats induced cardiovascular alterations that could be detected in conscious animals. These alterations, evident after only a few weeks supplementation, were noted as reduced heart rate, systolic blood pressure and diastolic blood pressure (Rousseau et al., 1998). Non-esterified EPA (n-3) and GLA (n-6) were administered inter-peritoneally via an osmotic pump to normotensive rats. EPA augmented heart rate reactivity while attenuating pressor⁴ reactivity to stress (50% as effectively as GLA) (Mills, 1990). GLA lowered resting blood pressure (after 2 weeks administration) and attenuated cardiovascular responses in rats with isolation induced stress. The rats showed a decrease in blood pressure⁵ throughout administration. Their resting heart rate also decreased for the control period. After 3 weeks the rats showed reduced stressed heart rates and after four weeks decreased responses to angiotensin⁶ (ANG) (Mills & Ward, 1986a, Mills & Ward, 1986b). In contrast, Mills et al. found that EPA, administered in triglyceride form and 100-fold the dose of the previous study, had no effect on reactivity while GLA again attenuated it (Mills, Ward, & Huang, 1989b). This suggests different mechanisms of action and sites for n-6 and n-3 EFAs (Mills, 1990).

1.5.2 Human studies

There is a paucity of research into the effects of EFAs on human stress reactivity, and that which has been published is inconsistent. Singer et al. (Singer, Wirth, & Voigt, 1985) found that 2-week periods of n-3 supplementation had no effect on blood pressure or norepinephrine⁷ reactivity to acute psychological stress, although a fish oil group (mackerel - high 20:5n-3 and 22:6n-6) exhibited lower resting blood pressure after 2-weeks. Similarly four weeks supplementation of 30 normotensive college

⁴ Pain endurance, evoked by submersing limb in ice water.

⁵ Unclear but it is thought to be systolic blood pressure, with no reference made to diastolic.

⁶ Hormone which raises blood pressure by constricting small blood vessels, releasing Aldosterone and stimulating the brain, kidneys and sympathetic nervous system. Can lead to increased blood pressure.

⁷ US term for noradrenaline, a hormone which raises blood pressure and heart rate.

students, with fish oil (20:5n-3 & 22:6n-3) had no effect on blood pressure, heart rate or catecholamine⁸ reactivity to psychological stressors (Mills, 1990).

In contrast, Mills found that supplementation with borage oil (18:3n-6) attenuated pressor and heart rate responses to tasks (Mills et al., 1989a). Mehta et al. found n-3 fish oil reduced blood pressure and heart rate responses to physical exercise while reducing resting systolic blood pressure (SBP), suggesting an attenuation of reactivity (Metha et al., 1988). An unpublished study (reported by Mills, 1990) revealed that, after 4-weeks of supplementation with borage oil, exposure to a lower body negative pressure (LBNP) of 40mm Hg resulted in augmented catecholamine and vascular responses. This suggests a shift in baroreceptor⁹ sensitivity with borage oil. However, fish oil (EPA & DHA) supplementation produced no effects on cardiovascular or neuroendocrine reactivity in response to LBNP, again suggesting differing mechanisms.

Furthermore, both Olive Oil and Sunflower Oil were found to reduce systolic and diastolic blood pressure (DBP) in healthy men after 4-weeks supplementation (Espino-Montoro et al., 1996). Three-weeks supplementation with n-3 fatty-acids have been shown to produce reductions in SBP, serum triglycerides (25%) and an increase in long-chain fatty acids in the serum phospholipids, in healthy men and women (Gustafsson et al., 1996). Several other studies have supported this reduction in blood pressure and heart rate in humans after omega-3 supplementation (Diboune et al., 1993, Hamazaki et al., 1998, Mills et al., 1989a, Yehuda et al., 2000).

⁸ Catecholamines are a group of hormones that derive from catechol. For example adrenaline and noradrenaline, which are secreted in response to stress and act to increase heart rate and blood pressure.

⁹ Receptors which respond to increases in blood pressure in the arteries, by sending parasympathetic input into the heart to slow it down.

1.6 *EFAs and their Effects on Coronary Heart Disease*

1.6.1 EFAs and Hypertension

Animal Studies, and Sodium Effects

Hypertension, induced in rats by replacing their drinking water with 1% saline was moderately attenuated by diets supplemented with either LA (n-6), EPA (n-3) or DHA (n-3) while evening primrose oil (LA & GLA) completely prevented the hypertension. In contrast, following administration of 1.5% saline solutions, fish oil augmented the pressor response in the rats. Retrospective studies of salt intake in human hypertensives suggest that sodium effects resting blood pressure and that n-3 fatty acids are unable to protect against the pressor effects of the sodium (Mills, 1990). However non-sodium induced hypertension can be reversed in rats by n-3 supplementation (Hui, St-Louis, & Falardeau, 1989).

Other studies have shown that the antiarrhythmic¹⁰ effects of free n-3 EFAs are associated with an inhibition of the sodium (Na⁺) channel. Kang et al. (Kang, Yunyuan, & Leaf, 1997) found that neonatal rat cardiac myocytes treated with Mexiletine (class I antiarrhythmic drug) had a 4-fold increase in toxic specific binding to cells while there was no significant effect on toxic binding in those rats treated with EPA. Rats treated with both EPA and Mexiletine showed a 40-50% reduction in toxic binding, compared to that of Mexiletine alone. This suggests that n-3 polyunsaturated fatty acids (PUFAs) / EFAs when used chronically do not mimic the toxic effect of class I antiarrhythmic drugs despite their similar blocking effect on the sodium channels. Hence chronic EPA treatment not only does not up-regulate the cardiac sodium channel expression but also reduces the mexiletine-induced increase in cardiac sodium channel expression. The practical implication here is that EPA may provide a therapeutic benefit but without the side effects of existing drug treatments.

¹⁰ Regulation of an irregular heart beat.

Human Studies

Essential fatty acids have also been shown to provide beneficial effects to the sufferers of coronary heart disease. Hypertensives treated with Alsepa fish oil (EPA & DHA) show reduced SBP and DBP. Repeated fasting and refeeding with Alsepa also facilitated the exchange of n-3 for n-6, suggesting that fish oil should be considered where rapid exchange of n-3 for n-6 is necessary (Yosefy et al., 1996). However, mild essential-hypertensives treated with 3grams (g) of EPA and DHA showed no significant changes in SBP, DBP or heart rate and no significant variations in blood pressure or heart rate variability. Yosefy concluded that **low** doses of n-3 as a single treatment may not be effective in altering variability nor lowering blood pressure or heart rate in mild hypertensives (Russo et al., 1995).

1.6.2 EFAs as Antiarrhythmic

Various animal and human studies have shown EFAs to have strong anti-arrhythmic properties (Kang & Leaf, 1996, Leaf & Kang, 1997, Weyland, Kang, & Leaf, 1996). Patients with low-grade ventricular premature complexes (VPC) were treated with a fish oil complex (1.5g EPA & 0.9g DHA) or sunflower seed oil (5g of LA defined as a placebo) for 16-weeks. The fish oil group had a 48% reduction in VPCs, with 44% of the patients having a 70% or greater reduction. The sunflower group had only a 25% reduction in VPCs. Couplets¹¹ and triplets¹² were also reduced by more than 80-90% in 54% of the fish oil patients as opposed to 44% of the sunflower group (Sellmayer, Lorenz, & Weber, 1996). A one-year trial of fish oil (EPA) compared to mustard oil (ALA), in suspected acute myocardial infarction patients, revealed that both reduced the total number of cardiac events. Reductions in arrhythmias¹³, left ventricular enlargement, angina pectoris¹⁴ and diene conjugates¹⁵ were found. The only significant

¹¹ Couplet refers to two ectopic (extra beats) heartbeats followed by a single normal heartbeat.

¹² Triplet refers to three ectopic heartbeats followed by a single normal heartbeat. Couplets and triplets should not be mistaken for bi- or tri-gemini beats, which refer to two and three ectopic beats followed by two and three normal beats.

¹³ An irregular heart beat.

¹⁴ Chest pain, due to insufficient oxygen being carried to the heart muscle in the blood supply.

difference between the groups was in cardiac deaths. The EPA group had fewer deaths than the ALA - 11.4% vs. 22%. Reductions in the blood lipoproteins were small and did not appear to be the cause. The "diene conjugate reduction suggests the benefit of n-3 may in part be due to a reduction in oxidative stress" (Singh et al., 1997).

Oxidation

Swislocki and Eason demonstrated that free fatty-acid levels are elevated in spontaneously hypertensive rats (SHR). Etomoxir, an inhibitor of fatty-acid oxidation, increased the levels and responses of the fatty acid in the SHR and produced a dramatic decrease in blood pressure. This suggests that inhibition of free fatty acid oxidation with etomoxir may lead to improved hypertension in the SHR (Swislocki & Eason, 1994).

Calcium

Studies have shown that calcium (Ca^{2+}) release often underlies cardiac arrhythmias. Extracellular application of EPA to adult and neonatal rat ventricular myocytes produced a prompt and reversible concentration-dependent suppression of voltage-gated L-type calcium current ($I_{\text{Ca,L}}$). It was concluded that PUFAs / EFAs may act as antiarrhythmic agents in vivo on normal and calcium-overloaded cells principally because they reduce calcium entry by blocking voltage-gated L-type calcium ($I_{\text{Ca,L}}$). They also directly decrease voltage-gated sodium current (I_{Na}) and $I_{\text{Ca,L}}$ but indirectly reduce the calcium transients and activate membrane current. Hence, it is clear that by reducing voltage-gated L-type calcium current ($I_{\text{Ca,L}}$), voltage-gated sodium current (I_{Na}) and calcium sparks, PUFAs can reduce spontaneous extra-systoles in the heart rhythm (Xiao, Gomez, Morgan, Lederer, & Leaf, 1997).

Furthermore, animal studies have shown that prolonged n-3 enrichment can almost completely prevent fatal ventricular fibrillation¹⁶ (VF) in most patients. Billman et al. (Billman, Hallaq, & Leaf, 1994) found that one hour after intravenous infusion of n-3, VFs were reduced from 100 to 13%. Thus indicating that circulating or lipoprotein-

¹⁵ Pair of carbon double-bonds linked by a single bond.

¹⁶ Uncontrollable twitching of the ventricle muscle fibre, which does not affect the entire muscle.

bound n-3 PUFA may be antiarrhythmic before it has been incorporated into membrane phospholipids. Weyland et al.'s (1996), antiarrhythmic study supports Billman, and concludes that n-3s are beneficial at a pre-phospholipid state (free acids).

Variability

Decreases in heart rate variability are strongly associated with increased mortality in postmyocardial infarction patients. Christensen et al. (1996) found that a supplement of omega-3 essential fatty acid (5.2g of eicosapentaenoic acid and docosahexaenoic acid) increased heart rate variability in these patients. Hence the increased parasympathetic cardiac tone, reflected in the increased heart rate variability, increases the ventricular fibrillation threshold and protects the myocardium against ventricular arrhythmias, improving prognosis. This illustrates n-3 EFAs antiarrhythmic effect as well as the reduction in the risk of cardiac arrest. Heart rate variability is positively associated with the content of n-3 PUFA / EFA in cell membranes, supporting the view that intake of n-3 PUFA may protect against sudden cardiac death (Christensen et al., 1997).

Paradoxically, Siegrist and Klein observed that decreases in heart rate variability can also occur after sustained autonomic activation *due to chronic stress* (Siegrist & Klein, 1990). This effect was observed when workers with high levels of chronic occupational stress exhibited lower maximal heart rate and blood pressure elevations under psychological challenge, irrespective of age, test performance, smoking status or hypertensive-state. It is possible then that an optimum level of stress exists, beyond which EFAs would have limited additional beneficial effects. A second theory is that EFAs¹⁷ may act to reduce reactivity by a reduction in central sympathetic activity (as suggested by Mills et al., 1989b) and an increase in parasympathetic activity through modification of baroreceptor functioning.

¹⁷ 18:3(n-6).

1.6.3 Suggested Mechanisms of EFAs

*Pressor Mechanisms*¹⁸

Various studies have investigated the effects of n-3 EFAs on cardiovascular reactivity to pressor hormones / agents as a possible mechanism of their effects on the cardiovascular system. Human studies appear to support this view. Supplementation with cod liver oil for 25-days decreased SBP responses to norepinephrine while producing no change in pressor reactivity to angiotensin (Mills, 1990). A second study administered mild hypertensives with a menhaden oil supplement (n-3) for 28-days. Menhaden also produced no significant change in pressor response to phenylephrine¹⁹, but control subjects (fish oil) tended to "develop bradycardia, suggesting the possible involvement of the central regulatory mechanisms". Evening primrose oil administered to pregnancy-induced hypertensives²⁰ attenuated diastolic pressor responses to ANG (Mills, 1990). Animal studies show that the effects of EPA (20:5n-3) on vascular responses to norepinephrine and serotonin are unclear. However, in rats EPA (as either fish oil or pure in form) "attenuates pressor responses to ANG II infusion" (Mills, 1990).

These results suggest that n-3 fatty-acid effects on vascular reactivity to pressor agents may play a role as a possible mechanism but, like blood pressure regulation itself, it is a highly complex process requiring the activity of many parts of the bodily system. More concrete is the antiarrhythmic effect of n-3 fatty acids and their possible antiarrhythmic mechanisms.

¹⁸Pain endurance often induced by submersing an arm or hand in a circulating bath of ice water.

¹⁹ An α -adrenergic agonist with minimal direct cardiac effects.

²⁰ Pre-eclampsia.

Antiarrhythmic Mechanisms

Omega-3 EFAs are believed to reduce cardiovascular mortality due to their antiatherogenic²¹, antithrombotic²² and vasodilatory²³ effects. It was long believed that this protective effect was due mainly to a slowing or regression of atherosclerosis²⁴. However, the beneficial effect of n-3 in humans and animals has been found to occur too quickly²⁵ to be the result of slowing of atherosclerosis - DART & Lyon Diet Heart Studies (Burr et al., 1989, DeLorgeril, Renauld, & Mamelle, 1994). Hence, their antiarrhythmic properties have been identified and the possible mechanisms derived from neonatal rat cardiac myocyte studies. These studies suggest that the mechanisms take the form of electrophysiological effects and increased myocardial oxygen supply. The n-3 electrophysiological effects involve the reduction of electrical excitability of cardiomyocytes; the increase in the threshold for action potentials and they also lead to a more negative resting membrane potential and prolong the refractory period twofold. These effects are associated with a change in ion transport across the plasma membrane and modulation of the L-type Ca²⁺ (calcium) channels. The secondary mechanism is that of increasing the myocardial oxygen supply. This is achieved by their ability to reduce the formation of vasoconstrictive eicosanoid thromboxane A₂; leaving unchanged vasodilatory prostacyclin I₂ and I₃ generation; increasing nitric oxide synthesis while decreasing prothrombotic factors e.g. platelet aggregability. Therefore the vascular response should shift towards that of an antiaggregatory, antithrombotic and vasodilatory state, increasing the myocardial oxygen supply (Sellmayer et al., 1996).

1.6.4 Summary

Findings to date on EFAs are complex and inconclusive. However, through the mist, the protective effects against coronary heart disease and occasionally the desired effect of reduced reactivity can be observed. Furthermore there is empirical evidence linking

²¹ The prevention of fatty deposits forming within inner lining of artery that obstructs blood flow.

²² The prevention of blood clots forming within the heart.

²³ Dilatation of the blood vessels walls.

²⁴ Degenerative disease of arteries, where fatty deposits form on the arteries inner linings.

²⁵ Weeks vs. months.

EFA levels with psychological dysfunction, such as schizophrenia, attention deficit hyperactivity disorder and depression.

Our understanding of the relationship between n-6 and n-3 fatty acids and the regulation of heart rate and blood pressure by the cardiovascular system is limited. We can surmise however that n-3 and n-6 fatty acids differ in their sites and mechanisms of action. Omega-6 fatty-acid mechanisms function in their modification of cardiovascular activity. Omega-3 fatty-acids include peripheral mechanisms and exert their effects on *baseline* cardiovascular function, while n-6 fatty-acids are more likely to affect cardiovascular reactivity in humans than n-3. It is evident from the research that any beneficial effects of EFAs on coronary heart disease and cardiovascular variability and reactivity to stress stem from a balance of n-6 and n-3 levels in the body. However, most studies have assumed that the biochemical effects of EFAs are direct chemical outcomes without consideration of the impact on psychological outcomes. It is possible that the beneficial effects of EFAs may be in part due to their ability to influence how the individual interacts with, and evaluates stressors in the environment. Just because something has a strong biochemical role this does not preclude it from also having a psychological one.

1.7 The Research Aims and Questions

1.7.1 The Research Aims

The first aim of this study was to investigate if omega-3 supplementation would reduce physiological and psychological responses to stress in humans.

The second aim was to investigate if these physiological and psychological responses are mediated or moderated by alterations in general coping style.

1.7.2 The Research Questions

The research questions were:

1. Is there an effect of omega-3 supplementation on the cardiovascular systems response to stress?
2. Is there an effect of omega-3 supplementation on psychological response to stress?
3. Is there a dissociation in the effects of omega-3 between physical (physiological) and psychological (mood) stress responses?
4. What is the correlation between the effects of omega-3 on laboratory stress responses and those evoked in more naturalistic environments?
5. If the omega-3 has an effect, what mediates/moderates it? Does the omega-3 work via a psychological or physiological mechanism? If the omega-3 has a desired effect (reduction) on reactivity to stress can it be said that the effect is due to:

Physiological dampening of the cardiovascular system alone?

Or physiological dampening in conjunction with an improvement in mood felt?

Or a factor of both the above and explained / mediated by improved coping styles?

1.7.3 The Supplement

The supplement will be eicosapentaenoic acid - (C20:5n-3), an omega-3 fatty acid. I chose to use an omega-3 acid instead of an omega-6 for several reasons. Although it is believed that omega-6 has a greater effect on cardiovascular reactivity than omega-3, which impacts upon cardiovascular functioning, it is the *balance* of both acids which produces optimal levels, for health and functioning. Hence too much omega-6 in the body could detrimentally influence cardiovascular functioning, by decreasing omega-3 levels. Although omega-3 acids are preferentially metabolised over omega-6s, the body preferentially retains omega-6 acids. Therefore if too much omega-6 is consumed the body's natural ability to metabolise it will become inadequate and deficiencies in omega-6 can occur. Furthermore, the evidence to date suggests that it is omega-3s that are implicated in psychological well being.

1.8 Glossary of Abbreviations

AA	Arachidonic acid
AD	Alzheimer's disease
ALA	Alpha-linolenic acid
ANG / ANG II	Angiotensin
CHD	Coronary heart disease
DBP	Diastolic blood pressure
DGLA	Dihomogamma-linolenic acid
DHA	Docosahexaenoic acid
EFA _s	Essential fatty acids
EPA	Eicosapentaenoic acid
GLA	Gamma-linolenic acid
LA	Linoleic acid
LBNP	Lower body negative pressure
n-3	Omega-3
n-6	Omega-6
PUFA	Polyunsaturated fatty acid
PVD	Peripheral vascular disease
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rat
VF	Ventricular fibrillation
VPC	Ventricular premature complexes

2 The Cardiovascular System and Stress

2.1 *The Cardiovascular System*

It is necessary to discuss how the research questions will be approached and to understand the relevant concepts used, such as the cardiovascular system, stress, reactivity, coronary heart disease, emotion and coping. Furthermore an introduction to the experimental techniques applied is required.

2.1.1 The Cardiovascular Cycle

The cardiovascular system functions to circulate blood around the body, to transfer waste products and to provide the oxygen and nutrients that are needed to sustain the living tissues contained within the body. This is achieved through a highly complex system. The following description provides a simplified picture.

The heart contains four chambers: the right and left atrium; and the right and left ventricle. Blood returning from circulation in the body enters the right atrium from where it is passed to the left ventricle. The blood is then forced into the pulmonary artery through which it is sent to the lungs to obtain oxygen. Once oxygenated it returns via the pulmonary vein to the left atrium of the heart. From there it is passed into the left ventricle and ejected into the aorta, and with that into the body's arteries. The regular sequence of contraction and relaxation of the heart muscle, which opens and closes the connecting heart valves, governs this entire motion of blood. Hence, the contraction and relaxation phases are what constitute the cardiac cycle, which produces the systolic²⁶ and diastolic²⁷ blood pressures. This cycle is commonly known as the heart beat, and the heart rate is calculated conventionally as the number of heart beats in

²⁶ Maximum pressure from contraction of heart, during which blood is pumped to the aorta and arteries.

²⁷ Minimum pressure from dilation of the heart chambers following contraction during which the chambers refill with blood.

a minute (bpm). A normal heart beats approximately 70 times per minute for 70 or more years: approximately 2.5 billion heartbeats in a lifetime (Turner, 1994).

2.1.2 Stress

Stress is a concept that has been of medical interest for decades but has only of late become a factor researched in everyday life. The terms stress and strain have long been used in the physical sciences. Cannon (1927, 1929, and 1935) was the first to use the terms in a non-engineering context. He viewed stress as a cause of medical problems postulating that emotional stress could cause considerable physiological alterations (Carroll, 1992). Hans Seyle (Seyle, 1956, Seyle, 1976) posited that any kind of stressor resulted in the same pattern of physiological activation which is called the general adaptation syndrome and consists of the three stages of alarm, resistance and exhaustion. However it is now known that humans do not adhere to such a rigid model, instead responses vary from individual to individual and situation to situation. Lazarus and colleagues (Lazarus, 1966, Lazarus & Folkman, 1984) concentrated on the psychological component of stress. Their research lead to the conclusion that in order to evoke a stress response an individual must perceive the situation as threatening. Therefore stress can be regarded as a process where environmental occurrences challenge a person's (or organisms) well being and evoke a response based on the person's interpretation of the challenge. Hence, the forgoing decades of research have lead to the conclusion that when referring to stress researchers must consider both the internal (biological and psychological) and external (stressors) occurrences to achieve the full picture (Turner, 1994).

2.1.3 Experiencing Stress

Stress is experienced when personal and situational demands exceed resources. Stress can take many forms including occupational stress, university and school achievement stress, family stress, personal stress and be physiological in nature, such as extreme hunger, exhaustion and thirst. Stress can lead to problems such as psychological and

physical illness, aggressive behaviour, conflict and anxiety (Perna, Schneiderman, & LaPerriere, 1997).

Originally *stress* was defined in the field of physics as the mechanical force acting on a body that causes strain or deformation. In psychophysiology, situations perceived as stressful or threatening lead to physiological arousal, a flight or fight reaction (Cannon). Lazarus and Folkman (1984), posited that stress occurs when demands exceed a person's ability to adjust. They found empirical evidence of physical stressors such as hunger and psychosocial stressors, for example anxiety. Minor daily hassles can also lead to a person experiencing stress (DeLongis, Coyne, Dakof, Folkman, & Lazarus, 1982, DeLongis, Folkman, & Lazarus, 1988). Physiological reactions to stress include increased blood pressure, tension headaches and stomach aches, especially when we focus on our basic anxieties: negative emotional experiences.

The concept of anxiety originated in the classical Greek period and can be conceptualised in a number of ways. Lewis (1970), defined anxiety "as an emotional state, with the subjectively experienced quality of fear or a closely related emotion", noting that the emotion is negative, unpleasant, out of proportion to the threat, and directed towards the future while involving subjective and manifesting bodily disturbances. Anxiety has been considered as a drive, a motive, a stimulus, a response or a trait. Spielberger (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) introduced some conceptual clarity by making the distinction between trait anxiety - an individuals' predisposition to respond; and state anxiety - a transitory emotion characterised by consciously perceived feelings of apprehension, tension and dread and accompanying physiological arousal. However, even with Spielberger's contribution, controversy over theories still exists, as do questions, especially considering the renewed interest in an interactionist model of personality, explained below.

2.1.4 Summary

To summarise, the foundation of our concept of stress and its effects is based on the belief that the machinery of our body has to defend itself against any adverse change in external conditions. Multiple control systems endeavour to preserve adaptive

homeostatic²⁸ mechanisms, which function to maintain life. During prolonged or severe periods of stress these mechanisms, which are adaptive in the short term, can become damaging in themselves, causing anxiety, aggression, physiological and psychological illness (Loyallo, 1997).

2.2 Quantifying Emotion and Coping in Stressful Situations

2.2.1 Emotion and Coping

Folkman and colleagues (Folkman, Lazarus, Gruen, & DeLongis, 1986b, Folkman & Lazarus, 1988a) believed that emotional state and coping style had a dynamic and mutually reciprocal relationship. Emotion and coping have traditionally been conceptualised in 2 distinct systems of thought: The Animal Model and the Psychoanalytic Ego Model.

The Animal model was derived from the belief that human stress had its roots in animal evolution. It emphasises learned behaviours for survival. Emotion is treated as drive and motivates behavioural responses to enable protection. The second, the Psychoanalytic Ego model views coping as cognitive processes, such as denial, repression, suppression and intellectualisation as well as problem-solving behaviours which are invoked to reduce anxiety and other distressing emotional states. Both models view coping as a response to emotion which functions as a tension/arousal-reducer (Folkman & Lazarus, 1988b). However it must be emphasised that the psychoanalytic model has little empirical support. Furthermore these traditional models suffer from being incomplete, static and unidirectional. Folkman states that a "model which fails to specify the nature of the cognitive activity in the emotion process is bound to be ambiguous and incomplete." In other words coping is not merely a response but is also strongly influenced by the appraised significance for well being of what is occurring, which is incorporated in the emotional arousal, and which effects the quality of the emotion. Furthermore, the relationship of emotion and coping is usually conceptualised as only a response, when in fact emotion both facilitates and interferes with coping, and over time

²⁸ The process of maintaining internal stability in the face of environmental change.

coping can also affect emotional reaction (Folkman, Lazarus, Dunkel-Schetter, DeLongis, & Gruen, 1986a).

Process-oriented approaches (Folkman & Lazarus, 1988b) have been developed whereby emotion is conceptualised as complex organised psychophysiological reactions consisting of cognitive appraisals, action impulses and patterned somatic reactions, which operate together to control the quality and intensity of emotion. Coping on the other hand consists of cognitive and behavioural efforts to manage specific external or internal demands that are appraised as exceeding the person's resources. This definition refers to two types of coping: problem-focused and emotion-focused. Antecedent personal characteristics and environmental variables influence cognitive appraisals of the situation. For example the impact of emotion on coping was highlighted in a study where students with high levels of personal motivation to study questioned their abilities and appraised an exam as more threatening than those students with low study motivation (Folkman & Lazarus, 1985). The changing character of what the person thinks and does during the unfolding of the specific person-environment encounter across encounters is considered the process of coping. Given the power of environmental conditions to shape reactions it is no surprise that emotion and coping are characterised by a high degree of variability among and within persons. Folkman and Lazarus (1988b), also state that existing measures of coping dispositions did not predict how people cope in specific situations, and furthermore that coping actually needs to be considered as a multidimensional process. They identified two forms of problem-focused coping and six forms of emotion-focused coping in their 67 item Ways of Coping measure.

2.2.2 Existing Measures of Coping Style

A wide variety of research suggests that when people are confronted with stressful or negative events, coping / problem solving plays an important role in their physical and psychological well being (Dixon, Heppner, & Anderson, 1991, Endler, 1988, Miller, Brody, & Summerton, 1988, Nezu, Nezu, Saraydarian, Kalmar, & Ronan, 1986). However, the role of coping in mediating both physical and psychological health is poorly understood and little examined. Little agreement exists regarding the optimal

concept of coping, hence numerous constructs have been suggested. Examples are problem- and emotion-focused coping (Folkman & Lazarus, 1980) hardiness (Kobasa, 1982), problem-solving appraisal (Heppner, 1988), social problem solving (D'Zurilla & Nezu, 1982), constructive thinking (Epstein & Meier, 1989), anti-depressive behaviours (Rippere, 1976), learned resourcefulness (Meichenbaum, 1977), and coherence (Antonovsky, 1979). Consequently a number of instruments have been developed in attempting to measure these aspects of coping and problem solving. Most measures have been empirically based with only a few derived from theory, and most are believed to have psychometric imperfections, including low reliability of test scores, unstable factor structure and ambiguous items.

Taking these criticisms into account researchers have attempted to create and validate reliable and comprehensive measures of coping. Various measures of coping exist, such as Amirkhan's Coping Strategy Indicator, CSI (Amirkhan, 1990, Amirkhan, 1994a, Amirkhan, 1994b, Amirkhan, Risinger, & Swickert, 1995), Folkman and Lazarus' Ways of Coping Revised, WOC-R (Folkman & Lazarus, 1988a), and Carver et al., COPE (Carver, Scheier, & Weintraub, 1989), each with their own positive and negative points. Clark et al. (Clark, Bormann, Cropanzano, & James, 1995) investigated the construct validity of the aforementioned measures. Clark found that each of the three scales had positive aspects but that none satisfactorily captured the whole picture of coping. Of the three scales the CSI was the best predictor of life satisfaction, while the COPE was the best predictor of physical symptoms and positive outcomes while the WOC-R best predicted the hassles and uplifts of life. Other comprehensive coping measures exist, such as Endler's Coping in Stressful Situations (CISS). Support for Endlers' CISS and EMAS scales (Endler Multidimensional Anxiety Scale, which is interlinked with his CISS) comes from Cook and Heppner (1997) who examined the psychometric properties of three coping inventories: the CISS, the COPE and the Coping Strategy Inventory (CSI). They examined the stability of factor structure (using confirmatory analysis) then ascertained which common constructs underlay the 3 scales. Their results indicated selective preference for particular factor structures for each coping measure, although none of the measures displayed a strong fit with the data in this study. Three common and general factor structures were found: problem engagement, avoidance, and social/emotional. Overall Cook et al. suggested a need for more complex conceptualisations of coping. They concluded that, although none of the

scales were complex enough to tap all the constructs that they believe exist, the CISS was the instrument that came closest.

2.2.3 The Multidimensional Interaction Model of Anxiety and Coping

Endler states that "personality is a person's coherent manner of interacting with one-self and with one's environment...". Concept definitions like this are often refuted by other researchers but all would agree that a comprehensive definition of personality must take into account traits, motives, abilities, emotions, cognitions, physiological factors, genetic factors and content, as well as processes (strategies) and roles of processing information and hence, behaving.

It is therefore evident that a situation that a person perceives as or appraises as threatening has the consequence of increasing state anxiety, which is a transitory experience. Individuals develop and learn various styles or strategies of coping for reacting to stressful situations and the consequent state anxiety. Endler's model distinguishes between state and trait anxiety and focuses on both interactions and the multidimensionality of anxiety, and has ultimately been expanded to include stress and coping.

The model is process-oriented. The basis is that we have what Endler termed 'phase 1': person variables. These are trait, cognitive style, heredity, and emotionality, some of which may be temperamental in nature and which may interact with one another. Additionally we have situation variables such as life events, hassles, pain, and crisis, which may interact with one another. Lastly we have an interaction of the person by situation variables. This interaction leads to the perception of danger or threat (phase 2). In turn this can affect both person and situation variables (phase 1) leading to changes in state anxiety (phase 3), and producing reactions (phase 4). The reactions in phase 4 can be coping responses, defences, illness and physiological reactions, hence a feedback loop and a continuous process transpires.

Measuring Anxiety

The Endler Multidimensional Anxiety Scales (EMAS) are used to assess trait anxiety (EMAS-T) in 4 situational realms: social evaluation (SE); physical danger (PD); daily routines (DR) and ambiguous routines (AR). EMAS-T consists of 4 sets of 15 items. State anxiety is measured via the EMAS-S and assesses cognitive worry (C-W) and autonomic-emotional (A-E) anxiety components as well as providing a total score. EMAS-S consists of 2 sets of 10 items. Endler also uses the EMAS-P to assess the individuals' perception of the stressful situation. EMAS-P consists of eight items (Endler & Parker, 1990a, Endler & Parker, 1990c).

Endler's interaction model of anxiety includes facets of personality, coping and stress going far beyond Spielberger's state / trait distinction of anxiety. Since Spielberger produced his model there has been renewed interest in an interaction model of personality, resulting in re-evaluations of existing models of personality and the state - trait distinction. Consequently it is within an interactionist focus that the state-trait distinction has gained its prominence. Endler's interactionist model assumes multidimensionality of state and trait anxiety and this is displayed in his use of anxiety facets: trait - SE, PD, DR and AR; state - C-W and A-E. Furthermore, Endler assumes that person-situation interactions occur and that these person-situation interactions induce changes in state anxiety when the threatening situation is congruent with the aspect of trait anxiety relevant to the individual. When situational stressors are incongruent no changes in state anxiety are expected (Endler & Parker, 1990b).

Support for Endler's model, in the form of construct validity of EMAS, comes from experimental paradigms in both the laboratory and the field. A person-situation (stressor) interaction was found only when the situational physical danger threat was congruent with physical danger trait anxiety: Military personnel were administered the EMAS-T in a non threatening situation and the EMAS-S and EMAS-P just prior to a parachute jump (Endler, Crooks, & Parker, 1992). Secondly, a person-situation interaction was found only when the situational social evaluation threat was congruent with the social evaluation trait anxiety: adolescent girls were assessed during practice and later during preparation for a competitive horse-jumping situation. Further, person-situation interaction was found only when situational ambiguous threat was congruent

with ambiguous trait anxiety: state and trait measures were completed by students awaiting a minor exam directly before and one week after a referendum. Those high on trait ambiguity scored high on the ambiguity of the referendum (Endler, Kantor, & Parker, 1994).

Further studies have been performed in diverse situations such as at dental surgeries, karate meetings, women undergoing laparoscopy²⁹ or a D and C (dilation and curettage), as well as with stressed bank managers and in psychotherapy settings. Endler claims that out of 34 tests of his interaction model of anxiety, differential hypotheses were confirmed in 28 cases and not in 6 cases, a success rate of 82.4% (Endler & Parker, 1990b).

Endler believes his scales to be highly reliable, quoting alpha reliabilities ranging from 0.89 - 0.93 for male undergraduates. Endler also suggests that the criterion (personality) validity of his scales is moderate to high: EMAS-S is highly correlated with Spielberger's State-Trait anxiety inventory (0.65-0.75). He notes that there are however important gender differences in the relationships between state anxiety and the various criterion measures. Additionally the strength of the association between the state and criterion measures varies across the anxiety sub-scales. He also quotes high association values for the Beck (1979) and Zung (1965) depression measures as well as the MMPI (Minnesota Multiphasic Personality Inventory, 1951). All facets of EMAS-T, except physical danger trait-anxiety, significantly correlate with the Spielberger STAI measures, Beck, and Zung measures of depression. Additionally he notes that daily routines are significantly correlated for all the criterion variables for both males and females, but for all other facets of trait-anxiety there are gender differences (Endler, Edwards, & Vitelli, 1991).

The above data support the multidimensionality of anxiety and the situational specificity, as well as attesting to the importance of assessing the two genders differently. Conceptually, under non-stressful conditions state and trait anxiety are empirically (relatively) independent. Endler found however that in such situations state

²⁹ Medical instrument consisting of a tube that is inserted through the abdominal wall to view the internal organs.

and trait were unrelated for males but that for females there was a correlation between total EMAS-S and social evaluation, and EMAS-T daily routines. Under a stressful examination situation EMAS-S correlated significantly with EMAS-T for both males and females. He suggests that perhaps females view non-stressful situations as having a social evaluation component (Endler & Parker, 1990c).

Measuring Coping Styles

Coping style means a characteristic or typical manner of approaching a stressful situation and dealing with it. Some situations lend themselves to specific coping behaviours, such as attempting to exit a burning building rather than just screaming in panic. In other situations, there may be a variety of coping responses possible and the specific one/s chosen relate to the individual's coping style or preferences. Situational demands may then override and interact with an individual's preferred coping style. There are three main personality styles of coping: task-oriented; emotion-oriented and avoidance-oriented. Avoidance can occur via distraction or social diversion.

To enable assessment of the interaction model of stress, anxiety and coping, a reliable and valid measure of the concept of coping was created. The Coping Inventory for Stressful Situations (CISS) is a multidimensional measure of coping with three 16-item factors that correspond with the above three. Respondents are asked to indicate on five-point intensity scales how they generally react to difficult, stressful or upsetting situations. Items include "schedule my time better", "become very upset" and "take time off and get away from the situation". Endler quotes high alpha reliability values for the CISS for the 3 factors: .90, .90 and .91 respectively for male students and .87, .89 and .82 respectively for female students. Comparable results were found for adults, adolescents and clinical psychiatric populations. Test-retest reliabilities after 6 weeks for the 3 factors were again relatively high: .73, .68 and .55 for male college students and .72, .71 and .60 for female college students.

Evidence for EMAS and CISS Strength

For validity Endler compared the CISS with Jackson's (1989) Basic Personality Inventory (BPI) scales. For both men and women he found that: emotion-oriented coping was related to psychiatric symptomology, depression and social symptomology;

task-oriented coping was negatively related to pathology, especially to depression. For avoidance coping Endler found that distraction was positively related to both psychiatric symptomology and social symptomology, while social diversion was negatively related to depression - thus depressed persons avoid situations involving others (Endler, 1997).

Correlations were made with the CISS and MMPI-2 scales, for 167 male airline pilots. Task-oriented coping negatively correlated with most of the MMPI content scales (especially low self-esteem, cynicism and anxiety content scales). Additionally pilot aptitude was positively correlated with task-oriented coping. Emotion-oriented coping positively correlated with all the MMPI content scales. Type A behaviour patterns³⁰ were strongly related to emotion-oriented coping and the distraction component of avoidance-oriented coping. Both emotion and avoidance-oriented coping were positively related to somatization. Task-oriented coping was unrelated to somatization. Gender differences existed but were not as marked as previously reported. With respect to state anxiety there was a positive correlation between EMAS-S and emotion coping and with distraction, but a negative correlation between EMAS-S and task-oriented coping (Hathaway & McKinley, 1989).

For patients undergoing cardiac bypass surgery their recovery was facilitated by personal and social resources such as perceived self-efficacy and social support. Patients who reported having high-perceived control solved more anagram tasks, showed less reliance on situation-specific emotion coping and displayed greater reliance on situation-specific task coping (than patients who perceived themselves as having low control over their recovery). Low-perceived control women relied more on task coping than men. High-perceived control men relied more on task coping than women. Task-oriented coping was found to be more efficacious in situations where one perceives oneself to have control, while emotion-oriented coping was used more often when control was not perceived (Endler & Parker, 1994).

³⁰ Self-critical and goal driven people who show behaviours of time urgency and anger/hostility.

2.2.4 Summary

Stress, anxiety and coping all involve complex interacting processes. There exists a multidimensional relationship among the various personalities and temperamental sub-factors, as well as a dynamic relationship between these factors and situational-stress factors. Endler expresses his opinion as: ".....to every complex problem there is a simple answer. And it is wrong." (Endler, 1993).

2.3 *Support for the Impact of Coping and Emotion in Stressful Situations*

2.3.1 Emotional Effects of Stress

Although, within the health care profession it is widely believed that coping influences emotion, the traditional research emphasis has been on the effects of emotion on coping. Folkman and Lazarus attempted to address this imbalance by evaluating to what extent emotions are mediated by coping during stressful encounters (Folkman & Lazarus, 1988a). The extent to which 8 forms of coping (confrontive, distancing, self-control, seeking social support, accepting responsibility, escape-avoidance, problem-solving, positive reappraisal) mediated 4 sets of emotions (worried/fearful, disgusted/angry, confident and happy/pleased) was evaluated. They found coping was associated with changes in all 4 sets of emotions, with some forms of coping associated with increases in positive emotions and other forms associated with increases in negative emotions.

DeLongis et al., (1988), investigated the somatic and psychological effects of everyday hassles. The study examined the daily stress processes among 75 married couples across 20 assessments during a 6-month period. Overall, there was a significant relationship between daily stress and the occurrence of both concurrent and subsequent health problems such as flu, sore throat, headaches, and backaches. The negative effects of stress on mood were limited to a single day, with the following day characterised by mood scores that were better than usual. Striking differences were found in the extent to which daily stress was associated with health and mood across time. Participants with

un-supportive social relationships and low self-esteem were more likely to experience an increase in psychological and somatic problems both on and following stressful days than those with high self-esteem and good social support. The data suggests that persons with low psychosocial resources are vulnerable to illness and mood disturbances when their stress levels increase, even if they generally have little stress in their lives.

Previous research has shown dispositional optimism to be a prospective predictor of successful adaptation to stressful encounters. A study by Scheier (Scheier, Weintraub, & Carver, 1986) attempted to identify possible mechanisms underlying these effects by examining how optimists differ from pessimists in the kinds of coping strategies they use. The results of two studies revealed modest but reliable positive correlations between optimism and problem-focused coping, seeking of social support, and emphasising the positive aspects of a stressful situation. Pessimism was associated with denial and distancing, and focusing on stressful feelings, as well as with disengagement from the goal with which the stressor was interfering. One of the studies found that when the situation was appraised as uncontrollable then optimism was positively associated with acceptance/resignation.

The studies mentioned above are testimony to the influence coping style and emotion can have on the healthy individual. The belief that emotional status and coping style can influence recovery from illness has also lead to the investigation of patient groups. A study of migraine sufferers indicated clearly the complex and varying nature of emotion and coping during stress. Physiological stress response specificity and the role of cognitive coping were investigated in migraine patients during cognitive tasks and subsequent recovery (Kroner-Herwig, Fritsche, & Brauer, 1993). Physiological measures were recorded during the task and recovery period. Migraine sufferers and non-headache controls were randomly assigned to one of two experimental conditions: a spontaneous processing condition and the "positive coping" treatment. The treatment condition pertained to a short training session in the conscious use of positive self-statements in stressful situations. The results found, irrespective of group, that spontaneous cognitive coping did not correlate with the patients physiological responses, while the positive coping patients were more relaxed with greater positive self-evaluation (subjective) and paradoxically more physiological arousal, than the

'spontaneous' group. It is no surprise then, that our emotional reaction to stress and more specifically how we cope with it affects our health.

2.3.2 Coping, Emotion and Cardiovascular Stress Reactivity

Bohen et al. (Bohnen, Nicolson, Sulon, & Jolles, 1991) used salivary cortisol secretion as an index of stress reactivity to 4-hours of continuous mental task performance to investigate whether or not the reactivity reflected individual differences in coping styles. Significantly higher levels of cortisol response were found to occur during the 4 hours of mental tasks than during a control period. However, individual variability of cortisol response was high. The correlational data further indicated that a significant negative relationship between the coping style "comforting cognitions" and the individual cortisol response during stress existed. That is to say, the more a subject employed a comforting cognition style the less cortisol reactivity occurred. During this type of cognitive stress, where the subject has no control over the experimental situation, comforting and emotion-focused coping may be effective because of the subject's efforts to reframe the inevitable situation in a positive and self-encouraging manner. In contrast, there was no significant relationship between trait anxiety and individual gluco-corticoid susceptibility to mental stress.

Fontana and McLaughlin, (1998) found that emotion-focused coping correlated with lower baseline heart rate levels; while distancing during conflict (avoidance) correlated with higher systolic blood pressure. Higgins and Endler, (1995) found that task-oriented coping was negatively related to distress in men while emotion-oriented coping was positively related to distress in both males and females. Higgins and Endler's findings show a paradox, where unlike the two previously reported papers this study suggested emotion-oriented coping related to increased stress levels. Gerin's normotensive study results further complicate the issue (Gerin, Litt, Deich, & Pickering, 1996). Gerin tested the proposition that, with effort left unconstrained, increased self-efficacy will increase cardiovascular response. Subjects were normotensive women between 18 and 21 years old. As predicted, the results indicated that systolic and diastolic blood pressure changes were smaller in the group where self-efficacy was manipulated (via false feedback from a psychological questionnaire) to be low, than the high self-efficacy group. The

percentage change scores were 17.9 vs. 25.2 for systolic ($p < 0.05$), and 8.7 vs. 13.0 for diastolic ($p = 0.07$). Heart rate was similar for the two conditions. The authors concluded that self-efficacy for a task may be an integral part of the active coping process, indirectly affecting blood pressure responses by acting on the effort involved in the coping response. They also reported that the more emotion-oriented coping occurred the more distress/stress resulted. However, it may be that the more distressed an individual is then the more they need to use emotion-oriented coping to attempt to reduce their distress.

Larson and Langer, (1997) investigated the effect of emotional anger on cardiovascular reactivity to a mental arithmetic task. Forty individuals were randomly assigned to complete the task with or without the threat of an electric shock, for incorrect responses. Participants also completed the Cook-Medley Hostility Inventory (Ho), the Marlowe-Crowne Social Desirability Scale (MC) and the Spielberger Anger Expression Scale. The results revealed that 'defensive hostile' subjects (high Ho / high MC) were significantly more physiologically reactive than any other subgroup. In addition, the combination of low Ho/high anger-out scores yielded a subgroup significantly less reactive than any other subgroup.

Although there is a large field of research into the impact of emotion and coping on stress responses, little is known about the physiological processes that may play a role. Coy and Dimsdale, (1998) investigated recent research which suggested that individuals with a repressive coping style demonstrate increased autonomic arousal, impaired immune function, and greater risk for some medical illnesses. The authors examined the effect of repressive coping style on heart rate, blood pressure and plasma catecholamine reactivity to stress. Based on self-reported scores from the Spielberger trait anxiety inventory and the Marlowe-Crowne Social Desirability Scale, subjects were grouped into one of four coping categories: low anxious, high anxious, defensive anxious and repressive coping. The stressor applied was a 3-minute public speaking task over which heart rate and blood pressure were measured. It was found that subjects who exhibited a repressive coping style, (defined by low anxiety and high social desirability) demonstrated significantly greater heart rate reactivity during stress ($p < .05$) than those classified as non-repressive. A statistical trend was noted, where subjects high on social desirability exhibited greater norepinephrine reactivity compared to low social

desirability subjects ($p=0.06$). Although repressors tended to show increased physiological reactivity on some indices, they demonstrated a trend to report less anxiety after stress compared to their counterparts. Subjects high on social desirability displayed a trend to report less anger after the stressor. This study supports previous findings that demonstrate increased physiological arousal among repressors. In addition, high social desirability, and the denial of hostility, was also associated with increased norepinephrine reactivity to stressors.

A study by Vogele and Steptoe, (1992) suggested that normotensives at risk for future hypertension are likely to show heightened stress-related cardiovascular responses if they also tend to inhibit the expression of negative emotions. Expression of humour as a coping mechanism was found to moderate emotion-oriented stressors. Lefcourt et al. (Lefcourt, Davidson, Prkachin, & Mills, 1997) found that females who were higher in coping humour exhibited lower systolic blood pressure than females lower on coping humour. Males who scored high on the coping humour scale exhibited higher systolic blood pressure, during stressor tasks, than males scoring low on coping humour. Furthermore, in the cold pressor task³¹, interactions were found between humour, gender and trials indicating a stress-moderator effect. The absence of interactions in the four other stress tasks examined suggested that humour may play more of a role in moderating the impact of uncontrollable (usually emotion-oriented) and passively experienced stressors. It may then be considered that negative emotions and a lack of humour during periods of stress could be detrimental to health status.

2.3.3 Coping, Emotion and Symptomology

Coping style and emotion can be shown to impact upon bodily function as well as influence cardiovascular responses to stressful events. A study by Shapiro et al. (Shapiro, Jamner, & Goldstein, 1997) found that emotional expression related to physiological alterations. Participants who reported frequently feeling angry during the day had higher levels of blood pressure, especially during sleep than those who did not report feelings of anger. Reports of sad feelings were positively correlated, while

³¹ Submersing an arm or hand in a circulating bath of ice water.

reports of pleasant or happy were negatively correlated with diastolic blood pressure during sleep. Participants who scored high on hostility and anxiety trait measures and low on defensiveness reported negative moods more frequently than participants who scored low on hostility and trait anxiety. Participants who were characterised by reports of negative moods plus anger had high scores on trait hostility and consistently higher levels of blood pressure, particularly diastolic during sleep. The results indicated that heart rate was not related to mood reports but that hostile and anxious behavioural dispositions may play a role in sleep disturbance and high levels of blood pressure, while positive mood may counter these effects.

In a series of experiments (Endler & Parker, 1994, Endler, 1997) it was reported that for both men and women task-oriented coping (non-emotion) negatively related to pathology, especially depression. In other words, those who relied more on emotion-oriented coping and less on task-oriented coping were more prone to pathologies such as depression. Further evidence revealed emotion-oriented coping was related to psychiatric symptomology, depression and social symptomology. These results directly support Higgins and Endler (1995) finding of increased distress with emotion-focused coping and Gerin et al. (1996) postulation that distress increases with the amount of emotion-oriented coping employed.

2.3.4 Summary

It appears then that coping and emotion play a vital role in our psychological and cardiovascular responses to stress. Coping styles and strategies mediate between antecedent stressful events and consequences such as anxiety, somatic complaints and psychological distress. In a controllable situation task-oriented coping is most productive, while in uncontrollable situations emotion-oriented coping is most effective. Avoidance-oriented coping can initially be appropriate but damaging in the long run, as task-oriented coping needs to be favoured.

2.4 *Modelling Stress and Assessing Reactivity in the Laboratory*

When researching the effects of stress on the cardiovascular system it is important to create a situation which people appraise as stressful. Therefore a considerable question is whether or not a stressful situation can be created in the experimental laboratory which will elicit the specific cardiovascular responses we wish to study. Although it must be borne in mind that stress responses must ultimately be studied in real-life, it is extremely useful in the early stages to work within a controlled setting.

The evolutionary purpose of the stress response is to prepare the body to minimise any damage sustained. However, in developed contemporary society, although the response pattern is the same (cardiovascular-hormonal change), the challenges and stressors tend to be less physically threatening and a little more sedate. This means that moderate levels of arousal are repeatedly elicited in daily-life and that it is possible to attempt to model such stress in the laboratory (Turner, 1994).

2.4.1 Example Stressor Tasks

The following are examples of the stressors, which have been developed and used within the laboratory. They can be categorised as psychological stressors, requiring continuous active engagement and minimal physical exertion.

Reaction time tasks - where the subject needs to respond typically with a button press as soon as a stimulus is detected. The basic paradigm can be altered to include avoidance and uncertainty, or more commonly, appetitive (monetary incentives) and competitive (head-to-head competition) versions are used (Light, 1981).

Video Games - Early studies showed video tennis and handball games to have impact on the cardiovascular system. Glass (1977, 1983) reported average increases of 16.3bpm and Dembroski et al. reported increases of 16.9bpm (Dembroski, MacDougall, Slaats, Eliot, & Buell, 1981). Video games provide uncertainty, novelty and avoidance and games such as "Space Invaders" also provide increasing difficulty, urgency and competition.

Presentations and Speech Tasks - These usually require the person to create and present a concise scenario, where they are marked and often have to compete with other participants (Al'Absi et al., 1997).

Mental Arithmetic - There is a long history of usage, which generally involves a verbal descending serial subtraction task. Versions often involve incentives. These tasks suffer from the drawback that they rely on arithmetic ability. Modern variations attempt to account for ability by altering level of difficulty (Al'Absi et al., 1997, Beh, 1998).

Other stressor tasks employed in cardiovascular research include information processing tasks, psychomotor tasks, affective conditions and aversive or painful conditions. The commonly used *cold pressor* passive stressor is an example of the latter, where the subject has to submerge their hand or foot in cold water (Steptoe & Vogele, 1991).

2.4.2 Quantifying the Physiological Stress Responses

Having selected the stressor it is necessary to measure the physiological reactivity which occurs. Considerable variations of physiological measures exist but the most commonly used are electrocardiographs (ECGs) to record heart rate, automatic or manual instruments (cuff and sphygmomanometer) to record blood-pressure, and impedance cardiograms to measure cardiac output.

Next it is necessary to define reactivity, which has two aspects: computational and conceptual. The computational definition relates to change, change from a previously recorded baseline to the current response. In other words heart rate reactivity would be the task heart rate minus the baseline heart rate. The conceptual definition can be regarded as a response. Sherwood defined it as ".....referring to the magnitude, patterns, and/or mechanisms of cardiovascular responses associated with exposure to psychological stress..... the propensity to exhibit an alteration in cardiovascular activity....." (quoted in Turner, 1994).

2.5 Disease and the Influence of the Stress Response

Diseases of the heart and blood vessels disable and kill more people than diseases of any other type. It is thought that the effects of psychological stress on cardiovascular (CV) activity may contribute to coronary heart disease (CHD) (Hart, Watt, Smith, Gillis, & Hawthorne, 1997, Johnston, Tuomisto, Chaudrey-Dijkerman, Koster, & Jain, 1997, Lawler, Rixse, & Allen, 1983). Psychological factors have been found to play a part in the aetiology and manifestation of cardiovascular disease (Johnston, 1997, Markovitz, 1998, Sundin, Ohman, Palm, & Strom, 1995). Measures of blood pressure (BP) and heart rate (HR) can demonstrate cardiovascular reactivity (CVR). Furthermore, there is evidence that CVR can be affected by the experience of stress. In other words, the heart rate and blood pressure responses of a given individual to laboratory stressors are used to define their CVR to stress. When people are asked to compete at video games, perform mental arithmetic or speak in public their cardiovascular reactivity increases in response to this stress (Al'Absi et al., 1997, Beh, 1998, Brody, Maier, Montoya, & Rau, 1994, Fauchoux et al., 1989, Korunka, Zauchner, Litchauer, & Hinton, 1997, Ohman, Nordy, & Svebak, 1985, Sharpley & McLean, 1991). Furthermore, this pattern is also found in everyday life (Jacob et al., 1999, Jain, Schmidt, Johnston, Brabant, & vonzurMuhlen, 1998, Turner et al., 1994, Siegrist & Klein, 1990). For example, heart rate and systolic blood pressure were found to increase at work, in subjects who appraised their jobs as high stress (Steptoe, Roy, Evans, & Snashall, 1995). In addition, Steptoe, Roy and Evans (1996) found that fire-fighters systolic blood pressure (SBP) increased in response to stress, both at work and at home.

2.5.1 Evidence for Stress Related Coronary Heart Disease

Human Research

There is evidence that cardiovascular reactivity to stress relates to coronary heart disease. For example, hypertensives show greater cardiovascular reactivity to stress than normotensives. Furthermore, research has shown that high rates of job stress are associated with high levels of blood pressure and abnormally enlarged hearts (DeLongis et al., 1988, Steptoe et al., 1995, Devereux et al., 1983, Ditto, 1993, Georgiades, Lemne,

Faire, & Fredrikson, 1995, Helmers & Krantz, 1994, Hinton & Hadapp, 1997, Hinton & Burton, 1997). Most of the evidence, which links cardiovascular reactivity to coronary heart disease, originates from cross-sectional studies although there is also prospective support. An example of this is a 45-year follow-up study, which showed that reactivity to a cold pressor in early adulthood was predictive of future hypertension development. Additionally, an exaggerated heart rate (tachycardic) response to psychological challenge has been shown to predict children's blood pressure development. Consequently, exaggerated cardiovascular responsiveness to behavioural and psychophysical challenge is associated with the development of certain disorders (e.g. hypertension and ischemic heart disease³², Mills, 1990). Short-term studies have also shown the effects of psychological stress, in the form of cardiovascular disorders in healthy patients. Paroxysmal atrial fibrillation³³ was triggered by psychological stress in two patients, both of whom had normal echocardiograms and coronary angiography. Neither patient was alcoholic or had ingested ethanol in relation to the onset of the atrial fibrillation and both were free of metabolic derangements (Houghton et al., 1990). It appears that both short- and long-term stress can lead to cardiovascular disorders and disease.

Animal Research

Further evidence for the role of stress in the development of coronary heart disease can be found in animal studies (Corley, Shiel, Mauck, & Greenhoot, 1973, Corley, Mauck, & Shiel, 1975, Corley et al., 1979). For example, Corley et al. (Corley, Shiel, Mauck, Clark, & Barber, 1977) found that monkeys submitted to shock avoidance scenarios developed abnormalities of heart functioning. Those monkeys which were able to avoid the shock, showed abnormal electrocardiogram (ECG) traces and heart muscle degeneration while the monkeys unable to avoid the shock showed bradycardia³⁴ and cessation of pumping of the left ventricle³⁵. Another example is from an extreme study with baboons (Lapin & Cherkovich, 1971). Under normal conditions cardiac pathology is extremely rare in baboons. However, after submitting the male baboons to stress

³² Lack of oxygen in the heart muscle.

³³ Sudden irregular twitching of the muscular wall of the upper heart chamber.

³⁴ Severe slowing of the heart.

³⁵ The left ventricle is the hearts major pumping chamber, forcing blood through most of the body.

(removing social dominance) 41 of the 57 developed cardiovascular disorders such as hypertension and abnormal ECG traces, while six had myocardial infarctions³⁶.

2.5.2 Laboratory vs. Real-life

The consistency and ability of laboratory stressors to reflect the effects of everyday, real-life stressful events is not clearly established. Parati et al. (1988) found laboratory tasks were limited in assessing cardiovascular reactivity to stress, and did not reflect the extent of blood pressure variations between night and day. They also found responses to psychological and physical stressors were unrelated. Van Egeren and Sparrow (1989) support this discrepancy between laboratory and real-life, with laboratory reactivity accounting for only 19% of diastolic blood pressure variance in real life environments at best. They also found that both laboratory and ambulatory measures were low in test-retest reliability. They posit that this disparity is related to inter-individual variability in activity on the day of ambulatory monitoring. However, Siegrist and Klein (1990) claim that cardiovascular reactivity during experimental tasks, such as the STROOP (Stroop, 1935), should be considered not only as a predictor of future risk of coronary heart disease, but also as an outcome of an individual's exposure to, and coping with, chronic stress in the real world (Siegrist & Klein, 1990).

Weidner et al.'s (1989) study is an example which displays the importance of combining both laboratory and real-life information when researching the influence of stress on coronary heart disease. They investigated the effects of hostility on cardiovascular reactivity to stress. The study compared reported day to day hostility levels with cardiovascular reactivity to stressors within the laboratory. They found that for both men and women, high hostility was a borderline predictor of increased blood pressure during laboratory stress tasks. However, although high hostility related to greater reactivity, heart rate during stress was not related to hostility. Weidner et al. claim that suspicion and mistrust rather than anger may be necessary to elicit increased blood pressure reactivity among high hostility scorers. From this they reason that highly mistrustful people may spend lots of time in a highly aroused state. This elevated

³⁶ Commonly known as the heart attack.

arousal could be associated with increased sympathetic nervous system activity, which in the long run may contribute to atherosclerosis and coronary heart disease (Weidner, Friend, Ficarrotto, & Mendell, 1989).

2.5.3 Summary

It can be shown that the experience of stress, whether prolonged or short term, affects the highly intricate and complicated set-up of the cardiovascular system. This reactivity to stress, the change from baseline to actual heart rate can be defined as the resulting response pattern. It is thought that prolonged exposure to stress and the resulting reactivity relates to coronary heart disease. Using stressor tasks, short-term effects can be evoked and recorded within the controlled conditions of the laboratory, however there is considerable disagreement as to how well laboratory responses relate to real-life.

2.6 *Ambulatory Monitoring Systems*

Individuals vary in their cardiovascular activity and reactivity within the laboratory setting. It is therefore important to determine if these individual differences predict measurements made in real-life. In other words, do people with exaggerated responses to laboratory stressors also show exaggerated responses to natural stressors? Ambulatory monitoring is however, a difficult procedure. The conditions are variable and cannot be controlled. In real-life people move around, talk, eat, drink, change posture and engage in exerting activities, all of which influence cardiovascular parameters and can obscure the actual cardiovascular effects of real-life stressors.

Ambulatory monitoring describes the process of recording an individual's cardiovascular activity while they go about their daily business outside the laboratory. Typically the individual attends the laboratory in the morning to have the lightweight recording devices fitted, then returns to his/her normal routine. Technological advances in ambulatory equipment have permitted the measurement of parameters, which were traditionally thought to be laboratory based, to be recorded in the field. An example is

the waist mounted Oxford MR-10 Medilog Tape Recorder, which is made of aluminium and powered by small batteries. This records ECG signals onto audiocassette, which at a later date can be displayed as minute epochs of heart rate (Turner, 1994).

Other monitors such as the Accutracker and Spacelab are able to record blood pressure at regular or quasi-random intervals. Subjects wear a cuff constantly, which is programmable to inflate and deflate to determine diastolic (DBP) and systolic blood pressure (SBP). The advantage of the quasi-random programming is that if the subject cannot pre-empt the event then they cannot alter their behaviour. Again the information recorded is stored within the unit and downloaded when the subject is scheduled to return to the laboratory.

Ambulatory recorders permit the study of interactions between the characteristics of the individuals environment or behaviour and their physiological functioning (Bussmann, Tulen, van Herel, & Stam, 1998, Hoehn-Saric, McLeod, & Funderburk, 1994, Jacob et al., 1999, Schmidt & Jain, 1996, Schneiderman, Weiss, & Kaufmann, 1989, Schwartz, Warren, & Pickering, 1994, Shapiro et al., 1997, Willemsen, Geus, Klaver, Doornen, & Carroll, 1996). This produces the problems of gathering the information concerning these interactions and of controlling for confounding variables in the situation. These difficulties are highlighted by the fact that many studies find physiological responses in the field and laboratory do not correlate well with each other (Johnston et al., 1994, Linden & Con, 1994, Spalding, Ribeiro, Lott, & Richards, 1997, Turner et al., 1994, vanEgeren & Sparrow, 1989, Warwick-Evans, Walker, & Evans, 1988). This may be due to research in the field not focusing on the same parameters as laboratory research.

The ambulatory monitor provides the physiological data but there is also a need to know what behaviour the subject is engaging in throughout the day, especially at each occasion when a physiological measurement is taken. At the simplest level there is a need to determine whether the subject is stressed or not at that point in time. There is also a need to know about other events that produce non-psychological cardiovascular activity such as consumption of food, caffeine and nicotine as well as level of physical activity and posture and social setting. This has led to the introduction of subject diaries. The diary provides subjects with a standardised set of questions to report their

current situation and behaviour. Subjects are instructed to complete an identical page of the diary every time a reading occurs, for example after the deflation of the Spacelab cuff (Chesney & Ironson, in Schneiderman et al., 1989, and Turner, 1994).

Although to date most studies with ambulatory monitors have investigated the laboratory-field generalisation of reactivity, ambulatory monitoring is an investigative technique in its own right. Medical research places great interest in how heart rate and blood pressure vary over the course of the day and night and how interventions affect this (Wenneberg et al., 1997, Tulen, Bussmann, Steenis, Peplinkhuizen, & Veld, 1997). There is evidence that ambulatory blood pressure levels, especially within the work environment, are better predictors of hypertensive problems, disease and mortality than blood pressure recordings in the clinic, or those causally taken at work (Brownley, West, Hinderliter, & Light, 1996, Devereux et al., 1983, Georgiades et al., 1995, Krantz et al., 1996, Leese, Savage, Chattington, & Vora, 1996, Shapiro, Jamner, & Goldstein, 1993).

2.6.1 Studies with Ambulatory Technology / Monitors

Research using ambulatory technology have developed from the work of Holter in the 1960's to permit investigative studies into the wide range of behaviours which affect cardiovascular functioning (Holter, 1961). The studies presented here centre around the effects of stress on cardiovascular functioning. The review begins with the origins of ambulatory monitoring moving onto investigations of behaviours that influence cardiovascular functioning. The review continues with comparisons of reactivity in the laboratory and in real-life, progressing to studies investigating the cardiovascular effects of psychological stress. Further, studies showing the use of ambulatory monitoring in understanding cardiovascular disease and appraising interventions are described before moving onto the topic of increasing ambulatory knowledge and technology.

Continuous ECG monitoring of ambulatory subjects has advanced since its origins in 1961. Holter's (1961) work began with radio telemetry and signal processing which identified changes in rhythm and rate by cycle-length dependent superimposition of ECG complexes. Soon Holter was able to observe cardiac irregularities in the otherwise

normal subject (Holter, 1961). By 1975 technology had advanced enough to permit 24-hour recording to occur. Lopez and colleagues (1975) were able to identify sporadic and variable ventricular and supra ventricular arrhythmias (summarised in Schneiderman et al., 1989). Further advances have permitted the detection of ventricular ectopy³⁷ in the otherwise apparently normal individual (Kligfield, 1984).

2.6.2 Ambulatory Studies in Their Own Right

Ambulatory monitoring was first introduced almost four decades ago and although it has become standard practice in clinical evaluations of arrhythmias it has been little used in behavioural studies until more recently. Jaquet et al. (Jaquet, Shapiro, & Uijdehaage, 1994) used ambulatory blood pressure and heart rate monitors to investigate the acute effects and possible intra-day tolerance of smoking on cardiovascular activity. Their subjects were fitted with the Accutracker and ECG monitors for 48-hours after an overnight deprivation from cigarettes. Baseline measures of heart rate and blood pressure had previously been taken in the laboratory. The results showed that the acute effect of smoking under natural conditions produced significant increases in blood pressure and heart rate, with no indication of intra-day tolerance. These results, unlike many, do reflect the results from laboratory studies. This type of research permits us to confirm what actually happens in everyday real-life. Additionally, the ambulatory study revealed that the effect of smoking was additive to the influence of posture on the cardiovascular system. Due to the natural situational variations in posture it is difficult to reproduce this within the laboratory.

Wenneberg et al. (1997) used ambulatory blood pressure monitoring to investigate the effects of a transcendental meditation³⁸ program on cardiovascular activity. The 4-month study of normotensive men revealed no differences between the transcendental meditation group and the controls in cardiovascular reactivity to stressors, while the experimental group did show a significant decrease in ambulatory DBP compared to the

³⁷ Contraction of the heart muscle which is out of normal sequence, in general it follows a normal heart beat and is followed by a longer than usual interval. After MI it can indicate damage to the heart conduction system.

³⁸ TM is the principle technology of consciousness of Maharishi's Vedic approach to health.

controls. Shapiro et al. (1997) examined the relationships between moods and ambulatory blood pressure and heart rate in 197 college students, as previously described. From the use of ambulatory technology Shapiro et al. were able to conclude that hostile and anxious behavioural dispositions may play a role in sleep disturbances and high levels of blood pressure.

2.6.3 Comparing Naturalistic and Laboratory Stressors

Returning to the laboratory-field generalisation, studies can adopt two basic strategies where the laboratory phase is unchanging, but the ambulatory assessments differ. One strategy is the approach of the naturalistic stressor where the experimenter selects the externally occurring stressor; the second is the open-ended approach (applied in the studies in the previous section) where the subjects are free to engage in their normal daily activities.

Obrist and Light (1988) investigated the heart rate responses of 18 subjects to laboratory stressors and two naturally occurring real-life situations (one non-stressful - attending a class, the second stressful - an examination). According to their laboratory responses subjects were divided into low or high heart rate reactor groups. The low reactor group had similar heart rate reactions to the two real-life situations, while the high reactor group had a greater heart rate response to the stressful real-life situation than the non-stressful. These results indicate a predictive validity from laboratory to real life stress responses. Obrist and Light concluded that the ambulatory data strongly indicated that the challenge of an examination influenced heart rate only in people with high heart rate reactivity in the laboratory.

Turner et al. (Turner, Carroll, Dean, & Harris, 1987) selected 3 extreme high and 3 low heart rate reactors from an original group of 24 on the basis of their responses to the two laboratory stressors of mental arithmetic and a video game. The 6 selected subjects were then monitored while they performed in a public speaking competition. Public speaking is a deliberately chosen real-life stressor. Turner commented that public speaking in an evaluative context is a common and stressful experience for us all whether it be making a speech to an audience, a panel interview or other stressful

communications. Turner and colleagues chose to use a structured 'balloon debate', where the subjects operated in pairs in front of the audience. They had to pretend they were in a balloon which was rapidly losing height and from which everything possible had already been jettisoned. The pair of course have to debate for 10 minutes as to who should jump to save the others life. The ambulatory data showed the 3 high heart rate reactors to have large real-life reactivity scores while the low reactors had much less of a response with the apparent exception of one. The structure of the ambulatory data permitted closer inspection of the reasons for this exception. The subject's task levels in the laboratory and real-life were very similar but his baseline values were very different. The laboratory value was 89bpm while his real-life value was only 46.8bpm. Although this subject had been classified as a low reactor in the laboratory it was due to the fact that he was actually reacting the entire time he was in the laboratory. This illustrates the difficulty in obtaining reliable baseline data in ambulatory investigations as well as the difficulty in reproducing real-life responses in the laboratory.

Further examples have found mental arithmetic stressors presented in the laboratory produce significant increases in heart rate, while exercise and examination anticipation in real-life produced significant systolic blood pressure increases (Warwick-Evans et al., 1988). Additionally, Warwick-Evans et al., found diet, regular daily exercise and type A personality had significant effects on reactivity. However, unlike Turner et al. who used only 6 subjects, no differences in real-life reactivity were found between high and low reactors in this larger group.

The alternate approach is that of open-ended investigation, where the subject is free to go about their normal activities. One example is Parati et al. (1988). They discovered a significant relationship between blood pressure reactivity to a mirror drawing task and blood pressure variability during daytime ambulation.

2.6.4 Ambulatory Stress Studies

Open-ended ambulatory monitoring has also been used to investigate the physiological effects of stress. Steptoe et al. (1995) examined whether cardiovascular reactivity to laboratory mental stressors interacted with job strain in predicting blood pressure at

work. They found SBP to be higher during work than non-work days, with DBP and heart rate higher on work mornings. These effects were partly factors of activity and posture differences between the two types of days. They found that neither job strain nor laboratory reactivity independently predicted ambulatory blood pressure, however work afternoon SBP (both ambulatory and in the laboratory) was significantly higher in the high strain-high systolic reactor group than any other. This result was independent of baseline SBP, activity and posture. Steptoe et al. (1995) concluded from this that individual differences in the appraisal of work stress modulate the relationship between stress reactivity and ambulatory blood pressure.

A slightly later study (Steptoe et al., 1996) investigated psychosocial influences on ambulatory blood pressure of male fire fighters over working and non-working days. Steptoe et al. (1996) found SBP was significantly higher on working days, with DBP being elevated in the smokers only. Systolic blood pressure was higher when the fire fighters reported feelings of anger and stress than when they did not, both at work and non-work. They also noted that emergency call-outs and daily stressors had little effect on blood pressure, concluding that raised SBP during work was affected by both physical activity and concurrent mood, specifically stress and anger.

2.6.5 Ambulatory Studies of Cardiovascular Disease

Ambulatory monitoring has become a useful tool in the investigation of cardiovascular diseases. Ambulatory monitoring of 63 stable coronary artery disease patients was able to reveal that exogenous factors such as physical and mental activity are the most potent triggers of ischemia³⁹ during the morning hours (Krantz et al., 1996). Krantz et al. also found evidence of the influence of postural change in the morning accounting for the increase in ischemia, as well as evidence for a morning activity-independent circadian influence. Studies like this permit greater insight and knowledge into the events and influencing factors of coronary heart disease, non-invasively and that were originally not possible.

³⁹ Lack of oxygen in the heart muscle.

Ambulatory monitoring used in conjunction with 20 minutes of static cycling in the laboratory examined the effects of acute aerobic exercise in borderline hypertensives compared to normotensives (Brownley et al., 1996). It was found that the 20 minutes of cycling produced significantly lower blood pressure at work in the borderline hypertensives. This effect lasted for up to 5-hours, and was not attributable to any changes in mood, total stress, posture or activity. Hence, borderline hypertensives who engage in aerobic exercise prior to the stress of daily life appear to experience a protective reduction in ambulatory blood pressure.

Johnston et al. (1993) used ambulatory monitoring to investigate the effects of stress management on blood pressure in mild primary hypertension. They were able to establish that the type of stress management recommended for treating mild primary hypertension was ineffective in lowering blood pressure in patients who were habituated to measuring their own blood pressure (Johnston et al., 1993).

Ambulatory monitoring also permits physiological investigation into the effectiveness of non-behavioural interventions such as supplements. Russo et al. (1995) used ambulatory techniques to investigate the effects of an omega-3 polyunsaturated fatty acid supplement on patients with mild essential hypertension. They found that 4-months of low dosage did increase the levels of polyunsaturated fatty acid (PUFA). However, contrary to their prediction, the PUFA when used as a single treatment was ineffective in lowering blood pressure or heart rate in the mild essential hypertensives. This evidence suggests that this specific PUFA may only be beneficial when used in conjunction with other interventions (Russo et al., 1995).

2.6.6 Increasing Ambulatory Knowledge

With the advancement of technology and understanding it has become evident that monitoring heart rate and/or blood pressure is not enough to provide the whole ambulatory picture. Osterhues et al. (1997) investigated the influence of physical activity on 24-hour measurements of heart rate variability of patients with coronary artery disease. They found that physical activity and posture significantly influenced the calculation of heart rate variability. This has implications for the way heart rate

variability is calculated. Since heart rate variability in patients with coronary artery disease is the main predicting factor of further cardiac events, the influence of physical activity and posture must now be taken into consideration (Osterhues, Hanzel, Kochs, & Hombach, 1997).

Concern has also been raised about whether or not the wearing of an ambulatory monitor alters normal behaviour. Blanchard and his colleagues compared the activities, locations and postural positions of 28 hypertensives when wearing an alarm watch and when wearing an ambulatory blood pressure monitor - Spacelab (Blanchard, Cornish, Wittrock, Jaccard, & Eisele, 1990). They found significant differences in behaviour between the two devices, suggesting that although the Spacelab was more accurate moment to moment, it may alter behaviour. However, they admit the study was limited. They did not counter balance for order effects, as all subjects wore the alarm watch before wearing the ambulatory Spacelab. Since wearing the watch and completing diary pages may be reactive and hence alter behaviour it cannot be concluded that the ambulatory device alters behaviour from the normal, only that behaviour differs between the two recording occasions and devices. Additionally, twice as many blood pressure measures were taken as alarm watch measures therefore behaviour was observed and related on a 2:1 basis. They also failed to control for natural differences in behaviour that may have occurred during monitoring.

Increasing Ambulatory Technology

More and more studies are discovering and acknowledging the, until now uncontrolled for, unmeasured factors influencing cardiovascular functioning. Sherwood and Turner, (1993) recognised the fact that stress is encountered in many postures in real-life, yet in the laboratory subjects are typically stressed while seated. They found no significant correlations of seated and standing blood pressure responses to stress. However they did discover that ambulatory monitoring of hemodynamic responses⁴⁰ during stress between postures was correlated. This suggests that according to the variable being researched, ambulatory hemodynamic monitoring may be more informative than blood pressure.

⁴⁰ Cardiac output, total peripheral resistance, systemic vasculature.

Further studies have shown ambulatory monitoring to be influenced by posture and respiration. For example, it has been found that the activity of postural change alters cardiovascular readings (Sipinkova, Hahn, Meyer, Tadlanek, & Hajek, 1997). Knowledge that posture and activity changes influence cardiac functioning during ambulatory monitoring should not be of surprise considering heart rate has long been considered a function of activity and posture. Instead, technology must be developed to measure these variables, which are essential determinants and also sources of unwanted variance.

Makikawa et al. (1994) developed a biosignal memory device using a microprocessor (MICRO - Medical Information Collection RObot). Their microprocessor-based memory device operates like real-time data processing, copes with data compression and automatic recording as well as on-line analysis. The MICRO monitors heart rate via an ECG amplifier and physical activity via a piezo-resistive accelerometer, which they believed suitable due to its low electrical power drain, high durability and wide frequency responses. The device has been found to successfully record changes in heart rate and physical activity in real-life (Makikawa et al., 1994).

Fahrenberg et al. (1996) also questioned the reliability and validity of diary completion in assessing activity during ambulatory monitoring. They evaluated the different accelerosensors available in conjunction with the positioning of the recording site in normal subjects. They found the electromyographic⁴¹ (EMG) measurement recorded from the lower leg, the tri-axial piezoelectric sensors and piezoresistive sensors in conjunction with the body position sensor were best at discriminating the task / activity. Heart rate within-subjects was best predicted by EMG at the lower leg and tri-axial actometer recordings, however after adjusting for subject and condition means only the accelerometers were found to significantly correlate with heart rate (Fahrenberg, Muller, Foerster, & Smeja, 1996). Similarly, Bouten et al. (1996) found the tri-axial accelerometer Tracmor was proficient at distinguishing among inter-individual and intra-individual levels of physical activity in free-roaming subjects (Bouten, DeVenne, Westerterp, Verduin, & Janssen, 1996).

⁴¹ EMG is a measure of general motor activity, which relates directly to the metabolic processes partly determining the level of cardiovascular activity.

At the same time Tuomisto et al. (1996) compared the ability of continuous accelerometric, EMG and hydrostatic posture⁴² measurements to discriminate variations in activity and posture, as well as their ability to predict heart rate variability. They found that although the EMG was a more sensitive measure than the accelerometer both explained comparable amounts of variance. Hydrostatic posture was found to differentiate between postures in a stable manner. It also explained a significant amount of heart rate variance although somewhat less than either the EMG or accelerometry (Tuomisto, Johnston, & Schmidt, 1996). These findings suggest that accelerometric and EMG measures of motor activity used either individually or in combination with hydrostatic posture are capable and valuable in discriminating activities and in controlling for the effects of activity and posture on heart rate during ambulatory monitoring.

Having shown posture and physical activity to be the main determinants of ambulatory heart rate the effort and impetus has been to develop physical activity and posture devices that are more and more sensitive, reliable, accurate and convenient for ambulation. More and more researchers are investigating the capability of bi- and tri-axial accelerometer devices. Bi-axial accelerometry has already been used in the field of psychopharmacology, where it was found to be a reliable and promising method for quantifying aspects of daily-life (postural and mobility activities) and for evaluation of psychological and cardiovascular effects of drugs (Tulen et al., 1997). Tri-axial studies have found that although the accelerometer is highly accurate at discriminating between most activities, its failing lies within the area of static exercise or sedentary activities, such as driving or typing, for which the device is not sensitive enough (Bouten, Koekkoek, Verduin, Kodde, & Janssen, 1997, Sugimoto, Hara, Findley, & Yonemoto, 1997). There are now wide bandwidth piezoresistive accelerometers which can record physical activity and posture even at sedentary levels. These devices consist of a direct current (DC) component and an alternating current (AC) component. The DC component assesses slow motion and change in position relative to the gravitational axis. The AC component represents acceleration along the sensitive axis of the device. Almost 100% accuracy in classifying activities was found for both the AC and the DC components (Fahrenberg, Foerster, Smeja, & Muller, 1997). It can be seen that the

⁴² Measured via a transducer and slim tube filled with sterilized water.

technology has advanced a long way since its origins 40 years ago, along with our understanding of ambulatory monitoring.

The Need for Interactive Ambulatory Heart Rate Monitoring

The technology behind ambulatory monitoring is advancing, however, there is still a need to improve the control of information retrieval and not just the quality of the situational information retrieval. Not only are the tri-axial accelerometers very expensive and not accessible to all, but without the DC component posture recording is poor (Fahrenberg et al., 1997). Additionally, it is tempting to rely on the accelerometer recordings to determine the wearers' activities, when in actual fact without the input of the wearer they can only provide gross discriminations. Instead, attention needs to focus on consideration and refinement of the issues of increasing the controls available during ambulation and improving the detection of psychological and environmental events which may significantly relate to cardiovascular reactivity, such as stress (Johnston, 1996).

The ability to detect significant physiological events, such as interestingly high heart rates, and elicit situational information from the wearer instead of on a random basis is boundless. It has implications for research into disease development, interventions and preventions, especially where the effects of stress on the cardiovascular system are important, as well as research into the understanding, management and treatment of disabilities, such as panic and agoraphobic attacks.

2.6.7 Attempted Validation of an Ambulatory Interactive Heart Rate Monitor

I attempted to validate an interactive ambulatory heart rate monitor. Nine subjects participated by each wearing a posture, activity and heart rate monitor continuously for 48-hours. It was assumed that posture and activity are predictive of heart rate, hence a regression equation for heart rate using these predictors was computed. This equation used data from day one of ambulatory recording, to program the 'Interactive monitor' for day two. When heart rate on day two exceeded that predicted by the equation, the device activated a tone to signal the wearer to provide interaction information in diary

format. It should be borne in mind that any such changes in heart rate could not be explained by locomotor or posture changes, suggesting they reflected “psychological” factors. This allowed us a method of retrieving data from the wearer when something significant occurred, rather than on a purely random basis.

However, although our monitor operated as programmed, it was plagued with technical problems, such as drift in the readings obtained from the posture devices and damage to the fragile activity devices. Moreover, there was little reliability in the relationship between posture/movement readings and heart rate on day two versus day one. This may have been due to the fact that subjects were participating in widely varying behaviours over the two days of recording. This study is fully described in appendix 28. Due to the problem of inconsistency between the day 1 and day 2 heart rate regression models, a more robust but less sophisticated ambulatory blood pressure monitor was employed in the main study.

2.6.8 Summary

Although not fully developed, ambulatory monitoring has greatly advanced since its origins and is now a common method used for investigation of physiological patterns and effectiveness of interventions and therapies in the medical, psychological, pharmacological and biochemical fields.

2.7 *Summarising the Issue in hand*

The issue here is not the effects of stress on cardiovascular reactivity but the possible effects of essential fatty acids on cardiovascular reactivity to stress within the laboratory and during ambulation, and what implications this has for coronary heart disease. Of further interest is the influence which EFA supplementation may have on the psychological mechanisms of emotion and coping, which researchers already know have a vital impact on the body’s ability to protect itself from the damage caused by the experience of stress.

2.8 Glossary of Abbreviations

AC	Alternating current
AE	Autonomic emotional
AR	Ambiguous routines
BP	Blood pressure
BPI	Basic Personality Inventory
BPM / bpm	Beats per minute
CHD	Coronary heart disease
CISS	Coping in Stressful Situations
COPE	Carver et al Coping Scale
CSI	Coping Strategy Indicator
CV	Cardiovascular
CVR	Cardiovascular reactivity
CW	Cognitive worry
DBP	Diastolic blood pressure
DC	Direct current
DR	Daily routines
ECG	Electrocardiograph
EMAS	Endlers Multidimensional Anxiety Scale
EMAS-P	EMAS-Perception
EMAS-S	EMAS-State
EMAS-T	EMAS-Trait
EMG	Electromyography
Ho	Cook Medley hostility inventory
HR	Heart rate
MC	Marlow-Crowe social desirability scale
MICRO	Medical Information Collection Robot
MMPI	Minnesota Multiphasic Personality Inventory
PD	Physical danger
PUFA	Polyunsaturated fatty acid
SBP	Systolic blood pressure

SE

Social evaluation

STAI

State and Trait Anxiety Inventory

WOC-R

Ways of Coping - Revised

3 Methods

3.1 Subjects

3.1.1 Selection Criteria

Participants were selected with as few exclusion criteria as possible. Male subjects were chosen to avoid the confounding effects of female menstrual cycle on results, and to avoid any possible implications that testing a drug supplement (albeit a natural nutrient) may have on women of child bearing age. Furthermore, the selection of male subjects served to maximise experimental power: Male animals require a higher intake of EFAs than females, which may be due to female's ability, in the presence of EFA deficiency, to more rapidly metabolise and retain the EFAs in tissues. (Pudelkewicz et al., 1968). Hence by only using male subjects, who require a greater amount of EFAs, then I would be sure that any effect of EPA supplementation was not confounded by females quicker up-take and retention of the acids. Subjects were also required to be healthy (not taking regular medication and having no known illnesses), of normal weight (weight ranged from 65 to 101.4 kg), with no self-reported history of heart disease. Their resting blood pressure readings had also to be within the range of normal (120/80 to 140/90 mm Hg). They were further required to be non-smokers, as smoking is known to influence cardiovascular function (Jaquet et al., 1994). They were required to eat oily fish fewer than 3 times a week, since more than this would elevate their levels of natural EFAs (Nelson & Ackerman, 1988, Yoshida, Ernani, Azar, & Fernandes, 1998). And lastly, to reduce the likelihood of confounding effects of possible undetected cardiovascular disease, they had to be within the age range 18–35 years.

3.1.2 Recruitment

All subjects were recruited from the vicinity of St. Andrews, Scotland, with the male student population at the University of St. Andrews targeted by a series of recruitment drives.

Initially, every male St. Andrews University postgraduate (around 400) was sent information regarding the experimental design and an invitation for them to take part in the study (see appendix 1 for example). An advertisement was also placed on every university department notice board to coincide with this mailing (appendix 2). Subject response was limited. Sixteen replied, of which only 5 conformed to the selection criteria described above.

In a second effort at recruitment, every male 1st, 2nd and 3rd year undergraduate (excluding medical students) was sent the same details and invitation. The information was also sent again to the male postgraduates. Advertisements were placed in every student hall of residence and permission was also obtained to advertise in the local National Health Service health centre (Pipeland Road Health Centre, St. Andrews, Scotland). Four subjects were recruited.

A third round of recruitment was initiated by placing an advertisement in the student newspaper, requesting subjects. Fliers were produced and left in student bars, departments, halls, the sports centre and other known gathering places for students. Both these approaches gave brief details and directed them to a web page, which gave study details in full and an automatic link to the researcher's email (see appendix 3).

The final round of recruitment focused on male medical undergraduates at the University of St. Andrews. These students were mailed with the relevant information and invitation to join the study, and posters were also placed in the School of Biomedical Sciences.

3.1.3 Interview

Respondents to the recruitment advertisements were invited to an informal meeting. The purpose of this meeting was fourfold: 1) To inform the subjects of the details of the study. 2) To confirm that they fulfilled the required criterion, including blood pressure readings. 3) To establish if they could fulfil the necessary time commitment to participate in the study. 4) To familiarise them with the laboratory environment and the equipment to be used.

3.1.4 Subject Demographics

The twenty-seven subjects were male undergraduates (n=19) or postgraduates (n=8) of St Andrews University. Of the undergraduates three were Medical students, two were Computing students, three were English students, two were History students, one was a History of Art student, three were chemistry students while the remaining five were psychology students. Two of the postgraduates were majoring in English, one in History, two in Chemistry, one in Computing and the remaining two were Management students. The subjects had an average education of 16.19 years (SD = 2.32), with an average age of 23.33 years (SD = 4.83). Their average height was 1.81 metres (SD = 0.05), and their average weight was 77.35 kg (SD = 9.83).

3.2 *Supplementation*

For each subject Laxdale Pharmaceuticals⁴³ provided a 12-week supply of either ethyl-EPA capsules (dose, please see below) or placebo, assigned to the subject on a pseudorandom basis. Each set of capsules arrived coded and split into three bottles, for ease of storage. Testing was performed without the subject or researcher knowing whether the subject had received ethyl-EPA or placebo.

Experimental capsules each contained 500 milligrams of, 98% pure, Ethyl-EPA (Eicosapentanoic Acid, 20:5n-3). Subjects were required to take 4 capsules per day providing them with a total of 2 grams EPA daily. The bottles were labelled with instructions to take the capsules with water and preferably after food. They were free to take all 4 capsules at once or space them throughout the day whichever suited them best. The flexibility in the supplement instructions was possible due to the fact that the half-life of EPA, in red blood cells, is 18 weeks (Brown et al., 1991). The long half-life also favoured the study in that the effects of an inadvertently missed dose would not be detrimental to the overall total of EFAs in the blood. *Placebo* capsules each contained 500 milligrams of Vitamin E, a mineral oil. Instructions were the same – 4 capsules per day with food and water.

⁴³ Company details can be found in appendix 4.

As a standard precaution and to assist in their monitoring Laxdale provided forms and a contact number for reporting any 'Serious Adverse Side Effects'. None occurred.

3.3 Cardiovascular Measures

Cardiovascular measures were taken from each subject, both in the laboratory and during daily activity.

3.3.1 Laboratory Measurements

Heart rate (HR) and blood pressure (BP) were recorded continually while the subject was under test conditions within the laboratory. Heart rate was monitored via a 3-electrode electrocardiogram (ECG) obtained from a Grass polygraph (Model 7PCP8, Grass Instruments, Co, 101 Old Colony Avenue, Quincey, MA). The polygraph consisted of an EEG AC pre-amp driver (Grass model 7P5B) and DC Driver (Model 7DAF, see appendix 5 for settings). Blood pressure was monitored via an Ohmeda finger cuff, and Ohmeda Finapres monitor (Ohmeda, 1315 West Century Drive, Louisville, CO). This system was used to display heart rate, diastolic and systolic values online and to produce an analogue signal (1 volt/100 mm Hg) representing blood pressure (Mulder et al., 1991).

The ECG data (from the Grass electronics) and the blood pressure data (from the analogue output of the Finapres) were digitised with a CED 1401+ data acquisition system (Cambridge Electronic Design, Cambridge, UK) using SPIKE 2 (version 3.14; Cambridge Electronic Design) as the data acquisition and analysis software. The data acquisition system was set to record 3 channels: Raw HR as a continuous waveform (sampled at 500 Hz); Raw BP as a continuous waveform (sampled at 250 Hz) and an additional keyboard channel, to mark the onsets and offsets of task epochs within the recording session (10 Hz). It should be noted that although termed 'continuous' there was intermittent loss of blood pressure data due to the Finapres' self-servo re-calibration period.

3.3.2 Ambulatory Measurements

Blood pressure was sampled at regular intervals by the Takeda Medical TM-2420 ambulatory blood pressure monitor (A&D Company Ltd, Frankfurt, West Germany). The monitor consists of an upper arm cuff and recording unit. The recording unit is compact and lightweight, weighing 390g. It consists of a battery for cuff inflation, a solenoid valve to control deflation speed and a processor to permit varied setting of recording times and frequency intervals. A separate TM-2020 processor, which is not carried by the subject, allowed programming and download of the recorder and its data. The cuff was secured to the subject's arm during ambulation, while the recorder unit was carried in a waist holster or on a plastic belt clip, whichever the subject preferred.

3.4 *Psychological Measures*

Mood can affect a subject's physiological responses (Abel, Larkin, & Edens, 1995, Higgins & Endler, 1995, Johnston et al., 1997, Lefcourt et al., 1997, Prkachin & Mills, to be submitted, Weidner et al., 1989). It can also be shown to be influenced by EFA levels (Horrobin & Bennett, 1999, Maes & Smith, 1998, Patterson, Gottdienerr, Hecht, Vargot, & Krantz, 1993, Peet, Murphy, Edwards, Shay, & Horrobin, 1997b, Peet et al., 1998, Stoney, Bausserman, Niaura, & Fahrenbach, 1994, Vitaliano, Russo, & Niaura, 1995, Vogelee, 1998). Therefore it was considered essential to assess the subjects' moods during the various stages of testing to assist in the precision of the study.

3.4.1 Laboratory Sessions

Endler Multidimensional Anxiety Scales (EMAS)

These are a set of three pen and paper self-report scales, produced by Norman S. Endler and published by Western Psychological Services, California. They measure different components of anxiety (see appendix 6 for example):

EMAS-S measures state anxiety. It consists of 2 sub-scales, *cognitive worry* and *autonomic-emotional* which are measured using a 20-item scale. Both sub-scale totals can be combined to provide an over-all State anxiety total.

EMAS-T measures trait anxiety. It consists of 4 scales of, *social evaluation*, *physical danger*, *daily routines* and *ambiguous*. Each scale has 15-items, providing a comprehensive breakdown of Trait anxiety.

EMAS-P measures subjective perception of the type and intensity of threat in the current situation. It consists of 8-items.

Measures of the psychometric properties for EMAS when administered to U.S. male undergraduates are as follows: Internal consistency alpha coefficient for EMAS-S = 0.92, and EMAS-T subscales range from 0.89-0.93. Construct validity for EMAS-S when correlated to Spielberger's STAI is 0.65-0.75 and for EMAS-T subscales range from 0.48-0.62. Test-retest reliability over 1 month for EMAS-S ranged from 0.35-0.46 and for the EMAS-T subscales over 2-weeks scores ranged from 0.60-0.79 and 0.50-0.62 over 4-weeks (Endler et al., 1991).

Coping Inventory for Stressful Situations (CISS)

The CISS is a pen and paper self-report rating scale of coping behaviour. The scale was produced by Norman Endler and published by Multi-health Systems Inc., Ontario. It consists of a 48-item scale requiring a response on a 5-point rating scale. It measures 5 styles of coping (see appendix 7):

Task oriented, consisting of 16-items.

Emotion-oriented, consisting of 16-items

Avoidance-oriented, consisting of 16-items, with two further sub-scales for *distraction* and *social diversion*.

Measures of the psychometric properties for CISS when administered to U.S. male undergraduates are as follows: Internal consistency alpha coefficients of the 3 factors are 0.90, 0.90 and 0.91. Construct validity for the five CISS subscales when correlated to Marlowe-Crowne Social Desirability Scale is -0.38-0.25 and -0.47-0.49 when

compared to Folkman and Lazarus, 1985 Ways of Coping Questionnaire. Test-retest reliability over 6-weeks was 0.73, 0.68 and 0.55 for the 3 factors (Endler et al., 1991).

Dietary Instrument for Nutrition Education (DINE)

The DINE assesses dietary intake of *fat, fibre and unsaturated fat*. It uses a frequency self-report approach, providing 3 sub-totals and an overall score (see appendix 8). Liane Roe and Margaret Thorogood, (ICRF General Practice Research Group) designed the DINE (Roe, Strong, Whiteside, Neil, & Mant, 1994) which has been previously used for research purposes (Wardle, Parmenter, & Waller, 2000). Psychometric properties as such are not available for the DINE which was designed to provide a quick and reliable indication of fat and fibre intake to enable monitoring of agreed changes in dietary habits.

Exercise Scale

No short simple questionnaire of level of exercise was readily available. Instead a list of 15 common forms of exercise was drawn up based on a measure used in the Cardia and Minnesota Heart Health program (Jacobs, Hahn, Haskell, Pirie, & Sidney, 1989). The measure was used to assess type and frequency of exercise on a 7-point self-report scale (see appendix 9). The Cardia measure itself has a correlation coefficient of 0.90 for males assessed exercise level and 2-week test-retest reliability coefficients ranging from 0.77-0.84 dependent of the level of exertion required.

Satisfaction with Life Scale (SWLS)

This is a short measure, consisting of 5 statements. Each statement pertains to subjective well being, and requires a rating to be made on a 7-point scale. (See appendix 10). Measures of the psychometric properties for SWLS when administered to male undergraduates are as follows: Internal consistency alpha coefficient for the SWLS is 0.87. Construct validity for the SWLS ranges from 0.61-0.81 when correlated with Adams (1996) Life Satisfaction Index. Test-retest reliability coefficients of a 2-month period was 0.82 (Diener, Emmons, Larsen, & Griffin, 1985).

Appraisal of Life Events Scale (ALE)

The ALE is not currently published but was provided by Eamonn Ferguson. This scale consists of 2 measures: subjective perception of current environment (general version); subjective perceptions of a specific event (specific version), via a list of 16 adjectives. It requires an indication on a 6-point rating scale according to each adjective's appropriateness (see appendix 11). Measures of the psychometric properties for ALE are as follows: Three stable factors of threat, challenge and loss exist with stability coefficients of the range 0.94-0.99, with internal consistency alpha coefficients of 0.74-0.86. Test-retest reliabilities for a single stressful event over a 1-month period range from 0.77-0.90. Test-retest reliabilities over a 3-month period for a 'life transaction' such as leaving home were 0.49 for threat appraisals, 0.48 for challenge appraisals and 0.59 for loss appraisals, $p < 0.01$ (Ferguson, Matthews, & Cox, 1999).

3.4.2 Ambulatory Measures

Diary

Subjects completed a small diary page after every cuff deflation, every 30 minutes throughout the 8-hour ambulation period. The diary provided data on *time, place, social situation, activity type, nutrition / stimulants, physical symptoms* and *mood*. These permitted a picture of the subject's environment and influencing factors, at the time of blood pressure recording, as well as a subjective indication of the subject's emotions, via a mini mood scale.

The mini mood scale used was adapted from the UWIST Mood Adjective Checklist - UMACL (Matthews, Jones, & Chamberlain, 1990) list of terms and MacKay's scaling technique (Mackay, Cox, Burrows, & Lazzarini, 1978). Matthews' UMACL was chosen due to its reliability and sensitivity to short term changes in mood, ideal for our half hourly readings. The UMACL measures dimensions of *energetic arousal (E)*, *tense arousal (T)* and *hedonic tone (H)*. Four terms were selected for each dimension - two negatively weighted and two positively weighted. Generally the term with the highest weighting was used, except in cases where possible ambiguity / confusion would occur,

between dimensions. Scoring occurred for a positive response to a positively weighted word, and for a negative response to a negatively weighted word. Matthews believed E reflected 'physiological energy mobilisation', T reflected 'emotional / stress reactions' while H may reflect 'motivational gratification'. Several studies show the UMACL scales to be sensitive to external stressors (Cumberbatch, Millner & Wragg - unpublished and Jacobson, 1938, in Matthews et al., 1990). Stressors evoking a general stress syndrome response are associated with reduced E and H and increased T. The order of word presentation was counterbalanced. The diary was designed to focus on the issues of specific interest to the aims and hypotheses (activity, place, and situation) and identify and measure the potential influence of current mood (see appendix 12).

3.4.3 Periodic Assessment over the Course of Supplementation

Beck Depression Inventory (BDI)

The BDI is a 21-item inventory designed to assess the severity of depression. Each item uses a 4-point rating scale scored on a 0 – 3 range of severity. It is a tool predominately used in a prognostic manner, to assess depression severity in previously diagnosed sufferers, however it has successfully been used to indicate depression levels in healthy individuals (Beck & Steer, 1979). The severity score ranges for the BDI are: 0 – 9 *minimal*; 10 – 16 *mild*; 17 – 29 *moderate* and 30 – 63 *severe*. The BDI was administered at 4-weekly intervals to determine if any changes occurred over the time of supplementation. See appendix 13. Measures of the psychometric properties for the BDI are as follows: An internal consistency alpha coefficient of 0.81 was found from 15 non-psychiatric samples. Validity for the BDI when correlated with the Beck Helplessness Inventory, Hamilton Psychiatric Rating Scale for Depression (Hamilton, 1960), SCL-90 and MMPI ranged from 0.60-0.76. Test-retest reliability for 9 non-psychiatric samples ranged from 0.60-0.90 (Beck & Steer, 1979), while Lightfoot and Oliver (1985) found test-retest reliability to be 0.90 for 204 undergraduates.

3.5 Tasks

The tasks were performed within the laboratory, under the conditions of cardiovascular monitoring described above. The 3 cognitive tasks were chosen to elicit a psychological stress response, while the physical task was selected as a physiological stressor.

3.5.1 Cognitive Tasks

Raven's Matrices (RM)

The Raven's Progressive Matrices are a standardised cross-cultural non-verbal test of intelligence (Raven, 1948). Raven's progressive matrices require the selection of the appropriate missing segment of a pattern for a correct score. For the purposes here a shortened computerised version was used, and presented on an Intel 80486 computer. The stimuli were 48 differing patterns, ranging in difficulty. Each stimulus was individually presented in a random order on screen, with an array of possible options (between 6 and 8, see appendix 14 for example). The subject was visually prompted to provide an answer within the time limit. The time limit was 30 seconds for complex, and 20 seconds for simple patterns. A count down tone was emitted to indicate running time. The subjects had up to 3 attempts at a correct answer, if time permitted. Subjects were given no break in presentation, each stimuli immediately followed the last. The instructions given to the subjects prior to the test can be found in appendix 15.

Choice Reaction Time Task (RT)

A choice reaction time task was conducted on the same computer used for the Raven's matrices. The stimuli consisted of the numbers 1, 2 and 3 briefly displayed on the computer screen. Each was presented one at a time in a random order. The task consisted of two conditions: the *compatible* condition, in which the subjects pressed the number corresponding to the stimulus they saw, and the *incompatible* condition, in which the subjects pressed button 3 for stimulus 1, button 1 for stimulus 2 and button 2 for stimulus 3. The task consisted of 6 blocks of 72 presentations. Presentation speed

(500 msec for the compatible and 800 msec for the incompatible) permitted a response by most people but did not wait for one before moving to the next stimuli. Incorrect or too slow responses elicited a harsh tone. The blocks alternated between compatible and incompatible responses, with a visual instruction at the start of each (see appendix 16).

Acoustic Startle Task (Startle)

The Startle task is widely used in animal research to measure psychological variability, (Koch & Hans-Ulrich, 1997, Lang, Bradley, & Cuthbert, 1990, Walker, Cassella, Lee, Lima, & Davis, 1997). It has also been used in human clinical populations for the same purpose (Blumenthal & Berg, 1982, Blumenthal, Chapman, & Muse, 1995, Cadenhead, Geyer, & Braff, 1993, Frankland, Dockstader, & McDonald, 1998, Kaviani, Gray, Checkley, Kumari, & Wilson, 1999, Panayiotou & Vrana, 1998).

The task was presented on an Apple Macintosh using PsyScope (Carnegie Mellon University, Cohen, MacWhinney, Flatt, & Provost, 1993) and required the subjects to press a button as quickly as possible after hearing a tone. Most (90%) of the tones were soft, requiring effort to detect them above background noise. The remaining tones (10%) were loud (full volume on the computer's audio system) and were designed to provoke a startle response. The tones were presented randomly and with varied presentation delays (200, 400, and 600 milliseconds). The subjects sat in front of the Macintosh computer while wearing a set of headphones. The subjects were required to listen for the random tones, via the headphones, and then to press a single key on the keyboard after every tone had ended (see appendix 17 for full subject instructions).

The computer screen was blank while the subjects listened for a tone. For every loud tone there was 8 soft tones presented in pseudorandom order. If the subject responded within 1500 msec then a green 'OK' was displayed on the screen for 1 second. The inter-tone interval (time between subject response and start of next random presentation delay) was 1 second, following soft tones and 12 seconds following loud tones. Incorrect responses could be one of two types and resulted in a red 'X' being displayed on the screen for 500 msec. The first type of incorrect response was if the subject failed to press the key within 1500 msec, and resulted in the trial being run again. The second

type of incorrect response occurred when the subject pressed a key other than the one designated in the instructions. If this occurred then the inter-tone interval began at the same time as the error display and the trial was re-run.

Digital transistor-transistor logic (TTL⁴⁴) pulses were sent by a PsyScope Button Box (Carnegie Mellon University) to the digital input port of the CED 1401+ so that the time stamping of relevant behavioural events (soft tones, loud tones and button presses) could be synchronised with the cardiovascular data.

A pilot subject successfully performed the acoustic startle task. Differences were seen in the subject's heart rate in response to soft and loud tones.

3.5.2 Physical Exertion Test

Static Cycling

The subjects were required to cycle for 8 minutes on a static exercise bicycle with a 1.5-kg weight resistance. They were instructed to 'cycle with effort, as you would for a workout, not as if you are out for a casual cycle. Try to achieve and maintain a speed of 30 km/h'. Subjects were prompted to speed up if they dropped below the 30km/h threshold. Subjects were also informed that they were free to stop at any time if they felt they became exhausted. None stopped before the 8 minutes were completed.

3.6 Biochemical Measures

Red Blood Cell analysis was necessary to determine quantity of EFAs in the subjects' blood before and after supplementation. A fully licensed physician collected a 10-ml venous blood sample from each subject. Samples were drawn into a tube with EDTA, as an anticoagulant. They were then spun in a centrifuge, at 1500gav⁴⁵ for 15 minutes at 4 °C. The plasma layer and buffy coat were separated from the red cells. The red cells

⁴⁴ Digital *ON* (+5V), *OFF* (0V) low voltage electrical pulses.

⁴⁵ Speed of spin relative to gravitation

were washed with an equal volume of 0.9% saline and centrifuged again for 15 minutes at 1500gav and 4 °C. The saline wash and buffy coat were discarded and the red cell fractions were transferred into a fresh tube. This fresh tube was then labelled and immediately frozen and stored at -70 °C, to await analysis.

3.7 Procedure

Potential subjects individually attended the initial interview, in which they were selected on the basis of the criteria described above. Those subjects who meet the criterion and wished to participate were then given a convenient start date and time to begin the study.

The procedure consisted of two days of testing at the beginning and end of a 12-week study period, with questionnaires being completed at home every 4 weeks (see appendix 18 for protocol).

3.7.1 Time One (Before Ethyl-EPA Supplementation)

Day One

The subject arrived at the laboratory on the morning of day one, where their written consent was obtained (see appendix 19 for example form). Their age, height and weight⁴⁶ were recorded. Their blood pressure was then measured using a Takeda electronic monitor. This enabled verification of the set up reading on the TM2420.

The following measures were then administered: DINE; Exercise level; EMAS-S; EMAS-T; EMAS-P; CISS; SWLS and ALE. The subjects were read each set of instructions first and asked to fill them in as honestly and accurately as possible. They were permitted to ask questions at any stage.

⁴⁶ Measured without shoes on a Salter electronic scale.

The ambulatory blood pressure cuff and monitor was then attached securely, calibrated and programmed. The monitor was programmed to inflate every 30 minutes between 2 p.m. and 10 p.m. Instructions on how to complete the diary page, after every deflation, were then given. They were instructed to avoid caffeine in excess⁴⁷, alcohol, contact sports and bathing / showering whilst wearing the monitor. Within these constraints, the subjects were asked to go about their normal daily routines. The subjects were permitted to remove the cuff and monitor after 10 p.m.

It should be noted that with this type of recording apparatus and half-hourly diary completion there would be a degree of intrusiveness, which may influence the subjects' behaviour during the recording event. The time taken to complete the diary page was minimised by keeping the diary short and simple.

Each subject was then supplied with an envelope containing the BDI and EMAS-S, with instructions to complete them during a quiet interlude that evening. Before leaving the laboratory the subject was provided with an office and 24-hour mobile phone number to use should he have any questions / worries during the course of that day.

Day Two

Subjects returned to the laboratory the next morning. The ambulatory BP data were downloaded and the diary and evening scales checked in. The subjects were then taken to the Biomedical Sciences Department, where Prof. M. Steel took a 10-ml blood sample from them. Once they recovered the subjects returned to the laboratory.

The subject was seated in a comfortable chair in quiet surroundings, where the ECG and Finapres blood pressure finger cuff⁴⁸ were attached and calibrated and recording began. The first epoch (A) of recording was a 15-minute baseline period where the subject sat alone. The subject was then asked to perform the Raven's matrices task (B) followed by a 3-minute break (C). They were then asked to perform the Reaction time task (D), again with a 5-minute break (E).

⁴⁷ Excess was considered 4 or more cups of tea/coffee/cola per day.

⁴⁸ Attached to middle finger of the subjects' non-dominant hand.

The subject was then moved to the static cycle where they sat still for 3 minutes to negate any effect of movement to the cycle (F). They were then required to cycle with effort for 8 minutes (G). After which they were given a 5-minute recovery break (H) and recording stopped.

The electrodes and cuff were removed and subject asked to complete the EMAS-S, EMAS-P and ALE. The subject was given his set of capsules with instructions and contact details.

3.7.2 Weeks 4 and 8

At this time the subject was sent the EMAS-S and BDI to complete that evening during a quiet period. They were asked to return the measures via internal mail.

3.7.3 Time Two (After Ethyl-EPA Supplementation)

Day One and *Day Two* followed the identical procedure, as at time one, up until epoch H: After completing their cycling (G) and 5-minute recovery break (H) the subjects were returned to the comfortable chair. Instructions for the Acoustic Startle (Tone response) task were given and headphones worn (I). On completion of this task (J) they had a 3-minute break (K). Recording was stopped and equipment removed. Again they were asked to complete the EMAS-S, EMAS-P and ALE.

Each subject was then debriefed (except for capsule type, which they would be informed of later) and permitted to ask any questions. They were thanked and given their £100 payment.

3.8 Study Design

The study is a mixed factorial design, with a repeated-measures factor of time of testing and a between group factor of supplementation (ethyl-EPA or placebo). The dependent measures taken at various time points were classified into four groups: biochemical measures; cardiovascular measures; psychological tests and cognitive tests. Data analysis for each of these constellations of measures will be presented in separate result sections.

3.9 Ethical Approvals and Safeguards

3.9.1 Subject Safeguarding

Crucial to the design of this study was the safeguarding of the subjects, which was performed in line with British Psychological Society (BPS) guidelines. Each subject was fully informed of the requirements, and their written consent was obtained at each test session. The subjects were advised that they were able to withdraw from the study at any point in time and without having to provide a reason. They were also informed that they would receive payment for their participation up to that point. The advertisements, information sheets and consent forms given to the subjects are included in appendix 2, 19 and 21. No subject exercised his right to withdraw.

3.9.2 Ethics Approval

The initial application was approved by the University of St. Andrews, School of Psychology Ethics committee, conditional on the right to withdrawal and payment to that point, being made explicit to the subjects. A later amendment regarding the inclusion of the acoustic startle task was submitted to the committee. Having confirmed that the 'loud' tone could not cause hearing damage the study was fully approved. See appendix 22 for the ethics letter of approval. Application was also made to the Fife Health Board Local Research Ethics Committee for approval of clinical research within

Fife. This permitted recruitment of the medical students and general public. See appendix 23 for letter of approval. Furthermore Banner Pharmacaps, who manufactured both the EPA and placebo, guaranteed that both sets of capsules were “in accordance with the principles and guidelines of good manufacturing practice for medical products for human use as is required by the directive 91/356/EEC.”

3.9.3 Insurance

A fully trained and insured physician (Prof. M. Steel, who has Medical Defence union cover as well as being on the Medical Register, and holding Honorary NHS clinical contracts in Lothian and Tayside) was engaged to draw the blood samples, using standard sterile techniques. Although EFAs are nutrients and have no known side effects, each subject was also given the ‘serious and adverse side effect’ forms and a 24-hour phone number to contact the researcher. Should any problems have arisen from the supplementation then the entire study was underwritten by the University Insurance brokers, Royal & Sun Alliance. A copy of the letter confirming this can be found in the appendix 24.

4 Biochemical Measures - Results

4.1 Essential Fatty Acids Explained

4.1.1 Essential Fatty Acid Structure

Essential fatty acids are carbon chains that have two or more double bonds. They can be placed into one of two groups, omega-3 ($n-3$) or omega-6 ($n-6$). This group allocation is according to the position of their first double bond, from the carbon atom at the methyl end of the chain. A simple shorthand notation can define the structure of an EFA. For example eicosapentaenoic acid is C20:5 $n-3$, while gamma-linolenic acid is C18:3 $n-6$. The letter C denotes that it is a carbon chain, but it is often omitted when writing as it is considered unnecessary. The first number defines the number of carbon atoms in the chain (20 and 18 in these examples). The second number after the colon specifies the number of double bonds (5 and 3 in these examples). The omega group of EFAs that it belongs to is defined by the $n-3$ or $n-6$ figure at the end. Fatty acids usually contain even numbers of carbon atoms in straight chains, hence they range⁴⁹ from C14 to C20:

C14:0	Myristic
C14:1	Myristoleic
C16:0	Palmitic
C16:1	Hexadecenoic
C18:0	Stearic
C18:1	Oleic
C18:2($n-6$)	Linoleic
C18:3($n-6$)	γ -linolenic
C18:3($n-3$)	α -linolenic
C18:4($n-3$)	Octadecatetraenoic
C20:0	Eicosanoic

⁴⁹ This is the most commonly defined and accepted range.

C20:1	Eicosenoic
C20:2(n-6)	Eicosadienoic
C20:3(n-9)	Dihomo- γ -linolenic
C20:3(n-6)	Dihomo- α -linolenic
C20:3(n-3)	Eicosatrienoic
C20:4(n-6)	Arachidonic
C20:4(n-3)	Eicosatetraenoic
C20:5(n-3)	Eicosapentaenoic
C22:0	Docosanoic
C22:1	Docosenoic
C22:4(n-6)	Docosatetraenoic
C22:5(n-6)	Docosapentaenoic
C22:5(n-3)	Docosapenteanoic
C22:6(n-3)	Docosahexaenoic
C24:0	Tetracosanoic
C24:1	Tetracosenoic

4.1.2 The Essential Fatty Acid Ratio

It has been established that n-3 and n-6 EFAs compete with one another during metabolism, and that the n-3s are preferential in this process and hence more effective at displacing the n-6s than vice versa (Sprecher, 1982). Optimal levels of each EFA are not known, but researchers are aware that there must be a balance or ratio between n-3s and n-6s within the body. In most tissues of the human body the n-6:n-3 ratio lies somewhere within the range of 3:1 to 9:1 (Leeds et al., 1990). Hence the exact influence that one EFA may have on another is not entirely clear. Studies do suggest that supplementation with omega-3 will lead to a decrease of omega-6 levels. For example, supplementation with omega-3 was found to lead to a marked increase in 20:5n-3 after 6 weeks, and 22:6n-3 after 12 weeks, while 20:4n-6 showed a large decrease after 6 weeks. Neither the 22:6n-3 nor the 20:4n-6 had returned to pre-treatment levels after a 24-week washout period (Marangoni et al., 1993). Similarly Prisco et al. (1996) found a depletion of n-6 levels after 4 months n-3 supplementation.

4.2 *Blood Biochemistry*

4.2.1 Transportation of Blood Samples

The frozen blood samples were removed from storage and packed into a polystyrene box with a minimum of 8lbs of dry ice to maintain their temperature during courier transportation for analysis. The fatty acid analysis was performed on the blood samples by Sheena Rowbottom at Mylnefield Research Services, Scottish Crop Research Institute, Invergowrie, Scotland.

4.2.2 Biochemical Analysis

Level One analysis (Manku, Horrobin, Huang, & Morse, 1983) was performed on the Red Blood Cell (RBC) samples. This procedure consists of a simple extraction of all lipids in the red blood cells and quantification of relative and absolute fatty acid composition. This method is considered sufficient for identifying EFA concentrations, as red blood cells largely contain phospholipids and free cholesterol, with only trace amounts of non-polar lipids, which would not be enough to contaminate the lipid extraction (Manku et al., 1983). Level One analysis consists of 3 stages:

Lipid Extraction

On thawing, the red blood cell fraction was suspended in NaCl/H₂SO₄ aq. (sodium sulphate, 17mmol/l NaCl, 1mmol/l H₂SO₄, 1.8ml), then shaken with methanol (3ml), Chloroform (6ml) and C17:0 (synthetic diheptadecanoyl-phosphatidylcholine). The C17:0 was used as an internal standard for calibration of the EFA assay. This mixture was stirred vigorously using a vortex mixer and then centrifuged at 2000g for 10 minutes. The lower layer containing the lipid extract was carefully removed and filtered through sodium sulphate before evaporation to dryness.

Preparation of Methyl Esters

Both the total lipid extract and the individual lipid classes were transesterified using H₂SO₄/methanol. They were purified by loading onto an isohexane-washed silica column prior to elution⁵⁰ with isohexane: diethyl ether (95:5).

Analysis by Gas Chromatography

The resulting methyl esters of the fatty acids were separated and measured using a Hewlett Packard HP5890 Series II Plus Gas Chromatograph (Cp-wax 52CB 25m capillary column, Chrompack UK). The carrier gas was hydrogen (1ml/min). Oven temperature was programmed to rise from 170 °C to 220 °C at 4 °C per minute. Detector temperature was 300 °C and injector temperature was 230 °C. Retention times and peak areas were automatically computed by Hewlett Packard HP3365 Chem Station software (Revision A.06.01). Peaks were identified by comparison with standard 17:0 methyl fatty acid ester (Sigma UK, Poole, Dorset).

4.3 Results

4.3.1 Sample Description and Exclusions

Twenty-eight subjects completed the study. The placebo and experimental groups were evenly matched in numbers (n=14) at the onset of the study. However, one *placebo* subject could not be included in the final analysis due to his commencing medication⁵¹ for depression mid-way through the study. He admitted withholding his diagnosis at the initial interview stage, as he had not accepted treatment at that time. One *EPA* subject failed to take over a third of his capsules (by his own admission) but was included in the analysis because of the long half-life and wash-out period of EPA (Brown, Pang, & Roberts, 1991, Marangoni et al., 1993, Prisco et al., 1996). However this did raise the issue that subject compliance to capsule consumption was not checked via a count of

⁵⁰ To wash out, by the action of a solvent.

⁵¹ Paroxetine 20mg was administered for treatment by his GP.

the capsules returned unused. Instead subjects were asked every four weeks if they had missed any capsules in the previous week. On average over the 12-weeks each subject reported missing between 2 and 4 days worth. Moreover the absolute levels of their RBC fatty acids were relied on, with no correlation made to the amount of capsules consumed. Each of the 27 subjects had a before and after RBC sample, producing a total of 54 samples which under went analysis.

4.3.2 EPA (20:5n-3)

Effect of Ethyl-EPA Administration

As shown in Table 4.1, the dosing regime increased the levels of EPA by more than a factor of two while the EPA levels in the placebo group were relatively unchanged. A repeated-measures ANOVA confirmed that the apparent differences between the EPA and Placebo groups after supplementation were not likely to be due to chance (Group x Time interaction [$F(1,25) = 39.65, p < 0.001$]; see figure 4.1).

RBC levels – EPA Only								
<i>EPA (n=14)</i>					<i>Placebo (n=13)</i>			
<i>Time</i>	<i>Min.</i>	<i>Max.</i>	<i>Mean</i>	<i>S.D.</i>	<i>Min.</i>	<i>Max.</i>	<i>Mean</i>	<i>S.D.</i>
<i>Before</i>	7.91	16.70	11.57	2.46	7.08	27.49	13.64	6.04
<i>After</i>	19.21	55.79	33.95	12.54	5.67	28.85	12.46	5.98

Table 4.1: Effects of ethyl-EPA administration versus placebo on the concentration of EPA detected in red blood cells. Units are expressed as $\mu\text{g/g}$ (micrograms of fatty acid per gram of RBC sample).

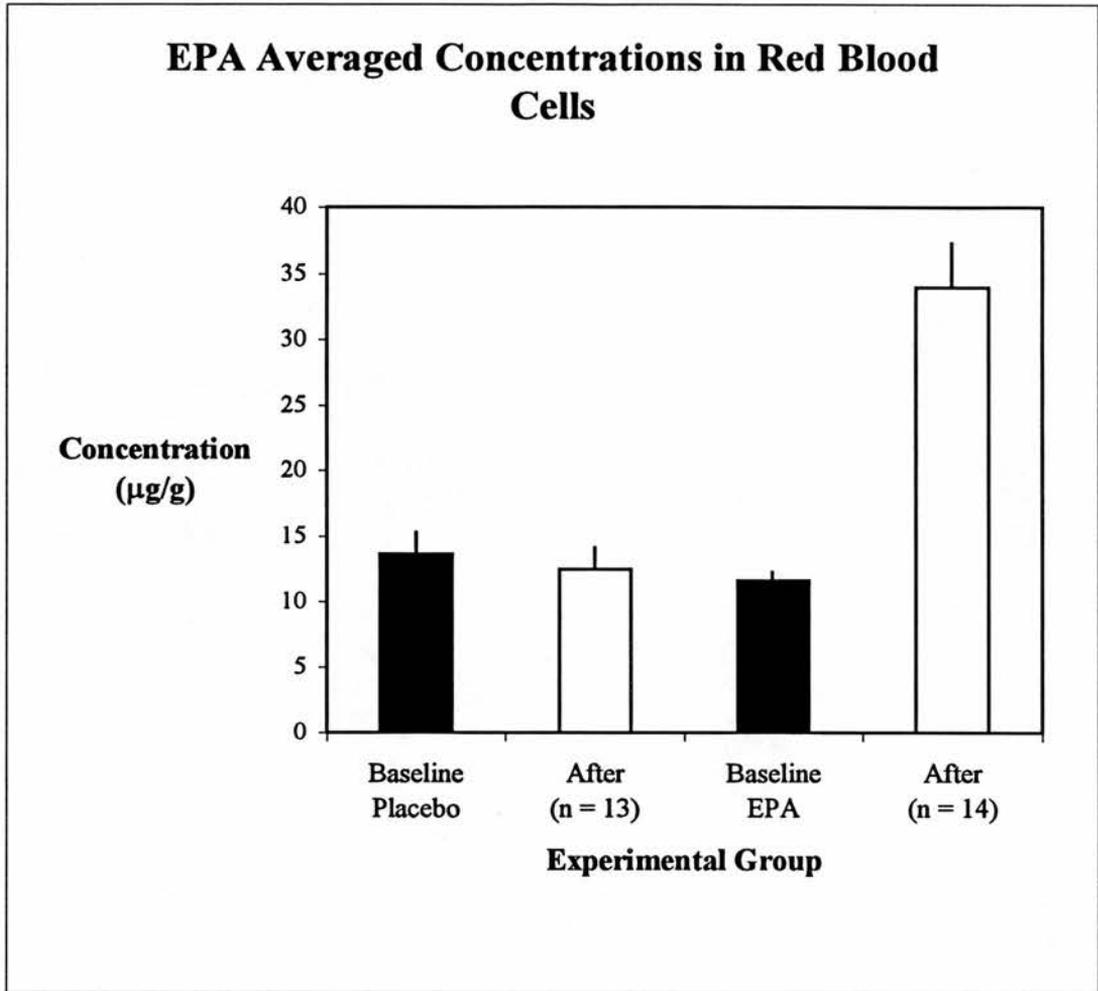


Figure 4.1: Group averages (\pm S.E.M) of concentration levels ($\mu\text{g/g}$) of Eicosapentaenoic acid at *Baseline* and *After* Supplementation. Red Blood Cell concentration analysis was based on methods in Manku et al. (1983), as described in section 4.1.

4.3.3 Essential Fatty Acids

The blood sample analysis looked at all the fatty acid components. The previous section looked only at the intervention fatty acid, EPA (C20:5*n*-3). Since there appear to be interactions between the biochemical pathways that determine the levels of n-3 and n-6 (or omega-3 and omega-6), I examined the effect of ethyl-EPA administration on all of the fatty acid components.

Table 4.2 below lists the average percentage change in the fatty acids analysed by the biochemical procedure. Although this table describes the differences between the EPA and Placebo groups, it does not allow us to identify objectively groups of fatty acids that covaried, although there appear to be groups of EFAs that do. For instance, although there was a large change in EPA levels for the EPA group (+193%) as expected, levels of other fatty acids changed by as much as fifty percent. Thus, these multivariate data were analysed with principal component analysis in order to identify constellations of fatty acids that covaried across the blood samples.

Fatty Acid	EPA group (n=14) % Change	Placebo group (n=13) % Change
C14:0	-7.24	8.02
C14:1	22.63	109.78
C16:0	-6.99	-2.24
C16:1	-13.47	17.48
C18:0	-4.53	-1.15
C18:1(n-9)	-9.00	-2.21
C18:1(n-7)	-4.80	-0.99
C18:2	-15.97	-4.20
C18:3(n-6)	-3.72	-9.11
C18:3(n-3)	-20.42	-14.20
C18:4(n-3)	-15.91	1.57
C20:0	-4.43	-3.06
C20:1	-22.71	-4.22
C20:2(n-6)	-15.10	-3.64
C20:3(n-9)	-25.10	-0.11
C20:3(n-6)	-23.23	-4.37
C20:4(n-6)	-18.66	-6.24
C20:3(n-3)	232.96	150.75
C20:4(n-3)	-39.96	-3.18
C20:5(n-3)	193.35	-8.64
C22:0	-2.05	-3.41
C22:1	52.60	54.19
C22:4(n-6)	-30.43	-7.91
C22:5(n-6)	-37.74	0.79
C22:5(n-3)	47.79	-9.32
C22:6(n-3)	-15.85	-7.08
C24:0	-1.13	-4.15
C24:1	-7.25	-9.91

Table 4.2: Shows percentage change $[100(\text{After}-\text{Before})/\text{Before}]$, per group relative to the pre-supplementation baseline, in RBC fatty acid components after the intervention. The row containing information regarding EPA (C20:5(n-3), the fatty acid supplement given to the EPA group, is highlighted in **bold**.

Principal Components Factor Analysis

To identify if groups of fatty acid components existed, the data reduction technique of factor analysis was employed. Each subjects' RBC fatty acid concentration levels, from both before and after, was standardised (z-scored). Thus each subject contributed two sets of data to the analysis. A principal components analysis was then performed on the z-scored data. Scree plot results show two dominant unrotated factors. Factor 1 accounts for 40.3% of the variance, while Factor 2 accounts for the next 12.1% of the variance (see figure 4.2).

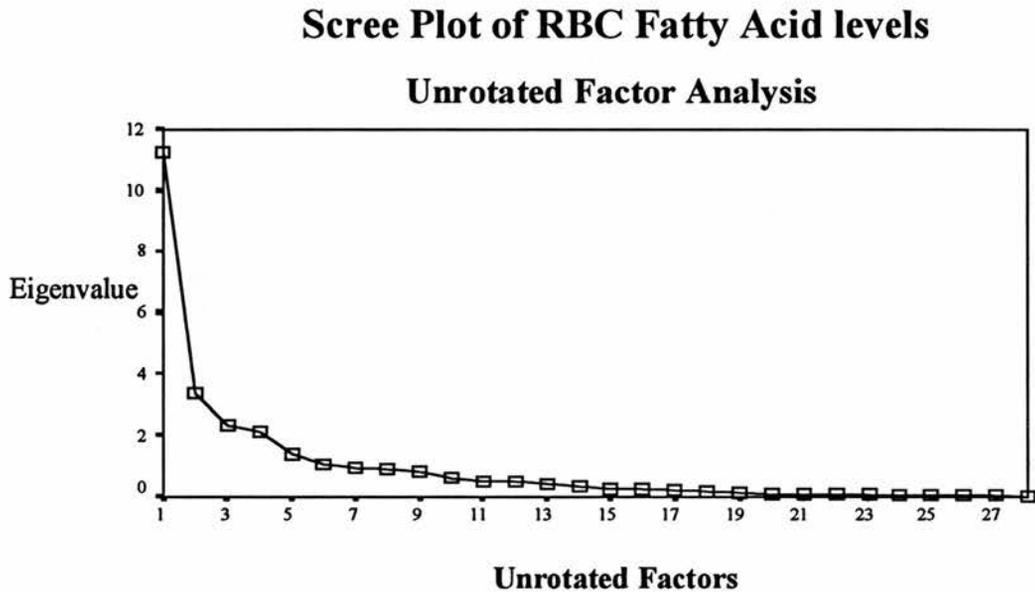


Figure 4.2: Scree Plot of unrotated factors determined from RBC fatty acid concentrations (both groups and time points included). Results from principle components factor analysis (z-scored). Factors 1 and 2 count for 52.4% of the variance.

The weightings of the component fatty acids within each factor are shown in figure 4.3. Factor 1, with all positive weightings, related to the total amount of fatty acid in the blood. Factor 2, although accounting for a smaller percentage of the variance, revealed the more interesting result. EPA was weighted highly in this factor, as were the other n-3 acids, whereas the n-6 acids were weighted negatively. Thus, the impact of EPA supplementation was not specific to the blood concentration of the compound itself, but it had a complex effect on other fatty acids.

RBC Fatty Acid Component Factor Matrix

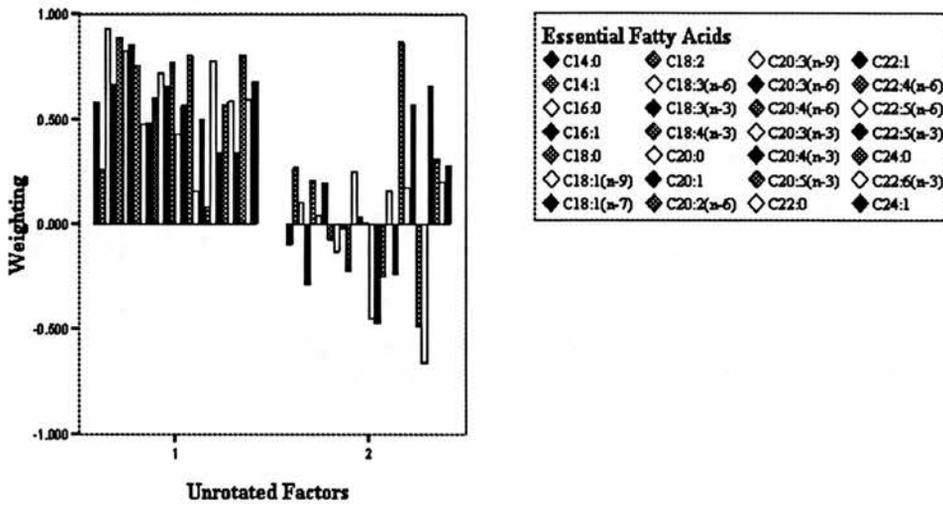


Figure 4.3: Unrotated factor weightings from the principal components analysis of standardised fatty acid concentration levels from each subject at each time period. Factor 1 and Factor 2, which account for more than 50% of the variance, are displayed here.

Factor 1 appeared to relate to the total amount of fatty acid in the red blood cells. Since I gave a fatty acid supplement (EPA) I would expect the total fatty acid content in the red blood cells to increase. Figure 4.4 shows the averaged levels of total fatty acids in the red blood cells before and after supplementation for both groups.

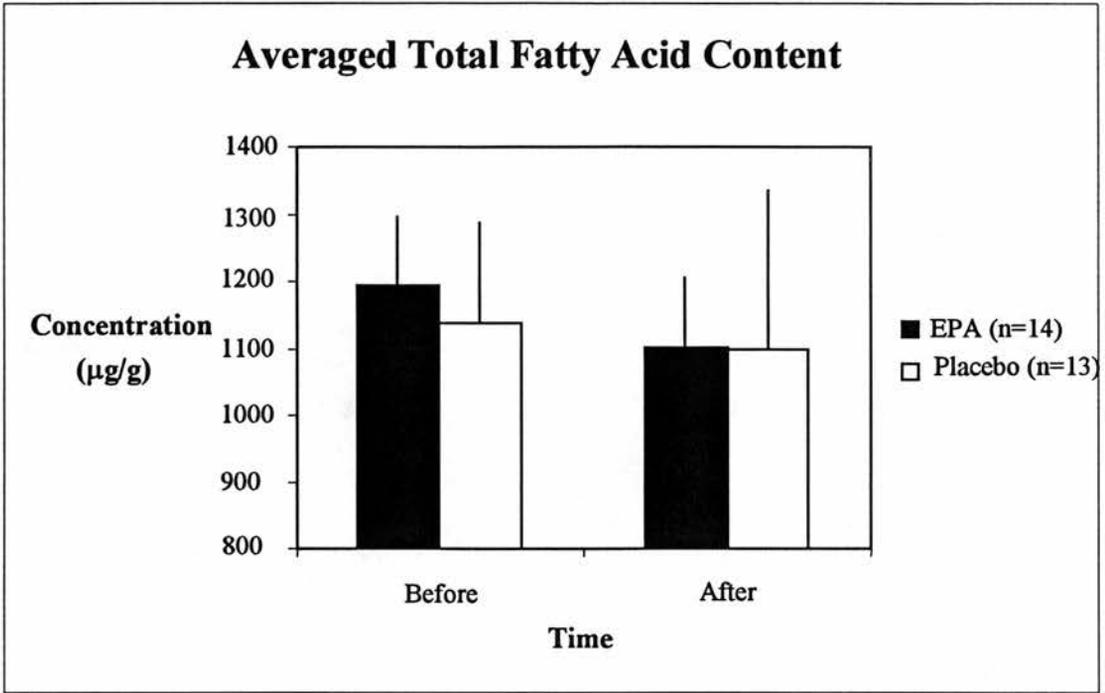


Figure 4.4: Averaged total fatty acid content (with standard deviations) in red blood cells before and after supplementation, for both experimental groups.

ANOVA

A mixed factorial ANOVA revealed no Group x Time interaction [$F(1,25) = 0.817, p = 0.375$]. This suggests that, with supplementation, other fatty acids must decrease in level. Therefore, this indicates that some form of active regulation of fatty acids occurs whereby an elevation in one reduces levels in others. This further supports the balance / ratio theory of omega-3 and omega-6 EFAs.

5 Cardiovascular Measures - Data Retrieval and Results

As described in the Methods section, all physiological data was collected by a computerised data acquisition system. In order to reject artifacts caused by electromagnetic noise, movement and the re-calibration procedure of the Finapres the acquired data was subjected to the algorithm described below:

5.1 Method of Artifact Rejection

Before the physiological data from a given session was analysed with respect to the experimental manipulations during the session, they were filtered to remove artifacts. The following flowchart (figure 5.1) demonstrates the routine used to extract the heart rate and blood pressure data from each data file. The process involved identifying putative heartbeats in the ECG, extracting the putative heart rate, systolic and diastolic values and then filtering these data to reject unrealistic values.

Cardiovascular Data Procedure

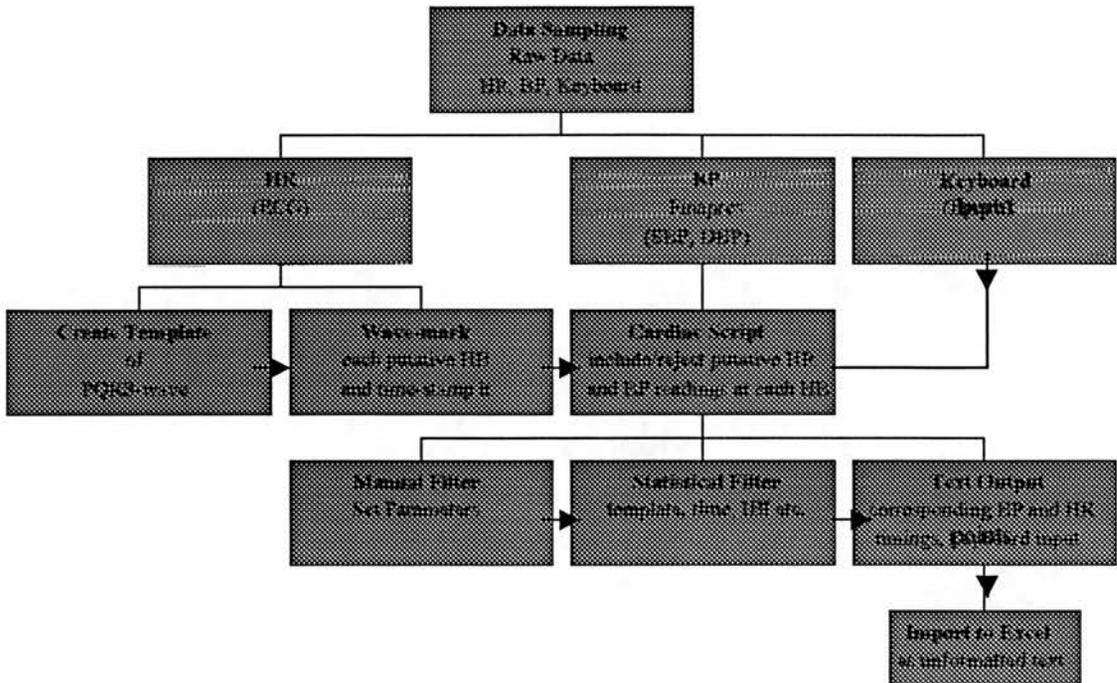


Figure 5.1: Flowchart demonstrating procedure for gathering, and including or rejecting laboratory cardiovascular raw data and outputting into Microsoft Excel text format.

5.1.1 Step 1: Identification of Cardiac Waveforms

A template-matching system, the “wavemarking” routine in Spike2 (Cambridge Electronic Design, Cambridge, UK), was used offline to characterise all waveforms exceeding a threshold voltage. The threshold was adjusted by the experimenter to enable detection of all cardiac waveforms while rejecting background noise. Once a waveform was detected, it was compared on a point-by-point basis to a template formed by the program. A waveform was assigned to a template if the number of points falling within the tolerance of the template exceeded a threshold percentage. Typically, more than one template of cardiac waveforms were formed by the program, since the cardiac potentials changed shape as the heart rate increased during exercise. Using the template-matching algorithm in this way permitted the filtering out of periods of AC noise, microphonic artifacts and electrical interference from the raw trace. With the noise

removed, each waveform corresponded with the time of each heartbeat. All waveforms that matched a template were time-stamped to a resolution of 2-milliseconds.

5.1.2 Step 2: Extraction of Blood Pressure Parameters and Rejection of Artifacts

A program was written using the Spike2 scripting procedures. The script served several purposes:

- The filtering out of all noise and artifacts from the trace that could not possibly be heartbeats.
- The computation of heart rate values from the inter-beat intervals between successive waveforms (bpm).
- The extraction of the systolic (SBP) and diastolic blood pressure (DBP) following each heartbeat (mm Hg).
- Reporting the timing of each heartbeat and the relative timing of the systolic and diastolic features of the BP waveform (to a resolution of 4 milliseconds).
- Identifying the task epoch in which each heart beat fell.
- Rejecting implausible values for heart rate and for the amplitude and timing of the systolic and diastolic features of the BP waveform.
- Outputting this in a text file format for transfer to a statistical package.

The cardiac script functioned by initial inclusion of a *manual filtering* stage in which data taken from each inter-beat interval were examined. This required the following parameters to be set: the range of heart rate (35 – 200 bpm); the range of blood pressure (35 – 275 mm Hg); the range of timing and of the amplitude of the systole and diastole; and the range of the difference between the diastolic and systolic BP. These parameters permitted the exclusion of epochs of noise or artifacts, such as during periods of Finapres re-calibration when the BP trace was flat. See appendix 26 for individual subject parameter settings.

After the manual filter each BP waveform passed through a *statistical filter*. The algorithm began by constructing an average BP waveform from the waveforms that had passed the manual filtering stage, and then correlating each waveform in turn with the

average. Only BP waveforms that correlated significantly ($p < 0.05$) were considered genuine.

As the filtering procedures moved through the data file, any rejected heart beats and blood pressure waveforms were signalled on new data channels (channels 10 – 18) which identified the reason for rejection. The exclusion criterion were:

- Channel 10 (-BPM): Putative heart rate was below specified parameters.
- Channel 11 (+BPM): Putative heart rate was above specified parameters.
- Channel 12 (-BP): Putative blood pressure was below specified parameters.
- Channel 13 (+BP): Putative blood pressure was above specified parameters.
- Channel 14 (-dBP): Difference between putative systolic and diastolic pressure was smaller than specified parameters.
- Channel 15: (dBP): Difference between putative systolic and diastolic pressure was larger than specified parameters.
- Channel 16 (t dia): Time of putative diastole was out-with the specified parameters.
- Channel 17 (t sys): Time of putative systole was out-with the specified parameters.
- Channel 18 (BPr): Putative blood pressure waveform did not significantly correlate with the average blood pressure waveform.

See appendix 25 for Script. The figure (5.2) below shows an example of a sampling script that has been processed through the Spike Script, showing periods of data drop out, with the corresponding criterion channels marked.

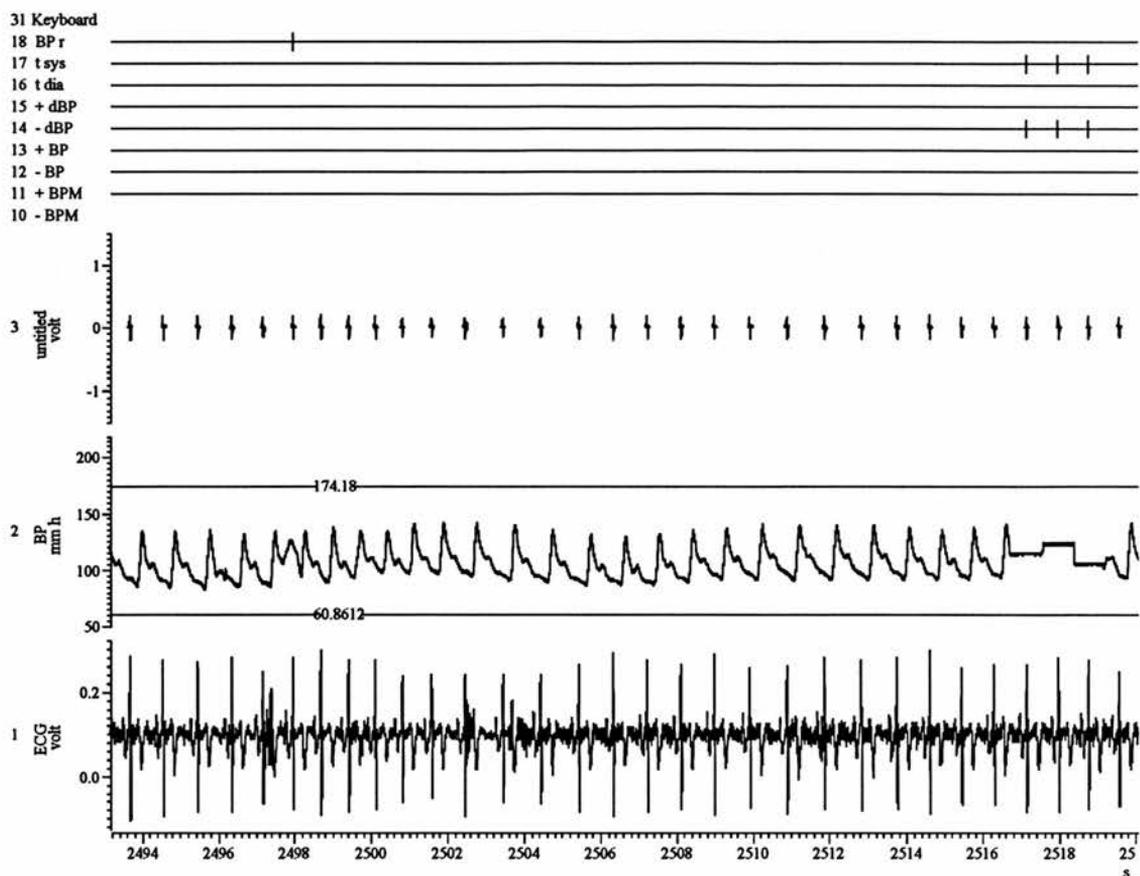


Figure 5.2: Spike Sampling example from laboratory session (subject 19, before supplementation) showing: channels 10 to 18 – wavemark exclusion criterion; channel 3 – wavemarked heart rate; channel 2 – raw blood pressure trace; channel 1 – raw Ecg. The first excluded wavemark (indicated by hash mark) on channel 18 corresponds with the abnormal blood pressure trace below. The cluster of 3 excluded wavemarks (channel 14 and 17) correspond with the blood pressure monitor’s self-recalibration period.

The extracted numerical data was then outputted into a text file. This file contained, in column format, the: time of each heart beat; inter beat interval; heart rate; diastolic blood pressure and its time from the corresponding heartbeat; systolic blood pressure and its time from the corresponding heartbeat; and the task epoch. The data output file was then read into Microsoft Excel and SPSS for analysis. Figure 5.3 below, shows the section of text file which corresponds to the above Spike example in figure 5.2.

Time	IBI	Heart rate	DBP	SBP	Relative time of diastole	Relative time of systole	Epoch
2493.675	0.878	68.33713	86.91406	134.5215	1.095	0.373	d
2494.553	0.904	66.37168	85.69336	134.7656	1.127	0.371	d
2495.457	0.9	66.66667	83.74023	136.7188	1.139	0.367	d
2496.357	0.818	73.34963	85.9375	132.0801	1.037	0.379	d
2497.175	0.786	76.33588	86.91406	135.2539	1.027	0.369	d
2497.961	0.736	81.52174					d
2498.697	0.718	83.56546	89.84375	139.4043	0.953	0.367	d
2499.415	0.698	85.95989	94.23828	135.2539	0.883	0.365	d
2500.113	0.704	85.22727	99.12109	135.2539	0.915	0.371	d
2500.817	0.772	77.72021	98.38867	140.8691	0.995	0.367	d
2501.589	0.874	68.64989	98.14453	142.5781	1.065	0.359	d
2502.463	0.99	60.60606	95.45898	142.3340	1.219	0.357	d
2503.453	0.984	60.97561	92.04102	140.1367	1.195	0.363	d
2504.437	1	60	90.08789	136.4746	1.211	0.371	d
2505.437	0.908	66.0793	87.40234	132.3242	1.131	0.375	d
2506.345	0.888	67.56757	88.13477	129.6387	1.123	0.375	d
2507.233	0.884	67.8733	88.13477	132.5684	1.115	0.375	d
2508.117	0.882	68.02721	90.08789	135.9863	1.117	0.371	d
2508.999	0.928	64.65517	92.77344	136.9629	1.153	0.373	d
2509.927	0.976	61.47541	92.28516	140.8691	1.189	0.369	d
2510.903	0.976	61.47541	92.04102	140.3809	1.205	0.373	d
2511.879	0.954	62.89308	92.28516	140.1367	1.183	0.377	d
2512.833	0.916	65.50218	92.28516	140.1367	1.151	0.367	d
2513.749	0.872	68.80734	92.28516	138.4277	1.111	0.375	d
2514.621	0.832	72.11539	92.77344	135.9863	1.079	0.379	d
2515.453	0.844	71.09005	92.77344	138.4277	1.083	0.379	d
2516.297	0.86	69.76744	94.23828	141.1133	1.087	0.375	d
2517.157	0.824	72.81553					d
2517.981	0.786	76.33588					d
2518.767	0.812	73.89163					d
2519.579	0.834	71.94245	93.50586	142.8223	1.055	0.369	d
2520.413	0.858	69.93007	92.77344	141.6016	1.081	0.371	d

Figure 5.3: Corresponding segment of text output from Spike Cardiac script (subject 19, before supplementation). The columns represent: time from recording start; inter-beat interval; heart rate (bpm); DBP; SBP; time of DBP and SBP since previous heart beat; current task epoch. It can be readily seen that the four missing BP data points (indicated in bold) correspond to the excluded data in figure 5.2.

5.2 *Laboratory Results*

The continuous nature of cardiovascular data means that it can lend itself well to analysis such as time series (Gottman, 1981, Mulder, 1988) and other algorithmic tracking variants (Heslegrave & Pigeau, 1985). Time series or spectral analysis as it is also known, is a general theory involving complex algorithmic tracking methods which can be used to examine the physiological components of heart rate and blood pressure. Time series would have been the natural ideal approach for a study with this nature of continuous physiological data had there not been regular periods of data drop out in each physiological data file. To clarify this point, time series theory along with the software packages used to perform it, such as CARSPAN, are unable to handle missing or interpolated data points well (Mulder, Meulen, vanDellen, & Opheikens, 1988). Therefore since there were multiple missing physiological data points (particularly resulting from the Finapres re-calibration) I felt I was unable to justify the employment of time series analysis. Instead, I simply used the mean values within a given testing epoch. To determine heart rate variability (HRV) I calculated the mean standard deviations of heart rate within a particular task epoch. It is understood and accepted that this approach to HRV produces values that covary with heart rate. Heart rate variability is commonly calculated using standard deviation. For example, Thornton and Hallas recently employed this method on heart rate inter-beat-interval (IBI) to define HRV in myocardial infarction patients (Thornton & Hallas, 1999). Heart rate, systolic and diastolic reactivity was calculated as the change between baseline and response levels during a given task, to illustrate: $SBP \text{ Reactivity} = \text{average SBP during raven's matrices} - \text{average SBP during baseline}$.

5.2.1 Subject Exclusions and Descriptive Statistics

Of the 28 subjects who completed the study, 27 were used for the laboratory analysis. The one excluded subject was again the participant who began anti-depressant medication.

The following tables (5.1 to 5.6) provide the group averages and standard deviation values for heart rate (HR), systolic and diastolic blood pressure (SBP, DBP) as well as the reactivity during the task epochs for both before and after supplementation. The epochs are Baseline, Raven's Matrices, and Reaction Time task, Seated Statically on Cycle, Cycling and Recovery.

	EPA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	63.65	9.63	66.81	8.69	64.79	12.91	66.10	11.67
SBP	117.60	14.57	122.29	23.63	118.67	9.80	121.83	9.58
DBP	68.50	12.98	69.23	15.28	68.51	7.12	72.51	9.59

Table 5.1: Baseline Epoch, group averages and standard deviation HR measured in beats per minute (bpm), SBP, DBP measured in millimetres of mercury (mm Hg).

	EPA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	69.72	11.07	70.49	9.48	69.31	10.24	69.22	10.07
HR R	6.07	5.02	3.68	4.80	4.53	6.66	3.13	4.79
SBP	137.94	20.12	130.56	21.46	134.41	19.15	128.38	10.84
SBP R	20.34	15.18	8.24	6.18	15.74	14.29	6.55	9.46
DBP	80.72	13.74	74.42	13.44	78.41	15.05	77.04	11.14
DBP R	12.22	8.82	5.19	3.72	9.90	10.58	4.53	5.80

Table 5.2: Raven's Matrices Epoch, group averages and standard deviations. The lightly shaded rows (HRR, SBP R and DBP R) highlight the reactivity values for each epoch.

	EPA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	72.72	11.70	72.76	9.51	72.58	11.36	72.81	10.34
HR R	9.07	8.33	5.92	9.03	7.79	8.83	6.48	10.52
SBP	145.81	18.28	137.07	20.60	144.70	18.01	137.46	10.02
SBP R	28.21	15.73	14.78	11.77	26.02	13.89	15.63	13.71
DBP	87.66	13.96	79.76	12.93	79.58	26.40	82.87	10.60
DBP R	19.16	10.09	10.53	6.46	11.05	25.19	10.35	6.00

Table 5.3: Reaction Time Epoch, group averages and standard deviations. The lightly shaded rows (HR R, SBP R and DBP R) highlight the reactivity values for each epoch.

	EPA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	71.56	11.33	75.32	13.76	71.70	11.25	72.64	10.78
HR R	7.91	3.92	8.51	9.98	6.91	5.45	6.54	6.11
SBP	151.08	24.67	145.89	20.89	151.18	20.97	147.72	13.43
SBP R	33.48	23.42	23.59	13.13	32.51	15.57	25.89	14.61
DBP	102.14	22.51	95.32	15.49	102.01	18.29	95.74	14.15
DBP R	33.64	21.05	26.09	10.46	33.51	14.65	23.23	13.87

Table 5.4: Seated Statically on Cycle Epoch, group averages and standard deviations. The lightly shaded rows (HRR, SBPR and DBPR) highlight the reactivity values for each epoch.

	EPA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	123.76	18.41	123.45	17.19	113.72	16.79	118.94	20.82
HR R	60.11	17.81	56.64	14.57	48.93	17.80	52.85	22.54
SBP	184.25	27.46	178.67	29.78	185.03	25.23	178.25	21.54
SBP R	66.65	22.46	56.38	16.46	66.35	22.53	56.42	23.57
DBP	109.28	29.24	102.67	19.17	109.21	19.26	102.02	15.33
DBP R	40.79	26.60	33.44	12.51	40.70	15.35	29.51	16.87

Table 5.5: Cycling Epoch, group averages and standard deviations. The lightly shaded rows (HR R, SBP R and DBP R) highlight the reactivity values for each epoch.

	EPA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	97.54	19.97	93.70	15.39	90.15	16.68	92.61	13.83
HR R	33.89	14.52	25.88	10.87	25.36	15.82	26.52	12.51
SBP	161.83	23.34	150.07	23.24	158.35	21.75	151.03	22.06
SBP R	44.23	19.06	27.78	11.88	35.93	19.30	30.23	22.74
DBP	103.05	25.19	95.27	17.23	100.50	16.23	95.98	16.81
DBP R	35.56	23.83	26.04	12.28	31.99	14.48	23.47	17.31

Table 5.6: Recovery Epoch, group averages and standard deviations. The lightly shaded rows (HR R, SBP R and DBP R) highlight the reactivity values for each epoch.

5.2.2 ANOVA of Task Epochs

The initial question from the cardiovascular data, was whether or not there was a global effect of EPA on cardiovascular function (heart rate, heart rate variability, systolic and diastolic blood pressure and the corresponding reactivity) during the laboratory period. Using SPSS (Statistical Packages for the Social Sciences, version 9), repeated-measures ANOVAs were performed, to investigate for any changes in cardiovascular functioning over time and between groups. Where Mauchly's test of sphericity was significant, the Greenhouse-Geisser correction was used (Brace, Kemp, & Snelgar, 2000). Data graphs can be found in section 5.2.3.

Heart Rate

An average heart rate (HR) for each task epoch was calculated for every subject both before and after supplementation (see tables 5.1 to 5.6 for values). The data was transferred to SPSS where a repeated-measures ANOVA was performed. Time (before and after supplementation) was the within group factor, with between Groups factor and task Epoch as the dependent variable. Mauchly's test of sphericity was significant therefore the Greenhouse-Geisser results were used. The results identified a main effect of Epoch within groups, $[F(1.649, 41.228) = 172.265, p < 0.001]$ which was expected considering the task epochs required different behaviours such as relaxing, cycling and cognitive tasks. However, the results indicated no significant differences between Groups, $[F(1,25) = 0.319, p = 0.577]$ and no significant interaction for Time x Epoch x Group $[F(3.03,75.85) = 2.155, p = 0.100]$. See figure 5.4, which consists of a graph for each group.

Heart Rate Variability

The standard deviation of heart rate was used to indicate heart rate variability, to the various tasks. The average heart rate standard deviations were calculated for each task epoch, for every subject both before and after supplementation. A repeated-measures ANOVA was performed. Greenhouse-Geisser results were used. As expected a main effect of Epoch was found $[F(2.102, 52.544) = 52.605, p < 0.001]$. No significant

differences, between Groups were found [$F(1,25) = 0.75, p = 0.395$]. No significant interaction was found for Time x Epoch x Group, [$F(2.584,64.61) = 0.375, p = 0.741$]. See figure 5.5.

Heart Rate Reactivity

Average heart reactivity was calculated for each task epoch, for every subject both before and after supplementation. A repeated-measures ANOVA was performed. Greenhouse-Geisser results were used. As expected a main effect of Epoch was found [$F(1.664,41.607) = 154.666, p < 0.001$]. No significant interaction was found for Time x Epoch x Group, [$F(2.892,72.309) = 0.76.725, p = 0.126$]. No significant differences, between Groups were found [$F(1,25) = 1.126, p = 0.299$]. See figure 5.6.

Systolic Blood Pressure

An average SBP, for each task epoch, was calculated for every subject both before and after supplementation (see tables 5.1 to 5.6 for values). A repeated-measures ANOVA was performed. Greenhouse-Geisser results were used. As expected a main effect of Epoch was noted within groups [$F(3.146, 78.655) = 132.795, p < 0.001$]. Results indicated no significant differences between Groups, [$F(1,25) = 0.019, p = 0.891$] and no significant interaction was found for Time x Epoch x Group, [$F(2.628,65.709) = 0.568, p = 0.615$]. See figure 5.7 for graphs.

Systolic Blood Pressure Reactivity

The average SBP, for each task epoch, was calculated for every subject both before and after supplementation (see tables 5.1 to 5.6 for values). A repeated-measures ANOVA was performed. Greenhouse-Geisser results were used. As expected a main effect of Epoch was noted within groups [$F(2.45,61.248) = 104.025, p < 0.001$]. A main effect of Time was also shown within groups [$F(1,25) = 10.465, p = 0.003$]. Figure 5.8 shows the after supplementation levels to be lower for both groups. Results indicated no significant differences between Groups, [$F(1,25) = 0.092, p = 0.764$] and no significant interaction was found for Time x Epoch x Group, [$F(2.645,66.119) = 0.628, p = 0.580$].

Diastolic Blood Pressure

An average DBP, for each task epoch, was calculated for every subject both before and after supplementation. A repeated-measures ANOVA was performed. Greenhouse-Geisser results were used. As expected a main effect of Epoch was noted within groups [F (2.165, 54.121) = 84.165, $p < 0.001$]. No significant Group differences, [F (1,25) = .004, $p = 0.95$] were found, and no significant Time x Epoch x Group interaction was shown, [F (2.557,63.919) = 0.746, $p = 0.509$]. See figure 5.9 for graphs.

Diastolic Blood Pressure Reactivity

The average DBP reactivity, for each task epoch, was calculated for every subject both before and after supplementation. A repeated-measures ANOVA was performed. Greenhouse-Geisser results were used. As expected a main effect of Epoch was noted within groups [F (1.828,45.691) = 62.397, $p < 0.001$]. A main effect of Time was also noted [F (1,25) = 6.275, $p = 0.019$]. Figure 5.10 shows reactivity after supplementation to be lower in both groups. No significant Group differences, [F (1,25) = 0.402, $p = 0.532$] were found, and no significant Time x Epoch x Group interaction was shown, [F (2.578,64.442) = 1.004, $p = 0.3788$].

5.2.3 ANOVA of Task Epochs – Figures

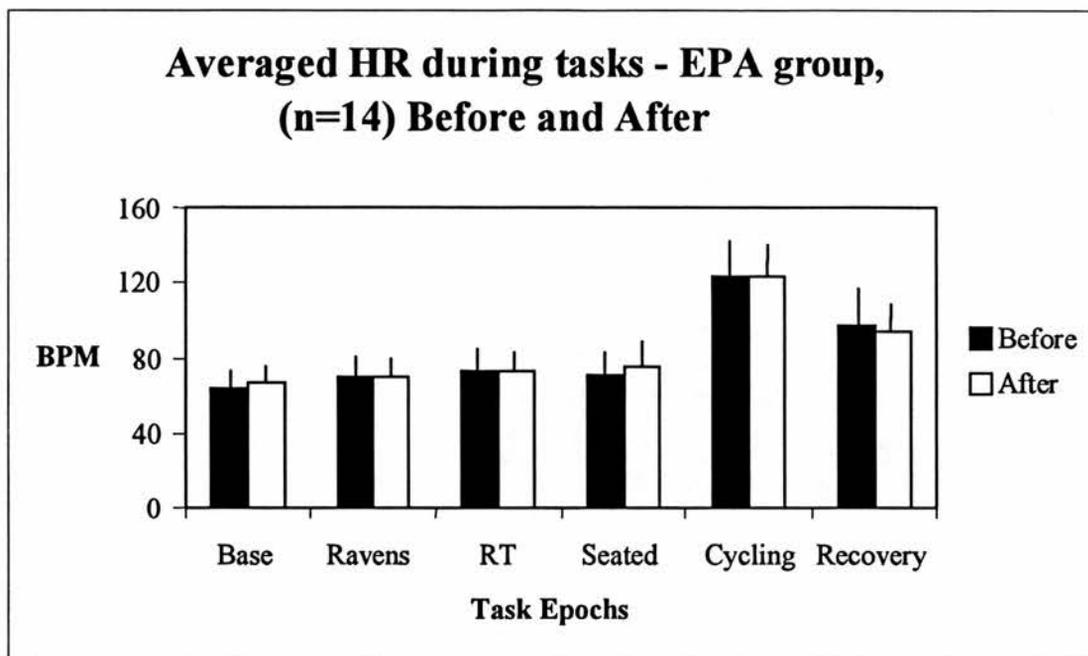
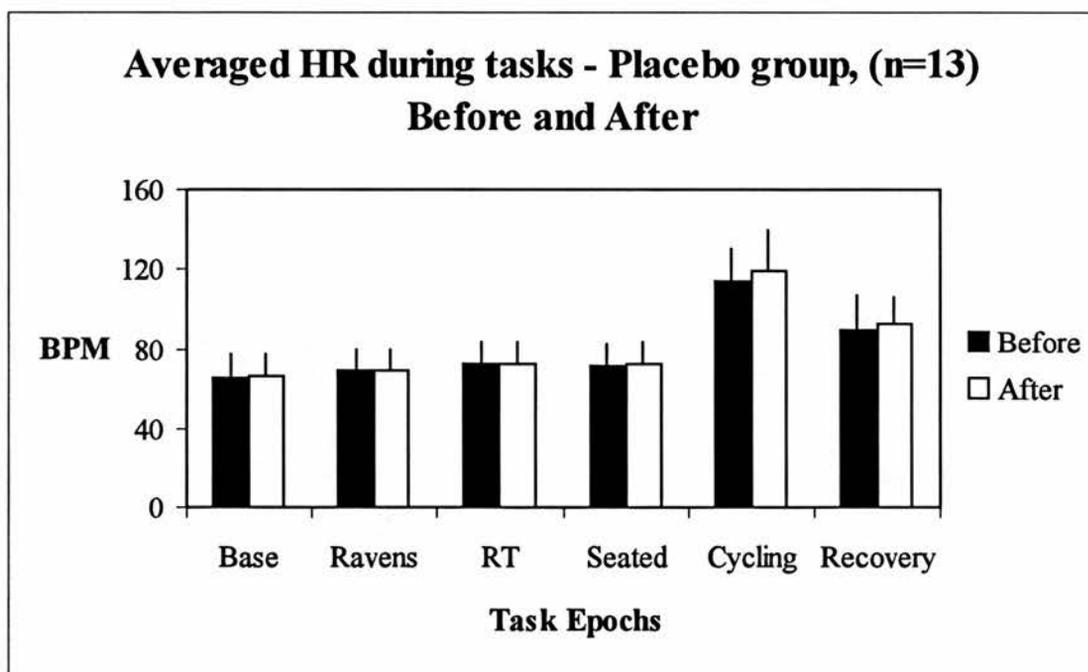


Figure 5.4: Graphs above (EPA) and below (placebo) show the averaged levels of HR (beats/minute or BPM) during task performance for each group, *Before* and *After* supplementation (with standard deviations). Neither a significant difference between Groups, [F (1,25) = 0.319, p = 0.577], nor a significant interaction was found for Time x Epoch x Group, [F (3.03,75.85) = 2.155, p = 0.100].



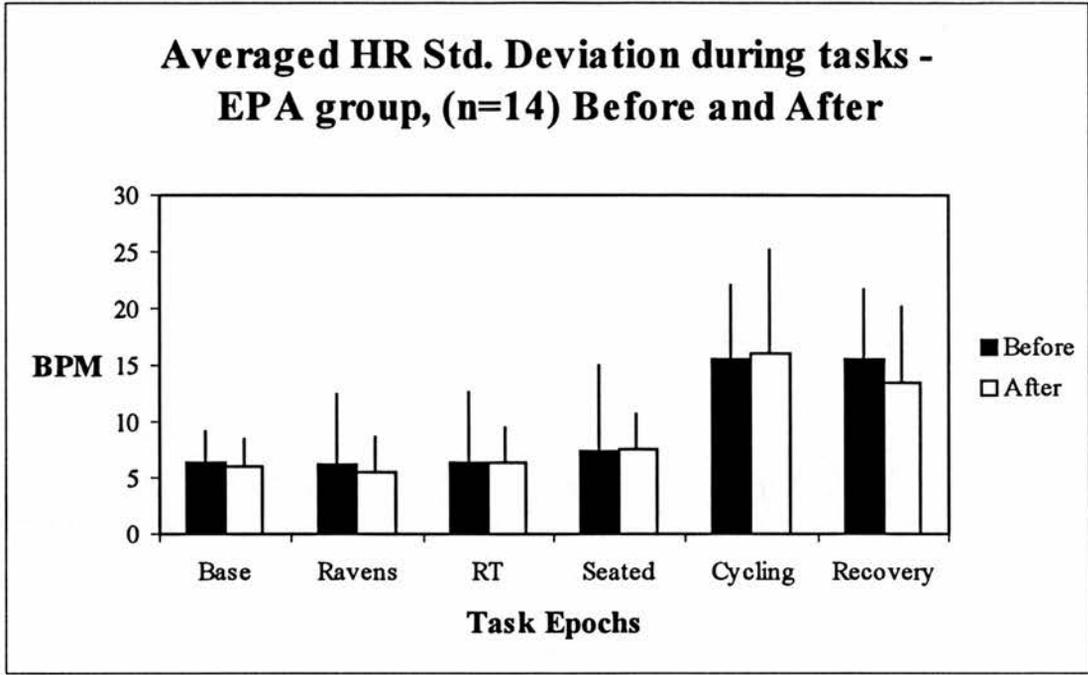
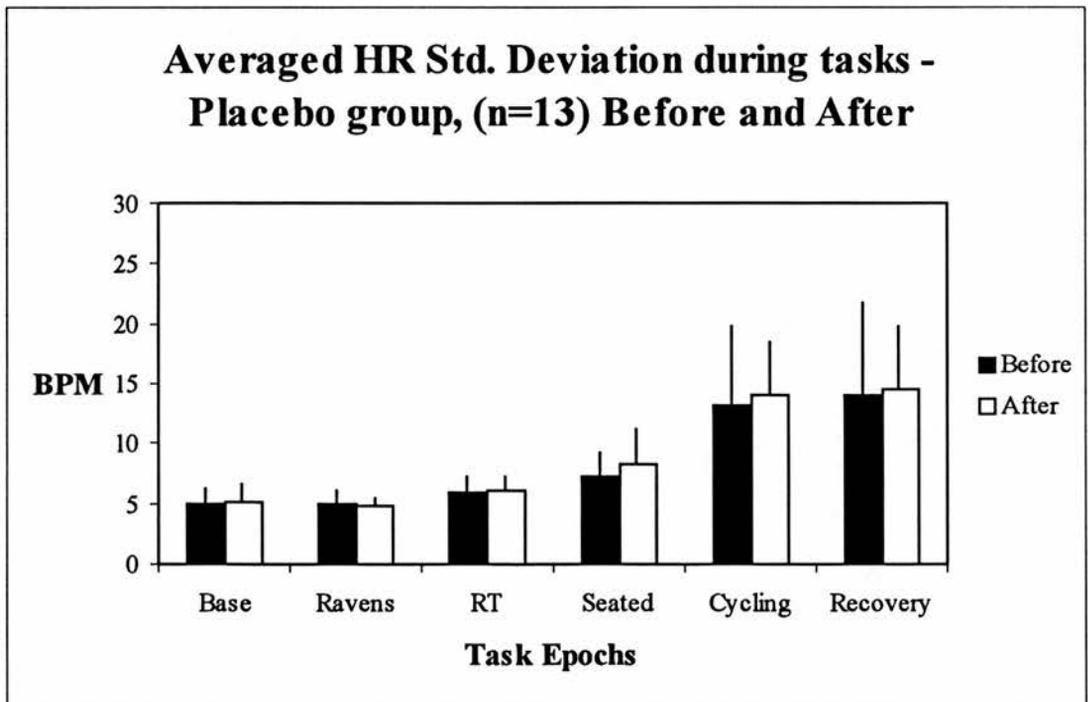


Figure 5.5: Averaged HR Variability (bpm) during task performance for each group before and after supplementation. No significant differences, between Groups were found [$F(1,25) = 0.75, p = 0.395$], and no significant interaction was found for Time x Epoch x Group, [$F(2.584,64.61) = 0.375, p = 0.741$].



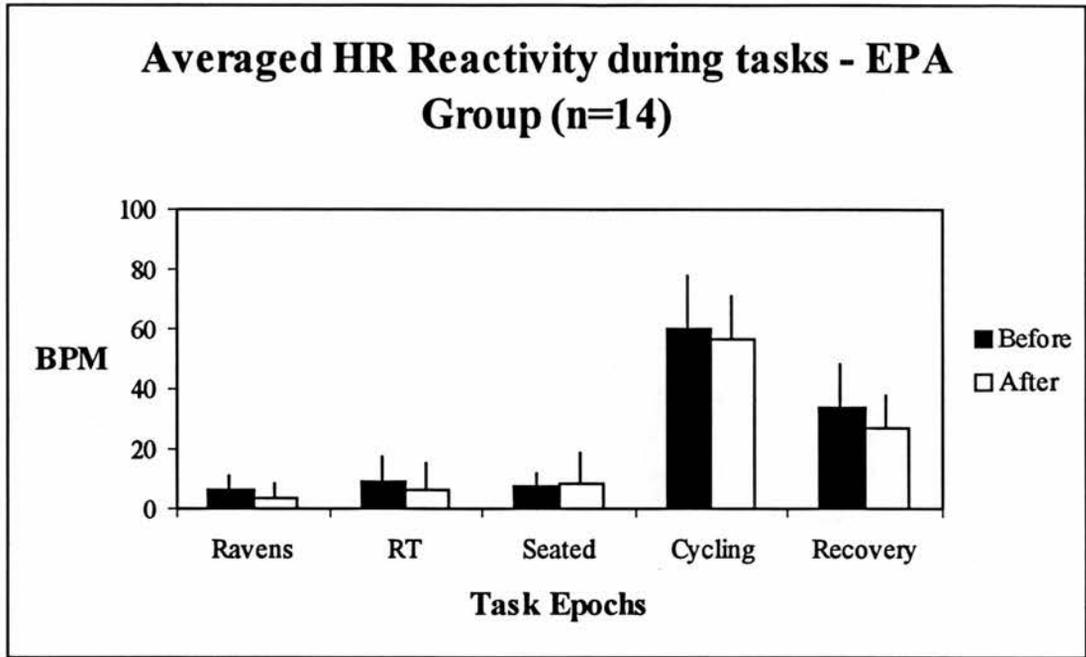
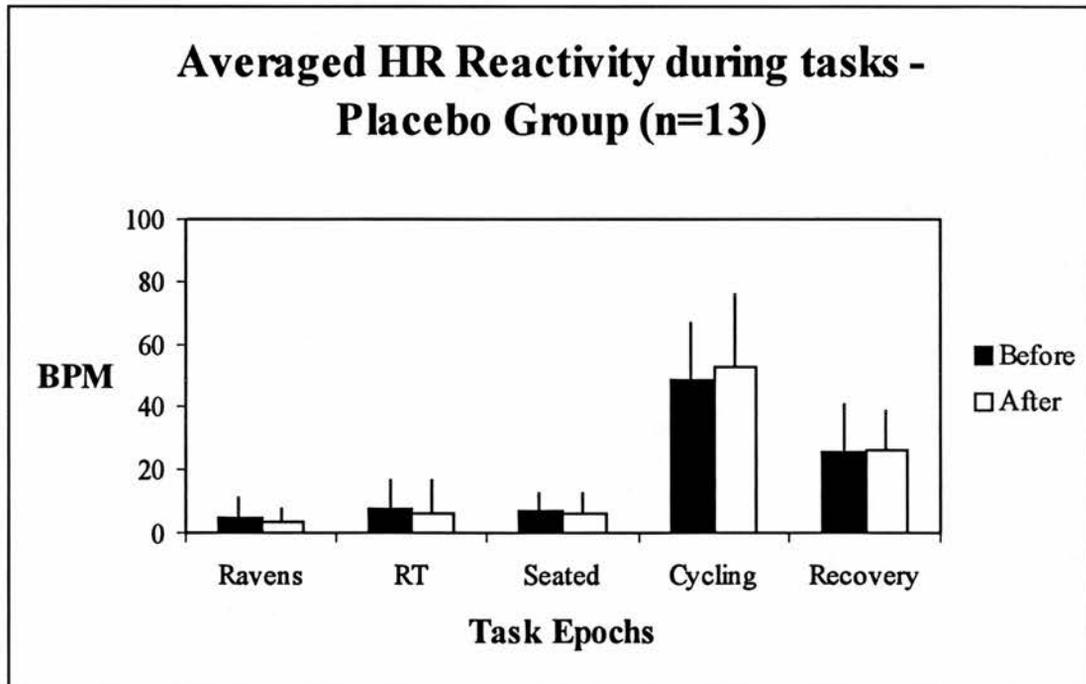


Figure 5.6: Averaged heart rate reactivity for both groups (above and below) before and after supplementation. ANOVA results showed no significant Time x Epoch x Group interaction [$F(2.892, 72.309) = 0.76.725, p = 0.126$] or significant difference between the Groups [$F(1, 25) = 1.126, p = 0.299$]. A main effect of Epoch was found [$F(1.664, 41.607) = 154.666, p < 0.001$].



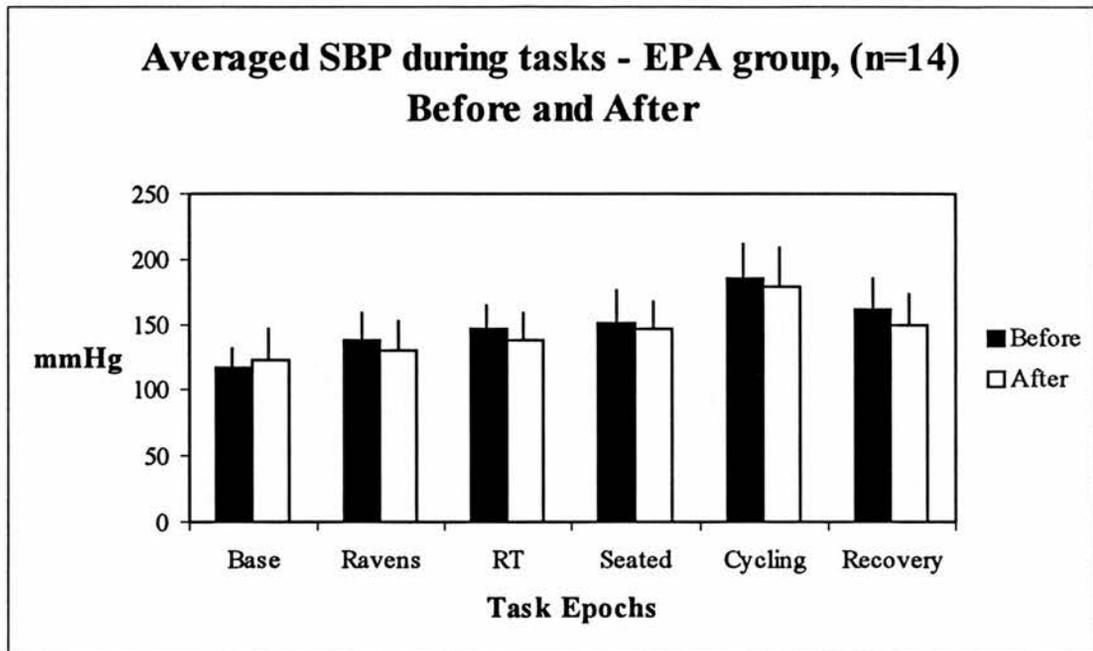
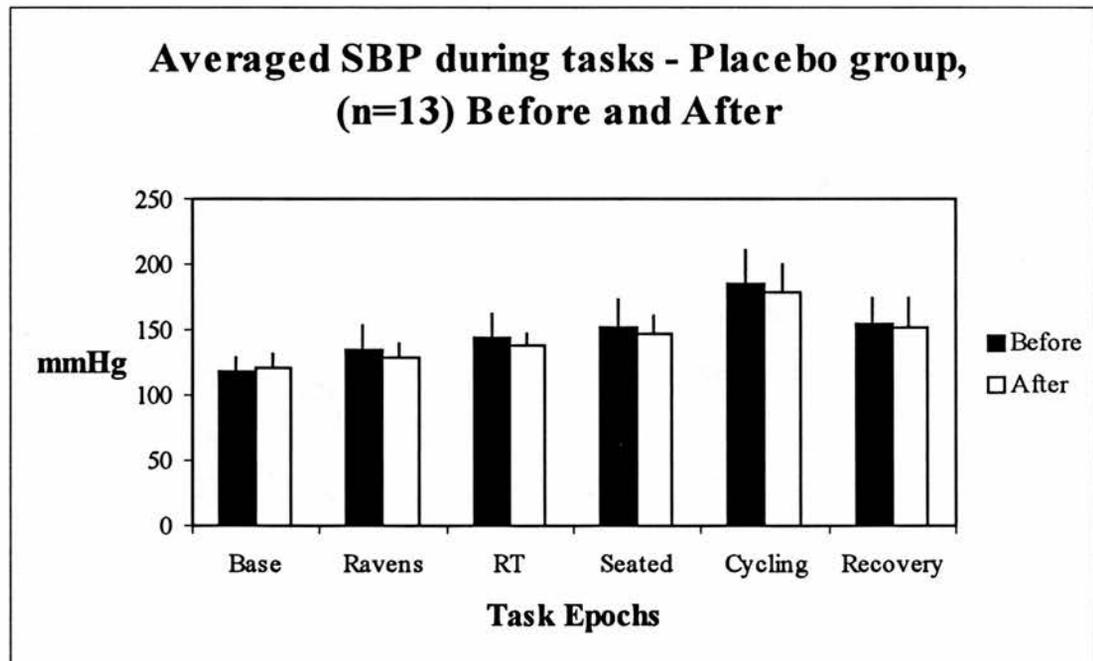


Figure 5.7: Averaged levels of SBP (and std. dev.) during task performance for each group, before and after supplementation. Repeated-measures ANOVA indicated no significant differences between Groups, $[F(1,25) = 0.019, p = 0.891]$. No significant interaction was found for Time x Epoch x Group, $[F(2.628,65.709) = 0.568, p = 0.615]$.



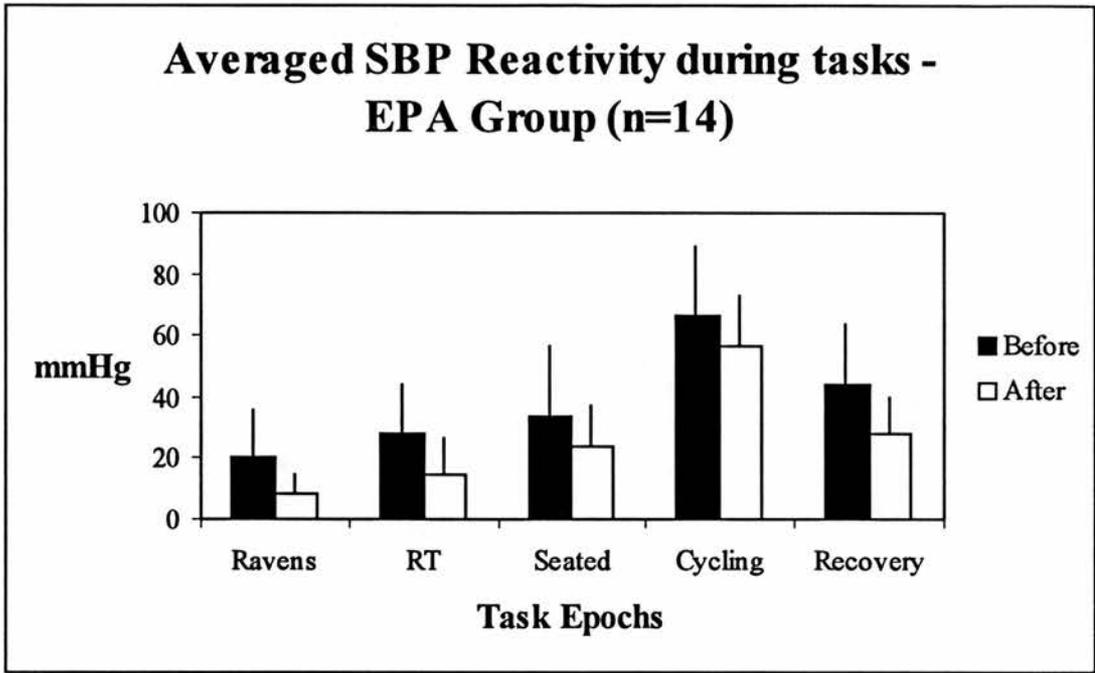
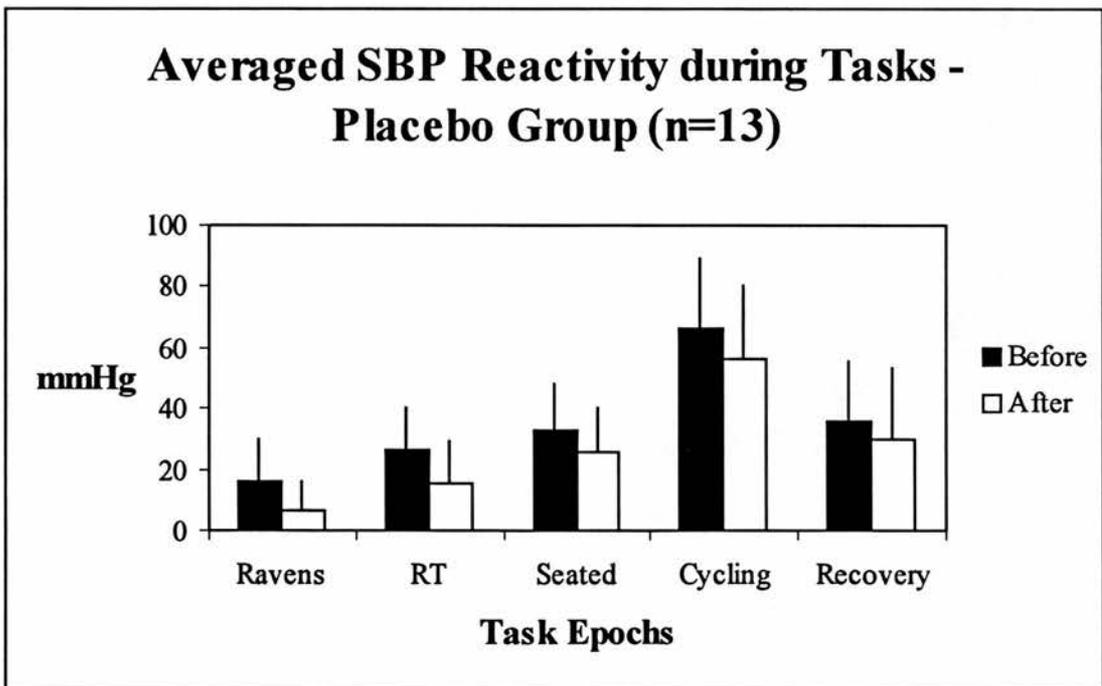


Figure 5.8: Averaged SBP reactivity (with std.dev) for both groups and both time points. ANOVA indicated a main effect of Time [$F(2.45, 61.248) = 104.025, p < 0.001$]. No significant Time x Epoch x Group occurred [$F(2.645, 66.119) = 0.628, p = 0.580$] and no differences were found between groups [$F(1, 25) = 0.092, p = 0.764$].



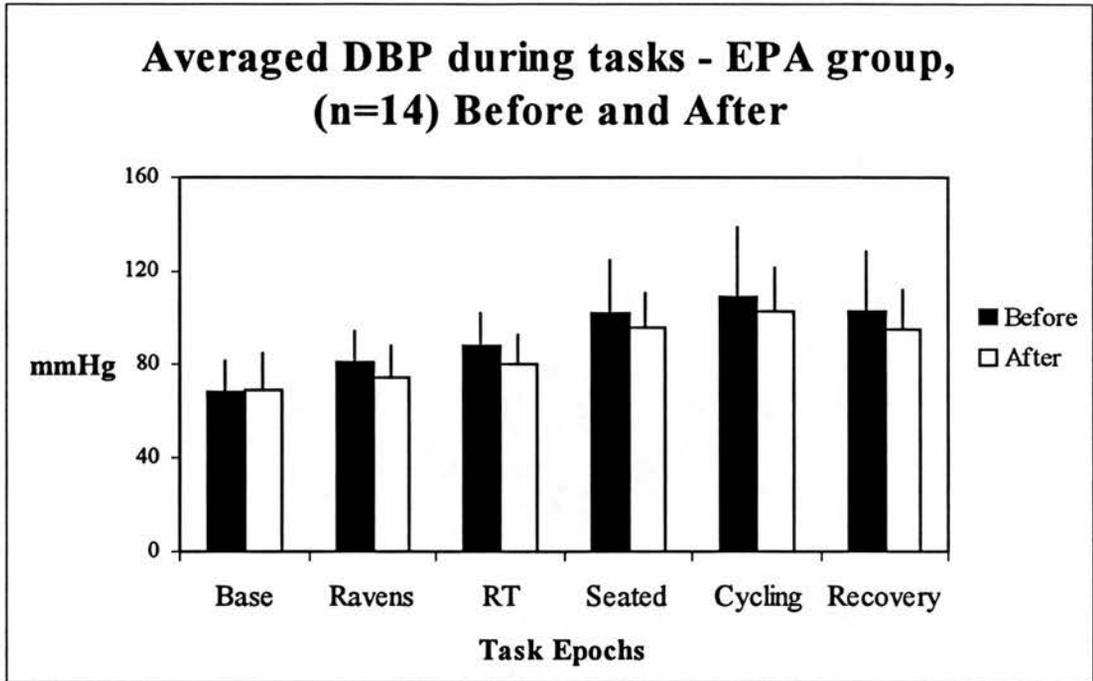
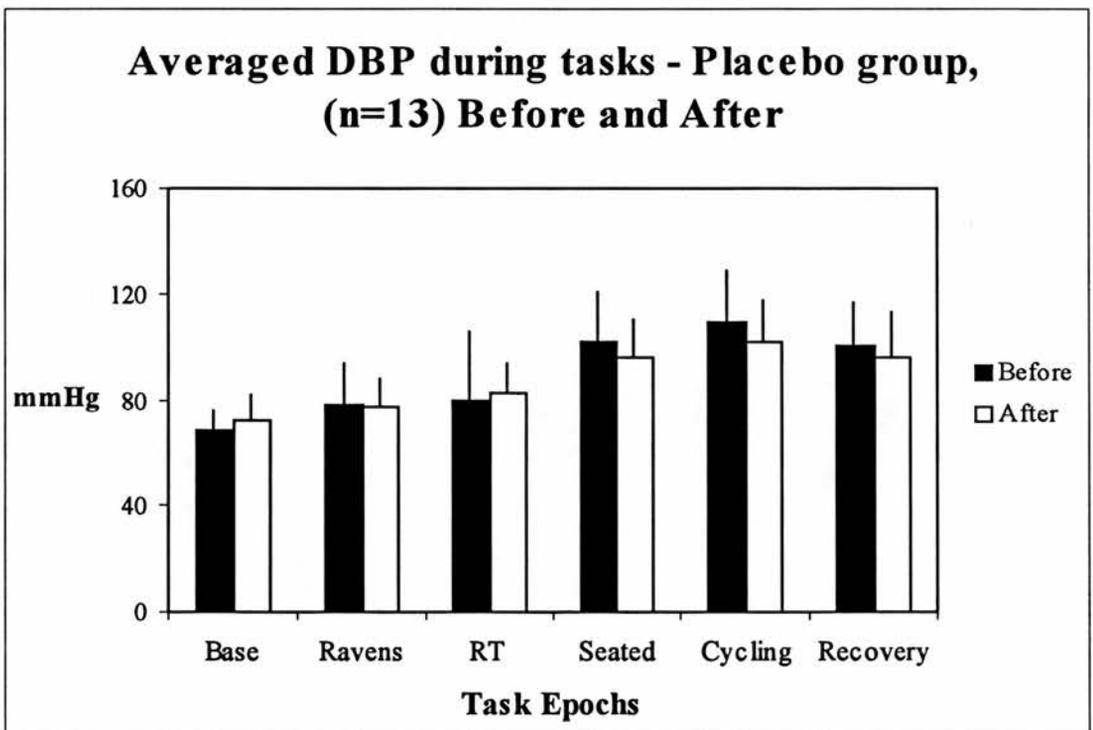


Figure 5.9: Averaged levels of DBP (and Std. Dev.) during task performance for each group, before and after supplementation. Repeated-measures ANOVA indicated no significant Group differences, $[F(1,25) = .004, p = 0.95]$. No significant Time x Epoch x Group interaction was found, $[F(2.557,63.919) = 0.746, p = 0.509]$.



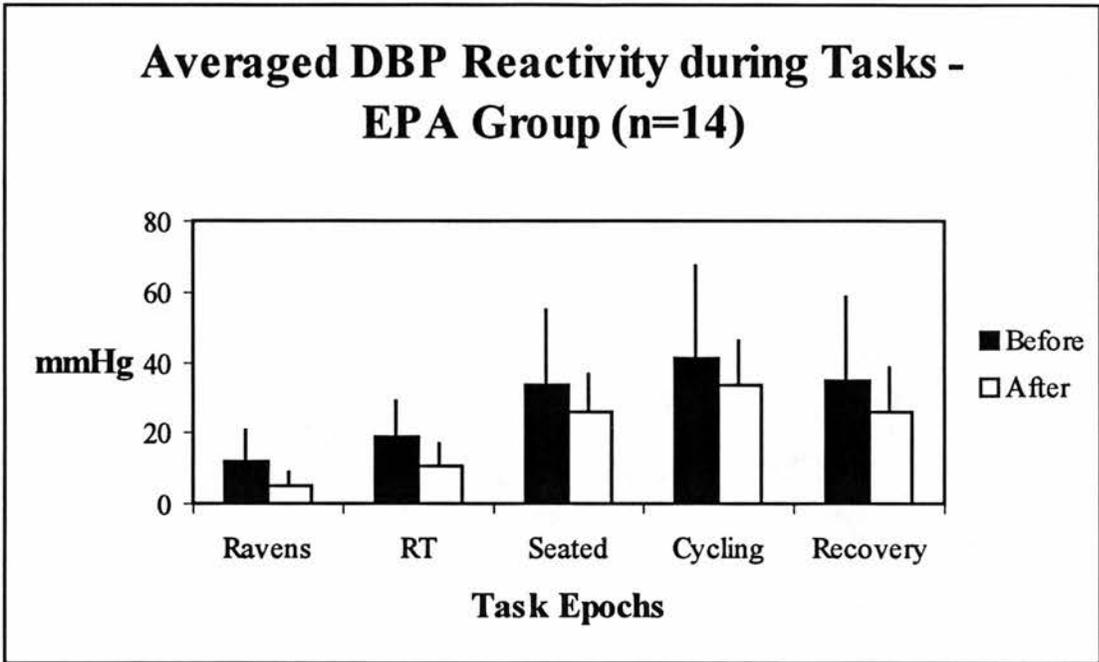
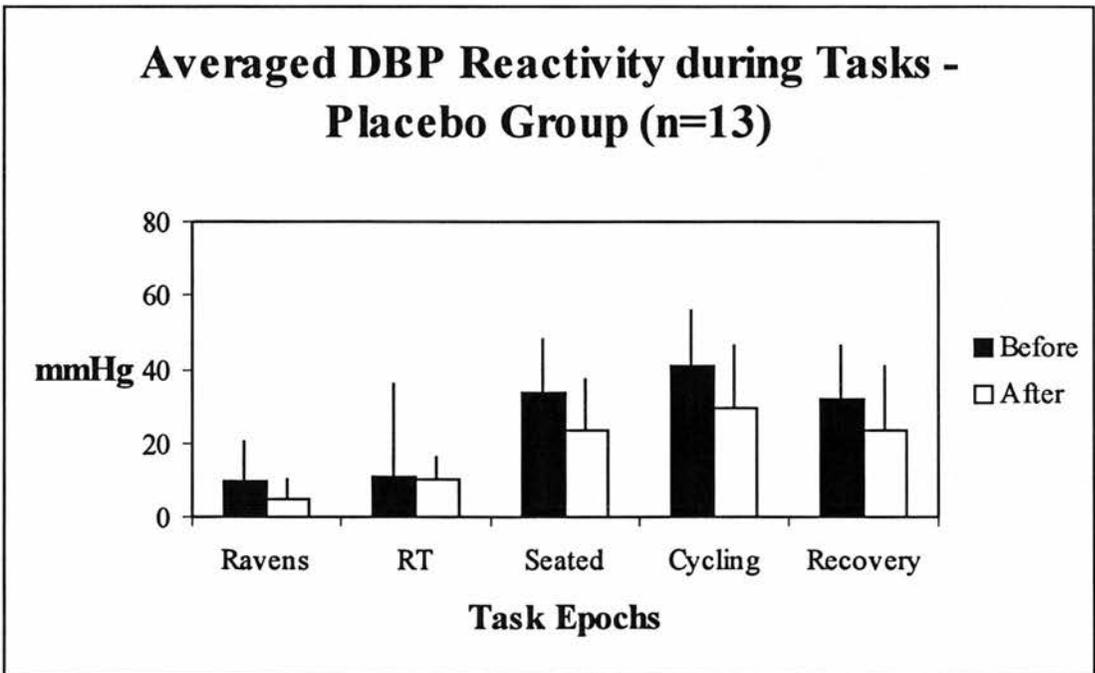


Figure 5.10: Averaged DBP reactivity for both groups and time points. ANOVA results found a main effect of Epoch [$F(1.828, 45.691) = 62.397, p < 0.001$] and Time [$F(1, 25) = 6.275, p = 0.019$]. No significant Time x Epoch x Group interaction was seen, [$F(2.578, 64.442) = 1.004, p = 0.3788$] or significant Group differences, [$F(1, 25) = 0.402, p = 0.532$].



5.3 Ambulatory Results

On the second morning of testing each subject returned with the ambulatory blood pressure monitor. The data from the monitor was downloaded at that time, via the TM2020 programming unit, to a paper printout. The printout consisted of: time of recording; SBP; DBP and heart rate values. The data was transferred to Excel files using standard duplicate procedures. Furthermore an impartial marker checked through the data transfer for errors.

5.3.1 Subject Exclusions and Descriptive Statistics

The subject on medication was again excluded. Further to this two more subjects (one from each group) had to be excluded due to the fact that they had no ambulatory readings for the second test session (*After*). Both subjects wore the ambulatory monitor as required but no data could be downloaded on their return. Both volunteered to a second monitoring attempt, but again the monitors failed to store the data. Of the remaining 25 subjects, several had missing data points where the device had either failed to trigger or was unable to take a reading. For this reason an average of each subject's ambulatory readings was calculated and used for the purpose of analysis. No true baseline period was recorded during ambulation therefore reactivity was calculated using each subject's laboratory baseline epoch. It should be noted that in general the laboratory epoch readings were higher than during ambulation. The ambulatory reactivity values may then be underestimated for each subject but will reflect the actual changes, which occurred during day to day activities. The group ambulatory averages, for both time points can be seen in table 5.7.

	EPA (n=13)				Placebo (n=12)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	72.22	14.37	74.64	11.67	68.21	10.62	69.81	11.10
HR R	7.34	13.24	7.56	11.77	4.29	8.89	4.55	5.07
SBP	137.45	18.82	131.67	11.31	129.79	11.36	131.70	17.52
SBP R	20.34	24.40	11.44	18.70	11.19	21.04	7.04	25.10
DBP	76.89	11.78	73.25	10.42	74.40	11.65	71.30	8.85
DBP R	10.73	14.75	3.48	15.68	3.77	21.56	5.48	16.08

Table 5.7: Group averages and standard deviations for ambulatory readings, from both time points, of heart rate (bpm), systolic and diastolic blood pressure (mm Hg). The lightly shaded rows highlight the reactivity for HR, SBP and DBP.

5.3.2 ANOVA of Ambulatory Cardiovascular Data

The initial question from the ambulatory cardiovascular data, was whether or not there was a global effect of EPA on cardiovascular functioning during day to day activities. SPSS repeated-measures ANOVAs were used to investigate for any changes in ambulatory cardiovascular functioning over time and between groups. Graphs can be found in section 5.3.3.

Heart Rate

The average heart rate for each subject, during ambulation, was calculated and the ANOVA performed, with Time (before and after) as the within group factor and Group as the between factor. ANOVA results found no main effect of Time [$F(1,23) = 0.608$, $p = 0.443$] and no Time x Group interaction [$F(1,23) = 0.026$, $p = 0.874$], within the groups. Similarly, the results indicated no significant differences between the Groups [$F(1,23) = 1.167$, $p = 0.291$]. See figure 5.11 for graph of group values.

Heart Rate Variability

The standard deviation from heart rate was used to define heart rate variability. For each subject average heart rate variability was calculated and used to perform the ANOVA (Group as between factor, Time as within factor). Results indicated no significant differences between the Groups' variability [F (1,23) = 1.987, p = 0.172]. The results also found neither an effect of Time [F (1,23) = 0.196, p = 0.662] nor an interaction of Time x Group [F (1,23) = 0.296, p = 0.591]. See figure 5.12 for graph of group values.

Heart Rate Reactivity

For each subject average heart rate reactivity was calculated and used to perform the ANOVA (Group as between factor, Time as within factor). Results indicated no significant differences between the Groups' reactivity [F (1,23) = 0.899, p = 0.353]. The results also showed neither an effect of Time [F (1,23) = 0.008, p = 0.929] nor an interaction of Time x Group [F (1,23) = 0.001, p = 0.994]. See figure 5.13 for graph of group reactivity values.

Systolic Blood Pressure

The average SBP, during ambulation, was calculated for each subject, and the ANOVA performed, with Time (before and after) as the within group factor and Group as the between factor. The results found no significant interaction of Time x Group [F (1,23) = 1.53, p = 0.229], nor a main effect of Time [F (1,23) = 0.386, p = 0.541]. Furthermore, the results indicated no significant differences between the Groups [F (1,23) = 0.536, p = 0.471]. See figure 5.14 for graph of SBP and DBP values.

Systolic Blood Pressure Reactivity

The average SBP reactivity, during ambulation, was calculated for each subject, and the ANOVA performed, with Time (before and after) as the within group factor and Group as the between factor. The results revealed no significant interaction of Time x Group [F (1,23) = 0.213, p = 0.649], nor a main effect of Time [F (1,23) = 1.611, p = 0.217].

Furthermore, the results indicated no significant differences between the Groups [$F(1,23) = 0.848, p = 0.367$]. See figure 5.15 for both the SBP and DBP reactivity graphs.

Diastolic Blood Pressure

The average DBP, during ambulation, was calculated for each subject, and the ANOVA performed, with Time (before and after) as the within group factor and Group as the between factor. No significant effect of Time [$F(1,23) = 0.3169, p = 0.088$] or Group x Time interaction was found [$F(1,23) = 0.021, p = 0.887$]. Furthermore results indicated no significant differences between Groups [$F(1,23) = 0.330, p = 0.571$]. The DBPs can be seen in figure 5.14.

Diastolic Blood Pressure Reactivity

The average DBP reactivity, during ambulation, was calculated for each subject, and the ANOVA performed, with Time (before and after) as the within group factor and Group as the between factor. No significant interaction of Group x Time interaction was found [$F(1,23) = 0.021, p = 0.887$]. A main effect of Time neared significance [$F(1,23) = 4.18, p = 0.053$], while results indicated no significant differences between the Groups [$F(1,23) = 0.683, p = 0.417$]. Figure 5.15 displays DBP reactivity values.

5.3.3 ANOVA of Ambulatory Cardiovascular Data – Figures

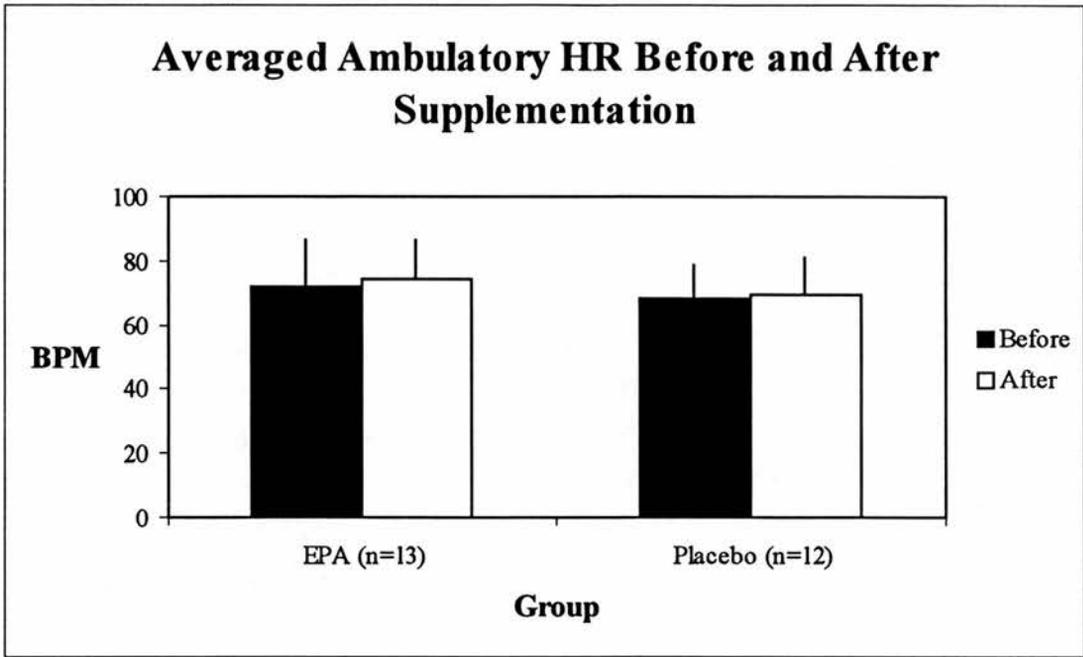


Figure 5.11: Averaged HR (bpm) levels during ambulatory day, for each group, before and after supplementation. Repeated-measures ANOVA displayed no significant differences between Groups [$F(1,23) = 1.167, p = 0.291$] and no Group x Time interaction [$F(1,23) = 0.026, p = 0.874$].

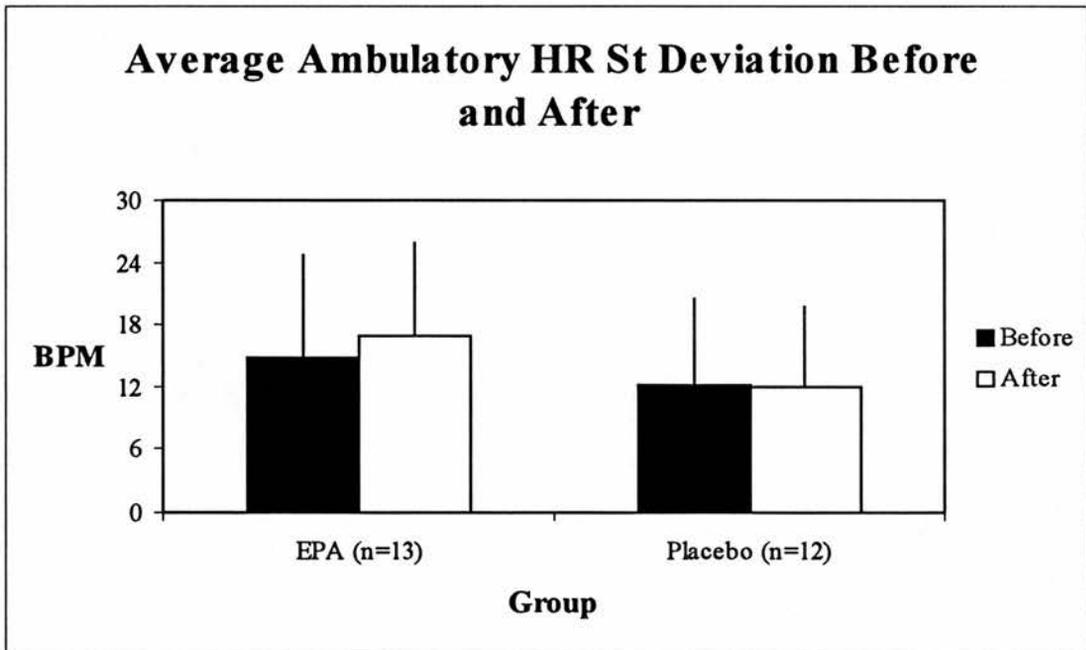


Figure 5.12: Averaged heart rate variability during ambulatory recording, for each group, before and after supplementation. Repeated-measures ANOVA results show no significant differences between Groups [$F(1,23) = 1.987, p = 0.172$] and no significant Group x Time interaction [$F(1,23) = 0.296, p = 0.591$].

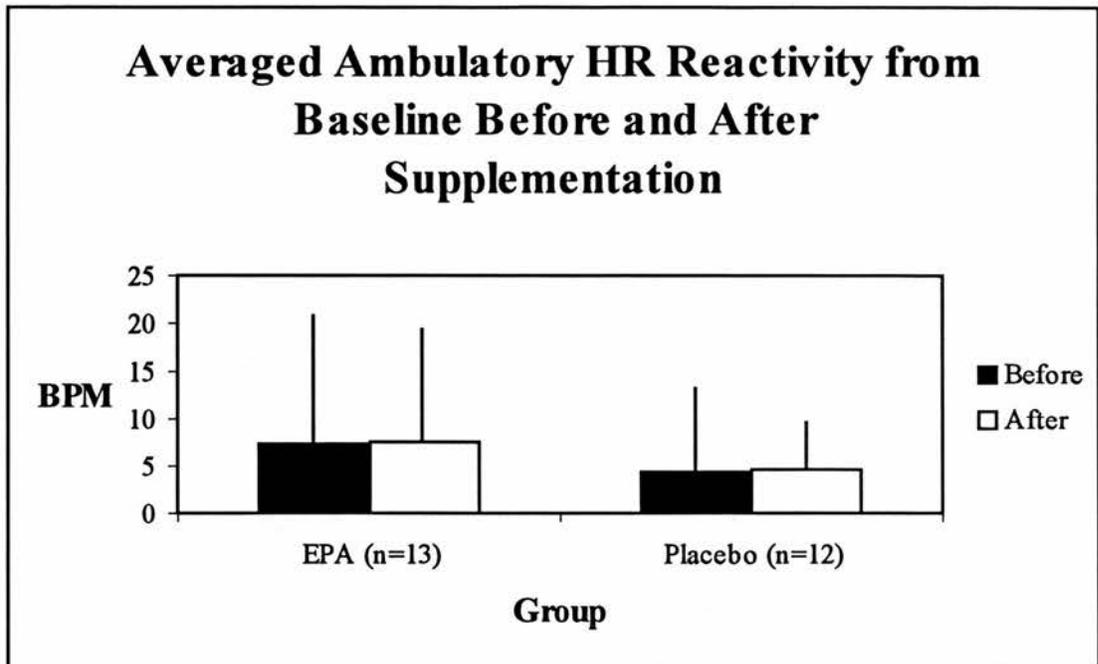


Figure 5.13: Averaged ambulatory HR reactivity before and after supplementation for both groups. ANOVA results found neither group differences [$F(1,23) = 0.899, p = 0.353$], nor an effect of Time [$F(1,23) = 0.008, p = 0.929$] nor an interaction of Time x Group [$F(1,23) = 0.001, p = 0.994$].

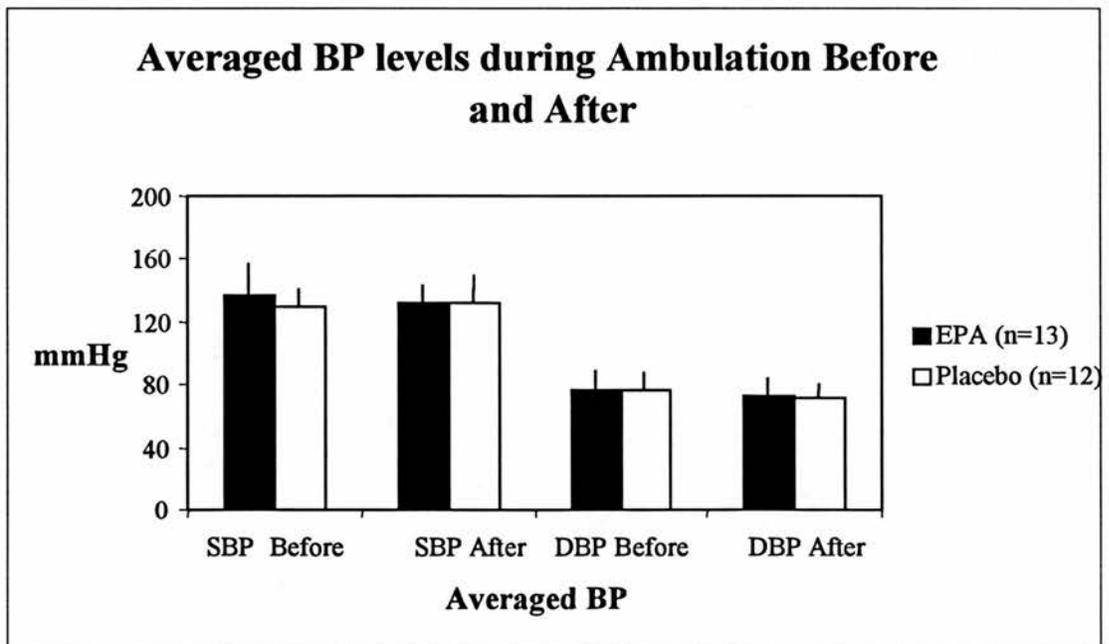


Figure 5.14: Averaged ambulatory SBP and DBP. ANOVA results show no significant group differences for SBP [$F(1,23) = 0.536, p = 0.471$] or DBP [$F(1,23) = 0.330, p = 0.571$]. No significant Group x Time interaction was found for SBP [$F(1,23) = 1.53, p = 0.229$], or DBP [$F(1,23) = 0.021, p = 0.887$].

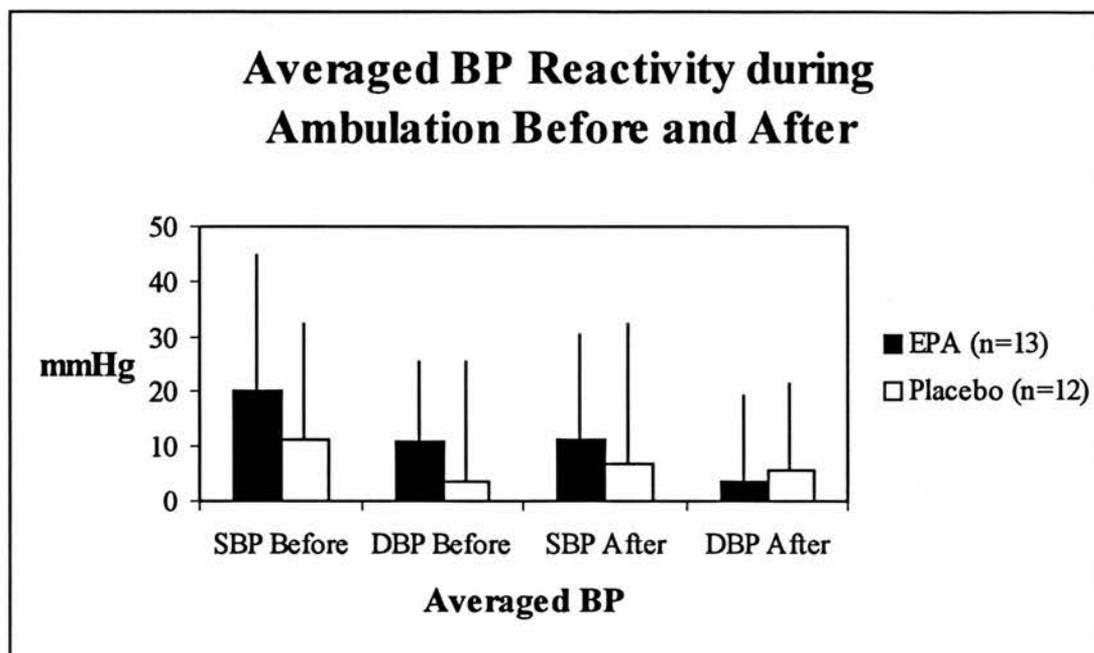


Figure 5.15: Averaged SBP and DBP reactivity before and after supplementation for both groups. ANOVA results indicated no significant interaction of Group x Time interaction [$F(1,23) = 0.021, p = 0.887$] and no main effect of Time [$F(1,23) = 4.18, p = 0.053$], or significant differences between the Groups [$F(1,23) = 0.683, p = 0.417$].

5.4 Laboratory vs. Ambulatory

One main purpose of measuring cardiovascular functioning both within laboratory conditions and during subject ambulation was to investigate whether or not laboratory data differed from ambulatory data, and whether or not this changed after supplementation with EPA. To examine this question, repeated-measures ANOVAs were used to compare cardiovascular levels within the laboratory and during ambulation.

5.4.1 Subject Exclusions and Descriptive Statistics

In total three subjects were excluded. One due to his medication and two others due to there being no ‘after supplementation’ ambulatory data for them.

The laboratory epochs were designed to mimic a range of normal daily activities, (where the raven's matrices and reaction time task were used to reflect mental workload). For this reason the average across the laboratory epochs was calculated, and used in comparison with the average of the ambulatory recordings, for each subject. Table 5.8 below presents the averaged epoch data for both groups at both time points. The averaged ambulatory data can be seen in table 5.7 and graphs can be found in section 5.4.3.

	EPA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	84.75	9.92	84.46	10.58	79.95	11.34	82.04	10.67
HR R	23.85	6.59	21.02	5.25	19.24	9.41	20.06	9.04
SBP	151.48	17.62	144.45	22.88	147.31	16.81	143.54	10.74
SBP R	39.81	14.43	22.25	11.25	30.29	11.71	29.36	10.14
DBP	93.05	17.46	86.34	15.16	90.69	14.26	86.14	9.26
DBP R	27.93	13.92	17.21	11.45	21.66	11.37	19.77	5.44

Table 5.8: Laboratory cardiovascular data averaged across epochs. Heart rate (bpm), systolic and diastolic blood pressures (mm Hg) shown with standard deviations. Lightly shaded rows highlight the reactivity values.

5.4.2 ANOVA of Laboratory and Ambulatory Cardiovascular Data

Heart Rate

The average heart rate, from ambulation and laboratory epochs was calculated, for each subject and used to perform a repeated-measures ANOVA. *Location* (defined as ambulation and laboratory) and *Time* were the within-subject factors, while *Group* was the between factor. Results indicated no significant differences between Groups [$F(1,23) = 1.414, p = 0.246$]. A main effect of *Location* was found [$F(1,23) = 25.074, p < 0.001$]. No significant *Group x Time* interaction was found [$F(1,23) = 0.058, p = 0.811$] or *Group x Time x Location* [$F(1,23) = 0.462, p = 0.504$]. Figure 5.16 shows

laboratory heart rates to be higher than ambulatory values. These results suggest that ambulatory measures of heart rate differ from those recorded in the laboratory, irrespective of supplementation.

Heart Rate Variability

The average standard deviations of heart rate were used to define heart rate variability. The averages from ambulation and laboratory epochs were calculated, for each subject and used to perform a repeated-measures ANOVA. Location and Time were the within group factors, while Group was the between factor. Results indicated no significant differences between Groups [F (1,23) = 2.684, p = 0.115]. A main effect of Location was found within groups [F (1,23) = 10.978, p = 0.003]. No significant Group x Time interaction was found [F (1,23) = 0.111, p = 0.742] or Group x Time x Location [F (1,23) = 0.545, p = 0.468]. Figure 5.17 shows laboratory heart rate variability to be larger than ambulatory values. These results suggest that ambulatory measures of heart rate variability differ from those recorded in the laboratory, irrespective of supplementation.

Heart Rate Reactivity

The reactivity averages from ambulation and laboratory epochs were calculated, for each subject and used to perform a repeated-measures ANOVA. Location and Time were the within group factors, while Group was the between factor. Results indicated no significant differences between Groups [F (1,23) = 2.794, p = 0.108]. A main effect of Location was found within groups [F (1,23) = 37.029, p < 0.001]. No significant Group x Time interaction was found [F (1,23) = 0.316, p = 0.579] or Group x Time x Location [F (1,23) = 0.571, p = 0.458]. Figure 5.18 shows laboratory heart rate reactivity to be greater than ambulatory reactivity, however this may be a factor of using the laboratory baseline epoch to calculate ambulatory values.

Systolic Blood Pressure

The average SBP from ambulation and laboratory epochs were calculated, for each subject and used to perform a repeated-measures ANOVA. Location and Time were the

within group factors, while Group was the between factor. ANOVA results indicated no significant differences between Groups [F (1,23) = 0.857, p = 0.364]. A main effect of Location was found within groups [F (1,23) = 11.577, p = 0.002]. No significant Group x Time interaction was found [F (1,23) = 1.064, p = 0.313] or Group x Time x Location [F (1,23) = 0.162, p = 0.691]. Figure 5.19 shows laboratory SBP to be higher than ambulatory values. The results suggest that irrespective of supplementation systolic blood pressure values differ according to the location of the recording.

Systolic Blood Pressure Reactivity

The average SBP reactivity, from ambulation and laboratory epochs was calculated, for each subject and used to perform a repeated-measures ANOVA. Location and Time were the within group factors, while Group was the between factor. ANOVA results indicated no significant differences between Groups [F (1,23) = 0.684, p = 0.417]. A main effect of Location was found within groups [F (1,23) = 28.127, p < 0.001] as well as a main effect of Time [F (1,23) = 6.427, p = 0.018]. No significant Group x Time interaction was found [F (1,23) = 2.950, p = 0.099] or Group x Time x Location [F (1,23) = 1.170, p = 0.291]. Figure 5.20 indicates that SBP reactivity, although lower during ambulation than in the laboratory, decreases for both groups in both locations over time.

Diastolic Blood Pressure

The average DBP, from ambulation and laboratory epochs was calculated, for each subject and used to perform a repeated-measures ANOVA. Location and Time were the within group factors, while Group was the between factor. ANOVA results indicated no significant differences between Groups [F (1,23) = 0.226, p = 0.639]. A main effect of Location was found within groups [F (1,23) = 30.753, p < 0.001]. No significant Group x Time interaction was found [F (1,23) = 0.193, p = 0.664] or Group x Time x Location [F (1,23) = 0.062, p = 0.805]. See figure 5.21, which identifies laboratory DBP as higher than ambulatory values. The results suggest that irrespective of supplementation diastolic blood pressure is higher during laboratory than during ambulatory recording.

Diastolic Blood Pressure Reactivity

The average DBP reactivity was calculated, for each subject and used to perform a repeated-measures ANOVA. Location and Time were the within group factors, while Group was the between factor. ANOVA results indicated no significant differences between Groups [F (1,23) = 0.891, p = 0.355]. A main effect of Location was found [F (1,23) = 25.545, p < 0.001] as well as a main effect of Time [F (1,23) = 7.566, p = 0.011]. No significant Group x Time interaction was found [F (1,23) = 2.057, p = 0.165] or Group x Time x Location [F (1,23) = 0.552, p = 0.465]. Figure 5.22 indicated that both groups DBP reactivity reduced over time of both locations.

5.4.3 ANOVA of Laboratory and Ambulatory Cardiovascular Data – Figures

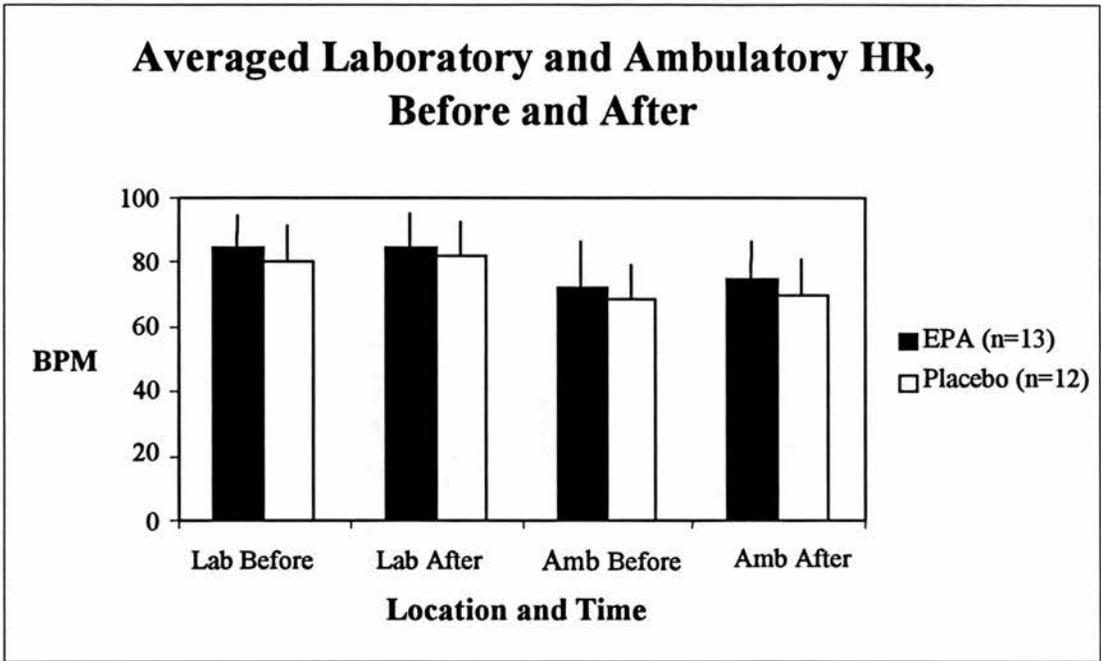


Figure 5.16: Averaged laboratory and ambulatory HRs (inc. Std. Dev.) before and after supplementation. Repeated-measures ANOVA results show no significant differences between Groups [$F(1,23) = 1.414, p = 0.246$]. A main effect of Location was found, [$F(1,23) = 25.074, p < 0.001$]. No significant Group x Time interaction was found [$F(1,23) = 0.111, p = 0.742$]

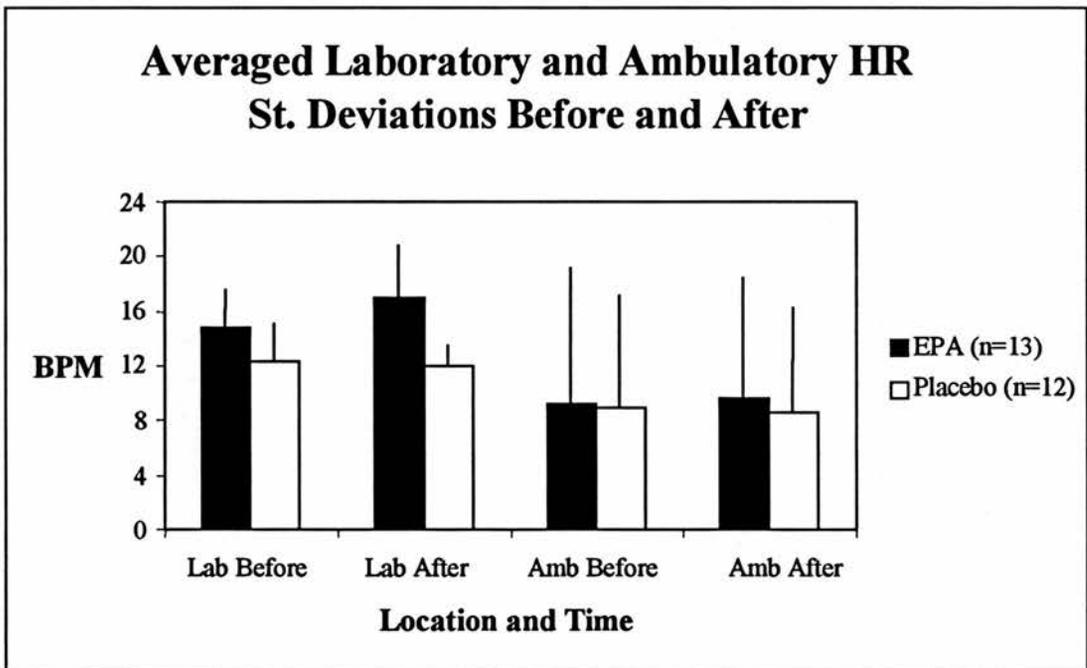


Figure 5.17: Averaged laboratory and ambulatory HRV before and after supplementation. Repeated-measures ANOVA results show no significant differences between Groups [$F(1,23) = 2.684, p = 0.115$]. A main effect of Location was found, [$F(1,23) = 10.978, p = 0.003$]. Group x Time x Location [$F(1,23) = 0.545, p = 0.468$].

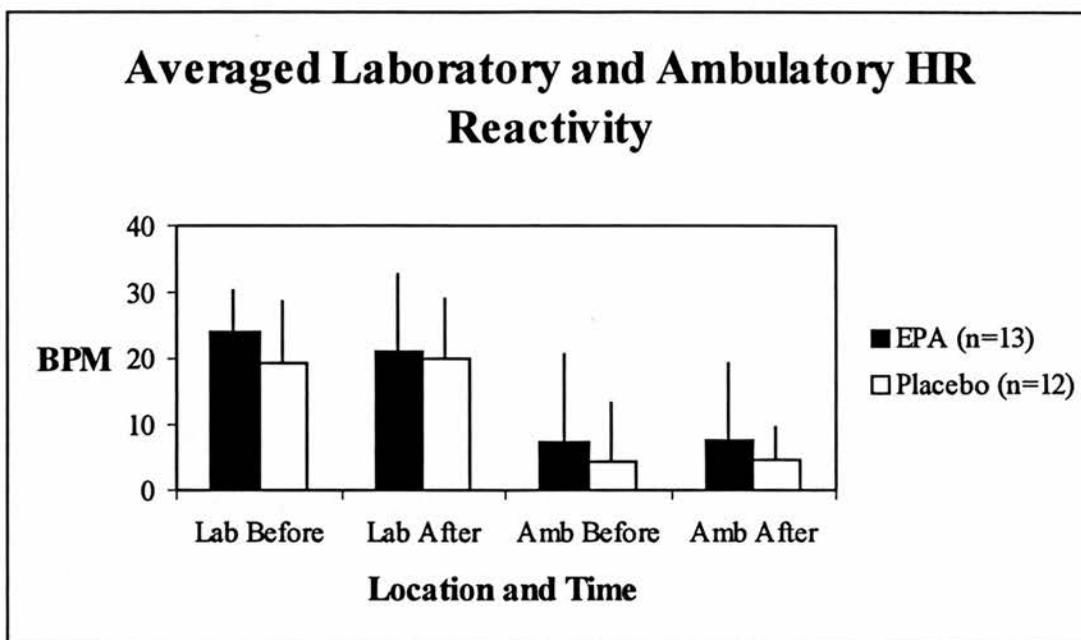


Figure 5.18: Averaged group HR reactivity before and after supplementation. ANOVA results indicate no differences between Groups [$F(1,23) = 2.794, p = 0.108$]. No significant Group x Time interaction was found [$F(1,23) = 0.316, p = 0.579$] or Group x Time x Location [$F(1,23) = 0.571, p = 0.458$]. A main effect of Location was found within groups [$F(1,23) = 37.029, p < 0.001$].

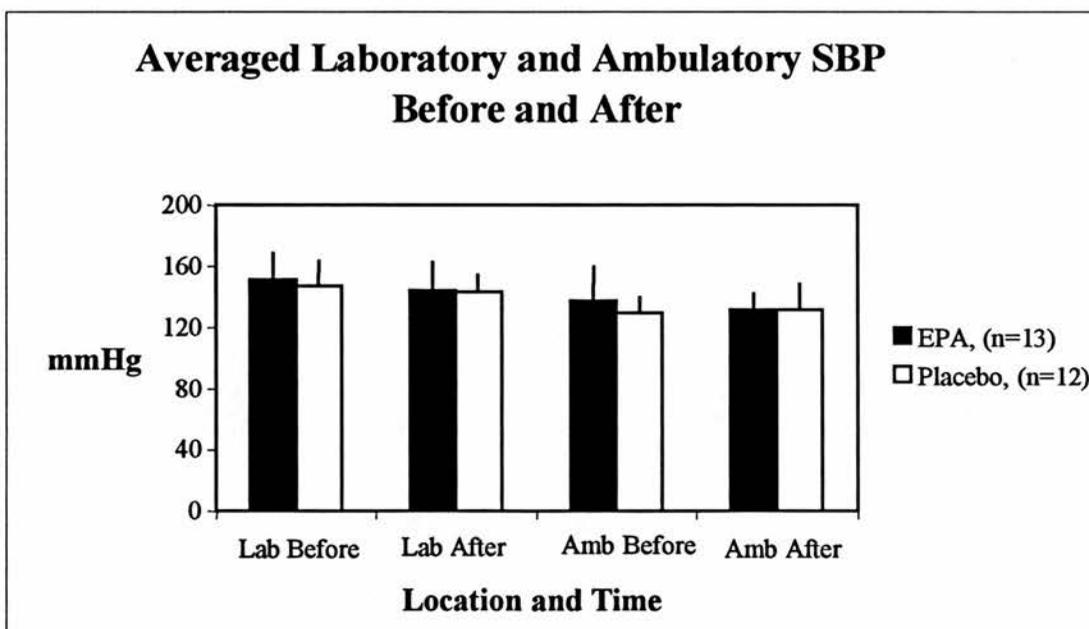


Figure 5.19: Averaged laboratory and ambulatory SBP (inc. Std. Dev.) before and after supplementation. ANOVA results indicate no significant differences between Groups [$F(1,23) = 0.857, p = 0.364$]. A main effect of Location was found, [$F(1,23) = 11.577, p = 0.002$]. No significant Group x Time interaction was found [$F(1,23) = 1.064, p = 0.313$].

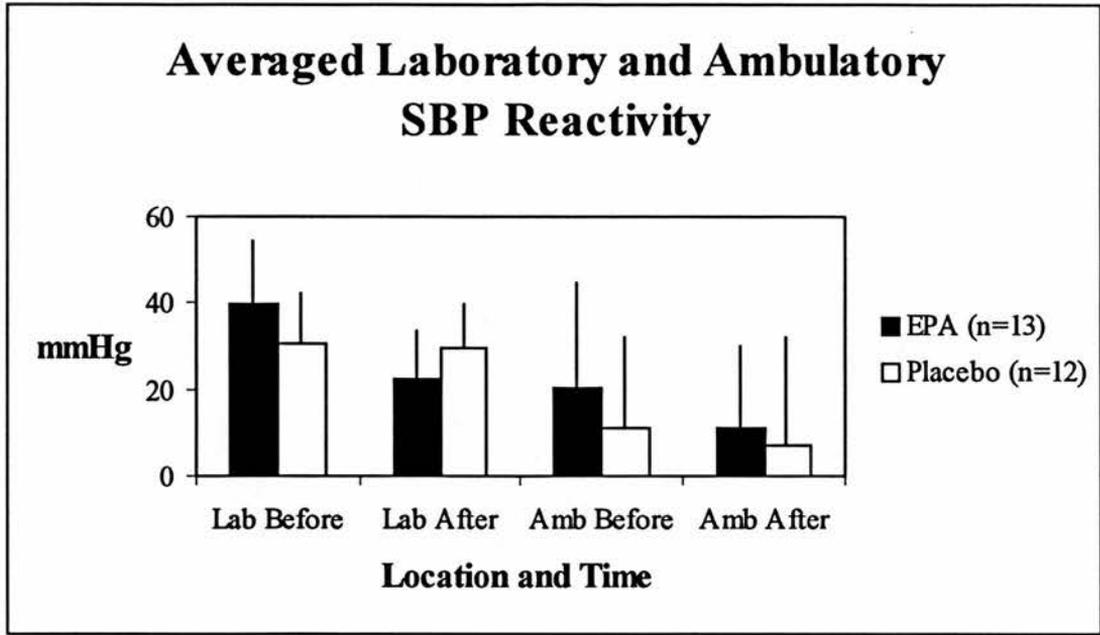


Figure 5.20: Averaged ambulatory and laboratory SBP reactivity before and after supplementation. ANOVA results found no significant differences between Groups [$F(1,23) = 0.684, p = 0.417$]. No significant Group x Time interaction [$F(1,23) = 2.950, p = 0.099$] or Group x Time x Location occurred [$F(1,23) = 1.170, p = 0.291$]. Main effects of Location [$F(1,23) = 28.127, p < 0.001$] and Time [$F(1,23) = 6.427, p = 0.018$] were found.

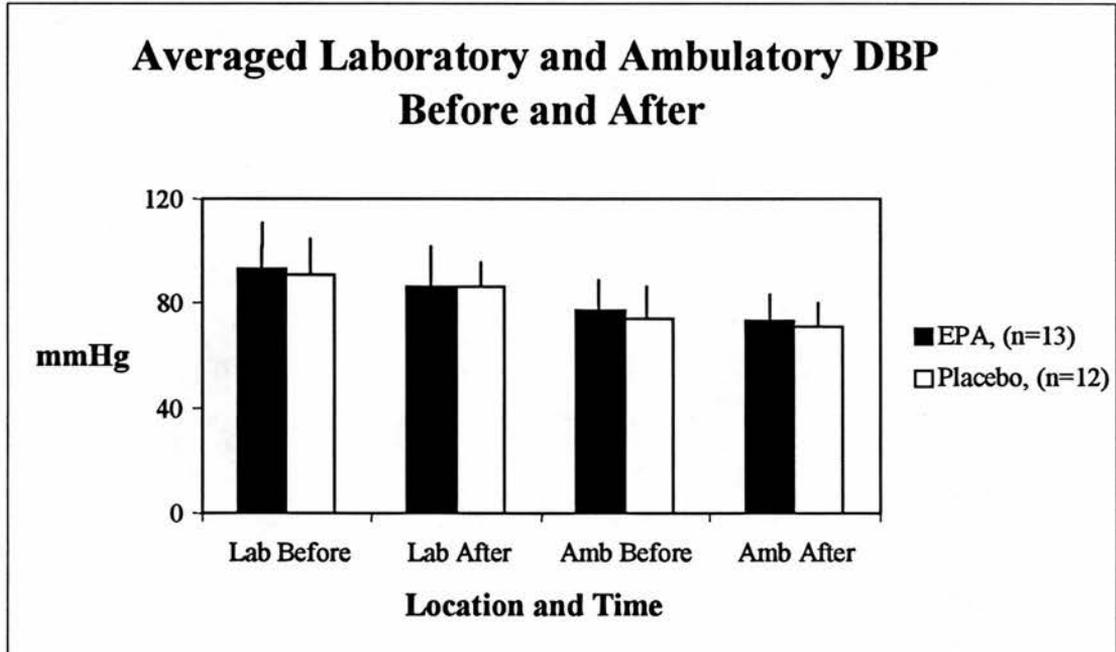


Figure 5.21: Averaged laboratory and ambulatory DBP (inc. Std. Dev.) before and after supplementation. ANOVA results show no significant Group differences [$F(1,23) = 0.226, p = 0.639$]. A main effect of Location was found, [$F(1,23) = 30.753, p < 0.001$]. No significant Group x Time interaction was found [$F(1,23) = 0.193, p = 0.664$].

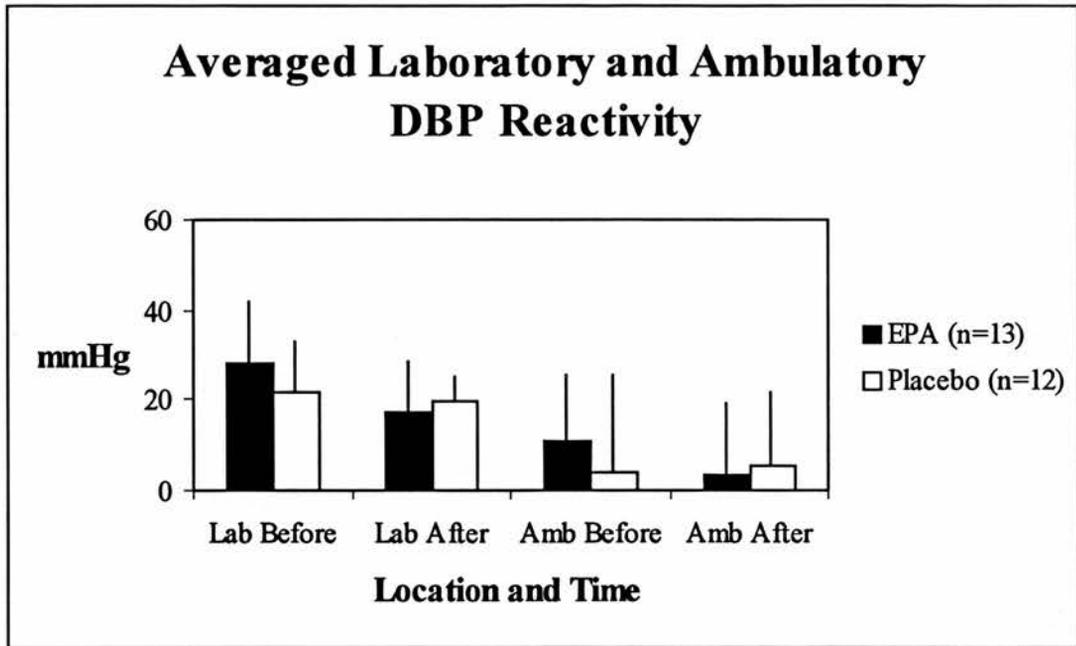


Figure 5.22: Averaged ambulatory and laboratory DBP reactivity before and after supplementation. ANOVA results found no significant Group differences [$F(1,23) = 0.891, p = 0.355$]. A main effect of Location [$F(1,23) = 25.545, p < 0.001$] and Time [$F(1,23) = 7.566, p = 0.011$] was found. No significant Group x Time interaction [$F(1,23) = 2.057, p = 0.165$] or Group x Time x Location [$F(1,23) = 0.552, p = 0.465$] was found.

5.4.4 Regression of Ambulatory and Laboratory Cardiovascular Data

ANOVA results indicate that laboratory and ambulatory data differed. This however may be due to many factors, for example, the use of different recording equipment with different bodily positioning (arm cuff and finger cuff) or the differing amounts of situational control available to the experimenter in each location. Therefore another important research question was whether or not a study performed within laboratory conditions, and the results gained, could be generalised to (are related to) real life situations. That is to say, would data recorded within the laboratory predict data recorded during ambulation? Linear Regression was used to investigate the likelihood that laboratory data predicted the ambulatory data at individual time points. Regression was performed on all time one (before) subjects, with no distinction made between groups, since all were supplement-free at this point. The regressions of time two (after)

may be confounded by factors such as supplementation, habituation (to task epochs) and order effects.

Subject Exclusions and Descriptive Statistics

One subject (medicated) was excluded from the ‘before’ regressions. However, a further two subjects were excluded from the ‘after’ regressions due to lack of ambulatory data. Table 5.9 provides the average values for both time points and locations, while the graphs can be found in section 5.4.5.

	Before				After			
	LAB n=27		AMB n=27		LAB n=25		AMB n=25	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	81.82	10.99	69.49	12.81	83.30	10.47	72.32	11.43
HR R	21.15	8.14	5.29	11.04	20.56	7.18	6.11	9.13
SBP	148.96	19.98	133.31	15.67	144.02	17.74	131.68	14.30
SBP R	37.01	14.76	15.19	23.35	25.66	11.12	9.32	21.65
DBP	90.84	15.60	76.49	11.78	86.25	12.42	72.32	9.55
DBP R	26.80	14.59	7.99	18.27	18.43	8.99	1.81	15.64

Table 5.9: Averages and standard deviations of laboratory and ambulatory cardiovascular data for the time points of before and after supplementation. HR (BPM), SBP and DBP (mm Hg) are shown. The lightly shade rows indicate reactivity values.

Heart Rate

Regressions were performed using the Ambulatory (AMB) heart rate data as the dependent variable and the Laboratory (LAB) heart rate data as the predictor variable (independent). Only the p-values for the correlations are displayed (below). Since r and R^2 are perfectly related it is unnecessary to include the F-test results⁵².

⁵² The p-value for r shows the probability that $r = 0$, while the F-test shows the probability that $R^2 = 0$.

Before: The correlation between Laboratory and Ambulatory data was found to be moderate [$r = 0.435$, $p = 0.012$ (one-tailed)]. The regression equation was:

$$\text{LAB} = 0.507(\text{AMB}) + 28.044$$

The results indicated a moderate to weak relationship, see figure 5.23 for scatter-plot.

After: The one-tailed correlation between Laboratory and Ambulatory data was found to be moderate [$r = 0.384$, $p = 0.029$]. The regression equation was:

$$\text{LAB} = 0.419(\text{AMB}) + 37.403$$

The results indicated a weak to moderate relationship, see figure 5.24.

Heart Rate Variability

Regressions were performed using the averaged standard deviations of Ambulatory heart rate data as the dependent variable and the Laboratory heart rate data as the predictor variable (independent).

Before: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = -0.208$, $p = 0.159$]. The regression revealed no significant relationship.

After: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = 0.195$, $p = 0.175$]. The regression revealed no significant relationship.

Heart Rate Reactivity

Regressions were performed using the averaged Ambulatory heart rate reactivity data as the dependent variable and the Laboratory heart rate reactivity data as the predictor variable (independent).

Before: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = 0.035$, $p = 0.431$]. The regression revealed no significant relationship.

After: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = -0.255$, $p = 0.110$]. The regression revealed no significant relationship.

Systolic Blood Pressure

Regressions were performed using the averaged Ambulatory SBP data as the dependent variable and the Laboratory SBP data as the predictor variable (independent).

Before: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = -0.018$, $p = 0.465$]. The regression revealed no significant relationship.

After: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = -0.148$, $p = 0.240$]. The regression revealed no significant relationship.

Systolic Blood Pressure Reactivity

Regressions were performed using the averaged Ambulatory SBP reactivity data as the dependent variable and the Laboratory SBP reactivity data as the predictor variable (independent).

Before: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = 0.275$, $p = 0.082$]. The regression revealed no significant relationship.

After: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = 0.209$, $p = 0.158$]. The regression revealed no significant relationship.

Diastolic Blood Pressure

Regressions were performed using the averaged Ambulatory DBP data as the dependent variable and the Laboratory DBP data as the predictor variable (independent).

Before: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = -0.150$, $p = 0.228$]. The regression revealed no significant relationship.

After: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = 0.261$, $p = 0.104$]. The regression revealed no significant relationship.

Diastolic Blood Pressure Reactivity

Regressions were performed using the averaged Ambulatory DBP reactivity data as the dependent variable and the Laboratory DBP reactivity data as the predictor variable (independent).

Before: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = 0.118$, $p = 0.279$]. The regression revealed no significant relationship.

After: The one-tailed correlation between Laboratory and Ambulatory data was not significant [$r = 0.139$, $p = 0.254$]. The regression revealed no significant relationship.

5.4.5 Regression of Ambulatory and Laboratory Cardiovascular Data – Scatter-plots

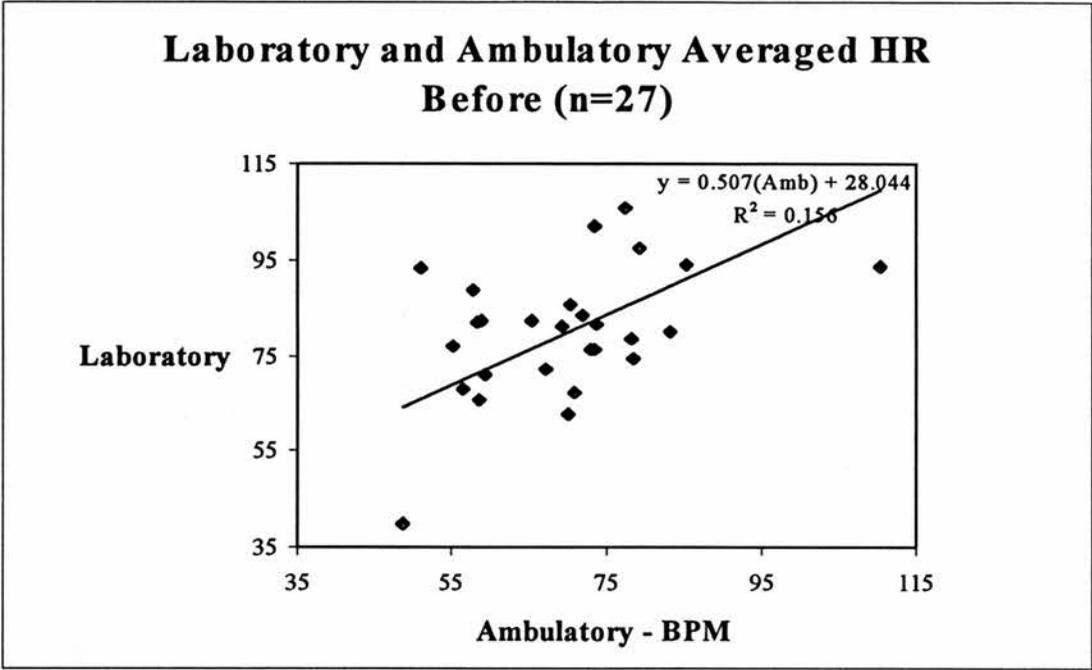


Figure 5.23: Averaged heart rate (BPM) from laboratory and ambulatory test sessions before supplementation. Linear regression indicated a moderate to weak correlation between Laboratory and Ambulatory recordings (trend-line shown), $r = 0.435$, $p = 0.012$ (one-tailed), with adjusted $R^2 = 0.156$.

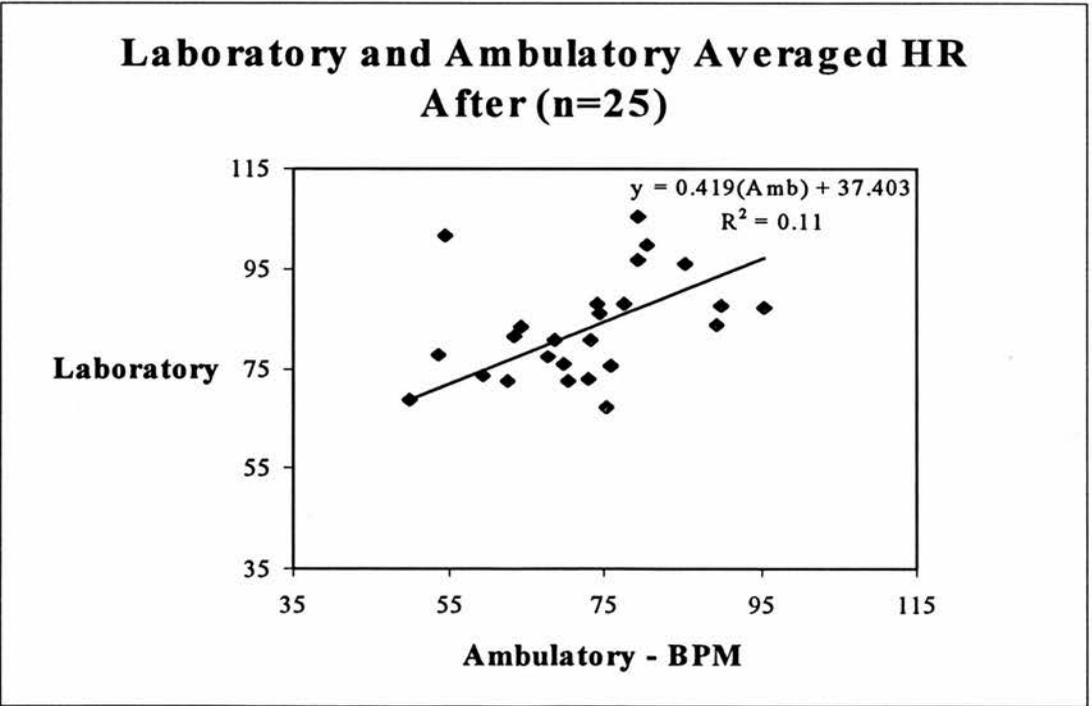


Figure 5.24: Regression of averaged HR after supplementation shows a weak to moderate relationship, $r = 0.384$, $p = 0.029$ (one-tailed), adjusted $R^2 = 0.11$.

5.5 *Acoustic Startle*

Each subject performed the acoustic startle task only once, after supplementation. Due to the nature of the acoustic startle task the cardiovascular data could not be treated similarly to the previous tasks, where epoch averages were used. In this event, with the distinction of *loud* and *soft* tone stimuli, calculating an average of the entire startle epoch, would be inappropriate. Instead, corresponding heart rate and blood pressure data was determined for a *time period* before and after each stimulus tone.

5.5.1 Extraction of Heart Rate and Blood Pressure Values

With the artifact rejection procedure (described in 5.1) completed, a final stage was added to the Spike2 script. The purpose of this stage was to:

- Specify the sampling time period required, before and after the stimuli
- Specify the resolution of the time period (bin size in seconds).
- Calculate and average of the physiological data over the given time periods.
- Output this in a text file format for transfer to a statistical package.

The script calculated the averages of the physiological data, over the specified time period according to the specified resolution, for each tone. This process took into account the fact that the interval between a given pair of successive heartbeats can straddle, be encompassed by, or encompass a specified time. It also took account of the fact that as heart rate slows down, each inter-beat interval contributes more to the average heart rate. Finally, where there were missing blood pressure data (e.g. during Finapres calibration), the script searched back in time to the last valid data point and used that as a best guess for the missing values. This final stage script was run on each subject's data file from the second testing session (after supplementation). See figure 5.17 for an example of the output file and appendix 27 for script.

Event	Number	Offset	BPM	Diastolic	Systolic
Soft	1	-2.000000	75.906856	56.182178	137.680396
Soft	1	0.000000	72.932776	59.126929	139.264307
Soft	2	-2.000000	74.271344	63.202307	140.180774
Soft	2	0.000000	66.425518	63.504236	143.690704
Soft	3	-2.000000	65.557615	60.994537	142.266632
Soft	3	0.000000	69.330372	59.022095	134.244995
Soft	4	-2.000000	70.031560	59.416193	133.321484
Soft	4	0.000000	71.824455	62.189905	133.118616
Soft	5	-2.000000	71.406109	63.252118	134.956921
Soft	5	0.000000	69.105435	65.486249	137.483661
Soft	6	-2.000000	65.709699	64.060211	140.438647
Soft	6	0.000000	75.011011	61.919543	135.708917
Loud	1	-2.000000	79.601690	65.024170	134.593872
Loud	1	0.000000	75.834266	71.317078	141.688477
Soft	7	-2.000000	75.051787	48.631958	105.747144
Soft	7	0.000000	74.230436	54.313208	116.049487
Soft	8	-2.000000	73.037561	58.174536	121.662671
Soft	8	0.000000	61.471213	58.158307	127.665454
Soft	9	-2.000000	67.468934	57.916132	126.910425
Soft	9	0.000000	63.289697	57.404425	126.825879
Soft	10	-2.000000	65.383086	56.377881	126.968286
Soft	10	0.000000	66.405358	59.035718	126.330127
Loud	2	-2.000000	68.472122	58.972656	129.064941
Loud	2	0.000000	72.227809	64.561401	134.815063
Soft	11	-2.000000	66.309886	57.021582	122.531567
Soft	11	0.000000	58.144879	59.814453	120.361328
Soft	12	-2.000000	62.747121	59.814453	120.361328
Soft	12	0.000000	61.037692	56.469019	121.341174
Loud	3	-2.000000	68.694369	56.760950	121.867639
Loud	3	0.000000	68.283880	64.363770	132.085376

Figure 5.17: Segment of text output from startle script of subject number 10, showing first 15 stimuli tones. The first column indicates the type of stimuli. The second column indicates the order of the stimuli. The third column (offset) indicates the beginning of the time period in seconds relative to the tone. The fourth, fifth and sixth columns show the averaged heart rate (bpm), diastolic and systolic blood pressure (mm Hg) respectively. For this example a time period and resolution of 2 seconds was used.

5.5.2 Subject Exclusions and Descriptive Statistics

Only the subject on anti-depressant medication was excluded from analysis. Every subject's data was passed through the Spike Startle script to extract the heart rate and blood pressure values. The sampling time period instituted was 2 seconds prior to each tone (*Pre*) and 6 seconds after (*Post*), with a resolution of 2 seconds. The *pre* time

period was chosen to avoid any influence from the previous tone on the results. The *post* time period specified was to permit a possible response in blood pressure levels to be observed, since blood pressure has a slight lag behind heart rate changes.

For every tone, for each subject an average for both pre and post periods was then calculated. See table 5.10 for the average values and standard deviations of the EPA group and table 5.11 for the placebo group values.

	Loud Tones				Soft Tones			
	Pre		Post		Pre		Post	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	71.05	11.45	69.80	11.40	70.46	11.57	70.53	11.60
SBP	135.26	14.67	138.23	15.23	134.54	14.59	134.67	14.42
DBP	80.97	12.88	80.74	13.19	80.70	12.90	80.86	12.80

Table 5.10: Averaged values and standard deviations for the EPA group (n=14) during the acoustic startle task. Pre and post values are shown for both the loud and soft tones. Heart rate is represented in bpm, while blood pressure is in mm Hg.

	Loud Tones				Soft Tones			
	Pre		Post		Pre		Post	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
HR	71.56	12.56	71.01	12.52	71.25	11.79	71.24	11.91
SBP	133.22	10.92	133.83	11.05	132.60	9.93	132.62	10.12
DBP	82.08	11.84	82.12	11.93	81.86	11.47	81.94	11.49

Table 5.11: Averaged values and standard deviations for the placebo group (n=13) during the acoustic startle task. Pre and post values are shown for both the loud and soft tones. Heart rate is represented in bpm, while blood pressure is in mm Hg.

5.5.3 ANOVA of Acoustic Startle Cardiovascular Data

The initial question to be asked from the acoustic startle data is, was there a difference between the two groups on cardiovascular functioning during the startle task? Subject averages were calculated (from the data outputted by the Startle Script) for both the dependent variables of *Tone Time* (pre and post) and *Tone Type* (loud and soft). Repeated-measures ANOVAs were executed on the averaged data to investigate for any differences between the two subject groups. Graphs can be found in section 5.5.4.

Heart Rate

An ANOVA was performed between Groups with Tone Type and Tone Time as the within group factors. Results indicated no significant differences between the Groups [$F(1,25) = 0.031, p = 0.861$]. A main effect of Tone Time was found within groups [$F(1,25) = 7.152, p = 0.013$], as was a Tone Type x Tone Time interaction [$F(1,25) = 1.338, p = 0.009$]. Although no differences in heart rate could be found between the groups these results suggest that the startle manipulation was a success. See figure 5.25 for a graph of the data.

Systolic Blood Pressure

An ANOVA was performed, on the SBP averages, between Groups with Tone Type and Tone Time as the within group factors. Results showed no significant differences between the Groups systolic blood pressures [$F(1,25) = 0.285, p = 0.598$]. A main effect of Tone Type [$F(1,25) = 8.718, p = 0.007$] and Tone Time [$F(1,25) = 4.502, p = 0.044$] was found within groups as well as a Tone Time x Tone Type interaction [$F(1,25) = 4.733, p = 0.039$]. Figure 5.26 shows graphic representation.

Diastolic Blood Pressure

A repeated-measures ANOVA was performed, on the DBP averages, between Groups with Tone Type and Tone Time as the within group factors. Results showed no significant effect of Tone Time [$F(1,25) = 0.003, p = 0.957$], or Tone Type [$F(1,25) =$

0.224, $p = 0.640$], or a Tone Time x Tone Type interaction [$F(1,25) = 0.233$, $p = 0.663$]. The ANOVA results also indicated no significant differences between the Groups diastolic blood pressures [$F(1,25) = 0.062$, $p = 0.805$]. These results suggest that the acoustic startle task had no effect on diastolic blood pressure values. See figure 5.27.

5.5.4 ANOVA of Acoustic Startle Cardiovascular Data – Figures

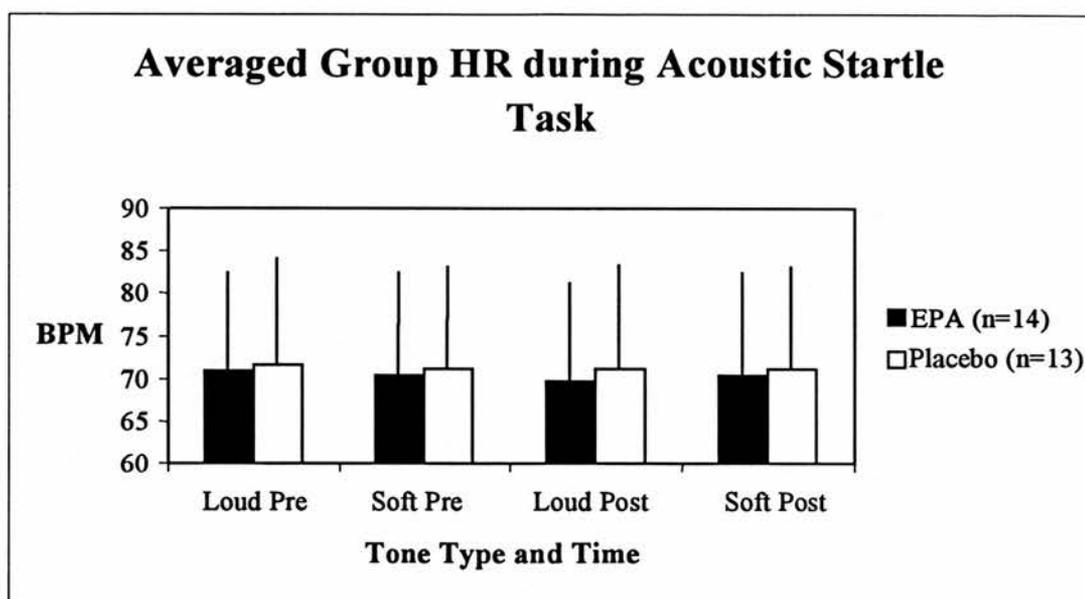


Figure 5.25: Averaged heart rate data (with standard deviations) for acoustic startle task indicated in BPM. Repeated-measures ANOVA found no significant Group differences [$F(1,25) = 0.031, p = 0.861$]. Within groups, a main effect of Tone Time was found [$F(1,25) = 7.152, p = 0.013$], as was a Tone Type x Tone Time interaction [$F(1,25) = 1.338, p = 0.009$].

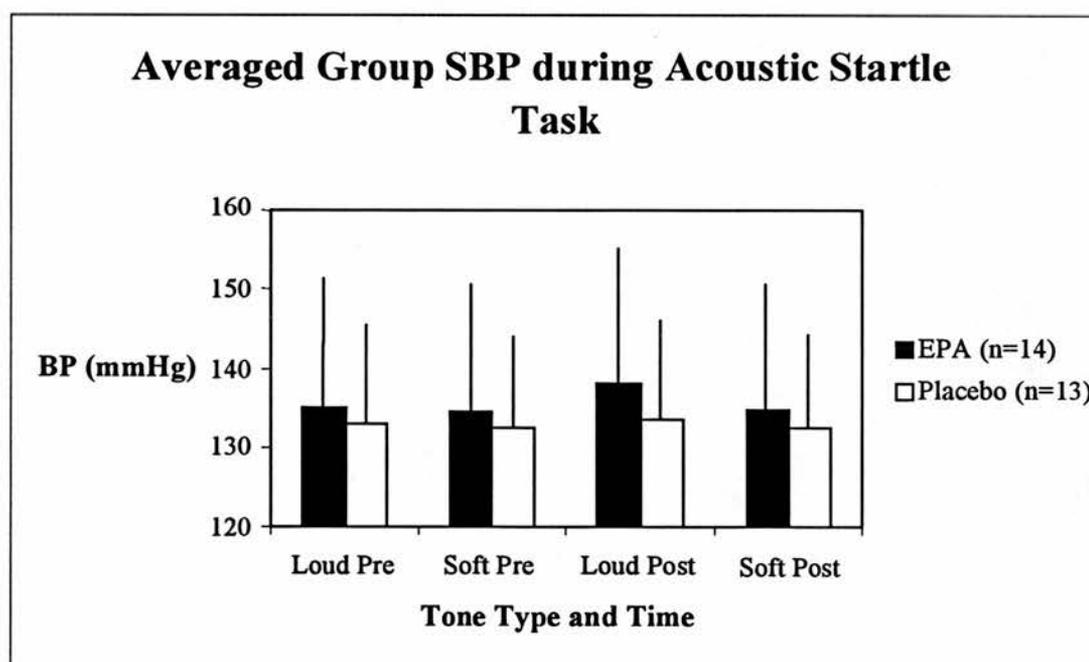


Figure 5.26: Averaged systolic blood pressure values (with standard deviations) for acoustic startle task (mm Hg). ANOVA indicated no significant Group differences [$F(1,25) = 0.285, p = 0.598$]. A significant Tone Time x Tone Type interaction [$F(1,25) = 4.733, p = 0.039$] was noted.

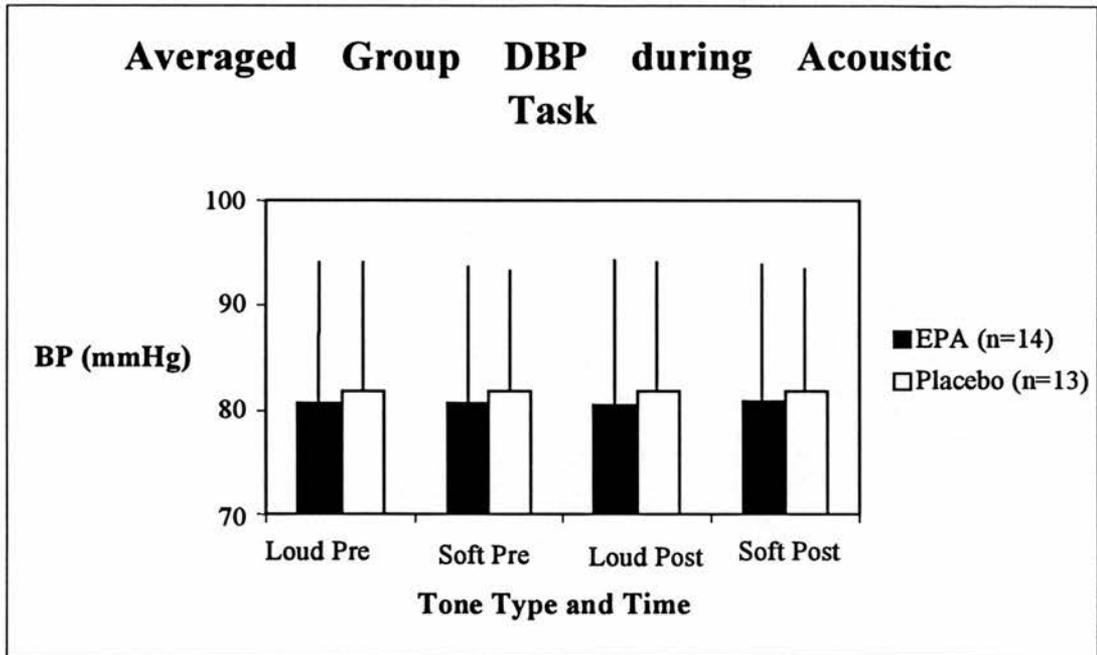


Figure 5.27: Averaged diastolic blood pressure values (with standard deviations) for acoustic startle task (mm Hg). ANOVA indicated no significant differences between the two Groups on DBP [$F(1,25) = 0.062, p = 0.805$], and no significant Tone Time x Tone Type interaction [$F(1,25) = 0.233, p = 0.663$].

6 Psychological Measures - Results

The non-physiological measures taken throughout the study fall into two main categories: subject characteristics and psychological characteristics.

6.1 *Subject Characteristics*

For the purposes of the study non-psychological characteristics were identified as: age, weight, and height, level of regular physical exercise and dietary intake of fat, fibre and unsaturated fats. They were measured, both before and after supplementation, for several reasons. Each of these characteristics is known to be a factor that can influence cardiovascular health and functioning (Carroll, 1992, Johnston, 1993). These characteristics could be considered potential confounding variables. Therefore it was necessary to measure them in order to eliminate the possibility that differences between the groups in these variables would produce a spurious “effect” of the ethyl-EPA or that such differences would mask a real effect of ethyl-EPA. Ideally the randomisation process should not have resulted in detectable differences in these variables between the groups at time one. Having confirmed this, it would be possible to isolate whether or not supplementation with EPA had any effect on the stress-related variables.

6.1.1 Subject Exclusions and Descriptive Statistics

Twenty-seven subjects were included in the analysis. Only the subject on medication was excluded. Age, level of exercise, and diet were all self-report measures, while weight and height was measured in the laboratory. The table (6.1) below shows the non-standardised average values and standard deviations, for each group both before and after supplementation. Age and height were static variables.

	Before				After			
	EPA n=14		Placebo n=13		EPA n=14		Placebo n=13	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
Age	23.50	5.57	23.15	4.10	--	--	--	--
Height	1.82	0.05	1.81	0.05	--	--	--	--
Weight	77.17	9.21	77.55	10.84	76.86	8.64	78.06	10.97
Activity	22.57	6.20	21.62	8.80	23.43	11.63	22.85	11.36
Fat	32.93	8.85	35.54	7.39	30.07	9.14	33.23	7.35
(Unsat.)	(8.43)	1.50	(8.46)	1.27	(9.14)	1.10	(9.08)	1.12
Fibre	31.00	11.46	35.85	13.11	30.28	10.95	35.00	9.06

Table 6.1: Group averages for the non-psychological characteristics both before and after supplementation. *Age* is measured in years. *Height* is in metres. *Weight* in kg. *Activity* is a score from a possible maximum of 84. *Fat* is a unit score for intake where 0-30 is low, 30-40 is moderate and 40+ high. *Unsat.* measured the unsaturated fat component of the total fat score, where 0-5 is low, 6-9 is moderate and 10+ is high, 12 being the maximum possible. *Fibre* is a unit score for intake where 0-30 is low, 30-40 moderate and 40+ high.

6.1.2 MANOVA of Subject Characteristics

Since all the measures used required different scales with different weightings it was necessary to standardise them. This eliminated the possibility of any scale unequally influencing the results. The z-scores were computed independently for each set of data (before supplementation data only and then after supplementation data only). To investigate for differences between the groups subject characteristics, separate MANOVAs were performed on the before and after z-scored data. Graphs can be seen in section 6.1.3.

Before Ethyl-EPA Supplementation

MANOVA results revealed no significant differences at time one between the Groups' subject characteristics [$F(7,19) = 0.537, p = 0.796$]. This suggests that the group randomisation was successful in avoiding bias, and the characteristics (see figures 6.1 to 6.5) were not acting as confounding variables.

After Ethyl-EPA Supplementation

Due to their stability the characteristics of age and height were excluded from this analysis, hence the z-scores were computed on the remaining five variables. MANOVA results revealed no significant differences at time two between the Groups' subject characteristics [$F(5,21) = 0.813, p = 0.554$]. This suggests that supplementation with EPA had no effect on level of activity, weight or diet.

6.1.3 Subject Characteristics - Figures

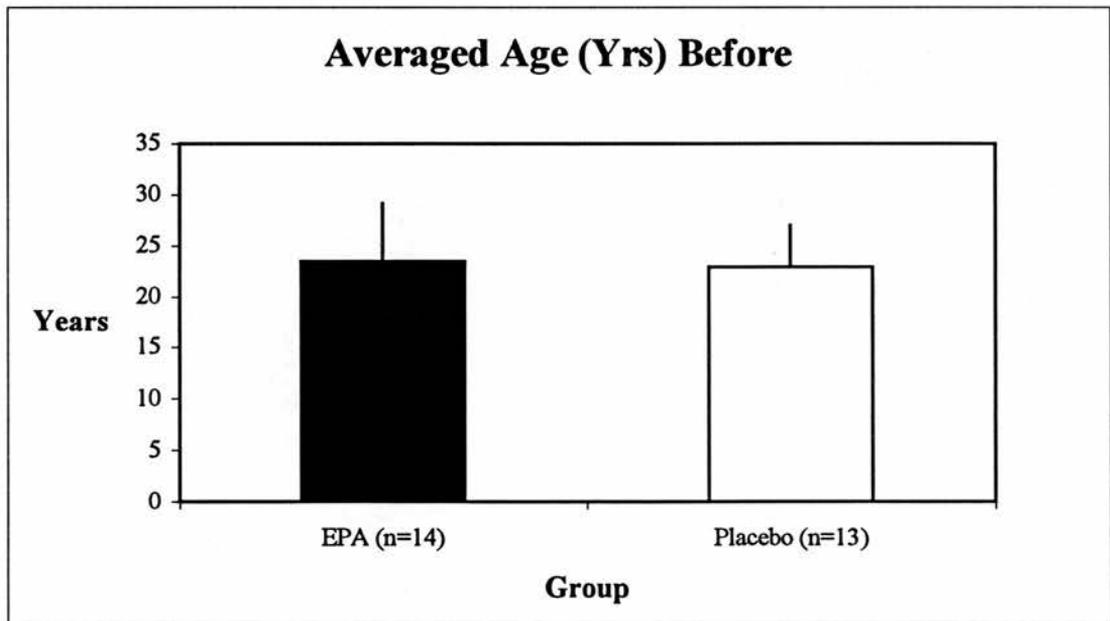


Figure 6.1: Average self-reported age of subjects, for each group, prior to supplementation. MANOVA indicated no differences between groups for Age, Exercise Weight, and Diet, [$F(7,19) = 0.537, p = 0.796$], shown in figures 6.1 to 6.5.

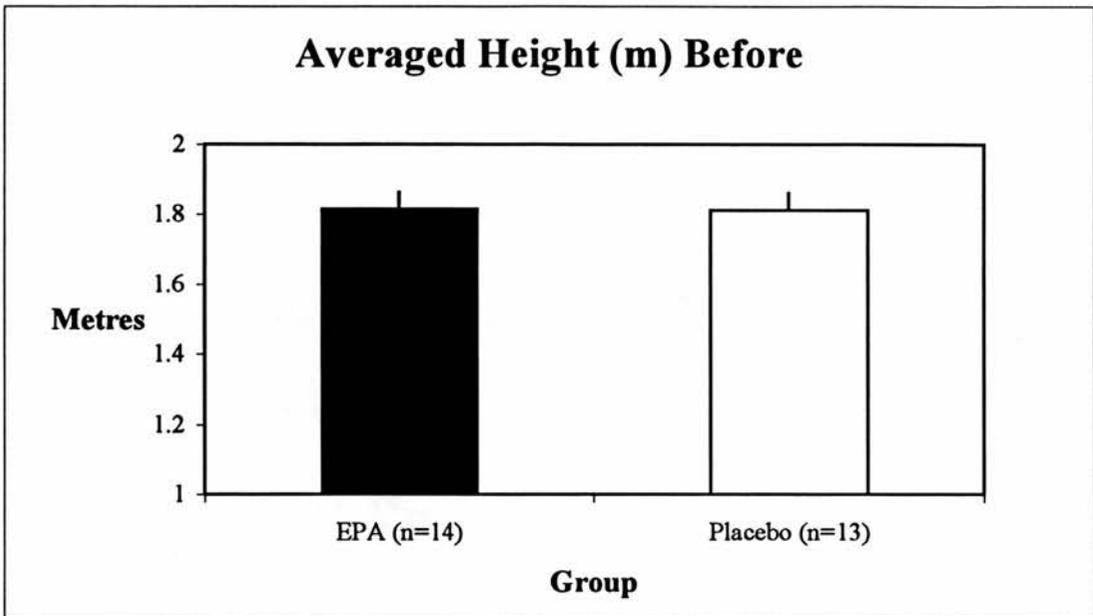


Figure 6.2: Averages of measured height, for each group, prior to supplementation. No Group differences were found.

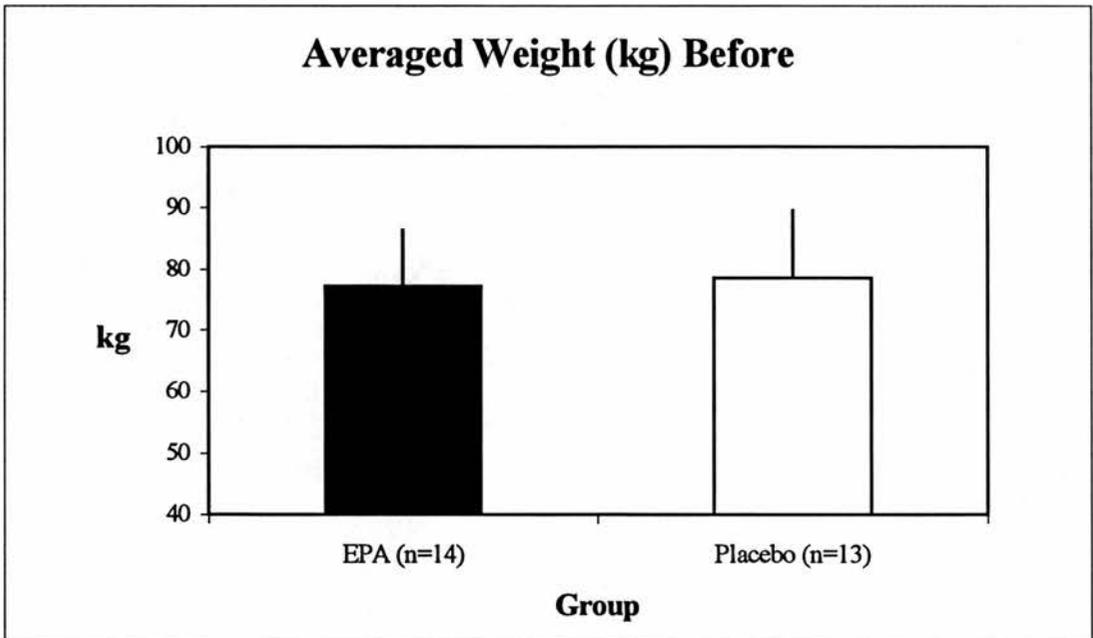


Figure 6.3: Averages of measured weight, for each group, prior to supplementation. No Group differences were found.

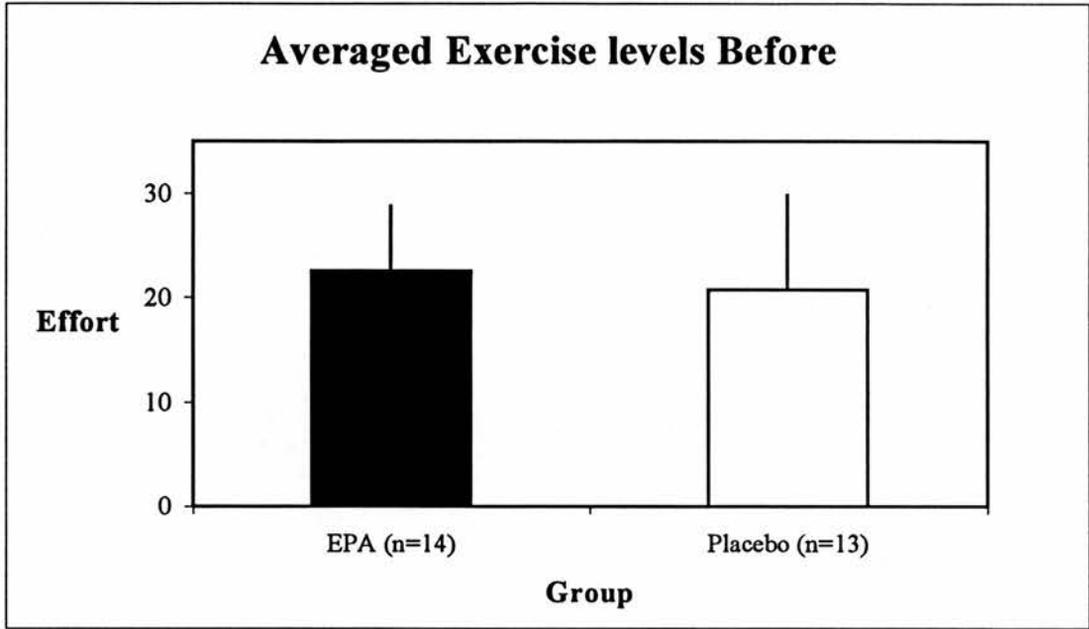


Figure 6.4: Averaged self-reported levels of activity, for each group, prior to supplementation. Maximum possible score of 90, although this would take superhuman ability. No differences were found between Groups.

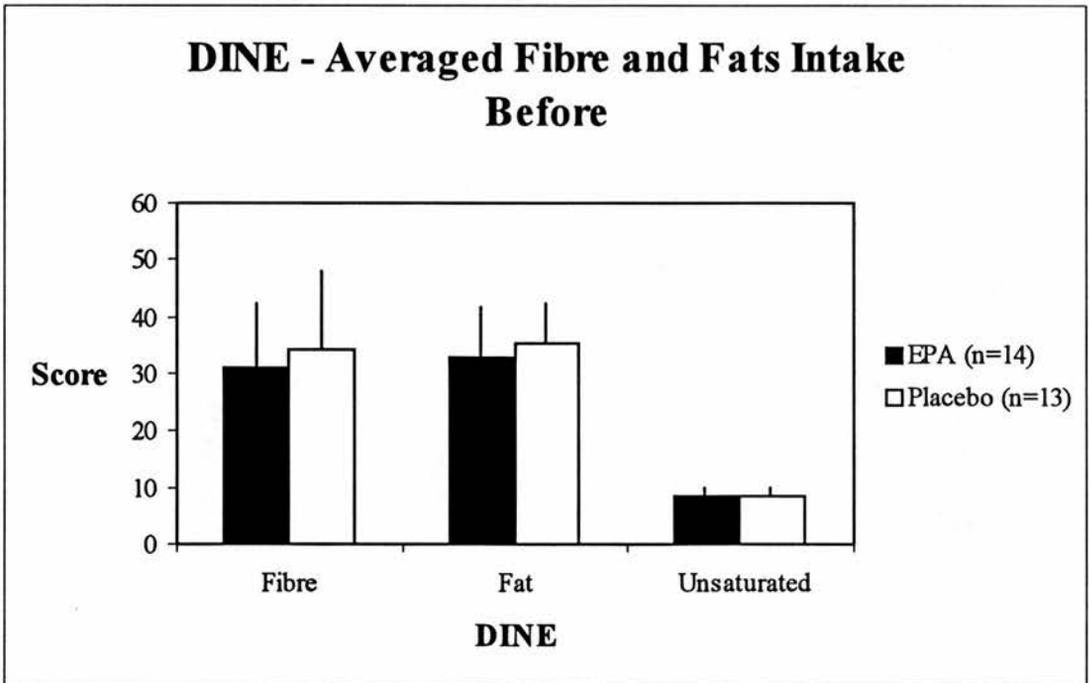


Figure 6.5: Averaged self-reported diet intake scores on the DINE (designed by L. Roe and M. Thorogood, ICRF General Practice Research Group), for both groups, prior to supplementation. No group differences were found.

6.2 *Psychological Characteristics*

The psychological characteristics were measured to determine if they differed between groups before or after supplementation. There were eight scales which produced 13 scores in total for each subject at each time point. The scales that produced multiple scores were: the EMAS-T, which had 4 sub-categories of trait anxiety – social evaluation, physical danger, daily routines and ambiguous; and the ambulatory Diary Mood Scale, which had 3 categories of arousal – energetic, tense and hedonic. The diary mood scales were averaged scores while all other scales produced a raw score.

The diary consisted of 17 mood scales (one for each blood pressure reading). Separately each page was divided into the 3 categories of arousal and scored as described in the methods. An average was then calculated for each category for every subject, where 4 was the maximum score. Averaging the scales was necessary to eliminate any effect of the occasional uncompleted diary page.

Once all the measures had been marked, for each subject, the 13 scores were standardised. Z-scoring the measures was necessary to prevent any scale unfairly influencing the results, due to different scoring techniques.

6.2.1 Subject Exclusions and Descriptive Statistics

The subject on anti-depressant medication was excluded. Additionally there were two subjects without ambulatory data from after the supplementation. Table 6.2 below shows the group averages and standard deviations on the measures before and after supplementation. Graphs of the group scores prior to z-scoring can be seen in section 6.2.6.

	Before				After			
	EPA n=14		Placebo n=13		EPA n=13		Placebo n=12	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
EMAS-S	28.07	8.01	26.77	4.17	25.57	7.19	24.69	3.99
EMAS-SE	48.71	9.67	42.54	10.88	47.07	9.74	41.23	8.96
EMAS-PD	54.36	9.87	52.69	10.43	50.57	10.85	53.38	9.91
EMAS-AM	39.07	6.91	41.08	5.79	39.14	7.05	37.00	6.18
EMAS-DR	23.00	4.40	25.92	8.09	25.00	4.67	24.31	6.22
EMAS-P	11.64	2.02	10.31	2.02	10.64	2.10	10.00	2.04
CISS	176.7	20.26	168.8	20.47	169.9	31.06	165.4	21.72
SWLS	21.43	6.12	27.00	6.45	22.64	7.02	20.08	5.92
DBI	4.07	3.93	3.54	3.04	4.00	4.20	3.58	4.91
ALE	15.43	6.07	16.62	5.30	15.14	9.22	16.23	6.78
Tense	0.30	0.34	0.42	0.70	0.45	0.47	0.30	0.46
Hedonic	3.50	0.48	3.60	0.30	3.60	0.44	3.55	0.50
Energetic	2.50	0.78	2.42	0.89	2.55	1.00	2.10	0.97

Table 6.2: Averaged group scores and standard deviation of psychological measures both before and after supplementation. Shaded areas denote subcategorised scores for trait anxiety and the diary mood scale.

6.2.2 Psychological Characteristics - Before Ethyl-EPA Supplementation

The subjects' scores were averaged into groups and standardised across time one. A MANOVA was performed on the z-scores, to investigate if there were any pre-existing differences between the groups that might mask the effects of EPA. Results found no significant Group differences on any of the psychological measures [$F(13,13) = 1.001$, $p = 0.499$]. See figure 6.6 for graphs. The result indicated that no pre-existing difference in psychological characteristics existed between the groups and therefore no effect of EPA would be masked.

6.2.3 Psychological Characteristics - After Ethyl-EPA Supplementation

The subjects' scores were averaged into groups and standardised across time two. A MANOVA was performed on the z-scores, to investigate for any effects of EPA. Results found no significant differences between the Groups on their psychological characteristics [$F(13,11) = 0.818, p = 0.639$]. The results show that psychological characteristics were similar within both groups after supplementation. See figure 6.7 for graph.

6.2.4 Changes in Psychological Characteristics

The previous two MANOVAs do not actually tell us whether or not psychological characteristics changed with supplementation, only that they did not differ from placebo group to EPA group. To investigate for any difference in score changes over time between the groups I looked at the change in scores from time one to time two. The *change scores* were calculated for every subject by subtracting their *before* scores from their *after* scores. These values were then standardised across both groups. A MANOVA was performed. The results indicate no significant differences between the two Groups [$F(13,13) = 1.113, p = 0.425$]. See figure 6.8 for graph of both groups change scores and figure 6.9 for presentation of EPA group scores before and after supplementation.

6.2.5 Periodic Assessment

The Beck Depression Inventory and EMAS-State were completed at 4-weekly intervals, during supplementation, to determine if any changes occurred during the course of the study.

BDI

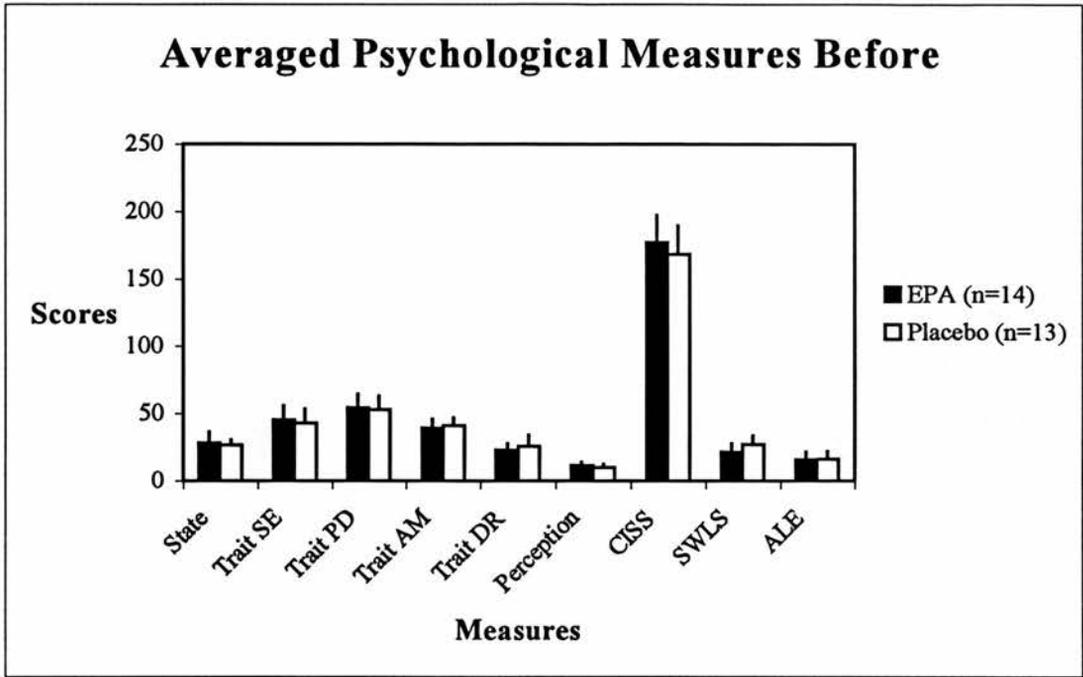
The subjects' scales were marked and group averages calculated. A repeated-measures ANOVA was performed, between Groups and within the factor of Time (4-weeks).

Four subjects were excluded from the analysis, three due to incomplete return of the BDI. The results found no significant differences between Groups [$F(1,22) = 0.002, p = 0.962$], and no significant effect of Time [$F(1.889,41.561) = 0.339, p = 0.702$] or Group x Time interaction [$F(1.889,41.561) = 0.223, p = 0.789$]. See figure 6.10 for graph.

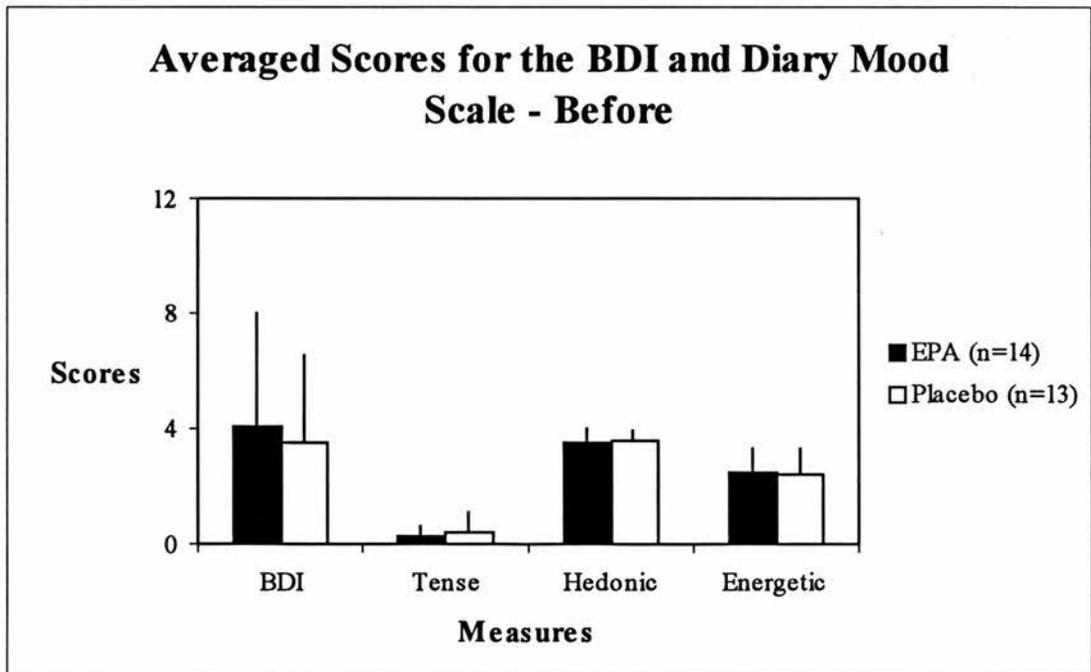
EMAS-S

The subjects' scales were marked and group averages calculated. A mixed ANOVA was performed, using Group and Time as factors. Three subjects were excluded from the analysis, two due to incomplete return of the scale. The results found no significant differences between the Groups [$F(1,23) = 0.23, p = 0.63$]. A significant main effect of Time was observed within groups [$F(3,69) = 4.872, p = 0.004$], but no Group x Time interaction occurred [$F(3,69) = 0.440, p = 0.725$]. Figure 6.11 shows that state anxiety scores decreased over time for both groups.

6.2.6 Psychological Characteristics – Figures



Figures 6.6: Averaged scores (with standard deviations) on psychological measures prior to supplementation (before) for both groups. MANOVA of z-scored data indicated no Group differences on the measures in figure 6.6 and 6.7 [$F(13,13) = 1.001, p = 0.499$]. Below, averaged scores for the BDI and Diary Mood Scale, for both groups, prior to supplementation. The BDI shows large standard deviations, which are within the *minimal* score range, indicating that although the subjects scores fluctuated none could be considered depressed.



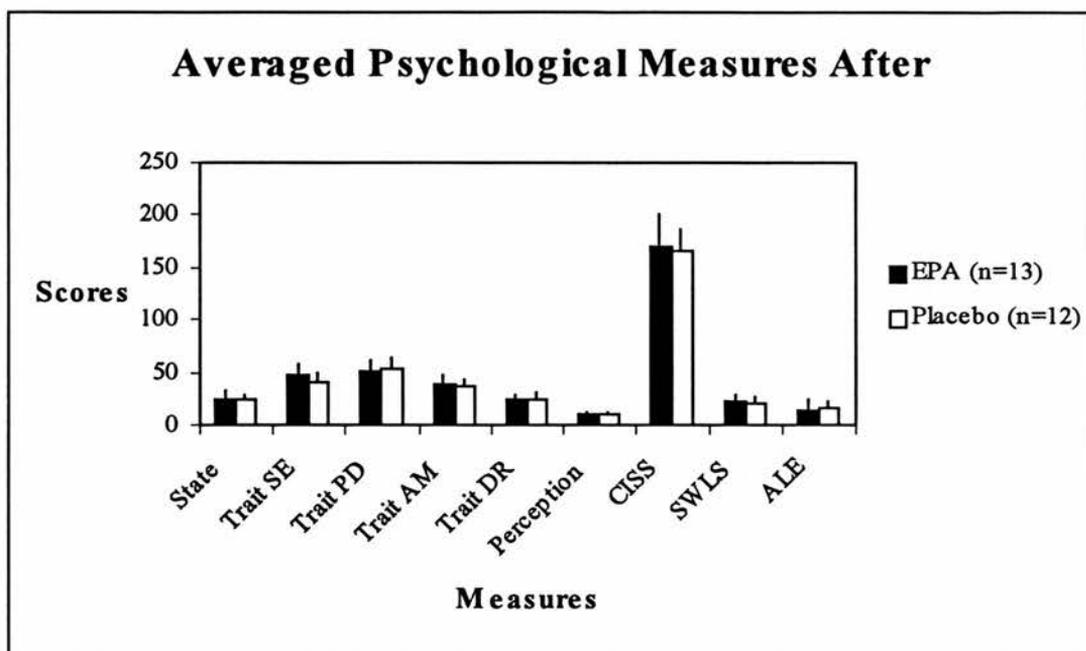
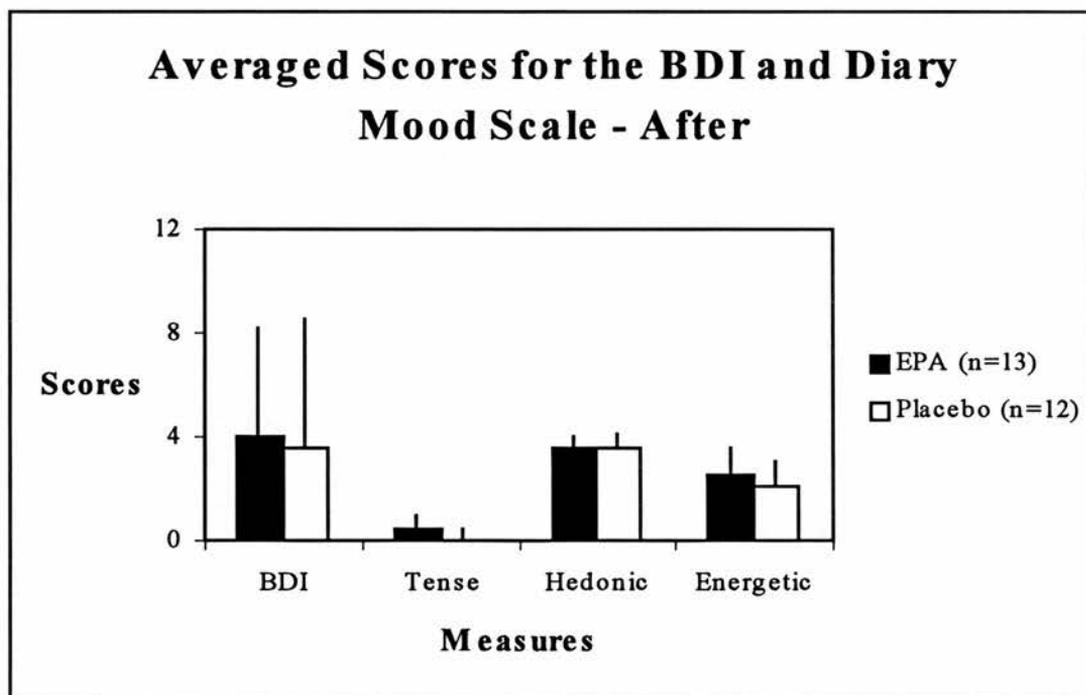
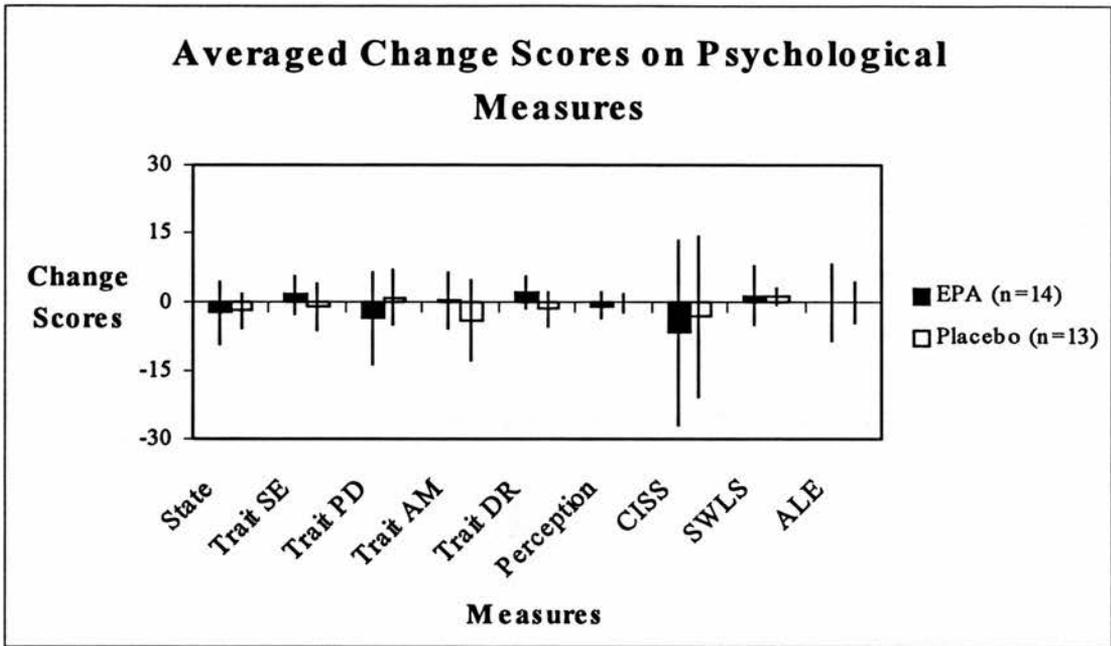
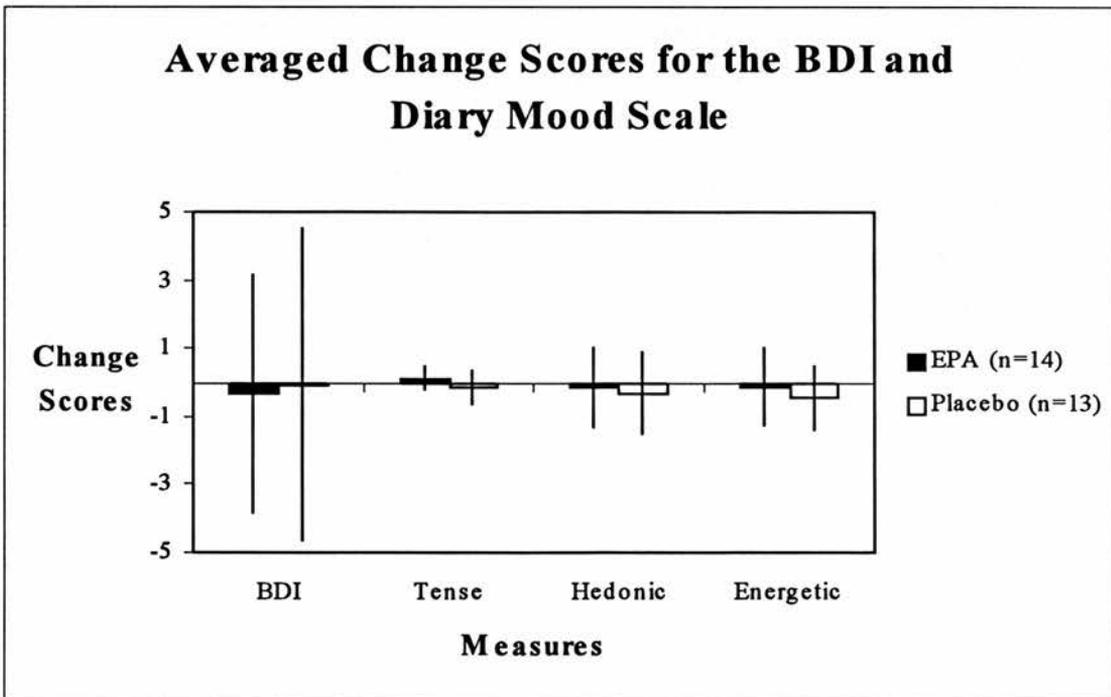


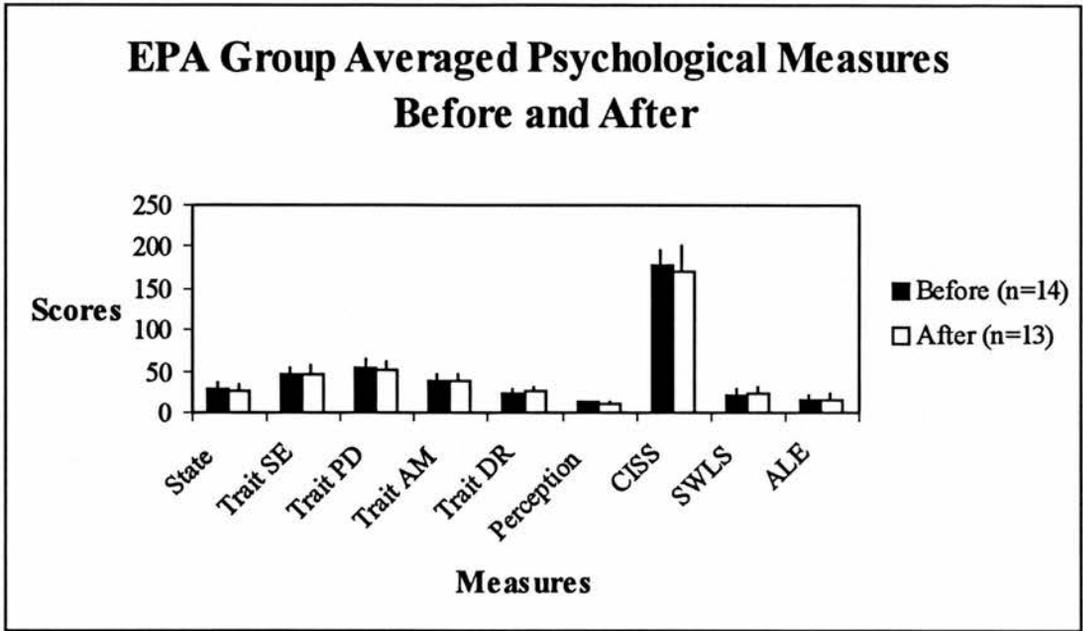
Figure 6.7: Averaged scores (with standard deviations) on psychological measures after supplementation for both groups. MANOVA of z-scored data indicated no Group differences on the measures in figure 6.7 [$F(13,11) = 0.818, p = 0.639$]. Below, averaged scores for the BDI and Diary Mood Scale, for both groups, after supplementation.



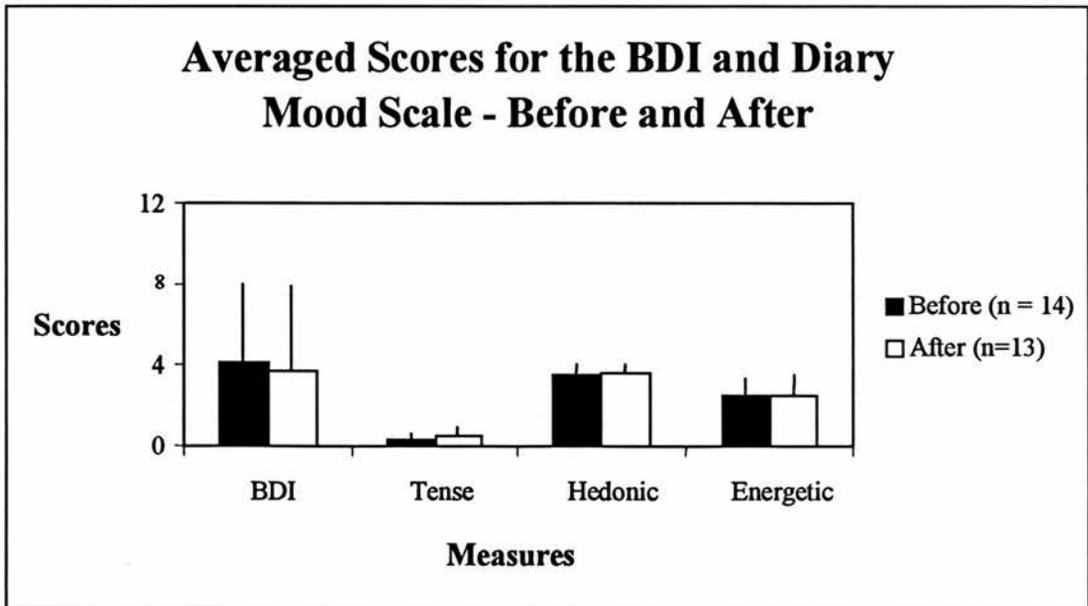


Figures 6.8: Averaged change in scores (with standard deviations) on the psychological measures, for both groups. MANOVA revealed no significant Group differences for any of the measures above and below, $[F(13,13) = 1.113, p = 0.425]$. Again the standard deviation shows the insensitivity in the ‘minimal’ range of the BDI.





Figures 6.9: Averaged scores on psychological measures, before and after supplementation, for the **EPA group** (above and below). MANOVA found no significant differences within the Groups. Refer to figure 6.7.



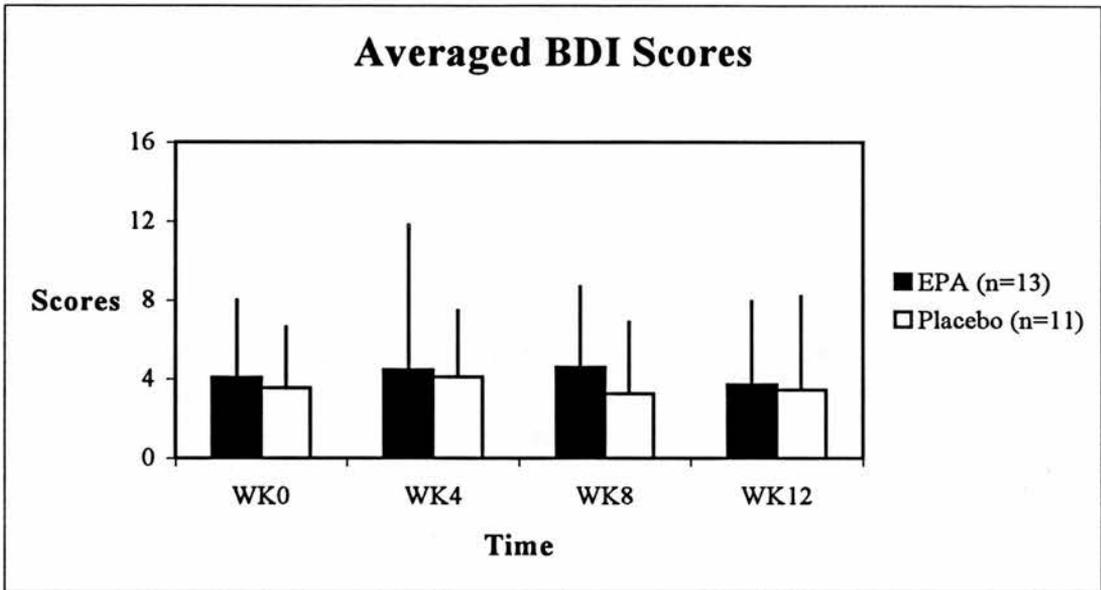


Figure 6.10: Averaged BDI Scores, per group, over the 12-week period. Repeated-measures ANOVA indicated no differences between Groups [$F(1,22) = 0.002, p = 0.962$], and no effect of time [$F(1.889,41.561) = 0.339, p = 0.702$] or Group x Time interaction [$F(1.889,41.561) = 0.223, p = 0.789$].

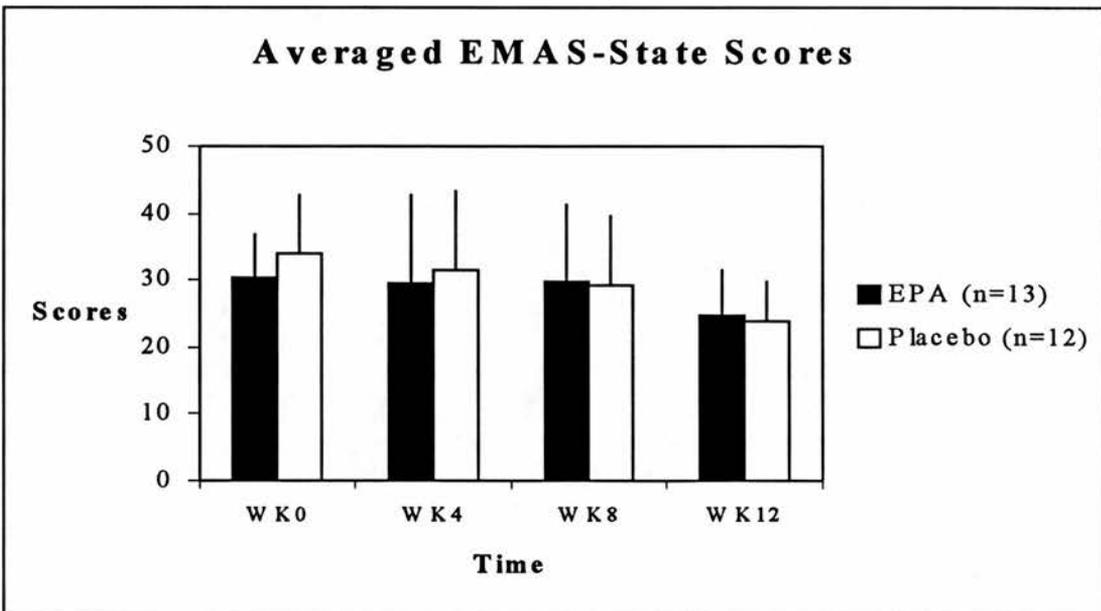


Figure 6.11: Averaged state anxiety scores, per group, over the 12-week period. Repeated-measures ANOVA indicated no differences between the Groups over time [$F(1,23) = 0.23, p = 0.636$]. A main effect of Time was observed within-groups [$F(3,69) = 4.873, p = 0.004$] but no Group x Time interaction occurred [$F(3,69) = 0.440, p = 0.725$].

7 Cognitive Task Performances - Results

The Raven's matrices and reaction time tasks were used to elicit a stress response in the subjects, while the acoustic startle task was used to probe for any cardiovascular changes in response to an unanticipated event. The cardiovascular responses to each task were described in chapter 5. The purpose here is to investigate whether or not performance varied between groups or over time.

7.1 Raven's Matrices

Subject responses were scored from a maximum of 48 correct. Their scores were then converted to 'percentage correct' for before and after supplementation. A repeated-measures ANOVA was then performed, between Groups with Time as the within group factor. Graphs can be seen in section 7.4.

7.1.1 Subject Exclusions and Descriptive Statistics

Two subjects were excluded from analysis. The subject on medication and an EPA subject whose time two performance was not saved due to a computer malfunction. Table 7.1 shows the average group scores, converted into percentage correct figures.

	EPA (n=13)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
% Correct	83.65	4.57	85.42	4.74	83.49	5.34	85.90	3.72

Table 7.1: Group percentage correct scores (and standard deviations) on Raven's Matrices task for before and after supplementation

7.1.2 ANOVA of Percentage Correct

As expected from the inspection of the means, which differ by less than 1%, the ANOVA results showed no significant differences between Groups on task performance [$F(1,24) = 0.011, p = 0.917$]. A significant main effect of Time was noted [$F(1,24) = 4.477, p = 0.045$] but no Group x Time interaction occurred [$F(1,24) = 0.106, p = 0.748$]. This result indicates that both groups performance improved slightly on the second test session, suggesting an effect of practice on the task (Figure 7.1).

7.2 Choice Reaction Time Task

For each subject the number of correct responses was recorded and converted into percentage correct scores. Additionally, for every correct response the subjects' reaction times were recorded and averaged for the before- and after-supplementation testing sessions. Repeated-measures ANOVAs were performed to investigate for any differences in performance between groups and over time.

7.2.1 Subject Exclusions and Descriptive Statistics

Only the subject on antidepressant medication was excluded. Table 7.2 below shows the group averages of percentage correct and averaged reaction times.

	EFA (n=14)				Placebo (n=13)			
	Before		After		Before		After	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
% Correct	44.73	12.35	55.03	16.92	52.28	15.50	59.10	18.60
RT (msec)	370.8	67.14	432.2	58.22	412.6	56.79	442.6	56.35

Table 7.2: Averaged (and standard deviations) group percentage correct and reaction time performance on choice reaction time task, before and after supplementation. Reaction time is shown in milliseconds.

7.2.2 ANOVA of Percentage Correct

A mixed ANOVA, between Groups with Time as within factor was performed. Results indicated a significant main effect of Time [$F(1,25) = 9.108, p = 0.006$] (see Figure 7.2), but no significant difference was found between the Groups [$F(1,25) = 1.136, p = 0.297$] or a Group x Time interaction [$F(1,25) = 0.377, p = 0.545$]. The results indicate that accuracy improved over time for both the groups, suggesting an effect of practice.

7.2.3 ANOVA of Reaction Time

A mixed ANOVA was performed with Group and Time as factors. Results indicated a significant main effect of Time [$F(1,25) = 17.133, p < 0.001$] but no significant difference was found between the Groups [$F(1,25) = 1.659, p = 0.209$]. No significant Group x Time interaction was found [$F(1,25) = 2.018, p = 0.168$]. See figure 7.3 for graph. The results indicate that both the groups' reaction times increased over time. This may suggest a speed accuracy trade off, where the subjects performed slower in an attempt to be more accurate.

7.3 *Acoustic Startle*

For every correct response to a loud or soft tone each subjects' reaction time was recorded. Each subject's reaction times were averaged according to *Tone Type* – loud or soft. An ANOVA was performed to investigate for any differences in reaction time performance between the groups.

7.3.1 Subject Exclusions and Descriptive Statistics

Only the subject on anti-depressant medication was excluded from the analysis. Table 7.3 below shows the group averages of reaction times to both the Tone Types.

	EPA (n=14)				Placebo (n=13)			
	Loud		Soft		Loud		Soft	
	Ave	SD	Ave	SD	Ave	SD	Ave	SD
RT	284.85	98.20	237.59	54.68	324.35	173.09	239.74	103.22

Table 7.3: Averaged group reaction times and standard deviation on acoustic startle task, for both Tone Types. Reaction time is shown in milliseconds.

7.3.2 ANOVA of Reaction Time

A mixed ANOVA was performed with Group and Tone Type as factors. Results indicated a significant main effect of Tone Type [$F(1,25) = 12.729, p < 0.001$]. Table 7.3 indicates that subjects were slower to respond to the loud tone. This result, in support of the startle cardiovascular data, suggests that the manipulation of loud and soft tones was successful. No significant Group x Tone Type interaction was found [$F(1,25) = 1.022, p = 0.322$]. Results found no significant differences between the Groups [$F(1,25) = 0.272, p = 0.606$]. See figure 7.4 for reaction time graph.

7.4 Cognitive Task Performance – Figures

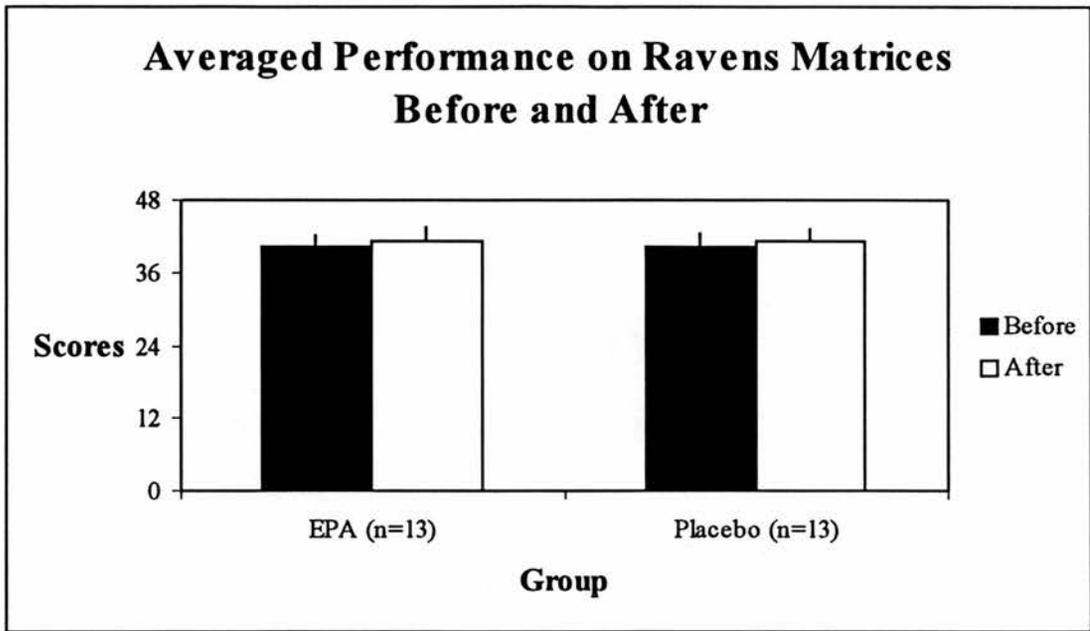


Figure 7.1: Averaged scores (maximum of 48, 15% chance) on Ravens Matrices task, for both groups, before and after supplementation. Repeated-measures ANOVA showed no significant differences between Groups [$F(1,24) = 0.011, p = 0.917$]. A main effect of Time was found [$F(1,24) = 4.477, p = 0.045$] but no Group x Time interaction occurred [$F(1,24) = 0.106, p = 0.748$].

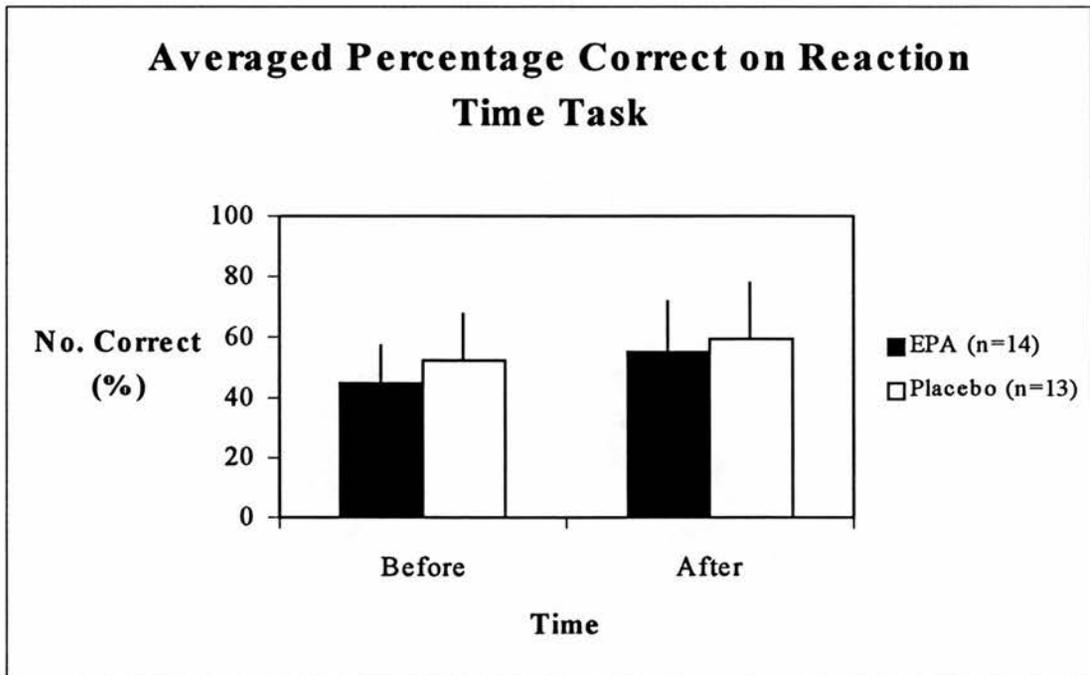


Figure 7.2: Averaged percentages correct scores. ANOVA indicated no significant differences between Groups [$F(1,25) = 1.136, p = 0.297$]. Again an effect of Time was found [$F(1,25) = 9.108, p = 0.006$]. No Group x Time interaction [$F(1,25) = 0.377, p = 0.545$] was found.

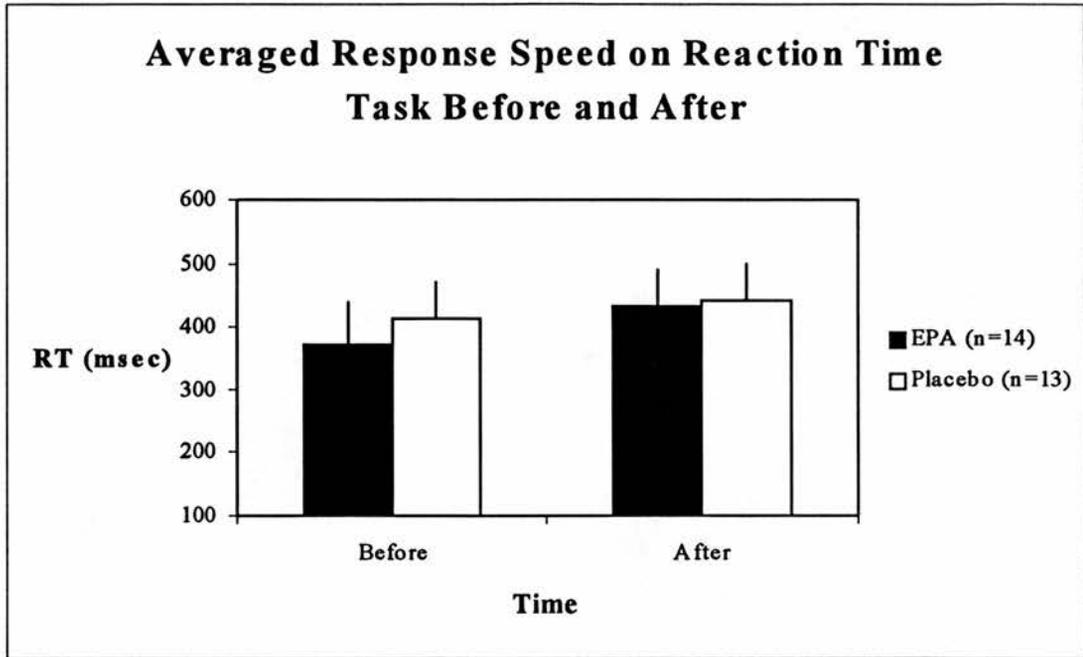


Figure 7.3: Averaged Reaction Times for both groups before and after supplementation. Repeated-measures ANOVA revealed no significant Group differences [$F(1,25) = 1.659, p = 0.209$]. However a main effect of Time was noted [$F(1,25) = 17.133, p < 0.001$]. No significant Group x Time interaction was found [$F(1,25) = 2.018, p = 0.168$].

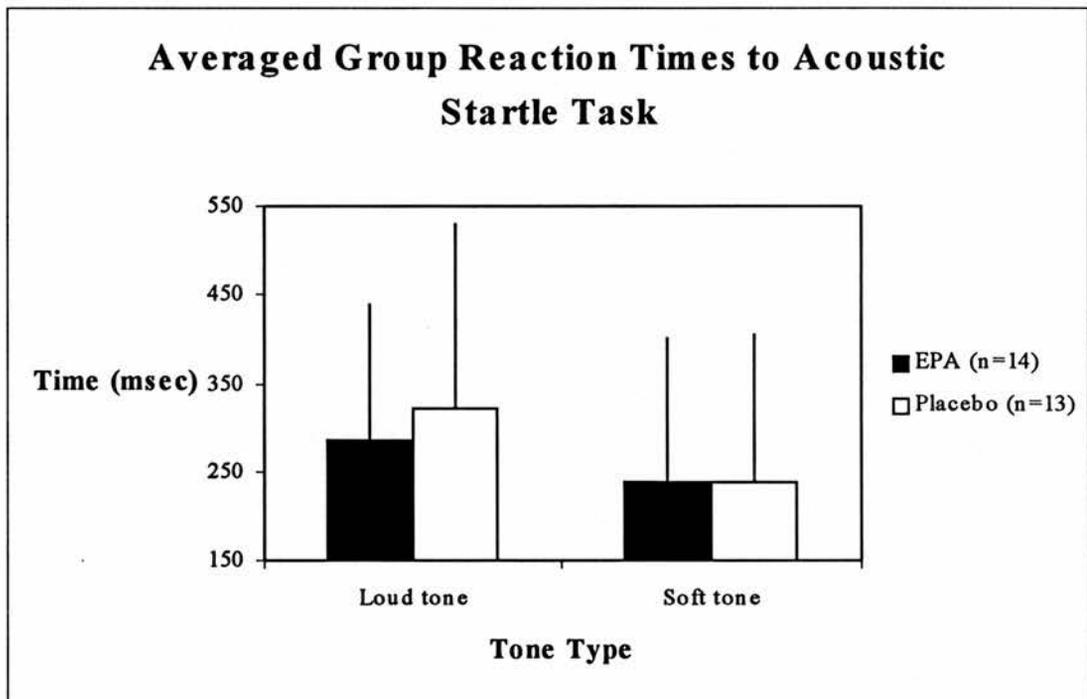


Figure 7.4: Averaged group reaction times for soft and loud tones on startle task. ANOVA results revealed a significant main effect of Tone Type [$F(1,25) = 12.729, p < 0.001$], but no significant differences between Groups [$F(1,25) = 0.272, p = 0.606$]. No significant Group x Tone Type interaction was found [$F(1,25) = 1.022, p = 0.322$].

8 Discussion

The most obvious findings from this research have been the apparent lack of effect of ethyl-EPA administration on the psychological, cardiovascular and performance measures. Since the results were negative, it is important to examine the experimental design for weaknesses that might have reduced statistical power. The sections that follow examine the potential causes of low power in the study. They lead to the conclusion that the lack of effects of ethyl-EPA administration in the subject population is unlikely to be due to flaws in the design or conduct of the experiment or due to insufficient numbers of subjects. The final section will discuss the relationship between this study, in which the subjects were young, healthy males, to previous studies of the effects of EFA administration in clinical populations.

8.1 Biochemical Efficacy of ethyl-EPA Administration

8.1.1 A Robust Change in Concentration Levels

It is known that the blood-brain barrier is more resistant to red blood cell changes in fatty acid levels than other areas of the body, such as the liver, and therefore some biochemists believe that phosphoglyceride levels should be used to gather fatty acid information (Ballabriga, 1994). This fact in itself does not detract from the use of red blood cell information, instead, red blood cell analysis provides a more conservative measure with adequate accuracy and detail for our purposes. The use of red blood cell concentration levels is a recognised and acceptable standard procedure of analysis. The more complex analysis of lipid levels does not provide any greater accuracy and is only thought necessary to employ when the red blood cells have been vulnerable to contamination, that makes red cell analysis problematical (Manku et al., 1983). Storage of the red blood cell samples at -70°C negated any problems of contamination, prior to analysis. I am confident that the analysis used was appropriate. The biochemical analysis of the red blood cell EFA contents and the subsequent ANOVA informed us

that there was a large change in fatty acid levels with supplementation. The EPA group showed a significant increase in EPA concentrations, with their natural levels increasing by more than a factor of two, while the placebo group levels remained relatively unchanged. I know for certain that one EPA subject failed to take over a third of his capsules, however with no formal returned pill count it is not possible to say how compliant the remaining subjects were regarding their daily consumption of the capsules. Therefore it is not possible to determine whether the change seen in RBC levels was the maximum that could be expected of an entire course of capsules. Yet despite this lack of certainty of capsule compliance by other subjects, the change in EPA levels was statistically significant indicating a robust effect.

However, it should be noted that although the EPA groups' RBC levels did alter, there is no way of knowing how or if the placebo influenced either the psychological or physiological findings. The placebo, vitamin E, is not completely inert and is known to benefit hair and skin conditions. However vitamin E is not known to have any similar properties to EPA. Therefore it is unlikely that vitamin E masked any effect of the EPA.

The biochemical data was further investigated for any influence which EPA supplementation may have on the other fatty acid components in our red blood cells. From examining each groups percentage change scores, (change from before to after supplementation) for all the fatty acids, it was clear that levels after supplementation were not static, and that some form of modification was occurring amongst the other fatty acid levels.

8.1.2 Active Regulation of Fatty Acids

To investigate if this 'modification' was a factor of interrelated components the data reduction technique of principal components factor analysis was employed to identify if any groups of fatty acids existed. Taking into account the small number of subjects in each group the data from both groups was combined at each time point to increase the reliability of the analysis. The data was standardised so that one acid did not have a greater influence than any other acid on the results. I found two dominant factors, which accounted for 52.4% of the variance in fatty acid component levels. The first factor

accounted for 40.3% of the variance and showed all positive weightings, indicating its relationship to the total amount of fatty acid in the blood. The second factor accounted for 12.1% of the variance but showed the more interesting result. EPA was highly and positively weighted in factor 2 along with all the other n-3 acids, whereas the n-6 acids were weighted negatively. This confirms that EPA supplementation does not only increase the blood concentration of the compound itself, but also has a complex impact on other fatty acids. In further support of this evidence a mixed factorial ANOVA found no Group x Time interaction in fatty acid levels. This suggests that no over all increase in fatty acid volume occurred with supplementation, indicating that some form of active regulation of fatty acids occurs whereby an elevation in one produces a reduction in others.

8.1.3 Continued Effects

Furthermore it is known from previous studies (Brown et al., 1991, Marangoni et al., 1993) that the efficacy of essential fatty acid supplementation is long lasting, due to a long half-life. Brown et al. provided twelve healthy young men with fish oil supplemented diets. They found that even six and twelve weeks after the fish oil supplemented diet ended, EPA and DHA levels were still higher than initial baseline levels. It was only after eighteen weeks that EPA had returned to baseline whereas DHA still had not.

8.1.4 Summary

A robust change was found in essential fatty acid levels with supplementation of 2 grams daily of ethyl-EPA, in healthy young men. The red blood cell level of EPA dramatically increased, as did the other omega-3 oils, however, the omega-6 oils decreased. Overall fatty acid levels remained static indicating a regulation between the omega types, while the previously determined half-life suggests that these effects of supplementation may be long lasting.

8.2 *Potential Sources of Variation that might Mask Effects*

Both physical and behavioural individual differences could mask the effects of ethyl-EPA administration if there were pre-existing differences between groups. The subject characteristic measures of age, height, weight, exercise level and dietary habits are factors known to influence cardiovascular health and functioning (Hotz, 1995, Block, Rosenberger, & Patterson, 1988, Lovallo, 1997). MANOVA results from the z-scored data found no significant differences between the groups either before or after supplementation. This demonstrates that the random allocation of subjects to groups was successful in avoiding bias, and therefore the subject characteristics were not acting as confounding variables. The finding that no differences existed after supplementation suggests that EPA had no effect on level of exercise, weight or dietary habits in this study.

Before discussing the psychological or cardiovascular measures it is worth mentioning that every subject was habituated to the laboratory environment, at the interview stage prior to beginning the study. This habituation / familiarisation to their surroundings and test equipment, produced the benefit of experience. In other words it provided the benefit that during test session One (before) none of the subjects should have been reacting primarily to their environment, which can act to mask their normal cardiovascular and psychological states. However, for future research, it may be more effective to habituate the subjects by attaching the apparatus to them to prevent any anticipation / concern occurring as a response to this procedure.

8.3 *Sensitivity of Psychological Measures*

8.3.1 Strength and Reliability of Experimental Manipulation

The psychological characteristics were measured to determine whether or not EPA supplementation had an effect on them, and therefore whether or not they could be possible moderators of cardiovascular functioning. Mood, anxiety, situation perception, and coping styles in stressful situations were measured before and after EPA

supplementation. A MANOVA of the time one psychological characteristics data was performed to investigate if there were any pre-existing differences between the two groups which might mask an effect of EPA. Again no differences were found indicating that group randomisation was appropriate and unlikely to mask an effect of supplementation. The MANOVA of the time two psychological characteristics again found no significant differences between the two groups after supplementation. This informs us that both groups reported similar psychological characteristics at each time point, confirming validity of the measures selected. However, it does not actually inform us if there was a change in both groups over time or if the scales were reliable. From the subject change scores their response levels were found to be unchanged throughout the entire 12-week study period. This, in addition to the small standard deviations noted, supports the test-retest reliability of the measures used.

The only measure of any concern was the BDI. It should be noted that the subjects' scores were within the BDI range of 'minimal', showing that the existing level of depressive feeling was already extremely low. Furthermore, the scores were so low that the standard deviations were of similar size to the actual scores. The lack of any effect of time or supplementation may be due to the originally intended use of the scale, which was not to grade healthy non-depressed individuals. Furthermore, the lack of effect relates to Gerin et al. (1996) and Higgins and Endler's (1995) findings that feelings of distress are increased with emotion-oriented coping, not task-oriented. The subjects here were only able to use task-oriented coping while in the laboratory therefore this restriction may have influenced the lack of effect on their BDI scores.

8.3.2 Possible Effects of EPA on Psychological Characteristics

To investigate if there was an actual change in both groups with EPA supplementation another MANOVA was performed on the change scores (differences in scores from before and after supplementation). No significant differences were found. These negative results confirm that not only did the two groups not differ in psychological characteristics, but that supplementation with ethyl-EPA failed to produce any effects on psychological characteristics.

Depression and state-anxiety measures were administered every 4-weeks throughout the study period to investigate for any changes in subjective well being during the period of supplementation. ANOVA of the Beck Depression Inventory scores showed no differences between the groups and no effect of time. This result suggests that the groups did not differ on their level of depression and that EPA supplementation did not have any effect. Although as stated above this lack of change may be a result of the type of scale used. However taking into consideration the overwhelming lack of effects seen in the rest of the results it is unlikely that an effect of EPA on these subjects' depression levels has been masked.

Endler's EMAS-S was completed at the same times as the BDI. ANOVA of the data showed neither a significant difference between the groups scores on state-anxiety nor and interaction of time by group with EPA supplementation. However, an effect of time was noted. The data show a significant drop in state-anxiety over time for both groups. This result may be an effect of habituation to the test procedure where, as time progressed the subjects became more relaxed with completing the EMAS-S.

Two points need to be noted here. No measure of significant life events was taken during the research. Therefore it may be that stressful life events occurring during the study, such as exam periods, bereavement, masked a possible effect of EPA. Furthermore no check was made for subject representativeness, especially on responses to the psychological measures. Ideally, since the Endler scales were only validated in the U.S.A and Canada, and no tables of U.K. data exist, the subjects' scores on these measures should have been compared to a group of non study subject responses to test for population representation. However, having noted these two points it is unlikely that any significant life events would have masked an effect of EPA to the extent of the negative results seen here. Furthermore, there is no question of the validity of Endler's scales when used in the U.K., although cultural differences suggest that a different response pattern may be found in U.K. respondents, the cultural similarities dictate that there should be no qualitative differences.

8.3.3 Considering the Sample and Population

I would like to pause for a moment and remain with the topic of sample representation. There is strong evidence that suggests students, as a group, are prone to high stress lifestyles. Looking at the normative data provided by Endler et al. (1991) and Spielberger et al. (1983) for their anxiety scales college students persistently score higher on state and trait anxiety than the adult population. Furthermore a myriad of experimental studies exist, that indicate that college, medical and university students suffer from stress (Humphrey & McCarthy, 1998, Guthrie, Black, & Shaw, 1997, Hamilton & Fagot, 1988, Bower, 1994, Fisher, 1993, Malathi & Damodaran, 1999, Uden, Krakau, Hogbom, & Romanus-Egerborg, 1995, Hudson & O'Regan, 1994, Dill & Henley, 1998, Jones & Johnston, 1997). The experience of university has even been compared to that of joining the army (Wintre & Ben-Knaz, 2000) and is considered akin to the stress suffered in the police force (Lennings, 1995). Students are subject to high performance pressures, continual deadlines and report feelings of stress accompanied by isolation and loneliness (Ponzetti, 1990), feelings of being academic impostors' (Henning, Ey, & Shaw, 1998), and problems of sleep disturbance (Hicks, Mistry, Lucero, Lee, & Pellegrini, 1989).

The constant high stress environment has lead student groups to show greater prevalence of anxiety, depression (Ockene, Ockene, Barrett, Ma, & Hebert, 1997, Camatta & Nagoshi, 1995, Wong & Whitaker, 1994, Johnson, Johnson, & Petzel, 1992), and burnout (Talbot, 2000, Chang, Rand, & Strunk, 2000, Goh, Cameron, & Mark, 1999, Guthrie, Black, & Bagalkote, 1998) as well as thoughts and acts of suicide (Tyssen, Vaglum, Gronvold, & Ekeberg, 2001, Furr, McConnell, Westefeld, & Jenkins, 2001, Westefeld, Whitchard, & Range, 1990, Hamilton & Schweitzer, 2000). One study of 737 university students reported that 43% had considered suicide with 20.4% attempting to act upon those thoughts (Rudd, 1989).

Although it would appear pertinent to study the effects of EFAs on stressed students the selection of this population was a result of the availability of young healthy males, and was not intentionally to study a high stress sample at this point in time. Rather the aim was to submit normal healthy adults to laboratory stressors and study the resulting

physiological and psychological arousal. I believe this was achieved. Despite the persuasive evidence for high anxiety, stress and depression in student populations the sample presented here may not be representative of this picture of students. The subjects displayed low levels of trait and state anxiety (when compared to the normative data from U.S students) and reported minimal levels of tension during ambulation. These low levels may in part be due to the fact that the subjects were volunteers who felt able to afford the time commitment and were therefore self selecting. A potential subject with typical student anxiety and stress may not have felt able to volunteer.

In future studies, requiring long-term commitment, the population choice should be considered in depth. Due to availability restrictions I was unable to consider using any other population than students. However, this has produced a dilemma that I cannot be sure whether my subjects were representative of students, or if they were more representative of the general adult population.

Although I have discussed this issue in depth and feel that it is important for future consideration I do not feel that its impact on this study goes beyond the common question of sample representation. The subjects were randomly allocated to their groups and neither group presented any pre-existing differences in their psychological characteristics, indicating any possible effect of EFA was not masked by subject differences.

8.3.4 Summary

The study results indicate that the experimental manipulation was appropriate. Although there was no measure of how well they represent the normal population, the subjects did not vary significantly from one another on their psychological characteristics before supplementation (or after) providing evidence of satisfactory group randomisation. Furthermore, the highly stable scores on each measure verify the reliability of the measures, underlining their usage as appropriate. The validity and reliability of the psychological measures used underpins the non-significant effect of EPA supplementation in this subject group.

8.4 *Sensitivity of Cognitive Measures*

Subject performance on the laboratory tasks was measured to ensure that any cardiovascular differences found between the groups during the tasks, before or after supplementation, was not in fact due to differences in task performance between the groups.

8.4.1 Strength and Reliability of Cognitive Tasks

For performance on the Raven's Matrices, scores were calculated as percentage correct and a repeated-measures ANOVA performed. Subjects performed at an average level of 83-86% correct, with small standard deviations and no floor or ceiling effects. This level of performance denotes that the difficulty of the task was suitable for the student population. A main effect of time was noted, indicating that both groups improved their performance at the second test session, which suggests that there may have been an effect of practice or memory second time round. The improvement in performance, although significant was not large (around 2%) attesting to the appropriate level of difficulty incorporated in the Raven's matrices task and the tasks reliability. The task increased heart rate by 3-6 bpm and systolic blood pressure by 8-20 mm Hg. Although these changes are not as large as Glass (1977) and Dembroski (1981) found in response to video games, they denote an alteration of cardiac function during the task that is not influenced by metabolic factors (e.g. activity, posture, caffeine).

Performance on the Choice Reaction Time task was measured as percentage correct and reaction time to every stimulus that was correctly responded to. The performance scores and reaction times were similar for each group and showed no floor or ceiling effects. ANOVA of the percentage correct scores revealed a significant effect of time but no significant differences between groups. The significant effect of time indicated that both groups performance improved at the second test session suggesting an effect of practice on the reaction time task. The ANOVA of reaction time again found an effect of time but no significant differences between groups. The data shows that reaction time increased for both groups at the second test session. These results may suggest a speed accuracy trade off, where having previously experienced the task, subjects performed

more slowly in order to be more accurate. Again, the task performance levels were well below ceiling at both time points and changes in reaction time and performance correct, although significant, were small and similar for both groups. The choice reaction time task showed changes in heart rate of 6-9 bpm and systolic blood pressure changes of 13.7-28.0 mm Hg indicating that the task successfully elicited a cardiovascular stress response.

These apparent effects of practise and prior experience denote a need to ensure that the task is as stressful the second time round as it was the first. This would require a validation study of task difficulty, for example shorter response times, quicker presentation of stimuli and more complex stimuli.

The final cognitive task was the acoustic startle paradigm which was administered after supplementation only, to prevent prior knowledge and anticipation of the task masking any effects of EPA. For each correct response the subject's reaction time was recorded, and averaged according to tone type (loud or soft). The ANOVA found a main effect of tone type. The data show that the loud tones resulted in slower responses from the subjects than for the soft tones. Considering the loud tones were easier to detect than the soft tones, yet elicited longer reaction times, in all subjects, it is acceptable to argue that the loud tones were psychologically disruptive. From these results the acoustic startle manipulation can be regarded as successful.

8.4.2 Possible Effects of EPA on Task Performance

The ANOVA results from the Raven's matrices showed no significant differences between the groups' performance nor did they reveal a group by time interaction with EPA supplementation. Although the subjects' performance improved with time it was not a factor of EPA supplementation. The choice reaction time percentage correct results indicated no time by group interactions with supplementation. Furthermore the subjects reaction time performances also indicated no group by time interaction (RT). It can be concluded that the subjects' performance on the choice reaction time task showed no effects of EPA supplementation. ANOVA of the subjects' reaction times evoked by the acoustic startle task found no significant differences between groups and

no interaction of group and tone type. Again, as with the other cognitive tasks, no effect of EPA supplementation was seen in subjects' reaction times.

8.4.3 Summary

The data show that the three cognitive tasks were successful and appropriate manipulations of subject performance. Although performance on the Raven's matrices improved, and the choice reaction time task showed a speed accuracy trade off, when tested for a second time, it was seen in both groups and hence was not an effect of EPA supplementation.

8.5 *Cardiovascular Measures*

8.5.1 Laboratory Measures Reliability and Possible EPA Effects

The physiological measurements employed produced highly valid and reliable data. For each subject the number of physiological data points ranged from 4200 to 5600 for 60-80 minutes recording at time one, and 5000 to 7500 for 70-85 minutes recording at time two. With such a large number of data points per subject I am confident that I was estimating very close to their true cardiac function. Therefore there was little noise in the data arising from poor estimates of each subject's cardiovascular data for each epoch. Hence most of the experimental error must therefore come from individual differences. Furthermore, the Spike script created for the purpose of artifact rejection and data extraction was successful, appropriately and reliably interpreting real and invalid data, according to the stipulated exclusion criteria.

Repeated-measures ANOVAs were performed to investigate for any changes, during the laboratory recording, in cardiovascular functioning over time and between groups. The averaged heart rate, heart rate reactivity, heart rate variability, systolic and diastolic blood pressures and blood pressure reactivity was calculated for each task epoch for every subject both before and after supplementation. A main effect of time was found

for both systolic and diastolic blood pressure reactivity. The reactivity data does show that after supplementation both groups' SBP reactivity and DBP reactivity was lower. This result is independent of supplementation and may indicate that the subjects found, on the whole, the laboratory session to be a less stressful experience the second time due to having previous knowledge of what to expect. The results indicated no significant differences between the groups on any of the cardiovascular variables. Taking heart rate as an example from the descriptive statistics it can be seen that the percentage differences between the groups, both before and after supplementation, is very small. For the Raven's matrices the difference in the group heart rates is 0.6% before and 1.8% after supplementation. For the choice reaction time task the difference in the group heart rates is 0.19% before and 0.07% after supplementation. For the seated epoch the difference in the group heart rates is 0.2% before and 3.7% after supplementation. For the cycling epoch the difference in the group heart rates is 8.8% before and 3.8% after supplementation. For the recovery epoch the difference in the group heart rates is 8.2% before and 1.2% after supplementation. Heart rate, reactivity and variability as well as systolic and diastolic blood pressure and the corresponding reactivity all showed a significant main effect of epoch. This result was to be expected considering the task epochs required different behaviours such as relaxing, mental workload and physical exertion. For example heart rate reactivity during the Raven's matrices ranged from 4.5% to 22.07 % for the two groups. For the choice reaction time task heart rate reactivity ranged from 8.1% to 12.5%. For the cycling epoch heart rate reactivity ranged from 43% to 48.6%. The strong effect of epoch also confirms that the manipulations were successful in eliciting cardiovascular responses. With such large changes observed in response to the epoch manipulations it is highly unlikely that I missed an effect of EPA supplementation.

Cardiovascular functioning during the acoustic startle task was examined separately from the other epochs. Due to the nature of the startle paradigm it was inappropriate to average the startle task data like the other task epochs. Instead the data was grouped by tone type and tone time. ANOVA results showed no significant group differences for heart rate, systolic or diastolic blood pressure. Results did reveal a main effect of pre-versus post-tone values for heart rate and systolic blood pressure, indicating a manipulation effect. A tone time x tone type interaction was found for both heart rate and systolic blood pressure. No significant differences were found in DBP. These

results suggest EPA supplementation had no effect on the acoustic startle task. However, the task produced cardiovascular alterations (effect of Tone Time and Tone Type, in HR and SBP) that support the decrement in reaction time performance. It can be seen that heart rate slowed after the tones, indicating an alteration in variability while systolic blood pressure increased after each tone indicating reactivity to the stimuli.

8.5.2 Ambulatory Measures Reliability and Possible EPA Effects

Each subject had a few missing data points from their days ambulatory recordings, where the maximum was seventeen. This data drop can only be expected during ambulatory monitoring. While the subject wears the blood pressure monitor and attends to their daily activities the experimenter has a lack of technical control, unparalleled in laboratory studies, and is unable to ensure a suitable environment while the recording is occurring. The data sets of two subjects' were lost, due to an intermittent memory and retrieval problem with the blood pressure monitor. For the remaining 25 subjects I used averages of their ambulatory cardiovascular data, to negate the effects of the occasional missing data point. It should be noted that since I was compelled to average the ambulatory physiological data that I also did the same with the diary mood scales, permitting them to be included in the psychological measures analysis, rather than a lengthy set of t-tests.

Returning to the physiological data, repeated-measures ANOVAs were used to investigate for any changes in ambulatory cardiovascular functioning over time and between groups. No significant main effects, interactions or differences between groups were found for heart rate, heart rate variability, heart rate reactivity, systolic and diastolic blood pressure or systolic and diastolic blood pressure reactivity. These results strongly suggest that EPA supplementation had no effect on ambulatory cardiovascular functioning.

8.5.3 Laboratory vs. Ambulatory Cardiovascular Measures

The laboratory and ambulatory physiological data were compared to each other to investigate for any differences between the two recording strategies and also to examine whether or not this relationship altered after EPA supplementation. The average of all the laboratory epochs was calculated and used to represent the laboratory measures. The averaged epoch data was considered an acceptable comparison to the ambulatory data since the laboratory tasks had been selected in an attempt to mimic, within the laboratory, the range of normal daily activities. ANOVA results found no significant differences between groups' levels of heart rate, heart rate variability, heart rate reactivity and systolic and diastolic blood pressures and corresponding blood pressure reactivity. Results did indicate a significant main effect of location (laboratory vs. ambulatory) for all the cardiovascular measures, where for both groups of subjects both before and after supplementation their laboratory measures were higher than their ambulatory. These data parallel Van Ergen and Sparrow's (1989) findings that results from laboratory testing differed from their ambulatory results. The only effect of time on ambulation was found for systolic and diastolic blood pressure reactivity, where both reduced after supplementation, reflecting the results found for the laboratory data. They support the suggestion that for all subjects the experiences of both the ambulatory and laboratory recordings was less stressful second time. These results suggest that EPA supplementation had no effect on the cardiovascular measures whatever their location, but that recording location, irrespective of EPA levels, is a large factor in data gained. Cardiovascular levels in this study were found to be significantly higher during laboratory recording than ambulatory monitoring. This result may be due to many factors, such as differing recording equipment with different bodily recording positions, continuous data acquisition in comparison to interval recordings, large differences in experimenter control, differing subject behaviours and the strong task manipulations available within the laboratory.

Clearly there was no effect of EPA on the laboratory and ambulatory measures but it is still unclear as to whether or not physiological data gained in the laboratory can predict or reflect physiological functioning in day to day life. Separate linear regressions, were performed with the laboratory cardiovascular data as the independent variables

(predictors) and the ambulatory cardiovascular data as the dependent variables, with no distinction made between groups. The linear regression of the data before supplementation indicated that when our subjects were supplement-free at the beginning of testing there was a moderate to weak correlation for heart rate only. Heart rate variability and reactivity, systolic and diastolic blood pressure and reactivity showed no significant relationship between data recorded in the laboratory and that recorded during ambulation. For the time two (after) data again no distinction was made between the groups. Effects of EPA supplementation may have confounded the results, but this is doubtful considering the results to date. However, more likely is that habituation to the task epochs during the laboratory session was a confounding variable. Time two results revealed a weak to moderate relationship between laboratory heart rate and ambulatory heart rate. No other cardiovascular measures were found to significantly relate. These results suggest that heart rate data gathered during the laboratory session show a weak relationship to ambulatory heart rate data. However, the negative regression results suggest that in general, cardiovascular data gathered in the laboratory are unrelated to ambulatory cardiovascular functioning. These findings further support Van Ergen and Sparrow (1989) who suggested that data from laboratory measures were only weakly correlated to those recorded in day to day life. These results suggest a continued need to experiment both within the laboratory and during ambulation. One method alone cannot provide the whole picture.

8.5.4 Summary

The data gathered within the laboratory was highly reliable and remarkably stable. Significant effects were found for task epoch, which confirmed the validity of the tasks used in the study, but no effect of EPA supplementation was evident. The ambulatory monitor performed satisfactorily, with stable data throughout, confirming its reliability. However, again no effect of EPA was noted. Although the laboratory and ambulatory data both indicated the same non-significant result of EPA supplementation it was found that the laboratory data did not predict the ambulatory indicating a need to continue experimenting both within the laboratory and in real life surroundings.

8.6 Addressing the Research Aims

The first aim of this study was to investigate *if ω -3 supplementation would reduce physiological and psychological responses to stress in humans*. Laboratory and ambulatory measures of cardiovascular functioning and psychological mood, anxiety, situation perception, subjective well-being, depression and coping styles were recorded during a 12-week period of supplementation with ethyl-EPA (C20:5n-3) or a placebo.

The second aim was *to investigate if these physiological and psychological responses are mediated or moderated by alterations in general coping style*. A comprehensive measure of coping with stressful situations was administered to subjects before and after supplementation, along with several other psychological scales, which may have influenced both psychological and physiological responses.

8.7 Answering the Research Questions

The research questions were:

1. Is there an effect of omega-3 supplementation on the cardiovascular systems response to stress?

In this study the data clearly indicate that there was no effect of EPA on cardiovascular responses to stress. These negative results are independent of recording location and are not masked by subject characteristics or their psychological propensities. Task performance was not a masking factor either, although it improved with habituation it did not differ between the groups. Furthermore, many of the results show $F < 1$, indicating that even with an infinite number of subjects a positive result would be improbable.

2. Is there an effect of omega-3 supplementation on psychological response to stress?

There were no significant changes in the subjects' psychological responses to stress after supplementation and no differences between to two experimental groups. Subjects responses, whether within the laboratory or during ambulation showed no effect of EPA

supplementation, with F values of less than 1, again indicating that subject numbers was not a factor influencing the negative results.

3. Is there a dissociation in the effects of omega-3 between physical (physiological) and psychological (mood) stress responses?

No dissociation occurred, the data failed to show any effect of EPA in these healthy young subjects' physiological or psychological responses to stress. These negative results may have resulted from the healthy, stress free status of the participating students, together with their comfortable familiarity with the environment of university testing.

4. What is the correlation between the effects of omega-3 on laboratory stress responses and those evoked in more naturalistic environments?

Overall there was no significant correlation, the data failed to show any effect of EPA in these healthy young subjects' physiological or psychological responses to stress either in the laboratory or during day to day activities. The interactions of Group by Time by Location were non-significant, with several F values of less than one. However, cardiovascular functioning (albeit heart rate did show a very weak correlation), irrespective of experimental group, did differ between the laboratory and ambulatory recordings, with the laboratory producing higher readings and responses to stress, which were not predictive of ambulatory levels.

5. If the omega-3 has an effect, what mediates/moderates it? Does the omega-3 work via a psychological or physiological mechanism? If the omega-3 has a desired effect (reduction) on reactivity to stress can it be said that the effect is due to:

Physiological dampening of the cardiovascular system alone?

Or physiological dampening in conjunction with an improvement in mood felt?

Or a factor of both the above and explained / mediated by improved coping styles?

I am unable to satisfactorily answer this question due to the negative physiological and psychological results. It can be said that coping style, along with the other

psychological measures administered remained static, in line with the subjects physiological and psychological functioning.

8.8 Reflecting on the Study and the Results

There is strong evidence from studies reviewed in the earlier chapters that clinical populations benefit from omega-3 supplementation. Coronary heart disease patients show decreased rates of secondary MI and morbidity, along with lowered blood pressure and antiarrhythmic properties, while schizophrenic patients show reductions in positive and negative symptoms. Furthermore, learning, cognitive, behavioural and attention development disorders, cognitive decline and depression all show decreased symptoms with omega-3 supplementation. Given that there were no significant effects of EPA in this study several causal possibilities must be considered. I can say with confidence that any possible effects of EPA were not masked by pre-existing differences between the groups. The statistical power of the study was such that even with an infinite number of subjects finding an effect would be unlikely / improbable. The physiological and psychological dependent variables used in this study showed high test-retest reliability. These variables were also sensitive to experimental manipulation exhibiting strong validity, further excluding this as a reason for the nil effects. Furthermore, the manipulations used resulted in the expected and desired responses from the subjects. However the subjects were not checked for population representativeness, nor were they asked to report any life events during the study, which may have altered their responses. Subject compliance to capsule consumption may have been a factor, as a return capsule count was not conducted. However non-compliance is doubtful considering the large change seen in red blood cell fatty acid composition of the EPA group. A further possible cause of negative results is often the lack of adequate data points per subject. This reason could not possibly be attributed to this study. Each subject had between 4000 and 5000 data points during each laboratory session, up to 17 recordings from each 8-hour period of ambulation and multiple comprehensive psychological measures during each test session and at regular intervals within the 12-week supplementation period.

During the study period both the experimenter and subjects remained blind to the capsule contents avoiding any effect of experimenter bias or subject manipulation. Although I can be sure that EPA dramatically increased in the EPA group, producing a regulatory effect on the other EFAs, I cannot be sure that the actual biological impact of the EPA was large enough to produce an effect. Neither can I be sure that 12 weeks of supplementation was adequate to reveal maximal effects. It must be considered that the placebo group was administered with vitamin E. Vitamin E may have acted to mask an effect of EPA. However vitamin E is not known to have any biochemical properties similar to EFAs. Although the emphasis of the study investigated for any significant changes between the two groups cardiovascular, psychological or cognitive functioning, change scores from before and after supplementation were also calculated. In addition, when taking into consideration the highly stable results from before and after (supplementation) and group to group it is very unlikely that the supplementation of vitamin E detracted from any possible effects of EPA.

Furthermore the sample population available may have given us a ceiling effect in regard to good health. The subjects were all young, healthy, non-smoking males with relatively “stress-free” lifestyles (as their low diary T scores testify to). Their cardiovascular health and attitude to life may have precluded anything other than minimal reactivity, which would not have permitted the effects of any intervention to be seen. This leads me to another possibility that the subjects were already at peak on the optimal levels of EPA, and hence by increasing them further there was nothing to gain. Perhaps what is important for cardiovascular well being is not the elevation of EPA, but the damage caused by lack of EPA in poor diets.

8.9 *Improving the Study*

With hindsight there were two minor points I would have liked to improve: a proper ambulatory baseline period, without the experimenter present and using the ambulatory monitor; recording of each subjects’ mileage on the static cycle for comparison with their time two distance. This was not available to be measured in this study instead subject speed was monitored, but did not act as a satisfactory measure of performance.

With hindsight I would have wished to test for sample representation. This would have involved another, larger subject group completing the psychological measures, providing a pattern of responses typical of the population. Secondly, I should have counted the unconsumed capsules back in, to give an indication of subject compliance.

Learning from this study several improvements are necessary for future research into this area, including those just mentioned. The initial suggestion is that a 35-49 age group sample is used, avoiding the ceiling of health that our subjects appeared to possess. In this age range subjects would be at a time in their lives where cardiovascular irregularities begin to appear, and a benefit of EPA supplementation may be found. A further population group of interest would be those who are regularly subject to high levels of stress. Although the ambulatory recorder used here was valid and reliable, the use of a more continuous monitoring method, such as the new ambulatory Portapres, may identify more interesting periods of cardiovascular activity in day to day life, which a monitor programmed at regular intervals may well miss. A third proposition is that the study be designed in a more longitudinal manner, permitting a longer supplementation period, more frequent re-tests and investigation into the longer-term effects. A crossover design with a suitable washout period may also be more fruitful if time restraints do not permit the longitudinal approach. The final proposal is that besides administering EPA and a placebo that, individuals with naturally low levels of EPA be investigated as well. This would permit investigation into whether it is EPA supplementation, which is optimal, or the reversal of EPA depletion.

8.10 Conclusions

The take-home message is that this study categorically failed to show any beneficial effects of EPA supplementation on either the physiological or psychological responses to stress in young male adults, within the laboratory or during a period of real life ambulation. It cannot be said that EPA supplementation does not benefit cardiovascular reactivity and variability, or psychological responses, only that in this sample of young healthy males 2-grams of ethyl-EPA per day for 12-weeks showed no effects in comparison to a placebo group on 2-grams of vitamin E.

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10 Appendices

10.1 List of Appendices

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Letter of Invite

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Dear Postgraduate

I am a fellow postgraduate doing my PhD. in Psychology. I very much hope that you will volunteer to take part in an experiment I am conducting for my thesis. It is on the possible beneficial effects of certain classes of foods on the heart. You will be paid £50 for your participation and at the end all the subjects will be entered into a prize draw for £100.

The experiment will run for 12 weeks between September 1999 and June 2000. It requires that you visit the laboratory on two consecutive mornings at the beginning and end of the 12 weeks. On the first day you will be required to complete a few simple questionnaires then return to your normal daily routine while wearing an ambulatory blood pressure recorder, which will be removed the next morning. The monitor is small, non-invasive and will permit you to carry out all normal activities, except swimming and showering/bathing.

On the second day you perform 2 general tasks and 8 minutes of static cycling as well as some more questionnaires, while your heart rate and blood pressure are monitored in the laboratory. It is estimated that the laboratory session will take 2-2.5 hours. After which you will be asked to give a blood sample, to assess the current level of fatty acid in your body.

During the following 12 weeks you **may** have to take a daily supplement. The supplement will be of the food class of essential fatty acids. The essential fatty acids will be in capsule form and are nutrients, which are non-harmful substances, required for normal functioning of the body. They can be found naturally in oily fish, amongst other foods.

Every third week (during the 12) a short questionnaire will be sent out for you to complete and return to me, in internal mail.

If you are interested in taking part then please contact me via the internal mail or preferably by email at **lap1@st-and.ac.uk**. I will then contact you to make arrangements and answer any queries.

Although you are free to withdraw from the experiment at any point, the nature of the research requires a **need for commitment**, for the 12 weeks and I ask that you be realistic about this consideration. Thank you for your time and I very much hope you will be in contact.

Lorraine

Advert

If You're Still Here In June

You Could Earn £100

1 - Are you male, under 35-years, healthy and a non-smoker?

2 - Are you available for 2 consecutive mornings?

then read on

I am a postgraduate student researching the possible beneficial effects of certain classes of foods on the heart. The experiment runs for 12 weeks requiring you to visit the laboratory on two consecutive mornings at the beginning and end of the 12 weeks. You will be required to perform 2 general tasks, a few minutes of static cycling and complete some mood questionnaires. During the 12 weeks you **may** be required to take a naturally occurring food supplement.

For further details,

see my web page: <http://psych.st->

and.ac.uk:8080/people/postg/lap1.html

Or contact Lorraine Paterson at : lap1@st-and.ac.uk

Or telephone : **46(2091)**

Not to Scale

Web Page – Unformatted text

**YOU CAN HELP
RESEARCH INTO THE FUTURE OF
HEALTHY HEARTS
AND EARN £100**

Dear Undergraduates and Postgraduates

Firstly thank-you for accessing our web page.

I am a postgraduate student doing my PhD. in Psychology. I very much hope that you will volunteer to take part in an experiment, which I am conducting, for my thesis. It is on the possible beneficial effects of certain classes of foods on the heart. You will be paid £100 for completing the study. You need to be **male, aged under 35, healthy and a non-smoker.**

The experiment will run for 12 weeks between September 1999 and June 2000. It requires that you visit the laboratory on two consecutive mornings at the beginning and end of the 12 weeks. On the first day you will be required to complete a few simple questionnaires then return to your normal daily routine while wearing an ambulatory blood pressure monitor, which you can remove at bedtime.

The monitor requires you to wear a blood pressure cuff and will record your blood pressure at pre-set times throughout the day. At these pre-set times you will be given a warning tone it indicate cuff inflation. After which you are required to complete one page of a diary indicating your activities and feelings over the previous five minutes. The data from this device will be stored on a small computer which is secured around your waist on a belt. The monitor is small, non-invasive and will permit you to carry out all normal activities, except swimming and showering/bathing while wearing it. I understand the inability to shower may put some people off volunteering but you will be able to shower that night (after 10pm) when you have removed the monitor.

You will be required to refrain from caffeine and alcohol during day 1 and day 2 testing. On the second day you perform 2 general tasks and 8 minutes of static cycling as well as some more questionnaires, while your heart rate and blood pressure are monitored in the laboratory. It is estimated that the laboratory session will take 2-2.5 hours. After which you will be asked to give a blood sample, to assess the current level of fatty acid in your body. This is no different than giving a blood sample at the doctors and will be taken by a professional.

During the following 12 weeks you will take four capsules once a day, which **may or may not** contain the supplement. The supplement will be of the food class of essential fatty acids. Essential fatty acids (EFAs) are **nutrients**, which are naturally occurring substances, required for normal functioning of the body. They can be found naturally in oily fish, amongst other foods. Half the subjects will receive the

capsules containing the EFA supplement while the other half will not. The other half of the subjects will take capsules containing an inert substance. You will not be told which you are taking. Both the capsules contain only non-harmful substances, which have no side effects.

Additionally, every fourth week (during the 12) a short mood questionnaire will be sent out to you along with the capsules, for you to complete and return to me, in internal mail.

The requirements:

that you are male

that you are under 35 years of age

that you are generally healthy

that you are not taking any medication for the heart e.g. beta-blockers, atorvastatin

that you are a non-smoker

that you are able to communicate in English

that you are available for the time period

The Test Session Times:

There are 4 slots for subjects per week. These are:

1st Slot:

Monday AND Tuesday 8:30am to 10:30/11am

2nd Slot:

Monday AND Tuesday 11am to 1/1:30pm

3rd Slot:

Wednesday AND Thursday 8:30am to 10:30/11am

4th Slot:

Wednesday AND Thursday 11am to 1/1:30pm

One of these test sessions will be allocated to suit you. You will need to attend the laboratory at these times, at the beginning and end of your 12 weeks. For example, if you are allocated the 2nd slot then you must attend the laboratory on a consecutive Monday and Tuesday at 11am, then exactly 12 weeks later you must attend these same session/slot times.

A final note, although you are free to withdraw from the experiment at any time, the nature of the research requires a **need for commitment**, for the 12 weeks and I ask that you be realistic about this consideration. Thank you for your time and I very much hope you will be in contact.

If you are interested in taking part then please:

contact me on 46(2091)

lap1@st-andrews.ac.uk

Laxdale Pharmaceuticals Ltd – Details

**Laxdale Ltd,
Kings Park House,
Laurelhill Business Park,
Polmaise Road,
Stirling
FK7 9JQ,
Scotland.**

Chairman: Dr David F Horrobin MA, DPhil, BM, BCh

Tel: +44 (0)1786 476000

Fax: +44 (0)1786 473137

Email: admin@laxdale.co.uk

Grass Polygraph settings - model # 7PCP8:

- **EEG Pre-amp Driver, model # :7P5B**

Cal = Use

G1 = 2

G2 = 3

0.5 amp low frequency = 0.04 – 3

Sensitivity uv/cm = 100

multiplication = * 10

- **DC Driver, model #: 7DAF**

Polarity = negative

0.5 amp high frequency = 0.75

High frequency filter = on

Driver sensitivity = 0.1

Example EMAS

Name: _____
 Age: _____ Sex: _____ Date: _____
 Ethnicity (optional): _____

EMAS

Norman S. Endler, Ph.D., F.R.S.C.
 Jean M. Edwards, Ph.D.
 Romeo Vite, Li, Ph.D.

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Directions

The 20 items to the right are descriptions of reactions to and attitudes toward a certain situation. Circle a number from 1 (not at all) to 5 (very much) to describe your reactions to and attitudes toward this situation.

Example:

You are getting ready to start the day.

1. Feel uncomfortable

1	2	3	4	5
---	---	---	---	---

 NOT AT ALL ← → VERY MUCH

If you feel very uncomfortable in this situation, circle the 5. If you feel somewhat uncomfortable, circle either the 2, 3, or 4, depending on how uncomfortable you are. If you do not feel uncomfortable at all in this situation, circle the 1.

If you have no questions, you may proceed to answer each item by circling the most appropriate response.

EMAS-S

For each of the following 20 items, please circle a number on the 5-point scale to indicate:

How you feel at this particular moment.

	1	2	3	4	5
1. Hands feel moist	1	2	3	4	5
2. Distrust myself	1	2	3	4	5
3. Breathing is irregular	1	2	3	4	5
4. Unable to focus on task	1	2	3	4	5
5. Have tense feeling in stomach	1	2	3	4	5
6. Heart beats faster	1	2	3	4	5
7. Feel helpless	1	2	3	4	5
8. Unable to concentrate	1	2	3	4	5
9. Perspire	1	2	3	4	5
10. Fear defeat	1	2	3	4	5
11. Mouth feels dry	1	2	3	4	5
12. Self-preoccupied	1	2	3	4	5
13. Feel uncertain	1	2	3	4	5
14. Feel tense	1	2	3	4	5
15. Feel inadequate	1	2	3	4	5
16. Hands feel unsteady	1	2	3	4	5
17. Feel flushed	1	2	3	4	5
18. Feel self-conscious	1	2	3	4	5
19. Feel incompetent	1	2	3	4	5
20. Feel lump in throat	1	2	3	4	5

Example CISS

CISS-Adult by Norman S. Endler, Ph.D., F.R.S.C. and James D.A. Parker, M.A.

Name: _____ Age: _____ Sex: _____ Date: _____

Occupation: _____ Education: _____ Marital Status: _____

Instructions: The following are ways people react to various difficult, stressful, or upsetting situations. Please circle a number from 1 to 5 for each item. Indicate how much you engage in these types of activities when you encounter a difficult, stressful, or upsetting situation.

Not at All					Very Much				
1	2	3	4	5	1. Schedule my time better.				
1	2	3	4	5	2. Focus on the problem and see how I can solve it.				
1	2	3	4	5	3. Think about the good times I've had.				
1	2	3	4	5	4. Try to be with other people.				
1	2	3	4	5	5. Blame myself for procrastinating.				
1	2	3	4	5	6. Do what I think is best.				
1	2	3	4	5	7. Preoccupied with aches and pains.				
1	2	3	4	5	8. Blame myself for having gotten into this situation.				
1	2	3	4	5	9. Window shop.				
1	2	3	4	5	10. Outline my priorities.				
1	2	3	4	5	11. Try to go to sleep.				
1	2	3	4	5	12. Treat myself to a favorite food or snack.				
1	2	3	4	5	13. Feel anxious about not being able to cope.				
1	2	3	4	5	14. Become very tense.				
1	2	3	4	5	15. Think about how I have solved similar problems.				
1	2	3	4	5	16. Tell myself that it is really not happening to me.				
1	2	3	4	5	17. Blame myself for being too emotional about the situation.				
1	2	3	4	5	18. Go out for a snack or meal.				
1	2	3	4	5	19. Become very upset.				
1	2	3	4	5	20. Buy myself something.				
1	2	3	4	5	21. Determine a course of action and follow it.				
1	2	3	4	5	22. Blame myself for not knowing what to do.				
1	2	3	4	5	23. Go to a party.				
1	2	3	4	5	24. Work to understand the situation.				
1	2	3	4	5	25. "Freeze" and don't know what to do.				
1	2	3	4	5	26. Take corrective action immediately.				
1	2	3	4	5	27. Think about the event and learn from my mistakes.				
1	2	3	4	5	28. Wish that I could change what had happened or how I felt.				
1	2	3	4	5	29. Visit a friend.				
1	2	3	4	5	30. Worry about what I am going to do.				
1	2	3	4	5	31. Spend time with a special person.				
1	2	3	4	5	32. Go for a walk.				
1	2	3	4	5	33. Tell myself that it will never happen again.				
1	2	3	4	5	34. Focus on my general inadequacies.				
1	2	3	4	5	35. Talk to someone whose advice I value.				
1	2	3	4	5	36. Analyze the problem before reacting.				
1	2	3	4	5	37. Phone a friend.				
1	2	3	4	5	38. Get angry.				
1	2	3	4	5	39. Adjust my priorities.				
1	2	3	4	5	40. See a movie.				
1	2	3	4	5	41. Get control of the situation.				
1	2	3	4	5	42. Make an extra effort to get things done.				
1	2	3	4	5	43. Come up with several different solutions to the problem.				
1	2	3	4	5	44. Take time off and get away from the situation.				
1	2	3	4	5	45. Take it out on other people.				
1	2	3	4	5	46. Use the situation to prove that I can do it.				
1	2	3	4	5	47. Try to be organized so I can be on top of the situation.				
1	2	3	4	5	48. Watch TV.				

Not at All Very Much

Example DINE

About how many times a week do you eat a serving of the following foods?

FAT SCORE

	less than 1 a week	1 - 2 a week	3 - 5 a week	6 or more a week
Cheese (any except cottage)	1	2	6	9
Beefburgers or sausages	1	2	4	6
Beef, pork or lamb (if vegetarian: nuts)	1	2	6	9
Bacon, meat pies, processed meat	1	2	5	6

Score

--	--

About how many times a week do you eat a serving of the following foods?

	less than 1 a week	1 - 2 a week	3 - 5 a week	6 or more a week
Chicken or turkey	0	1	3	5
Fish (NOT fried)	0	0	1	2
ANY fried food: fried fish chips, cooked breakfast, samosas	1	2	6	9
Cakes, pies, puddings, pastries	1	2	5	8
Biscuits, chocolate, or crisps	1	2	4	6

Score

--	--

About how much milk do you yourself use in a day, for drinking or in cereal, tea, or coffee? What kind of milk do you usually use? (choose only 1 if possible)

Milk	less than quarter pint	about a quarter pint	about a half pint	1 pint or more
Full cream (silver top) or Channel Islands (gold top)	1	3	6	12
Semi-skimmed (red striped top)	0	1	3	6
Skimmed (blue checked top)	0	0	0	0

Milk score

--	--

About how many pats or rounded teaspoons of margarine, butter or other spread do you usually use in a day, for example on bread, sandwiches, toast, potatoes, or vegetables?

Butter or margarine: Flora, Vitalite/Light, sunflower types, Blue Band, Golden Crown, Mello, Krona, Stork/Light Summer County pats times 4 = total

Low fat spread: Gold/Lowest Outline, Shape, Flora Extra Light, Clover Extra Lite, Delight, Half Fat Butter, Country Light pats times 2 = total

Spreading
Fat score

--	--

FAT RATING

Less than 30 = low fat intake
30 to 40 = moderate fat intake
More than 40 = high fat intake

**TOTAL
SCORE**

--	--

FIBRE SCORE

About how many pieces of bread or rolls (or chapatis) do you eat on a usual day?

Are they usually white, brown, or wholemeal? (choose 1 only, if possible)

Bread	less than 1 a day	1 - 2 a day	3 - 4 a day	5 or more a day
White bread	1	4	9	13
Brown or granary bread; Mighty White, soft grain	2	7	15	22
Wholemeal bread or 2 slices crispbread	3	8	18	26

Bread score

--	--

About how many times a week do you have a bowl of breakfast cereal or porridge?

What kind do you have most often? (choose 1 only, if possible)

Breakfast cereal	less than 1 a week	1 - 2 a week	3 - 5 a week	6 or more a week
Sugar type: Frosties, Coco Pop, Rice Krispies, Sugar Puffs				
Rice/Corn type: Corn Flakes, Rice Krispies, Special K	0	0	1	2
Porridge or Ready Brek <i>This is a source of soluble fibre</i>				
Wheat type: Shredded Wheat, Weetabix, Puffed Wheat, Fruit 'n Fibre, Nutri-Grain, Flat Krunchies, Star	1	2	5	7
Muesli type: Alpen, Jordan's				
Bran type: All-Bran, Bran Flakes, Buliana Bran, Team <i>High in sugar and calories!</i>	2	5	12	18

Cereal score

--	--

About how many times a week do you eat a serving of the following foods? (choose one on each line)

Vegetables etc.	less than 1 a week	1 - 2 a week	3 - 5 a week	6 or more a week
Pasta or rice	0	1	3	4
Potatoes	0	1	3	5
Peas	1	3	8	12
Beans (baked, tinned, dried) or lentils <i>These are a source of soluble fibre</i>	1	4	10	15
Other vegetables (any type)	0	1	2	3
Fruit (fresh, frozen or canned) <i>These are a source of soluble fibre</i>	0	1	3	5

Vegetables score

--	--

FIBRE RATING

Less than 30 = low fibre intake
30 to 40 = moderate fibre intake
More than 40 = high fibre intake

TOTAL SCORE

--	--

Exercise Level Measure

This questionnaire is designed to assess the type and frequency of any physical exercise programs, which you regularly engage in and to determine how physically active your working life is.

Using the 0-6 scale below please indicate the frequency with which you engage in the following activities:

- 0 = Never, or not regularly
- 1 = Once a Month
- 2 = Twice a Month
- 3 = Once a Week
- 4 = Twice a Week
- 5 = Three Times a Week
- 6 = More than 3 times a Week

- Jogging or running _____
- Vigorous racket sports _____
- Cycling faster than 10mph or exercise hard on exercise cycle _____
- Swimming - vigorously _____
- Vigorous exercise class or vigorous dancing _____
- Home or leisure activity (snow shovelling, moving, lifting) _____
- Strenuous sports (basketball, football, skating, skiing) _____
- Non-strenuous sports (softball, shooting baskets, volleyball, table tennis, swimming, cycling) _____
- Walks or hikes _____
- Bowling, golf _____
- Home exercises, callisthenics _____
- Weight training _____
- Home maintenance (gardening, carpentry, painting, raking, mowing) _____
- Vigorous job activity (lifting, carrying, digging) _____

• Are there any other activities not mentioned here, which you do regularly. If so please name them and indicate their frequency:

Would you rate your job as sedentary or requiring large amounts of physical effort?

Sedentary / Physically demanding (circle appropriate response)

If you indicated *physically demanding* then on a scale of 1 to 7, (7 being constant physical effort, 1 being only intermittent effort required) please define your work:

1 2 3 4 5 6 7

Time Two additional questions:

- In the last 12 weeks do you think your level / frequency of exercise has:

remained the same / increased / decreased (circle appropriate response)

If the level / frequency has altered please indicate in what manner: _____

- In the last 12 weeks do you think the amount of physical effort which your job requires has:

remained the same / increased / decreased (circle appropriate response)

If the amount of physical effort has altered please indicate in what manner: _____

Example SWLS

SATISFACTION WITH LIFE SCALE

Name:

Date: Record Number:

Below are five statements with which you may agree or disagree. Using a 1 to 7 scale, indicate your agreement with each item by placing the appropriate number in the box next to that item. Please be open and honest in your responses. The 7-point scale is:

- 1 = strongly disagree
- 2 = disagree
- 3 = slightly disagree
- 4 = neither agree nor disagree
- 5 = slightly agree
- 6 = agree
- 7 = strongly agree

In most ways my life is close to ideal.

The conditions of my life are excellent.

I am satisfied with my life.

So far I have got the important things I want in life.

If I could live my life again, I would change almost nothing.

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Code 4920 06 4



Appraisal of life events scale - General version

We would like you to rate your **perceptions** of your circumstances right now. Use the following six point scales (where 0 = not at all to 5 = very much so) to indicate the extent to which each of the adjectives best describes your **perceptions** now. Do this by circling the appropriate point on the scales. Please respond as quickly as possible as first responses are usually more accurate. Please make a response to each adjective.

THE CIRCUMSTANCES ARE:

Threatening:

0 1 2 3 4 5

Fearful:

0 1 2 3 4 5

Enjoyable:

0 1 2 3 4 5

Worrying:

0 1 2 3 4 5

Hostile:

0 1 2 3 4 5

Challenging:

0 1 2 3 4 5

Stimulating:

0 1 2 3 4 5

Exhilarating:

0 1 2 3 4 5

Painful:

0 1 2 3 4 5

Depressing:

0 1 2 3 4 5

Pitiful:

0 1 2 3 4 5

Informative:

0 1 2 3 4 5

Exciting:

0 1 2 3 4 5

Frightening:

0 1 2 3 4 5

Terrifying:

0 1 2 3 4 5

Intolerable:

0 1 2 3 4 5

Appraisal of life events scale - Specific event version

We would like you to rate your **perceptions** of the second day you spent in the lab. Use the following six point scales (where 0 = not at all to 5 = very much so) to indicate the extent to which each of the adjectives best describes your **perceptions** now. Do this by circling the appropriate point on the scales. Please respond as quickly as possible as first responses are usually more accurate. Please make a response to each adjective.

Example BDI



Date: _____

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

This questionnaire consists of 21 groups of statements. After reading each group of statements carefully, circle the number (0, 1, 2 or 3) next to the one statement in each group which **best** describes the way you have been feeling the **past week, including today**. If several statements within a group seem to apply equally well, circle each one. **Be sure to read all the statements in each group before making your choice.**

- 0 I do not feel sad.
- 1 I feel sad.
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad or unhappy that I can't stand it.

- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel that the future is hopeless and that things cannot improve.

- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

- 0 I don't feel particularly guilty.
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

- 8 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

- 9 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

- 10 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

- 11 0 I am no more irritated now than I ever am.
- 1 I get annoyed or irritated more easily than I used to.
- 2 I feel irritated all the time now.
- 3 I don't get irritated at all by the things that used to irritate me.

- 12 0 I have not lost interest in other people.
- 1 I am less interested in other people than I used to be.
- 2 I have lost most of my interest in other people.
- 3 I have lost all of my interest in other people.

- 13 0 I make decisions about as well as I ever could.
- 1 I put off making decisions more than I used to.
- 2 I have greater difficulty in making decisions than before.
- 3 I can't make decisions at all anymore.

Subtotal Page 1

CONTINUED ON BACK



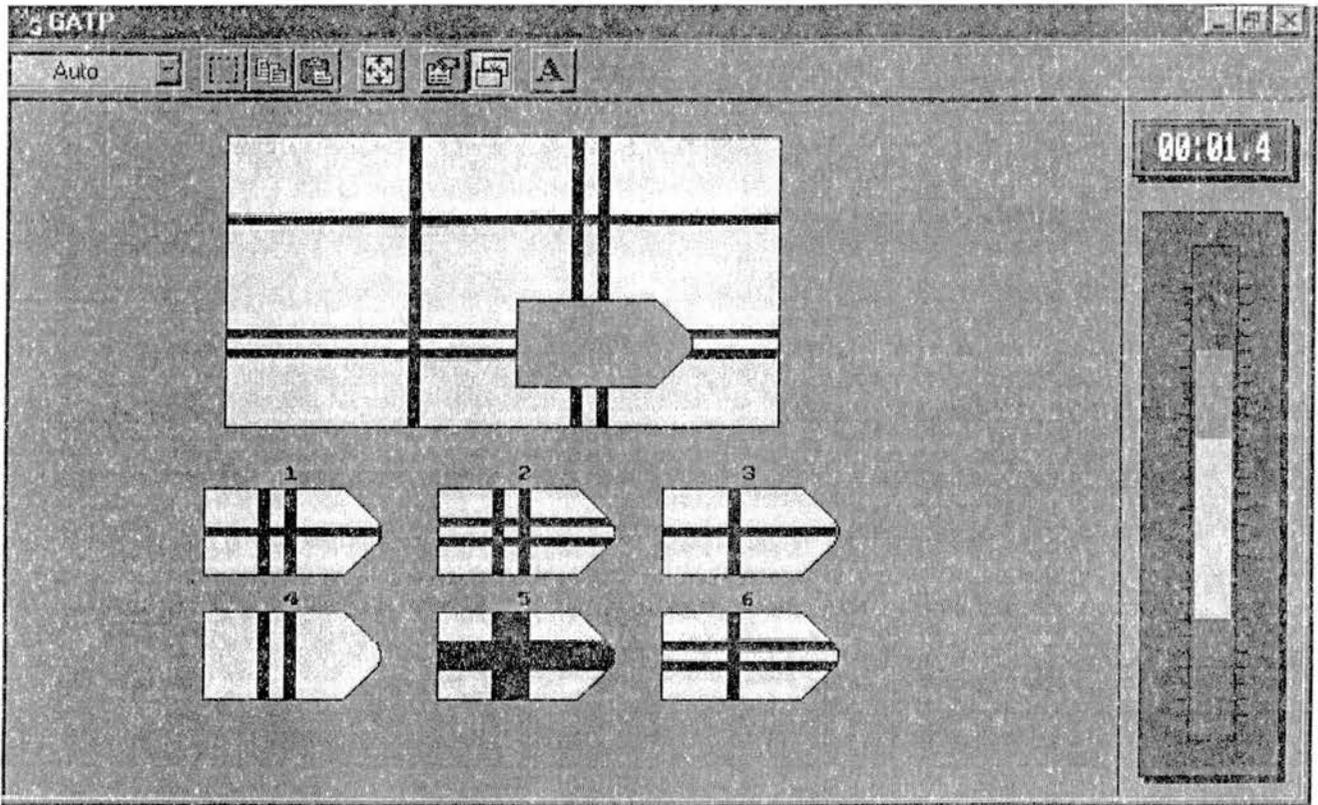
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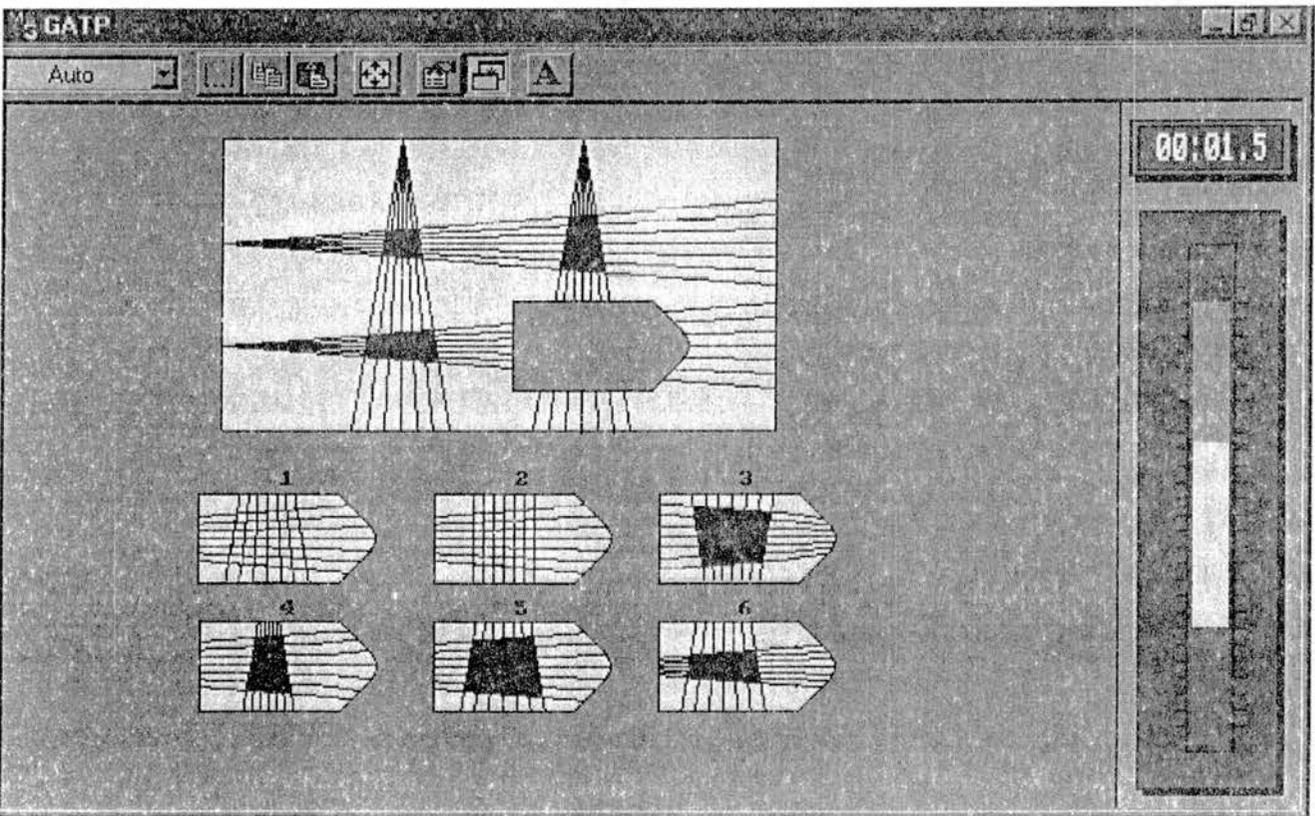
9-018359

Raven's Matrices Example Screen

A8



A12



Instructions for Raven's Matrices

I now require you to complete a pattern-matching task. It is a computerised version of the Raven's Matrices task, which you may be aware, is a non-verbal cross-cultural test of intelligence. We will be recording your speed and accuracy, which we will then use for group comparisons.

On the screen, in front of you, you will see a large pattern, with a small portion of it missing. Below this is an array consisting of pattern possibilities for this missing section. These options are numbered 1 to 6 or 8 from left to right on the screen.

Your task is to select the appropriate missing pattern, and type in its corresponding number, using the number pad. If you get it wrong you will be given the opportunity to repeat the trial. There will be 48 trials in total. Each trial will immediately follow the last. There will be no break in-between trials. Each trial will be timed, with an accompanying count down tone, also indicated on the right-hand side of the screen. Therefore do not waste any time but remember your objective is to be as accurate as possible, to enable measurement of your intelligence.

The first trial is an un-timed example. Any questions? Please begin when ready.

Choice Reaction Time Instructions

I now need you to complete a choice reaction time task. For this task you will only need to use the numbers 1, 2 and 3 on the number pad.

The numbers 1, 2 or 3 will appear the middle of the screen in front of you. Only one number will appear at a time, but another will immediately follow it. Your task is to respond as quickly and accurately as possible with the appropriate button presses. Incorrect or too slow responses will be indicated by a loud tone. Do not stop when you hear this, just continue as quickly and as accurately as possible. You will find that the rate of presentation has been set so that most people are able to respond before the next number is presented to them.

There will be 6 blocks of 72 trials. Three of the blocks require compatible responses. That is: when you see the number 1 you press number 1; when you see the number 2 you press the number 2; and when you see the number 3 you press the number 3.

However, the other 3 blocks require incompatible responses. That is: when you see the number 1 you press number 3; when you see the number 2 you press the number 1; and when you see the number 3 you press the number 2. In other words, everything has been shifted one place to the left.

The 6 blocks will be presented alternately: compatible then incompatible. At the beginning of each block instructions will be displayed on the screen to remind you of your task. The first block requires compatible responses. Remember your aim is to be as fast and as accurate as possible. We will be recording your speed and accuracy, for later comparisons.

Any questions? Please begin when ready.

At the point of every set of on screen instructions: You are now moving on to a block of in / compatible trials. Remember your aim is to be as fast and accurate as possible

Acoustic Startle Task – On Screen Instructions

To the subject:

In this task you will listen for a tone through a set of headphones. Your task is to react as quick as possible after the onset of the tones by pressing either the "z" key if you are left-handed or the "0" (zero) key if you are right-handed.

If you respond correctly, a "OK" will appear on the screen. If you make an error, then an "X" appears.

Some of the tones will be soft hums, while others will be loud buzzes.

When you and the experimenter are ready, press any key...

PROTOCOL

Time One (Before)

Day One:

- Measure, weight, height. Confirm D.O.B non-smoking, and health status
- Measure BP
- Fit ambulatory BP Cuff
- Measure dietary Fat and Fibre intake using the D.I.N.E. – self report.
- Measure State and trait anxiety as well as situation perceptions using EMAS-S, EMAS-T and EMAS-P: Endler
- Measure of Exercise level – frequency questionnaire
- Measure general coping strategies used in difficult/upsetting /stressful situations, using CISS: Endler
- Short measure of satisfaction with life - SWLS: Diener
- Subject returns to normal routine wearing cuff – cuff measures every half-hour between 2 p.m. and 10 p.m., after which they are free to remove the cuff. Subjects are required to complete a short diary page assessing situational details and mood, with each measurement.
- In the evening at home the subjects are required to complete another EMAS-S and BDI.

Day Two:

- Subject returns and is taken to have blood sampled – EDTA
 - In Laboratory subject is attached to a 3-electrode ECG and Finapres finger cuff.
 - A 15 minutes baseline is recorded
 - Subjects complete a computerised version of the Ravens Matrices IQ task
 - Subjects are given 3 minutes quietly for CV recovery
 - Subjects complete a computerised choice reaction time task, requiring compatible and incompatible responses
 - Subjects are given 5 minutes quietly for CV recovery
 - Subjects are moved to a static cycle and seated there for 3 minutes.
 - Subjects are required to cycle with some effort for 8 minutes.
 - Subjects are given 5 minutes quietly for CV recovery
 - Recording is stopped and equipment removed.
 - Subjects are given the EMAS-S, EMAS-P.
 - Intervention - Subjects are be provided with either the EFA supplement or the placebo and instructed to take four capsules every day, until their return in 12 weeks.
- Additionally, subjects will be administered the EMAS-S and BDI (Scales to complete at home during weeks 4 and 8).

Time Two: 12 weeks after beginning supplementation

Day One:

- Measure, weight.
- Measure BP

- Fit ambulatory BP Cuff
- Measure Fat and Fibre intake using the D.I.N.E.
- Measure State and trait anxiety as well as situation perceptions using EMAS-S, EMAS-T and EMAS-P: Endler
- Measure of Exercise level – frequency questionnaire
- Measure general coping strategies used in difficult/upsetting /stressful situations, using CISS: Endler
- Short measure of satisfaction with life - SWLS: Diener
- Subject returns to normal routine wearing cuff – cuff measures every half-hour between 2 p.m. and 10 p.m., after which they are free to remove the cuff. Subjects are required to complete a short diary page assessing situational details and mood, with each measurement.
- In the evening at home the subjects are required to complete another EMAS-S and BDI.

Day Two:

- Subject returns and is taken to have blood sampled – EDTA
- In Laboratory subject is attached to a 3-electrode ECG and Finapres finger cuff.
- A 15 minutes baseline is recorded
- Subjects complete a computerised version of the Ravens Matrices IQ task
- Subjects are given 3 minutes quietly for CV recovery
- Subjects complete a computerised choice reaction time task, requiring compatible and incompatible responses
- Subjects are given 5 minutes quietly for CV recovery
- Subjects are moved to a static cycle and seated there for 3 minutes.
- Subjects are required to cycle with some effort for 8 minutes.
- Subjects are given 5 minutes quietly for CV recovery
- Subjects are moved back to chair and given another 3 minutes for CV recovery.
- Subjects complete Startle task – 5 minutes of responding to loud and soft tones.
- Subjects are given 3 minutes quietly for CV recovery
- Recording is stopped and equipment removed.
- Subjects are given the EMAS-S, EMAS-P.
- Subjects are Debriefed and thanked.
- Subjects are paid.

SUBJECT CONSENT FORM

School of Psychology
University of St Andrews

Title: *Essential Fatty Acids, Stress Reactivity and Coping*

Experimenter: Lorraine A. Paterson

Supervisor: Eric Bowman

The purpose of this form is to ensure that you are willing to take part in this study and that you understand what it entails. Signing this form does not commit you to anything you do not wish to do.

- Have you read and understood the subject information sheet? Yes/No
- Have you had the opportunity to ask questions and discuss the study? Yes/No
- Have you received satisfactory answers to your questions? Yes/No
- Do you understand that you are free to withdraw from the study
* at any time
* without having to give a reason? Yes/No

I agree to participate in this study.

Subject

Signature _____ Name _____

Witness

Signature _____ Name: Lorraine A. Paterson

Date _____

Doctors Letter for Medical Students

Ms L. A. Paterson
School of Psychology
University of St Andrews
St Andrews
KY16 9JU
01334 462091

St Andrews Health Centre

Dr _____

Dear Dr _____

Please be advised that your patient _____ has volunteered to take part in research involving investigation of the possible beneficial effects of Essential fatty Acids. The research occurs in the school of Psychology, University of St Andrews and requires that your patient take a supplement for 12 weeks. The supplement will be either Ethyl-EPA or a placebo consisting of vitamin E an inert mineral oil. Ethyl-EPA as you are aware is a natural substance required of normal bodily functioning while the placebo is inert. There are no known side effects to either of these substances.

I have enclosed a subject information sheet outlining the study, and I will be available on the above number to answer any queries you may have. Please be advised that your patient is free to withdraw from this research at any time, without giving reason.

Yours sincerely

L.A.Paterson

SUBJECT INFORMATION SHEET

**School of Psychology
University of St Andrews**

Title: *Essential Fatty Acids, Stress Reactivity and Coping*

The experiment will run for 12 weeks. It requires that you visit the laboratory on two consecutive mornings at the beginning and end of the 12-week period. On the first day you will be required to complete a few simple demographic, mood and coping questionnaires then return to your normal daily routine while wearing an ambulatory blood pressure monitor which you can remove at 10pm that night.

The monitor will record your blood pressure at intervals throughout the day. You will feel the cuff gently inflate and deflate while a reading is being taken. Please relax your arm and sit down while this is occurring if possible. Directly after a recording has occurred you are required to complete one page of a diary indicating your activities and feelings over the previous five minutes. You will be able to carry out all normal activities, except swimming and showering/bathing. Although once you have removed the cuff at 10 p.m. you are of course free to shower then.

You will be required to return to the laboratory on the second morning where you will be required to perform a reaction-time task, a non-verbal IQ test and 8 minutes of static cycling while your heart rate and blood pressure are monitored, in the laboratory. You will also be asked to complete more mood and coping questionnaires. It is estimated that the laboratory session will take 2-2.5 hours. After which you will be asked to give a simple blood sample, to assess the current level of fatty acid in your body.

During the following 12 weeks you will take 4 capsules a day, which **may or may not** contain Essential Fatty Acids (EFAs). Half the subjects will receive the capsules containing EFAs while the other half will not. You will not be told which you are

taking. Both the capsules contain non-harmful substances. The EFA is a nutrient essential for normal functioning of the body. The other capsule contains Vitamin E and mineral oil, both inert substances.

Additionally, every fourth week (during the 12) a simple short mood questionnaire will be sent out for you to complete and return to me, in internal mail.

Although you are free to withdraw from the experiment at any time, the nature of the research requires a **need for commitment**, for the 12 weeks and I ask that you be realistic about this consideration. On completion of the experiment you will receive £100 payment.

If you have any queries, or feel you cannot take part in the experiment for any reason please discuss these with the experimenter.

School of Psychology Ethics Committee Approval



UNIVERSITY OF ST ANDREWS
SCHOOL OF PSYCHOLOGY
ST. ANDREWS, FIFE, SCOTLAND, KY16 9JU

From:

Switchboard: (01334) 476161

Extension:

Direct line: (01334) 46 2012

Fax: (01334) 463042

UNIVERSITY OF ST ANDREWS
SCHOOL OF PSYCHOLOGY ETHICS COMMITTEE

27th July 1999

Lorraine Paterson
School of Psychology
University of St Andrews

Dear Lorraine

Re: Addendum to "Essential Fatty Acids, Stress Reactivity and Coping"

Thank you for submitting the further information that the Ethics Committee requested. This project has been approved.

If, during the course of the proposed research, any important condition were to alter, then the Committee would wish to be informed.

Yours sincerely

ff Dr Hugh Morris
Convener

Dictated but not read

Fife Health Board Research Approval

Letter of Insurance



Springfield House
Cupar
Fife
KY15 5UP

Your Ref:
Our Ref: DE/JG/Ethics/Letters/0102010
Enquiries to: Mr D. Elder
Ext: D.D. 01334 421004
Dept Fax: 01334 652210
E-Mail: -

Tel: (01334) 656200
Fax: (01334) 652210
Text: 0345 626799

8 February 2000

Ms. L. Paterson
School of Psychology
University of St. Andrews
ST. ANDREWS
KY16 9JU

Dear Ms. Paterson

ESSENTIAL FATTY ACIDS, STRESS REACTIVITY AND COPING

I refer to your faxes dated 24 and 25 January 2000 in response to concerns previously expressed by the Committee in relation to this study.

I write to confirm that the Fife Local Research Ethics Committee at its meeting on 1 February 2000 noted and accepted the responses received and, accordingly, approved your application.

The Committee noted that the study started in September 1999 and I was asked to stress to you the importance of such applications being submitted to them prior to the commencement of a study.

The remit of the Committee requires that they follow up projects they have approved to determine the success or otherwise of such studies.

I would be pleased, therefore, if you would provide a copy of any final reports produced as a result of your study or alternatively to receive from you written confirmation of the results of your study for submission to the Committee.

Yours sincerely

MR D ELDER
Secretary
Fife Local Research Ethics Committee

c.c. Ethics Committee, University of St. Andrews, St, Andrews, Fife, KY16 9JU.



**University of St Andrews
Financial Accounts
College Gate
St Andrews
KY16 9AJ**

**Professor Ronan O'Carroll
School of Psychology**

10/03/2000

Dear Professor O'Carroll,

Project Title : Essential Fatty Acids, Stress Reactivity and Coping

Recently, I was contacted by a member of your staff, on a matter of insurance, concerning the project titled above.

The details of the study were then forwarded to Marsh (UK) Ltd., who act as Insurance Brokers for the University, and subsequently passed to Royal & Sun Alliance Insurance Plc.

We have now been advised by Marsh (UK) that Royal & Sun Alliance have agreed to underwrite the project, and confirmed that insurance cover is now in place for the study to commence.

Yours sincerely,

Kenneth G N Stewart

**Phone: 01334 46 2465
Fax : 01334 46 2672
E-mail: kgns@st-and.ac.uk**

Spike 2 Cardiac Script

```

'EMB Added filter lines
'EMB Saved filter lines

'Name of data file
'Set size of memory array for putative heartbeats
'Handle for input data file
'Handle for file containing log of filtering process
'Maximum time of wavemark channel

'Time window after heart beat in which min BP & max BP are searched
'Dimension an array to hold parameters for filtering
'Number of intervals into which to sample BP between successive HBs
'Array to hold average BP waveform. [x][0]=n cases; [x][1]=sum; [x][2]=mean

'Array to hold BP data from individual HBs
'Critical value of correlation coefficient with d.f.=23, p=0.05, 1-tailed
'Array holding channel information for filter events

'Array to hold BP data from individual HBs

'Generated toolbar code
DoToolBar(); 'Try it out
Halt;

Func DoToolBar() 'Set your own name...
ToolBarClear(); 'Remove any old buttons
ToolBarSet(6, "&All", DoAll%);
ToolBarSet(5, "&Data", DoData%); 'Link to function
ToolBarSet(4, "&Extract", DoExtract%); 'Link to function
ToolBarSet(3, "&Filter", DoFilter%); 'Link to function
ToolBarSet(2, "&Save output", DoSave%);
ToolBarSet(1, "&Quit", DoQuit%); 'Link to function
ToolBarEnable(4,0);
ToolBarEnable(3,0);
ToolBarEnable(2,0);
return ToolBar("Cardiac analysis: EMB 6/00 Version 3.0", 13);
end;

Func DoAll%()
DoData%();
DoExtract%();
DoFilter%();
DoSave%();

```

```

end;
return(1);

Func DoData%() 'Button 1 routine
var DataFiles%[2],file%,x%,y%;
  ToolbarEnable(4,0);
  ToolbarEnable(3,0);
  ToolbarEnable(2,0);
  file%=0;

file%=CheckFiles%(DataFiles%[]);
if file%≠1 then
  DataView%:=DataFiles%[1];
  FrontView(DataView%);

  DataFile$=FileName$(3)+FileName$(4);
  ToolbarText("File: "+DataFile$);
  for x%=0 to (sizeHBs%-1) do
    if (x% mod 100=0) then ToolbarText("File: "+DataFile$+" Clearing HBs[]: "+str$(x%)); endif; 'Display progress in toolbar
    for y%=0 to 10 do
      HBs[x%][y%]=0;
    next;
  next;
  totalHBs%=0;

'Reset heart beat count
'Message("Mark sure that WarkMark has been performed before using Extract button");
  ToolbarEnable(4,1);

"extract" button
  ToolbarText("File: "+DataFile$);
endif;

return 1; 'This leaves toolbar active
end;

Func DoExtract%() 'Button 2 routine
var keys%,channels%[2],beats%;

'Get key presses
FrontView(DataView%);
window to front
keys%:=Count(31,0,MaxTime(31))+2;
running out of memory
var keymarks[key%][2];
array to save keypresses
GetKeyPresses(keys%,keymarks[[]]);

'Extract physiological data

'Find data window
'Bring it to

'Get name of data file
'Display it in toolbar
'Zero heart beat array

'Find data window
'Bring it to

'Bring data

'Get the number of key presses and add 2 to prevent
'Dimension

'Find and save keyboard presses

```

```

ChanList(channels%[,1:16];
if (channels%[0]>0) then
channel open?
    WMmaxT:=MaxTime(channels%[1]);
maximum time of wavemark
beats%:=Count(channels%[1],0,WMmaxT);
'Debug();
if (beats%>=sizeHBs%) then
    Message("Data overflow: Heart beats (" +str$(beats%)+") >= sizeHBs% constant (" +str$(sizeHBs%)+") for HBs[]");
halt;
'Abort script
endif;
'End data overflow error
totalHBs%:=GetPhysioData%(channels%[],keymarks[],keys%,beats%);
ToolbarEnable(3,1);
button
    ToolbarEnable(2,1);
output" button
    ToolbarText("File: "+DataFile$)
else
    'No wavemark data
    Message("No WaveMark channel found! Create wavemark channel and hit extract button again");
endif;

return 1; 'This leaves toolbar active
end;

Func DoFilter%() 'Button 3 routine
var ok%,handle%;
'Set the filtering parameters
ok%:=filterParams%();
if (ok%/=1) then
    'Open the error file
    handle%:=OpenErrorFile%();
    if handle%>=0 then
        FilterFileView%:=handle%;
        FilterData();
        ToolbarText("File: "+DataFile$);
    endif;
else
    Debug();
endif;

return 1; 'This leaves toolbar active
end;

```

'Poll for any wavemark

'Is there a wavemark

'Set global variable to hold value of

'Determine maximum number of heart beats

'Are there more heart beats

'Extract physiological data and return number of HB's

'Enable "Filter"

'Enable "Save

'Display file title in toolbar

'Save handle of filter log to global variable

'Must follow statement above

'Display file name

```

Func DoSave%0 'Button 3 routine
var handle% ,n%,datum%,stage$,lastStage$,printHBs%,path$,
    path$:=FilePath$0;
n%:=FilePathSet("C:\My Documents");
if n%<0 then
    Message("Error in setting file pathway in DoSave%0: "+str$(n%)); 'Set save directory
    Debug(); 'Get current directory
endif;
handle%:=FileOpen(DataFiles$+"_HB.txt",8,1,"Save cardiac data to:"); 'Print error message
dowcase
case handle%<0 then
    Message("Data output file not opened! Aborting saving of cardiac data...");
else
    Print("Time\t");
    Print("IB\t");
    Print("Hz\t");
    Print("MinBP\t");
    Print("MaxBP\t");
    Print("tMinBP\t");
    Print("tMaxBP\t");
    Print("Stage\t");
for n%:=0 to (totalHBs%-1) do
    if (n% mod 100=0) then ToolbarText("File: "+DataFiles$+" Save HB: "+str$(n%)); endif; 'Display progress in toolbar
    if HBs[n%][8]=0 then
        printHBs%:=printHBs%+1;
        for datum%:=0 to 7 do
            dowcase
            case datum%=7 then
                stage$:=Chr$(HBs[n%][datum%]);
            dowcase
            case stage$="-" then
                Print("%c\n",lastStage$);
            case stage$="+" then
                Print("%c\n",lastStage$);
            case stage$>="a" and stage$<="z" then
                Print("%c\n",stage$);
        endcase
    endcase
endfor
there are no flags to skip all data from this "HB"
'Increment counter of number of heart beats printed
for datum%:=0 to 7 do
    dowcase
    case datum%=7 then
        stage$:=Chr$(HBs[n%][datum%]);
    dowcase
    case stage$="-" then
        Print("%c\n",lastStage$);
    case stage$="+" then
        Print("%c\n",lastStage$);
    case stage$>="a" and stage$<="z" then
        Print("%c\n",stage$);
    endcase
endcase
'Print the stage of the putative HB
'Start of user defined recording pause
sampling problems during
'End of user defined recording pause
valid stage
current stage
'For total number of putative heartbeats
'Print only if
'For each datum
'Store the current stage
'Denote
'Print the last
'If stage is a lowercase letter
'Print the

```

```

'Record last stage
lastStage$:=stage$;
else
'Unrecognized character
Print("?n");
lastStage$:="?";
endcase;
case datum%>=3 and datum%<=6 then
if (HBs[n%][9]+HBs[n%][10]=0) then
if datum%<=5 then
Print("%f\t",HBs[n%][5]+HBs[n%][1]);
'Add IBI to time of diastolic to get true relative
time of diastolic
else
'Datum is not the time of the diastolic
Print("%f\t",HBs[n%][datum%]);
'Print data
endif;
else
'Do not print
Print("\t");
'Print status to toolbar
endcase;
'End of check regarding time of diastolic
else
'Rest of data should be OK
Print("%f\t",HBs[n%][datum%]);
'Print datum
endcase;
next;
next;
endcase;
endcase;
next;
ToolBarText("File: "+DataFile$+" Printed HBs: "+str$(n%));
FileClose();
endcase;
n%:=FilePathSet(path$);
directory to old value
if n%<0 then
Message("Error in setting file pathway in DoSave%0: "+str$(n%)); 'Print error message
Debug();
endif;
SaveFilterChannels();
return 1; 'This leaves toolbar active
end;

```

Func DoQuit%() 'Button 4 routine

'Restore working

```

'Your code in here...
return 0; 'This stops the toolbar
end;

Funcn CheckFiles%(Array%[])
files
var files%,flag%;
flag%:=0;
'Set flag to no files open
files%:=ViewList(Array%[],1);
if (files%<=0) then
open
Array%[0]:=FileOpen("",0,7,"Open a cardiac data file:"); 'Open a file
if Array%[0]>0 then
flag%:=1;
successfully loaded
'Flag file open
else
'Data file not open, user hit cancel or there was an error:
Message("Warning: Data file not open");
'Debug();
endif;
else
'There is a file open
flag%:=1;
'Flag file open
endif;
'Debug();
return flag%;
end;

Proc GetKeyPresses(keys%,array[])
testing stages
var n%,time,code%{4};
n%:=0;
time:=1;
repeat
'Set current keypress to 0
'Set to ensure start of search at very beginning
repeat
'Loop through keypresses
time:=NextTime(3,1,time,code%[]);
if (time>=0) then
'If a genuine time
array[n%][0]:=time;
'Save the time of the key press into the array
array[n%+1][1]:=code%[0];
ASCII code of the key press into the array

```

'Determine if there are open

'Get open files

'No data files

'If a file is

'Get times of key presses marking

'Get time and information re: next keypress

'Save the

```

n%:=n%+1;
'Increment count of key presses
endif;
'End if a genuine time
until (time<0);
'Until no more key presses
array[keys%-1][0]=MaxTime();
end;

'Save last time of channel

Func GetPhysioData(channels%[],KP[][],keys%,beats%)
var points%,prePoints%,SFR,offset;
var stage%,stageExpires,time,n%,hb%,codeLast,timeLast;
var code%[4],BP>window,result,minBP,maxBP,tminBP,tmaxBP;

points%:=Markinfo(channels%[1],prePoints%);
SFR:=Binsize(channels%[1]);
offset:=prePoints%*SFR;

peak
stage%:=0;
stageExpires:=KP[stage%][0];
time:=0;
prior to any possible time in the data
n%:=0;
hb%:=0;
number is zero to start
codeLast:=0;
(0=artifact or initial
repeat
extraction for all heart beats
if (hb% mod 100=0) then ToolbarText("File: "+DataFile$+" Extracting HB: "+str$(hb%)); endif;
time:=NextTime(channels%[1],time,code%[1]);
dowcase
artifact or true cardiac potential?
case code%[0]=0 then
codeLast:=0;
not true cardiac potential
case code%[0]>0 then
if (codeLast=1) then
HBs[hb%][0]=timeLast+offset;
HBs[hb%][1]=time-timeLast;
HBs[hb%][2]=60/(time-timeLast);
and peak
'Artifact or first HB
'Record that last event was
'Belongs to wavemark template (i.e., true cardiac
'If last heart beat was filter[
'Save time of first heart beat, accounting for lag in wavemark between start
'Save inter-beat interval
'Save heart rate (BPM) for the interval
'Calculate time lag between start of wavemark and
'Get sampling resolution
'Determine number of points and point prior to peak
'Find expiry time before first stage
'Set stage of session to first
'Start at time
'Event number
'Heart beat
'Code for last heart beat
'Repeat
'Wavemark
'Display progress in toolbar
'Get next heart beat

```

```

too long for complete search of IBI
'If IBI is less than constant SearchBP
'Use IBI as window
'IBI

'Search for systolic BP
if HBS[hb%][1]<searchBP then
  BPwindow:=time;
else
  BPwindow:=timeLast+SearchBP;
endif;
result:=Minmax(2,timeLast,BPwindow,minBP,maxBP,tminBP,tmaxBP); 'Get blood pressure values
if (result=0) then
  HBS[hb%][4]:=maxBP;
  HBS[hb%][6]:=maxBP-timeLast;
  HBS[hb%][3]:=minBP;
  HBS[hb%][5]:=tminBP-timeLast;
else
  Message("Minmax() function returned an error! [BP]");
  'End of Minmax()error check
endif;
if (timeLast<stageExpires) then
  HBS[hb%][7]=KP[stage%][1];
else
  repeat
    stage%:=stage%+1;
    stageExpires:=KP[stage%][0];
  until timeLast<stageExpires or stage%=keys%-1;
  HBS[hb%][7]=KP[stage%][1];
endif;
'Debug();
hb%:=hb%+1;

'Keypress(es) occurred before this heart beat
keypresses until keypress following HB is found

determining which stage the heart beat belongs to
counter
  endif;
  timeLast:=time;
  codeLast:=1;
  Message("Error: Wavemark code <0");
  'Oops - not good
  endcase;
  'Debug();
  n%:=n%+1;
  event counter
    until (time<0) or (n%>=beats%-1);
  'This should never happen
  'Wavemark id
  'Used to debug extraction
  'Increment
  'Finished extracting information from heart beats?
  'Save time of the heart beat
  'Designate the last event as
  'Increment heart beat
  'Add one to stage
  'Determine when stage ends
  'Set current stage
  'End of
  'Go through
  'Set to current, ongoing stage
  'Record maximum blood pressure
  'Record relative time offset of BP maximum
  'Record minimum blood pressure
  'Record relative time offset of BP minimum
  'An

```

```

end;
return hb%;
Func OpenErrorFile%()
var n%,handle%,path$;
'Set error file
path$:=FilePath$();
n%:=FilePathSet("C:\My Documents");
if n%<0 then
    Message("Error in setting file pathway in DoFilter%(): "+str$(n%)); 'Print error message
    Debug();
endif;
handle%:=FileOpen(DataFile$+"_X.txt",8,1,"Save error data to:"); 'Open a file to output data
if handle%<0 then
    Message("Error output file not opened!");
endif;
return handle%;
end;
func filterParams%()
var ok%;
'Set the variables above for initial values
filter[0]:=40;
filter[1]:=200;
filter[2]:=35;
filter[3]:=275;
filter[4]:=20;
filter[5]:=160;
filter[6]:=0.0;
filter[7]:=searchBP;
filter[8]:=0.20;
filter[9]:=searchBP;
DigCreate("Cardiac data filter parameters"); 'Start new dialog
DigReal(1,"Minimum heart rate (BPM)",0,100);
DigReal(2,"Maximum heart rate (BPM)",50,250);
DigReal(3,"Minimum blood pressure (mm Hg)",0,150);
DigReal(4,"Maximum blood pressure (mm Hg)",50,300);
DigReal(5,"Minimum [max-min] BP (mm Hg)",0,100);
DigReal(6,"Maximum [max-min] BP (mm Hg)",20,180);
DigReal(7,"Minimum time relative to next beat [min BP] (secs)",0,0.25);
DigReal(8,"Maximum time relative to next beat [min BP] (secs)",0.2,SearchBP);
DigReal(9,"Minimum time [max BP] (secs)",0.15,0.35);
DigReal(10,"Maximum time [max BP] (secs)",0.3,SearchBP);

```

'Get current directory

'Set save directory

'Error flag was returned

'Minimum heart rate
'Maximum heart rate
'Minimum BP
'Maximum BP
'Minimum BP delta
'Maximum BP delta
'Minimum time for BP low
'Maximum time for BP low
'Minimum time for BP high
'Maximum time for BP high

```

ok% := DigShow(filter[0],filter[1],filter[2],filter[3],filter[4],filter[5],filter[6],filter[7],filter[8],filter[9]); 'ok%=0 if user cancels
return ok%;

end;

Proc FilterData()
var beat%,n%,x%,minT,maxT;
MakeFilterChannels();
FrontView(FilterFileView%);
for beat%=0 to (totalHBs%-1) do
    if (beat% mod 100=0) then
        ToolbarText("File: "+DataFile$+" Manual filtering HB: "+str$(beat%));
    endif;
    for n%=8 to 9 do
        HBs[beat%][n%]=0;
    next;
    'Check to see how many beats are in window
    x%=0;
    n%=beat%-1;
    minT:=HBs[beat%][0]-filter[10];
    while (n%>=0) do
        if (HBs[n%][0]>=minT) then
            x%=x%+1;
        else
            n%=n%-1;
            n%=-1;
        endif;
    wend;
    counter to negative to stop loop
    UserDefinedCheck(beat%);
next;
'Statistical filtering - build up average waveform for BP
FrontView(DataView%);
for beat%=0 to (totalHBs%-1) do
    if (beat% mod 100=0) then
        ToolbarText("File: "+DataFile$+" Building average of BP waveform: "+str$(beat%));
    endif;
    if (HBs[beat%][8]+HBs[beat%][9]=0) then
        minT:=HBs[beat%][0];
    endif;
    'Display progress in toolbar
end;

'Make filter channels for displaying filter data
'Make filter file log focus of output
'Display progress in toolbar

'Clear screening flags
'Set all flags to OK

'Start with counter = 1 for current HB
'Calculate start of window relative to current beat
'Search backward in array for heartbeats until window limits
'Increment number of HBs found in
'Set counter to search for next previous beat
'Early limit of window reached - set

'Bring data window to front
'Check using manual parameters
'If manual checked passed
'Calculate time start of window

```

```

maxT:=min T+HBs[beat%][1];
SumBP(beat%,minT,maxT);
endif;
'Calculate end of window (first HB + IBI)
'Routine for statistics check
'End if manual check passed

next;
for n%:=0 to BPsamples%-1 do
  BPavg[n%][2]=BPavg[n%][1]/BPavg[n%][0];
  'Calculate mean BP waveform a point at a time
  'Mean = sum of mm Hg / number of HBs
  'Statistical filtering
  'Now correlate BP waveform HB-by-HB to average BP waveform
  for beat%:=0 to (totalHBs%-1) do
    if (beat% mod 100=0) then
      ToolbarText("File: "+DataFile$+" Correlating BP waveform with average: "+str$(beat%));
    endif;
    if (HBs[beat%][8]+HBs[beat%][9]=0) then
      StatCheck(beat%);
      'If manual checked passed
    endif;
  next;
  'Routine for statistics check
  'End if manual check passed

end;

Proc SumBP(beat%,t1,t2);
var delta,interval,n%,t,BP,flag%,binsz;
delta:=2-t1;
interval:=delta/BPsamples%;
for n%:=0 to (BPsamples%-1) do
  t:=1+(n%*interval);
  BP:=ChanValue(2,t,flag%);
  if (flag%=0) then
    Debug();
  else
    BPavg[n%][0]+=1;
    BPavg[n%][1]+=BP;
    BPs[beat%][n%]:=BP;
  endif;
next;
'Bring data view to front
'Create a filter channel
'Error

Proc MakeFilterChannels()
var x%;
FrontView(DataView%);
for x%:=0 to 8 do
  FilterChannels[x%]=MemChan(3,0);
  if FilterChannels[x%]=0 then
    Debug();
  else

```

```

ChanShow(FilterChannels%(x%));
docase
    case x%=0 then ChanTitle$(FilterChannels%(x%)," - BPM");
    case x%=1 then ChanTitle$(FilterChannels%(x%)," + BPM");
    case x%=2 then ChanTitle$(FilterChannels%(x%)," - BP");
    case x%=3 then ChanTitle$(FilterChannels%(x%)," + BP");
    case x%=4 then ChanTitle$(FilterChannels%(x%)," - dBP");
    case x%=5 then ChanTitle$(FilterChannels%(x%)," + dBP");
    case x%=6 then ChanTitle$(FilterChannels%(x%)," t dia");
    case x%=7 then ChanTitle$(FilterChannels%(x%)," t sys");
    case x%=8 then ChanTitle$(FilterChannels%(x%)," BP r");
    else Debug();
endcase;
endif;
DrawMode(FilterChannels%(x%),2);
next;

end;

Proc FilterShow(flag,beat%)
var CurrentView%;

CurrentView%:=View();
FrontView(DataView%);
MemSetItem(FilterChannels%(flag),0,HBs[beat%][0]); 'Show in data file
FrontView(CurrentView%);

end;

Proc StatCheck(beat%)
var n%,count%,SumX,SumXX,SumY,SumYY,SumXY,SumXnum,den,r;

FrontView(FilterFileView%);
'Debug();
Print("n: %d\t: %d\t: Statistical\n",beat%,HBs[beat%][0]);
for n%=0 to BFsamples%-1 do
    count%+=1;
    SumX+=BPs[beat%][n%];
    SumXX+=(BPs[beat%][n%]*BPs[beat%][n%]);
    SumY+=BPavg[n%][2];
    SumYY+=(BPavg[n%][2]*BPavg[n%][2]);
    SumXY+=(BPs[beat%][n%]*BPavg[n%][2]);
next;

num:=(count%*SumXY)-(SumX*SumY);
den:=Sqrt(((count%*SumXX)-(SumX*SumX))*((count%*SumYY)-(SumY*SumY)));

r
'Numerator for

```

'Show Channel

'Draw as vertical ticks on a line

'Indicate on filter channel that beat failed a test

'Get current view handle
'Bring data view to front

'Restore view

'Calculate r between individual BP waveform and overall average

'Set current view to filter log file

'Calculate intermediate statistics

'Increment count of points

'Sum of individual BP data

'Sum of average BP data

'Sum of squared individual BP data

'Sum of average BP data squared

'Sum of products

'Denominator for r

num:=(count%*SumXY)-(SumX*SumY);

den:=Sqrt(((count%*SumXX)-(SumX*SumX))*((count%*SumYY)-(SumY*SumY)));

r

'Numerator for

```

r:=num/den;
'Calculate r

if (r>=Rcrit) then
'Accept as valid BP waveform if correlation above threshold
  HBs[beat%][10]=0;
'Flag as OK
else
  HBs[beat%][10]=1;
'Correlation is too low, waveform is treated as artifact
'Flag as artifact
  Print("\n r < critical value [ %f , %f ]\n", r,Rcrit);
  FilterShow(8,beat%);
'Show tick mark in filter channel
endif;

end;

'Print out error message

'Statistical BP check

Proc UserDefinedCheck(n%)
var deltaBP;
Print("\n: %d\t\t: %f\t User\n",n%,HBs[n%][0]);

'Check heart beat
if HBs[n%][2]>=filter[0] then
  if HBs[n%][2]>filter[1] then
    HBs[n%][8]=1;
    Print("\nBPM too high [%f %f]\n",HBs[n%][2],filter[1]);
    FilterShow(1,n%);
  endif;
else
  HBs[n%][8]=1;
  Print("\nBPM too low [%f %f]\n",HBs[n%][2],filter[0]);
  FilterShow(0,n%);
endif;

'Flag to skip

'Flag to skip all

'BPM is < min

'Check BP
if (HBs[n%][8]=0) then
  if HBs[n%][3]<filter[2] then
    HBs[n%][9]=1;
    Print("\nBP too low [%f %f]\n",HBs[n%][3],filter[2]);
    FilterShow(2,n%);
  endif;
  if HBs[n%][4]>filter[3] then
    HBs[n%][9]=1;
    Print("\nBP too high [%f %f]\n",HBs[n%][4],filter[3]);
    FilterShow(3,n%);
  endif;
endif;

'Check BP only if BPM is valid

'min BP < min allowed

```

```

allowed
endif;
deltaBP:=HBS[n%][4]-HBS[n%][3];
if deltaBP<filter[4] then
  Hbs[n%][9]=1;
  Print("\deltaBP too low [%f %f]\n",deltaBP,filter[4]);
  FilterShow(4,n%);
endif;
difference allowed
if deltaBP>filter[5] then
  Hbs[n%][9]=1;
  Print("\deltaBP too high [%f %f]\n",deltaBP,filter[5]);
  FilterShow(5,n%);
endif;
difference allowed
if ((HBS[n%][5]<filter[6]) or (HBS[n%][5]>filter[7])) then
  Hbs[n%][9]=1;
  Print("\Time of diastolic [%f %f %f]\n",Hbs[n%][5],filter[6],filter[7]);
  FilterShow(6,n%);
endif;
acceptable
if ((HBS[n%][6]<filter[8]) or (HBS[n%][6]>filter[9])) then
  Hbs[n%][9]=1;
  Print("\Time of systolic [%f %f %f]\n",Hbs[n%][6],filter[8],filter[9]);
  FilterShow(7,n%);
endif;
acceptable
'Debug();
endif;
end;
'User defined heart beat parameter check

Proc SaveFilterChannels()
var x%,y%;
for x%=0 to 8 do
  y%=10+x%;
  MemSave(FilterChannels[x%],y%);
  DrawMode(y%,2);
  ChanShow(y%);
  ChanDelete(FilterChannels[x%]);
next;
end;
'Calculate high - low BP
'max BP > max
'max-min BP < min
'max-min BP > max
'Time of min BP not
'Time of min BP not
'For each filter channel
'Calculate new channel id #
'Draw as vertical ticks on a line
'Save channel in data file

```

Spike Script Parameters

Manual Parameter Settings

Default Parameters:

1. Minimum HR (bpm)	40
2. Maximum HR	200
3. Minimum BP (mm Hg)	35
4. Maximum BP	275
5. Minimum SBP-DBP	20
6. Maximum SBP-DBP	160
7. Min time to next HB (sec)	0.00
8. Max time to next HB	0.45
9. Min time of SBP from HB	0.2
10. Max time of SMP from HB	0.45

Subject parameters were set at default except for the following cases:

Time One

- Subject 008: parameter 6 = 170
- Subject 012: parameter 4 = 280
- Subject 022: parameter 6 = 170
- Subject 023: parameter 1 = 35
- Subject 028: parameter 5 = 10

Time Two

- Subject 001: parameter 5 = 10
- Subject 007: parameter 5 = 10
- Subject 013: parameter 9 = 0.18
- Subject 024: parameter 9 = 0.18
- Subject 028: parameter 6 = 180

Acoustic Spike 2 Script

```

'Modifications:
'15/06/00
'22/06/00
'25/10/00

'Declare global variables
var DataFile$;
const sizeHbs%=10000;
var HBS[sizeHBS%][11],totalHBS%;
heart beats, n of heart beats
var DataView%;
var FilterFileView%;
var WMmaxT;
var searchBP:=0.45;
var filter[11];
const BPsamples%:=25;
var BPavg[BPsamples%][3];
'Array to hold average BP waveform. [x][0]=n cases; [x][1]=sum; [x][2]=mean

indicates BP at x/BPsamples% of the interval 'tween HB's
var BPs[sizeHBS%][BPsamples%];
individual HBS
const Rcrit:=0.337;
var FilterChannels%[9];

startle[3];
const sizeStartleEvents%=500;
var StartleEvents[sizeStartleEvents%][2];
'Array holding channel information for filter events
'Array holding pre-trigger offset; post-trigger offset and bin time
'Size of array used to hold time of events during startle task
'Array to hold events (soft, loud tones) during startle testing

(0=soft; 1=loud)

'Name of data file
'Handle for input data file
'Maximum time of wavemark channel with ECG
'Dimension an array to hold parameters for filtering
'Array to hold BP data from
'[][]=time; [][]=type

'Set size of memory array for putative heartbeats
'Handle for file containing log of filtering process
'Time window after heart beat in which min BP & max BP are searched
'Critical value of correlation coefficient with d.f.=23, p=0.05, 1-tailed

```

EMB Added filter lines

EMB Saved filter lines#

EMB Added analysis of startle data

'Save memory space for 50k

(x above

'Array to hold BP data from

' [][]=time; [][]=type

```

'Generated toolbar code
DoToolBar0; 'Try it out
Halt;

Func DoToolBar0 'Set your own name...
ToolBarClear0; 'Remove any old buttons
ToolBarSet(7, "&All", DoAll%);
ToolBarSet(6, "&Data", DoData%); 'Link to function
ToolBarSet(5, "&Extract", DoExtract%); 'Link to function
ToolBarSet(4, "&Filter", DoFilter%); 'Link to function
ToolBarSet(3, "&Save output", DoSave%);
ToolBarSet(2, "&Startle analysis", DoStartle%);
ToolBarSet(1, "&Quit", DoQuit%); 'Link to function
ToolBarEnable(5,0);
ToolBarEnable(4,0);
ToolBarEnable(3,0);
ToolBarEnable(2,0);
return ToolBar("Cardiac analysis: EMB 10/00 Version 4.0", 13);
end;

Func DoAll%0
DoData%0;
DoExtract%0;
DoFilter%0;
DoSave%0;
return(1);
end;

Func DoData%0 'Button 1 routine
var DataFiles%(2),file%,x%,y%;
ToolBarEnable(5,0);
ToolBarEnable(4,0);
ToolBarEnable(3,0);
ToolBarEnable(2,0);
file%:=0;
file%:=CheckFiles%(DataFiles%[]);
if file%≠ 1 then
DataFiles%:=DataFiles%[];
FrontView(DataView%);
DataFile$:=FileName$(3)+FileName$(4);
ToolBarText("File: "+DataFile$);
'Get name of data file
'Bring it to front
'Find data window
'Display it in toolbar

```

```

"+str$(x%); endif;

count
    'Display progress in toolbar
    for x%:=0 to (sizeHBs%-1) do
        'Zero heart beat array
        if (x% mod 100=0) then ToolbarText("File: "+DataFile$+" Clearing HBs[]:
        for y%:=0 to 10 do
            HBs[x%][y%]=0;
        next;
        totalHBs%=0;
        next;
        'Reset heart beat

        'Message("Mark sure that WarkMark has been performed before using Extract button");
        ToolbarEnable(5,1);
        ToolbarText("File: "+DataFile$);
        'Display file in toolbar
    end;

return 1; 'This leaves toolbar active
end;

Func DoExtract%() 'Button 2 routine
    var keys%,channels%[2],beats%;
        'Get key presses
        FrontView(DataView%);
        keys%:=Count(31,0,MaxTime(31))+2;
        'Get the number of key presses and add 2 to prevent running out of memory
        var keymarks[keys%][2];

        GetKeyPresses(keys%,keymarks[][]);

        presses
        'Extract physiological data
        ChanList(channels%[],16);
        if (channels%[0]>0) then
            WMmaxT:=MaxTime(channels%[1]);
            beats%:=Count(channels%[1],0,WMmaxT);
            'Determine maximum number of heart beats
            Debug();
            if (beats%>=sizeHBs%) then
                'Set global variable to hold value of maximum time of waveform
                'Poll for any waveform channels
                'Is there a waveform channel open?
                'Find and save keyboard
                'Bring data window to front
                'Dimension array to save keypresses
                'Are there more heart beats than reserved in HBs array

```

```

("+str$(sizeHbs%)+") for HBs[]);

Message("Data overflow: Heart beats (" +str$(beats%)+") >= sizeHBs% constant
halt;

'Abort script
endif;

'End data overflow error
totalHbs%:=GetPhysioData%(channels%[],keymarks%[],keys%,beats%);
ToolBarEnable(4,1);
ToolBarEnable(3,1);
ToolBarText("File: "+DataFile$)
'Display file title in toolbar

else
    'No wavemark data
    Message("No WaveMark channel found! Create wavemark channel and hit extract button again");
endif;

return 1; 'This leaves toolbar active
end;

Func DoFilter%( ) 'Button 3 routine
var ok%,handle%;
'Set the filtering parameters
ok%:=filterParams%(0);
if (ok%=1) then
    'Open the error file
    handle%:=OpenErrorFile%(0);
    if handle%>=0 then
        'Save handle of filter log to global variable
        FilterFileView%:=handle%;
        FilterData0;
        'Must follow statement above
        ToolbarText("File: "+DataFile$);
        ToolbarEnable(2,1);
        'Enable startle button
    endif;
else
    Debug0;
endif;

'Display file name

return 1; 'This leaves toolbar active
end;

```

```

Func DoSave%() 'Button 3 routine
var handle%,n%,datum%,stage$,lastStage$,printHBs%,path$,
    path$:=FilePath$0;
n%:=FilePathSet("C:\My Documents");
if n%<0 then
    Message("Error in setting file pathway in DoSave%(): "+str$(n%));
    Debug();
endif;
handle%:=FileOpen(DataFiles$+"_HB.txt",8,1,"Save cardiac data to:");
docase
case handle%<0 then
else
    'Error flag was returned
    Message("Data output file not opened! Aborting saving of cardiac data...");
    Print("Time!");
    Print("IB!");
    Print("Hz!");
    Print("MinBP!");
    Print("MaxBP!");
    Print("tMinBP!");
    Print("tMaxBP!");
    Print("Stage!");
    for n%:=0 to (totalHBs%-1) do
        'For total number of putative heartbeats
        if (n% mod 100=0) then ToolbarText("File: "+DataFiles$+" Save HB:
        if HBs[n%][8]=0 then
            'Print only if there are no
            printHBs%:=printHBs%+1;
            'Increment
            for datum%:=0 to 7 do
                'For each datum for a given
                docase
                case datum%=7 then
                    'Print the stage
                    'Store the current stage
                    docase

```

Func DoSave%() 'Button 3 routine

var handle%,n%,datum%,stage\$,lastStage\$,printHBs%,path\$;

path\$:=FilePath\$0;

n%:=FilePathSet("C:\My Documents");

if n%<0 then

Message("Error in setting file pathway in DoSave%(): "+str\$(n%));

Debug();

endif;

handle%:=FileOpen(DataFiles\$+"_HB.txt",8,1,"Save cardiac data to:");

docase

case handle%<0 then

else

" "+str\$(n%)); endif;

'Display progress in toolbar

flags to skip all data from this "HB"

counter of number of heart beats printed

putative heartbeat

of the putative HB

stage\$:=Chr\$(HBs[n%][datum%]);

'Get current directory

'Set save directory

'Print error message

Message("Data output file not opened! Aborting saving of cardiac data...");

Print("Time!");

Print("IB!");

Print("Hz!");

Print("MinBP!");

Print("MaxBP!");

Print("tMinBP!");

Print("tMaxBP!");

Print("Stage!");

for n%:=0 to (totalHBs%-1) do

'For total number of putative heartbeats

if (n% mod 100=0) then ToolbarText("File: "+DataFiles\$+" Save HB:

if HBs[n%][8]=0 then

'Print only if there are no

printHBs%:=printHBs%+1;

'Increment

for datum%:=0 to 7 do

'For each datum for a given

docase

case datum%=7 then

'Print the stage

'Store the current stage

docase

```

then
defined recording pause
    case stage$="-"
    'Start of user

then
defined recording pause
    'Denote sampling problems during
    case stage$="-+"
    'End of user

stage$>="a" and stage$<="z" then
    'Print the last valid stage
    case
    'If stage is a lowercase letter
    'Print the current stage
    'Record last
    else
    'Unrecognized character

stage
    Print("%c\n",lastStage$);
    Print("%c\n",lastStage$);
    Print("%c\n",stage$);
    lastStage$:=stage$;
    Print("?n");
    'Print ? in the column
    lastStage$:="?";
    'Record last stage as unknown

(HBs[n%][9]+HBs[n%][10]=0) then
then
    'Special procedure for time of diastolic (have to add IBI)
    Print("%\t",HBs[n%][5]+HBs[n%][1]);
    Print("%\t",HBs[n%][datum%]);
    'End of check regarding time of diastolic

'Datum is not the time of the diastolic
'Print data
end;

'Add IBI to time of diastolic to get true relative time of diastolic
else
    'If flags to skip BP data are false
    if datum%=5
    'Print BP data from putative HB
    if
    case datum%/>=3 and datum%/<=6 then
    endcase;
    'Do not print
end;

```

```

Print("\t");
endif;
endif;
else
'Print datum
endcase;
next;
endif;
next;
ToolBarText("File: "+DataFiles+" Printed HBs: "+str$(n%));
FileClose();
'Restore working directory to old value

'Print nothing in the column

Print("%\t",HBs[n%][datum%]);

'Print status to toolbar
endcase;
n%:=FilePathSet(path$);
if n%<0 then
Message("Error in setting file pathway in DoSave%0: "+str$(n%));'Print error message
Debug();
endif;
SaveFilterChannels();

var ok%,events%;
ok%:=startleParams%0;
if (ok%/=1) then
events%:=GetStartleEvents%0;
DoStartleAnalysis(events%);
Message("Analysis of startle data cancelled!");
else
endif;

return 1; 'This leaves toolbar active
end;

Func DoStartle%0;

events%:=GetStartleEvents%0;
DoStartleAnalysis(events%);
Message("Analysis of startle data cancelled!");

return 1;
end;

Func DoQuit%0 'Button 4 routine
'Your code in here...
return 0; 'This stops the toolbar

```

'User hit OK in dialog box

'Get timing parameters

'Get number of soft and loud tones

```

end;
Func CheckFiles%(Array%[])
    'Determine if there are open files
    'Set
    var files%,flag%;
    flag%:=0;
    files%=ViewList(Array%[],1);
    'Get open files
    if (files%=0) then
        Array%[0]=FileOpen("",0,7,"Open a cardiac data file:");
        'Open a file
        if Array%[0]>0 then
            flag%:=1;
            'If a file is successfully loaded
        else
            'Data file not open, user hit cancel or there was an error
            Message("Warning: Data file not open");
            'Debug0;
        endif;
    else
        'There is a file open
        flag%:=1;
    endif;
    'Flag
end;
Proc GetKeyPresses(keys%,array%[])
    var n%,time,code%[4];
    n%:=0;
    time:=-1;
    'Set to ensure start of search at very beginning
    repeat
        'Get times of key presses marking testing stages
        'Set current keypress to 0
    until key%>0
    'Loop through keypresses
end;

```



```

start
    hb%=0;
    codeLast=0;
    repeat
        if (hb% mod 100=0) then ToolbarText("File: "+DataFile$+" Extracting HB: "+str$(hb%)); endif; 'Display progress in toolbar
        time:=NextTime(channels%[1],time,code%[]);
    do case
        cardiac potential?
            'Heart beat number is zero to
            'Code for last heart beat (0=artifact or initial)
            'Repeat extraction for all
            'Wavemark artifact or true
            case code%[0]=0 then
                'Artifact or first HB
                codeLast:=0;
                'Record that last event was not true
            case code%[0]>0 then
                'Belongs to wavemark template (i.e., true cardiac potential)
                if (codeLast=1) then
                    'If last heart beat was filtered
                    HBs[hb%][0]:=timeLast+offset;
                    'Save time of first heart beat, accounting for lag in wavemark between start and peak
                    HBs[hb%][1]:=time-timeLast;
                    'Save inter-beat interval
                    HBs[hb%][2]:=60/(time-timeLast);
                'Save heart rate (BPM) for the interval
            else
                'Search for systolic BP
                if HBs[hb%][1]<searchBP then
                    'If IBI is less than constant SearchBP
                    BPwindow:=time;
                    'Use IBI as window
                else
                    'IBI too long for
                    BPwindow:=timeLast+SearchBP;
                    'Use SearchBP constant to set maximum window
                endif;
            if (result=0) then
                'If Minmax() did not return a negative error code
                HBs[hb%][4]:=maxBP;
                'Record maximum blood pressure
    result:=Minmax(2,timeLast,BPwindow,minBP,maxBP,tminBP,tmaxBP); 'Get blood pressure values

```

complete search of IBI

```

    HBs[hb%][6]:=tmaxBP-timeLast;
    HBs[hb%][3]:=minBP;
    'Record minimum blood pressure
    HBs[hb%][5]:=tminBP-timeLast;
    'Record relative time offset of BP minimum
    else
        'An error has
        Message("Minmax() function returned an error!");
    endif;
    'End of

    if (timeLast<stageExpires) then
        HBs[hb%][7]:=KP[stage%][1];
    'Set to current, ongoing stage
    else
        'Keypress(es)
        repeat
            'Go through keypresses until
            stage%:=stage%+1;
            'Add one to stage
            stageExpires:=KP[stage%][0];
        until timeLast<stageExpires or stage%=keys%-1;
        HBs[hb%][7]:=KP[stage%][1];
    'Determine when stage ends
    'Set current stage
    endif;
    'End of determining which
    'Debug();
    hb%:=hb%+1;
    'Increment heart beat counter
    endif;
    timeLast:=time;
    'Save time of the heart beat
    codeLast:=1;
    'Designate the last event as having been
else
    Message("Error: Wavemark code <0");
    'Oops - not good

```

'If the heart beat occurred between keypresses

'This should never happen

occured
[BP]");
Minmax()error check

occured before this heart beat
keypress following HB is found

stage the heart beat belongs to

a true cardiac potential

```

extracting information from heart beats?
end;
Func OpenErrorFile%()
    endcase;
    'Wavemark id
    'Used to debug extraction routine
    'Increment event counter
    'Finished

    until (time<0) or (n%>=beats%-1);
        return hb%;
    var n%,handle%,path$;
    'Set error file
    path$=FilePath$0;
    n%:=FilePathSet("C:\My Documents");
    if n%<0 then
        Message("Error in setting file pathway in DoFilter%(): "+str$(n%));
        Debug();
    endif;
    handle%:=FileOpen(DataFiles+"_X.txt",8,1,"Save error data to:");
    if handle%<0 then
        'Error flag was returned
        Message("Error output file not opened!");
    endif;
    return handle%;
end;
func filterParams%()
    'Set the variables above for initial values
    filter[0]=40;
    filter[1]=200;
    filter[2]=35;
    filter[3]=275;
    filter[4]=20;
    filter[5]=160;

    'Get current directory
    'Print error message
    'Error flag was returned
    Message("Error output file not opened!");

    'Minimum heart rate
    'Maximum heart rate
    'Minimum BP
    'Maximum BP
    'Minimum BP delta
    'Maximum BP delta

```

```

filter[6]=0;
filter[7]=searchBP;
filter[8]=0.20;
filter[9]=searchBP;

'Minimum time for BP low

'Maximum time for BP low

'Minimum time for BP high

'Maximum time for BP high

DigCreate("Cardiac data filter parameters"); 'Start new dialog
  DigReal(1,"Minimum heart rate (BPM)",0,100);
  DigReal(2,"Maximum heart rate (BPM)",50,250);
  DigReal(3,"Minimum blood pressure (mm Hg)",0,150);
  DigReal(4,"Maximum blood pressure (mm Hg)",50,300);
  DigReal(5,"Minimum [max-min] BP (mm Hg)",0,100);
  DigReal(6,"Maximum [max-min] BP (mm Hg)",20,180);
  DigReal(7,"Minimum time relative to next beat [min BP] (secs)",0,0.25);
  DigReal(8,"Maximum time relative to next beat [min BP] (secs)",0.2,SearchBP);
  DigReal(9,"Minimum time [max BP] (secs)",0.15,0.35);
  DigReal(10,"Maximum time [max BP] (secs)",0.3,SearchBP);

ok% := DigShow(filter[0],filter[1],filter[2],filter[3],filter[4],filter[5],filter[6],filter[7],filter[8],filter[9]);
return ok%;

var beat%,n%,x%,minT,maxT;
  MakeFilterChannels();
'Make filter channels for displaying filter data
  FrontView(FilterFileView%);
for beat%=0 to (totalHBs%-1) do

log focus of output

'Make filter file

if (beat% mod 100=0) then
  ToolbarText("File: "+DataFile$+" Manual filtering HB: "+str$(beat%));
endif;
'Display progress in toolbar

for n%:=8 to 9 do
  'Clear screening flags
  HBs[beat%][n%]=0;
'Set all flags to OK
next;

```

```

'Check to see how many beats are in window
x%=0;

'Start with counter = 1 for current HB
n%=beat%-1;
'Start with last beat
minT:= HBs[beat%][0]-filter[10];
while (n%>=0) do
'Search backward in array for heartbeats until window limits reached
if (HBs[n%][0]>=minT) then
  x%=x%+1;
  'Increment number of HBs found in window
  n%=n%-1;
'Set counter to search for next previous beat
else
  n%=n%-1;
  'Early limit of window reached - set counter to
  wend;
endif;

UserDefinedCheck(beat%);

ToolbarText("File: "+DataFile$+" Building average of BP waveform: "+str$(beat%));
'Display progress in toolbar
'If manual checked passed
minT:=HBs[beat%][0];
'Calculate time start of
maxT:=minT+HBs[beat%][1];
SumBP(beat%,minT,maxT);
'Routine for statistics check
'End if manual check passed
'Calculate mean
'Mean = sum of mm Hg / number of HBs

```

'Calculate start of window relative to current beat

'Check using manual parameters
next;

'Statistical filtering - build up average waveform for BP
FrontView(DataView%);
'Bring data window to front
for beat%=0 to (totalHBs%-1) do

if (beat% mod 100=0) then
endif;

if (HBs[beat%][8]+HBs[beat%][9]=0) then

'Calculate end of window (first HB + IBI)

endif;

next;
for n%=0 to BPsamples%-1 do

BPavg[n%][2]=BPavg[n%][1]/BPavg[n%][0];

next;

BP waveform a point at a time

negative to stop loop

window

```

"+str$(beat%);

'Now correlate BP waveform HB-by-HB to average BP waveform
for beat%:=0 to (totalHBs%-1) do
    if (beat% mod 100=0) then
        'Statistical filtering
        ToolbarText("File: "+DataFile$+" Correlating BP waveform with average:");
    endif;
    if (HBs[beat%][8]+HBs[beat%][9]=0) then
        'Display progress in toolbar
        'If manual checked passed
        StatCheck(beat%);
        'Routine for statistics check
        'End if manual check passed
    endif;
    next;

end;

Proc SumBP(beat%,t1,t2);
'Perform intermediate calculations to get average normalized BP waveform
var delta:interval,n%,t,BP,flag%,binsz;
    delta:=t2-t1;
    interval:=delta/BPsamples%;
    for n%:=0 to (BPsamples%-1) do
        'Calculate current time of segment
        BP:=ChanValue(2,t,flag%);
        t=t1+(n%*interval);
        if (flag%=0) then
            'Get the time between the heartbeats
            'Divide the IBI
            'Step through each segment
            'Get
            'Something went wrong
            Debug();
            'Should not happen
            'Value returned
            BPavg[n%][0] += 1;
            'Increment numbers of cases
            BPavg[n%][1] += BP;
            'Add to running sum
            BPs[beat%][n%] := BP;
            'Save data from individual beat for later comparison to mean
            'End if no BP value returned
        else
            'Get
        endif;
    next;

end;

```

```

Proc MakeFilterChannels()
    var x%,
    FrontView(Data View%),
    'Bring data view to front
    for x%=0 to 8 do
        'Make channels for filter events
        FilterChannels[x%]:=MemChan(3,0);
        if FilterChannels[x%]=0 then
            'Create a filter channel
            Error
        else
            'Show Channel
            ChanShow(FilterChannels[x%]);
            docase
                case x%=0 then ChanTitle$(FilterChannels[x%], "- BPM");
                case x%=1 then ChanTitle$(FilterChannels[x%], "+ BPM");
                case x%=2 then ChanTitle$(FilterChannels[x%], "- BP");
                case x%=3 then ChanTitle$(FilterChannels[x%], "+ BP");
                case x%=4 then ChanTitle$(FilterChannels[x%], "- dBP");
                case x%=5 then ChanTitle$(FilterChannels[x%], "+ dBP");
                case x%=6 then ChanTitle$(FilterChannels[x%], " t dia");
                case x%=7 then ChanTitle$(FilterChannels[x%], " t sys");
                case x%=8 then ChanTitle$(FilterChannels[x%], " BP r");
            else Debug();
            endcase;
        end;
        ticks on a line
        DrawMode(FilterChannels[x%],2);
        next;
    end;
    Proc FilterShow(flag,beat%)
    filter channel that beat failed a test
    var CurrentView%;
    CurrentView%:=View();
    'Get current view handle
    FrontView(Data View%);
    'Bring data view to front
    MemSetItem(FilterChannels[flag],0,HBs[beat%][0]);
    FrontView(CurrentView%);
    'Restore view
    end;
    Proc StatCheck(beat%)
    'Calculate r between individual BP waveform and overall average
    'Draw as vertical
    'Indicate on

```



```

var deltaBP;

Print("n: %d\tt: %f\t User\n", n%, HBS[n%][0]);

'Check heart beat
if HBS[n%][2]>=filter[0] then

    'If BPM>=min
    'If BPM>max

    HBS[n%][8]=1;

    Print("\tBPM too high [%f %f]\n", HBS[n%][2], filter[1]);
    FilterShow(1, n%);

    'BPM is < min

    Hbs[n%][9]=1;
    Print("\tBP too low [%f %f]\n", HBS[n%][3], filter[2]);
    FilterShow(2, n%);

    'min BP < min allowed

    Hbs[n%][9]=1;
    Print("\tBP too high [%f %f]\n", HBS[n%][4], filter[3]);
    FilterShow(3, n%);

    'max BP > max allowed
    'Calculate high -

    Hbs[n%][9]=1;
    Print("\tdeltaBP too low [%f %f]\n", deltaBP, filter[4]);
    FilterShow(4, n%);

    'max-min BP < min difference allowed

    Hbs[n%][9]=1;
    Print("\tdeltaBP too high [%f %f]\n", deltaBP, filter[5]);
end if;

else

    'Flag to skip all
    HBS[n%][8]=1;

    Print("\tBPM too low [%f %f]\n", HBS[n%][2], filter[0]);
    FilterShow(0, n%);

    'Check BP
    'Check BP only if BPM is valid
    if (HBS[n%][8]=0) then

        'Check BP
        if HBS[n%][3]<filter[2] then

            'Check BP only if BPM is valid
            if HBS[n%][3]<filter[2] then

                'min BP < min allowed

                Hbs[n%][9]=1;
                Print("\tBP too low [%f %f]\n", HBS[n%][3], filter[2]);
                FilterShow(2, n%);

                'max BP > max allowed
                'Calculate high -

                Hbs[n%][9]=1;
                Print("\tBP too high [%f %f]\n", HBS[n%][4], filter[3]);
                FilterShow(3, n%);

                'max-min BP < min difference allowed

                Hbs[n%][9]=1;
                Print("\tdeltaBP too low [%f %f]\n", deltaBP, filter[4]);
                FilterShow(4, n%);

                'max-min BP < min difference allowed

                Hbs[n%][9]=1;
                Print("\tdeltaBP too high [%f %f]\n", deltaBP, filter[5]);
            end if;

        end if;

    end if;

end if;

low BP

```

```

FilterShow(5,n%);
endif;
if ((HBS[n%][5]<filter[6]) or (HBS[n%][5]>filter[7])) then
    'max-min BP > max difference allowed
    Hbs[n%][9]=1;
    Print("\tTime of diastolic [%f %f]\n",Hbs[n%][5],filter[6],filter[7]);
    FilterShow(6,n%);
endif;
if ((HBS[n%][6]<filter[8]) or (HBS[n%][6]>filter[9])) then
    'Time of min BP not acceptable
    Hbs[n%][9]=1;
    Print("\tTime of systolic [%f %f]\n",Hbs[n%][6],filter[8],filter[9]);
    FilterShow(7,n%);
endif;
'Debug();
end;
'User defined heart beat parameter check
Proc SaveFilterChannels()
    'For each filter channel
    var x%,y%;
    for x%:=0 to 8 do
        y%:=10+x%;
        MemSave(FilterChannels%(x%),y%);
        DrawMode(y%,2);
    end;
    ChanShow(y%);
    'Show the channel
    ChanDelete(FilterChannels%(x%));
    'Delete temporary channel
    next;
end;
'Calculate new channel id #
data file
func startleParams%()
    var ok%;
    'Set the variables above for initial values
    startle[0]:=-10;
    startle[1]=30;
    startle[2]=1;
    'Pre-trigger offset
    'Post-trigger offset
    'Increment

```

```

DigCreate("Startle parameters"); 'Start new dialog
DigReal(1,"Pre-trigger offset (secs prior to tone)",-60.0);
DigReal(2,"Post-trigger offset(secs after tone)",0,300);
DigReal(3,"Time of each bin (secs)",0,30);

ok% := DigShow(startle[0],startle[1],startle[2]); 'ok%=0 if user cancels
return ok%;

end;

Func GetStartleEvents%(
    'Get times of soft/loud tones during startle testing

    'Initialise local variables

    'Bring Data channel to front

    n%:=0;
    'Set current number of tones to 0
    for x%:=0 to 1 do
        time[x%]:=-1;
        flag%[x%]=0;
    end for
    'Set for start of search

    next;
    t:=-1;
    repeat
        'Loop through tones
        for channel%:=4 to 5 do
            x%:=channel%-4;
            if ((flag%[x%]=0) and (time[x%]=-1)) then
                'If search for next event for this channel is required
            end if
        end for
    end repeat

    'Offset in arrays (channel 4 = offset 0, etc.)

    finished flag to 0 (0=not finished)

    time to -1 (search)

    'Set
    'Set

    'For each
    'For each channel

```

```

time[x%]:=NextTime(channel%,t,code%[]);
'Get time and information re: next tone in channel
docase
  case time[x%]=-1 then flag[x%]=1;
  'Data exhausted, no further events available
  case time[x%]<0 then DebugO;
  'NextTime has returned an error
endcase;
endif;

'Next channel
docase
  case time[0]<time[1] then
    'Channel 4 appears to be
    docase
      case flag[0]=0 then x%:=0;
      'Channel 4 time is valid
      else x%:=1;
    endcase;
  case time[1]<time[0] then
    'Channel 5 appears to be
    docase
      case flag[1]=0 then x%:=1;
      'Channel 5 time is valid
      else x%:=0;
    endcase;
  else x%:=-1;
endcase;

'End if search on channel is required
next;

docase
  'Which of two channel events is earliest (ignore them if they are =)
  'Channel 4 is finished and hence channel 5 is earliest
  'Channel 5 is finished and hence channel 4 is earliest
  'Both channel times are equal, an artifact found at onset of testing
endcase;

'Which channel is earliest
docase

```

```

case x%=-1 then
    t:=time[0];
    time[0]:=-1;
    time[1]:=-1;
else
    if (time[x%]>=0) then
        StartleEvents[n%][0]:=time[x%];
        StartleEvents[n%][1]:=x%;
        n%:=n%+1;
        t:=time[x%];
        time[x%]:=-1;
    else
        'Increment count of tones
        'Set searching time
        'Need a new time for this channel
        until (flag%[0]*flag%[1]>0);
        return n%;
    endif;
    'End if a genuine time
    'End of check for times being equal, an artifact
    'Until no more tones
end;

'Times on both channels were equal, an artifact to be discarded
'Set search time to time of artifact
'Set flag to search channel 4
'Set flag to search channel 5

the array

Proc DoStartleAnalysis(events%)
    var PreBins%,PostBins%,binOnset,bin%;
    Var event%,nBin%,bin%,t,PhysioData[3],x%,offset%;

```

```

prior to tones
number of bins after tones
data (Hz; diastolic; systolic are last index)

PreBins%:=1*(startle[0]/startle[2]);
PostBins%:=startle[1]/startle[2];
bins%:=PreBins%+PostBins%;
'Array must be set up after statements above
var StartlePhysio[events%*bins%][3];
for event%:=0 to (events%-1) do
    nBin%:=0;
    for bin%:=(PreBins%*-1) to PostBins%-1 do
        'Reset the ordinal number of the bin
        each bin
        event
        physiological parameter
        ordinal number of bin
        "+str$(binOnset);
        'Calculate number of bins
        'Calculate
        'Reserve memory for startle
        'For each event
        binOnset:=bin%*startle[2];
        'Calculate onset of bin relative to startle
        t:=StartleEvents[event%][0]+binOnset;
        'Calculate time of start of bin
        GetStartlePhysioData(t,PhysioData[]);
        'Get physiological data
        offset%:=(event%*bins%)+nBin%;
        'Calculate offset in first dimension of StartlePhysio[][]
        for x%:=0 to 2 do
            StartlePhysio[offset%][x%]:=PhysioData[x%];
            'For each
            next;
            nBin%:=nBin%+1;
            'Increment
            ToolbarText("File: "+DataFile$+" Startle event: "+str$(event%)+ " Offset:
            next;
            'Next bin
            'Next event
            SaveStartleData(events%,bins%,PreBins%,PostBins%,StartlePhysio[][]);
        end;
    end;
Proc GetStartlePhysioData(t,array[])

```



```

diastolic:=HBs[i%][3];
systolic:=HBs[i%][4];

case (IBIstart>binEnd) then
case (IBIend<binStart) then
case (IBIstart<binStart) then
do case
case (IBIend<=binEnd) then
t:=IBIend-binStart;
else
'IBI encompasses entire bin
t:=binEnd-binStart;
end case;
case (IBIstart>=BinStart) then
do case
case (IBIend<=binEnd) then
t:=IBIend-IBIstart;
else
'IBI straddles end of bin
t:=binEnd-IBIstart;
end case;
'If IBI falls within bin
pBin:=i/(binEnd-binStart);
'Calculate proportion of bin taken up by IBI
array[0]:=array[0]+(pBin*BPM);
array[1]:=array[1]+(pBin*diastolic);
array[2]:=array[2]+(pBin*systolic);

until (HBs[i%][9]+HBs[i%][10]=0);
do case
'IBI straddles start of bin

'IBI entirely within bin

end case;
if ((IBIstart<=binEnd) and (IBIend>=binStart)) then
'Calculate weighted averages of physio parameters
endif;
beat%:=beat%+1;

until ((IBIstart>binEnd) or (IBIend>binEnd));

end;

Proc SaveStartleData(events% bins% pre% post% data[])
var path$n% handle% event% type% nBin% binOnset% offset% bin% Nevenis%[2];
path$:=FilePath$();

```

'Get current directory

```

n%:=FilePathSet("C:\My Documents");
if n%<0 then
    Message("Error in setting file pathway in SaveStartleData( "+str$(n%)); 'Print error message
    Debug();
endif;
handle%:=FileOpen(DataFile$+"_ST.txt",8,1,"Save startle data to.");
do case
case handle%<0 then
    'Error flag was returned
    Message("Data output file not opened! Aborting saving of startle data...");
    Print("Event");
    Print("Number");
    Print("Offset");
    Print("BPM");
    Print("diastolic");
    Print("systolic");
    for event%:=0 to events%-1 do
        nBin%:=0;
        type%:=StartleEvents[event%][1]; 'Determine type of startle events (0=soft;
        Nevents%(type%]:=Nevents%(type%]+1; 'Increment number of presentations
        for bin%:=(pre%*-1) to post%-1 do
            'For each bin
            binOnset:=bin%*startle[2]; 'Calculate onset
            offset%:=(event%*bins%)+nBin%; 'Calculate offset in first dimension of
        do case
        case type%:=0 then Print("Soft");
        case type%:=1 then Print("Loud");
        else
            Message("Error is SaveStartleData( ");
            Debug();
        end case;
        Print ("%d\t",Nevents%(type%]);
        Print ("%d\t",binOnset);
        for n%:=0 to 2 do
            do case

```

l=loud)

of bin relative to startle event

StartlePhysio[]

```

Print("%f\n", data[offset%][n%]);

case n%==2 then
else Print("%f\t", data[offset%][n%]);
endcase
next;
nBin%:=nBin%+1;

Increment ordinal number of bins

next;
ToolbarText("File: "+DataFile$+" Printed startle events: "+str$(event%));
FileClose();
'Restore

'Print status to toolbar
endcase;
n%:=FilePathSet(path$);

if n%<0 then
Message("Error in setting file pathway in SaveStartleData(): "+str$(n%));
Debug();
endif;

working directory to old value

end;

```

Attempted Validation of An Interactive Heart Rate Monitor – 1998 Study

**Validating An Ambulatory
Interactive Heart Rate
Monitor**

**Modified from submitted 1st Year report,
which was a requirement for the PhD. I Lorraine A. Paterson,
hereby certify that this thesis, which is approximately 13,500 words in length,
is solely my work.**

Abstract

Research indicates that cardiovascular reactivity to stress studied within the laboratory may not reflect actual cardiovascular functioning in real-life. Therefore it is necessary to develop ambulatory monitoring equipment which is capable of providing the wealth of information which can be achieved within the laboratory. The aim of this study was to increase the ability to detect and record significantly high heart rates and the accompanying behaviour of subjects during ambulatory monitoring. Nine subjects had their ambulatory heart rate, activity and posture values recorded over 48-hours. A linear regression equation was fitted to each subject's data collected during the daytime of the first day of testing. For each subject this model was used to reprogram the Interactive Heart rate monitor the second day. The Interactive Heart rate monitor continued to record heart rate, activity and posture while applying the heart rate model. The Interactive monitor calculated any day-2 heart rates which were higher ($1.5 \times SD$ of residual heart rate) than that predicted by the day-1 heart rate model, and then emitted a tone to the subject. This tone signalled that the subject should complete a self-report diary page concerning their activities prior to the signal. Each diary page retrieved information on time, place, social situation, activity and subjective mood. The results indicate that in this study the subjective mood scales were unrelated to heart rate and heart rate changes. As predicted, heart rate was significantly higher during the elicited / interactive prompts than the randomly set ones. However, inspection of the models using activity and posture to predict heart rate revealed that there were significant differences between the data collected on the first versus the second days. The results indicate that over the two days of recording subjects engaged in very different behaviours. In conclusion, the Interactive Heart rate monitor operated well, retrieving information during significantly high heart rates, however the subject's heart rate models differed significantly over the course of the 2 days.

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1 Aims and Research Questions

1.1 *The Aims of the Study*

- To examine whether the interactive monitor is accurate and hence valid in predicting high heart rate from assessed activity and posture.
- To determine if the interactive monitor will trigger when the subject is stressed.

1.2 *The Research Questions*

1. Does the regression model of heart rate on activity and posture predict ambulatory heart rate?
2. Can we generate a model of heart rate from the 'mimicking' tasks, which will correlate to a model from the ambulatory data?
3. Does the day 1 model of ambulatory heart rate predict the day 2 ambulatory heart rate model?
4. Can heart rate recordings above that predicted, by the ambulatory heart rate model, be reliably accepted as related to stress and not metabolic activity.
5. When heart rate is high does it relate to differences in Mood State?
6. Does the ambulatory heart rate model predict heart rate response to a known stressor?

1.3 *Comment*

This study is presented for technical background. It must be noted that for the appendix the study has been modified, consequentially it is presented without the original two introductory chapters.

2 Methods

Two pilot subjects were employed to identify any equipment and design problems prior to the main study. The methods and procedure used for the pilots are presented first.

2.1 *Pilot Study*

2.1.1 Participants

The pilot subjects were both female. Their mean age was 26.5 years, mean weight was 67.2kg and their mean height was 1.63m. They were both non-smokers in good general health with no family history of hypertension.

2.1.2 Measures

Monitoring Devices

The monitoring devices were attached to the subject at the start of the experiment and not removed until the end, permitting everything to be recorded. The interactive ambulatory recorder emitted a tone, at pre-set intervals. This tone was programmed to turn off between 10 p.m. and 8 am to permit normal sleep patterns. The subjects used three predefined marker buttons; marker one indicated when a diary page was completed; marker two when the subject felt they were encountering a very stressful situation; marker three when they experienced their, agreed, individual stressor (see later).

The equipment used was a digital RM-10 recorder (Parametric Recorders, Ltd.) which comprised of a recorder unit (weight about 500g) with interchangeable plug-in

modules for the measurement of different physiological parameters, a marker channel, and operating software (RMOS 4.0) for IBM compatible PCs.

Heart rate was measured using a three electrode ECG, attached to the chest. Activity¹ was measured from a small movement detector (shock detector) taped to the thigh and Posture² from a slim water filled tube which ran from the calf to the shoulder. The data from each device was stored on the RM-10 ambulatory recorder secured around the subject's waist by a belt. The data from day 1 was averaged over 60 seconds and then fitted into the regression model. This model predicted heart rate on day 2. A high heart rate on day 2 was considered to be $1.5 \times SD$ of the residual heart rate predicted from the day 1 model. When heart rate was high for 3 out of 4 consecutive minutes a signal was emitted (Interactive Prompt). It should be noted that the day 2 heart rate values were also calculated at the time as an average of 1-minute epochs. Therefore, both days operated on a 1-minute average of heart rate avoiding any incongruity of sampling rate.

Pre-set prompts (PPs) were determined from a random number table between 0 and 90 to simulate a random prompt occurring on average every 1.5 hours. An interactive prompt (IP) was defined as a high heart rate, which has occurred for 3 out of 4 minutes. Further to this the IP was programmed to occur only when the activity level was below the 90% limit since high activity is normal reason for high heart rate. Additionally IPs were inhibited for 4 minutes following physical activity to permit heart rate to return to near normal. Only one prompt of any kind could occur in a 20-minute period. Lastly, no prompts occur during sleep. See Appendix 4 for PP times.

¹ ACT is measured via a single axial, shock detector. Later studies hope to use tri-axial.

² POST is measured via a hydrostatic device consisting of a transducer that records changes between the top and bottom positions (height) of the water column.

The interactive ambulatory monitor allowed study of the interactions between physiological functioning and the characteristics of the subject's behaviour and environment. Therefore information of these interactions must be gained, via means of a convenient and easy to use diary.

Diary

Subjects completed a diary page in response to each tone emitted by the interactive ambulatory monitor. On the first day the tone sounded at predetermined intervals³ (PP). On the second day the tone sounded additionally when heart rate increased beyond that predicted by the day one data (IP). The diary assessed responses to the time, place, social situation, activity type, nutrition / stimulants, physical symptoms and mood. These responses permitted a picture of the subject's environment and influencing factors at the time of the tone, as well as a subjective perspective of the subject's emotions. Mood is known to affect a subject's physiological behaviour. Therefore a mood scale was included in an attempt to increase the accuracy of situation information. This was adapted from the UMACL (Matthews et al., 1990) list of terms, which is reliably sensitive to short term changes, and MacKay's scaling technique (1978). Matthews' UMACL measures dimensions of energetic arousal, tense arousal and hedonic tone. Four terms were selected for each dimension - two negatively weighted and two positively weighted. Generally the term with the highest weighting was used, except in cases where possible ambiguity / confusion would occur, between dimensions. Scoring occurred for a positive response to a positively weighted word, and for a negative response to as negatively weighted word. Matthews believed E reflected 'physiological energy mobilisation', T reflected 'emotional / stress reactions' while H may reflect 'motivational gratification'. Several studies show the UMACL scales to be sensitive to external stressors (Cumberbatch, Millner & Wragg - unpublished and Jacobson, 1938 in Matthews et al., 1990). Stressors evoking a general stress syndrome response are associated with reduced E and H and increased T. Order of word presentation was counterbalanced. Questions regarding threat and challenge have been included in response to Joe Tomakas' (1997)

³ The tone is determined by an equation for randomisation of numbers between 0 and 90 (i.e. a 1.5 hour average).

finding that a threat appraisal produced only moderate cardiac reactivity; while a challenge appraisal produced maximal cardiac reactivity. The diary was designed to focus on the issues of specific interest to the aims and hypotheses (activity, place, and situation) and identify and measure the potential covariates.

Mimics

Each morning the subject was put through a light regime of activity chosen to attempt to mimic the type of behaviour participated in, in real life. It included sitting still, standing and lying down, enabling calibration of the monitoring devices. The mimics also required subjects to walk, climb and descend stairs and listening to music⁴. Requiring the subjects to listen to music was an attempt to replicate relaxing and angst times in the day, whilst taking posture into account. They were asked to rate the music to account for differences in individual taste. The mimics were to act to map out a brief but comparable / equivalent period of realistic activity and reactivity while within the controls of the laboratory.

Stressor

The stressor used was the P.A.S.A.T. (Paced Auditory Serial Addition Test) a standard neuroscience test that has an arousing effect on heart rate and blood pressure. The P.A.S.A.T. was administered while seated. After this the subjects were also required to verbally repeat the P.A.S.A.T. numbers to counter any effects of vocalisation on CV reactivity. The P.A.S.A.T. lasted for 8 minutes and involved only numbers between 1 and 9, with a total of 284 trials. The presentation rate of the trials increased from 2.4 seconds per trial to 2.0 to 1.6 and lastly to 1.2 seconds per trial. It is known to produce an average heart rate increase of around 15bpm, SBP rises by around 18 mm Hg while DBP rises by around 12 mm Hg.

Individual Stressor

Cardiovascular reactivity can be elicited by idiosyncratic events. For this reason it is often not clear why a subject appeared so stressed at a particular time. To help clarify

⁴ Calming instrumental and agitative heavy metal music.

these personal tendencies and permit more accurate information gathering each subject was asked on day one and a note made of their idiosyncratic stressor. This was in the confines of something that could occur in any day. Each individual was then asked to press the third marker button and fill out a specific diary page if they experienced / choose to expose themselves to this event.

2.1.3 Pilot Study Procedure

Subjects were screened for health (lack of known heart disease and non-smoker) and availability of time. Having received their informed consent a morning appointment was arranged. The subject arrived on day one where information was relayed again and written consent obtained. The monitoring devices were attached to the subject and calibrated. After a 5 minute rest period the subject was required to participate in the mimics (light regime of activity) which took them round a small area of the department continuing in the laboratory where the subject was required to listen to music while seated and standing. There were 3-minute rest periods throughout and at the end to permit heart activity to return to normal (near normal) between the different mimics.

The subject was then given instructions on ambulation, tone emission and honest and complete diary usage as well as a contact number for difficulties / problems. They were then asked for their individual stressor. This was left to last so as not to have been on the subject's mind throughout the laboratory testing. The subject then left to attend to their normal daily life, with diary completion, while the interactive monitor recorded.

Subjects returned on day two where the stored data was downloaded and subsequently a regression analysis was performed. The monitor was reprogrammed with the HR model and the devices were recalibrated. Problems and diary were checked over. The subject then completed the same process of mimics as occurred on

day one. After which subjects completed the stressor task. A new diary was provided and the subject left as before with the monitor in place. This time the interactive monitor emitted a tone according to the heart rate equation attained from the previous days data, as well as at predetermined intervals. The subjects were not informed as to this alteration so as not to be concerned that their heart rate was 'high', nor to create a biofeedback effect.

Subjects returned on day three where the same procedure as day one was applied (no stressor). However this time after the mimics the monitor and devices were removed. The subject was thanked, paid and debriefed.

2.1.4 Pilot Study Design

The design of the study was within subjects. Each subject was tested individually. Subjects wore the interactive monitor, continuously, for 50 hours. Within this time the subjects were assessed in the laboratory on three successive mornings: where daily activity was reproduced (mimicked) and monitored. Additionally on the second morning their response to a stressor task was monitored. To prevent order effects the task was counterbalanced. Subjects having worn the interactive monitor for the rest of day one returned on day two where it was reprogrammed using the previous days data. They continued to wear the monitor for another 24 hours. Diary measures were taken throughout to provide information of the subject's behaviour and daily actions.

2.2 *Validation Study*

2.2.1 Participants

Participants were identified with as few exclusions as possible to enhance pragmatic value and generalizability of the study. They had to be healthy (no history of heart disease) and preferably young to minimise the confounding effects of possible undetected cardiovascular disease. They were also all non-smokers, as smoking is known to influence cardiovascular reactivity and variation. Participants who conformed to the above criterion were selected. All were undergraduate or postgraduate students.

2.2.2 Measures

Monitoring Devices

The same as for the pilot study.

Diary

The same as for the pilot study.

Mental Stressor

The same as for the pilot study.

Physical Stressor

At this stage it was decided that nothing was to be gained from continuing to include the mimics in the validation study. Hence the mimics were dropped and 4 minutes of 'effortful' static cycling were included. Two minutes with a 1kg weight then two minutes with a 1.5kg weight resistance, to occur continuously. This provided a record

of high activity in controlled conditions, for reference purposes. This was conducted on day one only.

Individual Stressor

The same as for the pilot study.

2.2.3 Validation Study Procedure

The procedure follows the same format as the pilot study with a few changes:

Subjects were screened for health and their informed consent gained. The subject arrived on day one where the monitoring devices were attached and calibrated. After a 5-minute rest period the subject was required to complete 4 minutes of effortful cycling since the mimics have been totally removed. Instructions on ambulation, tone emission and honestly in completing diary pages as well as a contact number for difficulties / problems were given. They were then asked for their individual stressor. The subject then left and returned to their normal daily life, with diary completion, while the interactive monitor recorded.

Each subject returned on day two where the stored data was downloaded and used to reprogram the interactive monitor. Problems and diary were checked over. The subject completed the stressor task. A new diary was provided and the subject left as before with the monitor in place.

The subject returned on day three where the monitor and devices were removed. The subject was thanked, paid and debriefed. See appendix 7 for protocol.

2.2.4 Validation Study Design

The design of the study was within subjects. Each subject will be tested individually. Subjects wore the interactive monitor, continuously, for 48 hours. Within this time the subjects attended the laboratory on two successive mornings. The first morning consisted of 4 minutes of physical activity while the second morning consisted of the stressor. Subjects having worn the interactive monitor for the rest of day one returned on day two to have it reprogrammed using the previous days data. They continued to wear the monitor for another 24 hours. Diary measures were taken throughout to provide information of the subject's behaviour and daily actions.

3 Results

3.1 Pilot Subjects

The data and subsequent results from the pilot subjects are presented prior to the Validation study, which can be seen in section 3.2.

3.1.1 Heart Rate Models

We measured activity and posture, as there is an accepted assumption of proportionality between the said activity and posture and heart rate. This assumption permits us to advance a model of heart rate, and heart rate reactivity by a means of a regression equation. This takes the form of:

$$HR = k + (\alpha * ACT) + (\beta * POST)$$

where k , α and β are the coefficients gained from the days data.

A linear relationship would be the simplest way of expressing and explaining a model of heart rate (HR). Therefore by using such a linear equation it permits us to assume proportionality between ACT, POST and HR as linear. Typically, we have found that using regression, provides a HR model which explains 50 - 70% of the variance. The variance accounted for in the pilot study ranged from 55 - 71% while in the following study it ranges from 31% to 76%.

The models of ambulatory heart rate were derived from fitting a linear equation to the days data. In other words, creating a regression equation from the HR, activity and posture values for the day. HR, activity and posture were sampled 5 times a second. This raw data was then checked for equipment artifacts and averaged per minute. Minute averages were used to provide data that retained the accurate details without losing anything important and while not being over-cluttered. The averaged HR, activity and posture data was then scaled and downloaded into an ASCII file. Sleep

data was removed from analysis as it would lead to unrealistic ambulatory HR models due to low HR and activity during sleep periods. The ASCII file was then pulled into SPSS 7.5 (Statistical Package for the Social Sciences) as a freefield file where a linear regression analysis was run to determine the coefficients for the ambulatory HR model.

Below are each subject's HR models: one from the day 1 data and one from the day 2 data. Ideally we require the HR models from both days to be similar.

V. acc. = % Variance accounted for by amb. models

Table 3.1a: Subject Tas

Models	Coefficients			95% Confidence Intervals			V. acc.
	k	ACT	POST	K	ACT	POST	
D1 - Amb.	58	.18	.16	55 - 61	.17 - .20	.13 - .18	57
D2 - Amb.	58	.20	.18				71
D1 - Mimics	50	.27	.18	39 - 61	.23 - .32	.09 - .28	
D2 - Mimics	66	.24	.15				

From the 95% confidence intervals (CI) it can be seen that for Tas:

The HR constant and the ACT and POST coefficients from the ambulatory HR model of day 2 all fell within the CIs from the day 1 ambulatory model. This suggests the two ambulatory HR models are similar.

The HR constant and POST coefficient from the HR model of the mimics on day 1 fall within the CIs from the day 1 ambulatory model. The ACT coefficient lies out with these same CIs, suggesting that the mimic HR model although similar in tendency to the ambulatory HR model was unrelated in the ACT variable.

The ACT and POST coefficients from the HR model of the mimics on day 2 fall within the CI of the HR mimic model of day 1, the constant however, was out with the CI, suggesting a dissimilarity of HR mimic models over the 2 days.

Table 3.1b: Subject Tcc

Models	Coefficients			95% Confidence Intervals			N _{acc.}
	k	ACT	POST	k	ACT	POST	
D1 - Amb.	57	.20	.18	54 - 60	.18 - .21	.16 - .21	70
D2 - Amb.	73	.32	.01				55
D1 - Mimics ⁵	37	.20	.35				
D2 - Mimics	42	.24	.31	7 - 77	.10 - .38	.04 - .62	

From the 95% confidence intervals (CI) it can be seen that for Tcc:

The HR constant and ACT and POST coefficients from the ambulatory HR model of day 2 all fall out-with the CIs from the day 1 ambulatory model. This suggests that the 2 models were unrelated over the 2 days.

Only the ACT coefficient from the HR model of the mimics on day 1 falls within the CI of the day 1 ambulatory HR model. This suggests no similarity between the ambulatory and the mimics HR models.

The HR constant and both the ACT and POST coefficients from the day 1 HR model of the mimics fall within the CI of the day 2 mimics model. This suggests a similarity of HR mimic models over the 2 days, however it must be noted that the CI for the constant was very large, therefore showing a large variance and Std. error.

⁵ In the case of Tcc the mimic HR model included a 3min rest period. Day 2 CIs were used due to the day 1 file being corrupted.

The pilot subjects' data suggest that although ambulatory HR models were not necessarily similar over 2 days it is promising that the Tas models were similar. The results of the HR mimic models were contradictory but they do point towards the direction of being dissimilar, when it is considered that the protocol for the mimics over both days was exactly the same. Lastly, however, it is clear that the ambulatory and the mimic models of HR are dissimilar. The mimics have failed to provide a brief but comparable period of realistic activity and reactivity while within the controls of the laboratory.

3.1.2 Diary Mood Scales

Interactive Prompts (IPs) vs. Pre-set Prompts (PPs)

Subjects' diary pages from day 2 ambulation were first categorised into IPs or PPs, then each page was separated into the three mood variables and scored. The separate scores for T, H and E interactive prompt diary pages were then compared with scores from the pre-set prompt pages.

Subject: Tas

Table 3.2a: Tas - Means of Interactive Prompts

Mood	No.	Min.	Max.	Mean	Std.D
Tense	4	3	4	3.5	0.58
Hedonic	4	2	2	2	0
Energetic	4	0	4	1.5	1.73

Table 3.2b: Tas - Means of Pre-set prompts

Mood	No.	Min.	Max.	Mean	Std.D
Tense	4	3	4	3.75	0.5
Hedonic	4	1	3	2	0.82
Energetic	4	3	4	3.5	0.58

Although all results were non-significant there was a very general trend towards decreased E during IPs as predicted.

Subject: Tcc

Table 3.3a: Tcc - Means of Interactive Prompts

Mood	No.	Min.	Max.	Mean	Std.D
Tense	12	0	4	1.83	1.34
Hedonic	12	2	4	3	0.95
Energetic	12	0	1	2.42	1.38

Table 3.3b: Tcc - Means of Pre-set prompts

Mood	No.	Min.	Max.	Mean	Std.D
Tense	4	0	1	0.75	0.5
Hedonic	4	2	4	3.5	1
Energetic	4	1	4	2.75	1.26

Again there was a very general trend towards decreased E and increased T during IPs as predicted. The results are disappointing but with only 2 subjects it was difficult to reject the value of the diary in this situation therefore the diary will be left as is for the validation study.

3.1.3 Conclusions Drawn and Changes Made from the Pilot Subjects

Having run two pilot subjects it has become apparent that the laboratory mimics do not portray a comparable record of cardiovascular reactivity in the laboratory equivalent to that of daily life. In other words the models of HR produced from the mimics are totally dissimilar to the HR models of the ambulatory data.

This provides support for the previous studies that have found very different results between the laboratory and the field. At this stage it has been decided that nothing would be gained from continuing to include the mimics in the validation study. The mimics were subsequently dropped and replaced by 4 minutes of 'effortful' static cycling.

This refinement resulted in the experiment no longer having to run over a third day. Furthermore, we were unable to compare the results of ambulatory monitoring with that recorded in the laboratory (with the same ACT, POST and ECG devices). Additionally, the elimination of the mimics ruled out the ability to look for a constant response / reactivity to laboratory recording over the period of three days / times. In practical terms this meant a reduction of performance pressure on the subjects, as well as enabling a record of high physical stress (cycling) to be made along with the original record of high psychological stress (P.A.S.A.T.).

3.2 *Validation Study*

3.2.1 Subject Exclusions and Descriptives

13 subjects were run in total, but 4 were excluded from analysis: one was excluded due to a wire breakage in the posture device; a second had no day 2 data due to difficulties modelling day 1 HR; a third had drift in the posture measurement, which varied greatly over the two days and problematic channel recording; the fourth was excluded due to day 2 data loss resulting from battery removal. Subject val11 was also excluded from all the prompt and diary analysis due to an incorrectly completed diary.

Of the 9 subjects 6 were male and 3 were female, although in this experiment no distinction was between gender⁶. The mean age was 21.8 years (Std.dev.=2.28), ranging from 19 to 25 years. The mean height was 1.71m ranging from 1.63 to 1.94m. The mean weight was 71.7kg ranging from 57 to 84kg.

The following is a descriptive table of mean heart rate (HR), mean activity levels (Activity), mean posture values (Posture), mean HR during IPs and PPs, as well as the amount of variance accounted for by the subjects ambulatory HR models on day 1 (d1) and day 2 (d2).

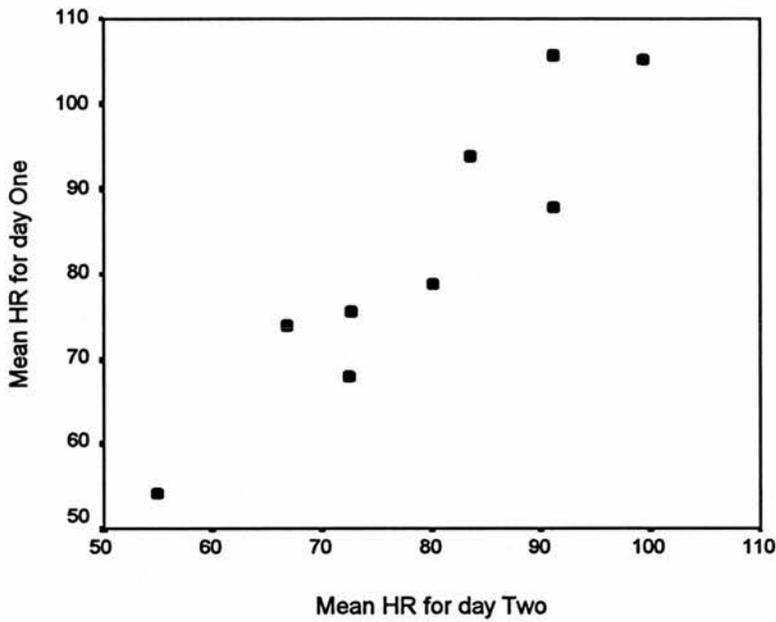
	No.	Min.	Max.	Mean	Std. Dev
Mean HR d1	9	54.09	105.73	82.59	17.19
Mean HR d2	9	54.95	99.51	79.14	13.94
Mean Activity d1	9	4.14	22.81	12.50	5.52
Mean Activity d2	9	2.01	21.60	9.12	5.77
Mean Posture d1	9	67.28	146.68	120.42	24.60
Mean Posture d2	9	60.40	118.50	101.13	18.75
IP mean HR d2	7	73.72	112.38	92.81	16.80
PP mean HR d2	7	61.50	98.86	79.92	13.29
% V. Acc. d1	9	35	76	56.00	15.12
% V. Acc. d2	9	31	70	51.44	14.81

Table 3.4: Mean values for day one and two HR (bpm), ACT, POST, and variance accounted for.

⁶ Gender differences were not analysed for as the emphasis of the study was how well the device functioned as an interactive monitor.

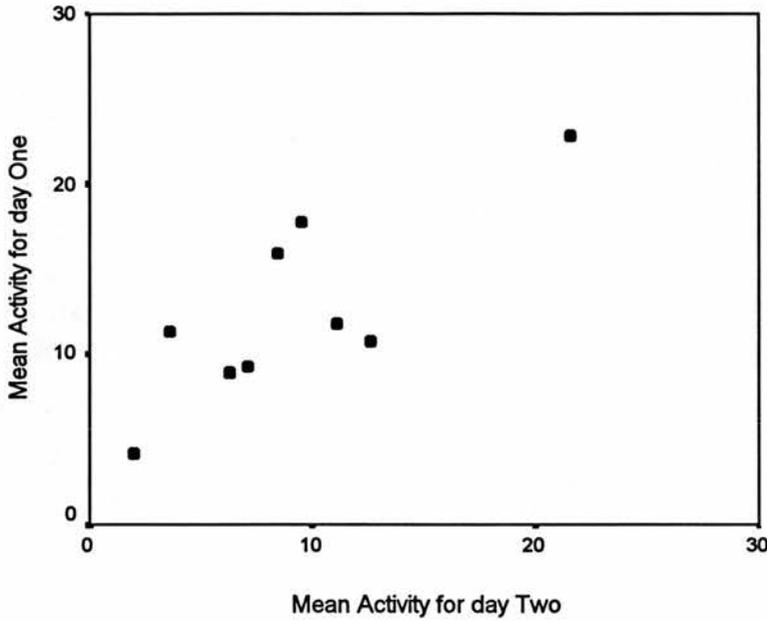
3.2.2 Reliability of physiological measures

Figure 3.1: Heart Rate - scatterplot of HR means (bpm)



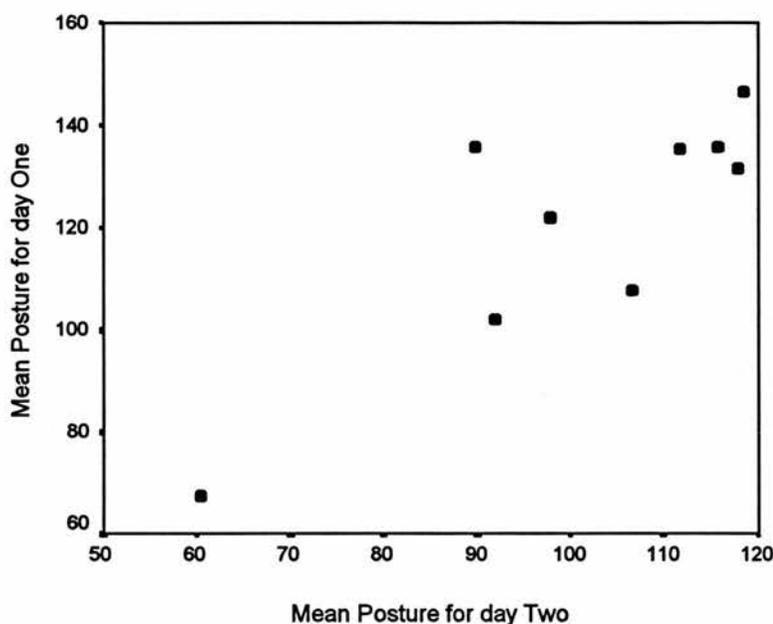
The groups mean heart rate from day 1 was found to significantly correlate with the mean HR from day 2 ($r=0.93$, $p<0.05$). Additionally the paired samples t-test found no significant differences between the two [$t(8)=1.59$, $p=0.15$]. These results suggest that HR was highly reliable - those with high HRs at time one continued to have high HRs. Furthermore there was consistency in HR levels over the two days.

Figure 3.2: Activity levels - Scatterplot of Activity means



The groups mean activity level from day 1 was found to significantly correlate with the mean activity level from day 2 ($r=0.80$, $p<0.01$). However the paired samples t-test found significant differences between the two [$t(8)=2.83$, $p<0.05$]. The correlations show that the activity device had high reliability, but the t-tests show a significant difference in levels between the two days.

Figure 3.3: Posture values - Scatterplot of Posture means



The groups mean posture values from day 1 was found to significantly correlate with the mean posture values from day 2 ($r=0.84$, $p<0.005$). However the paired samples t-test found significant differences between the two [$t(8)=4.34$, $p<0.005$]. The correlations show that posture device had high reliability, but the t-tests show a significant difference in levels between the two days.

3.2.3 Individual Analysis of Ambulatory Heart Rate Models and CIs

Below are each subject's heart rate models: one from the day 1 data and one from the day 2 data. Ideally we require the HR models from both days to be similar.

Confidence Intervals (CI) can be used as a guide to whether the second set of coefficients are similar to / fall within the range of the first set of coefficients. In other words is the day 2 HR model within the 95% CI of the day 1 HR model.

✓ = within 95% CI

× = outwith 95%CI

V. acc. = % Variance accounted for by amb. models

Table 3.5: val01

Models	k	Coefficients		95% Confidence Intervals			V. acc.
		ACT	POST	K	ACT	POST	
D1 - Amb.	40	0.25	0.28	37 - 43	0.2 - 0.31	0.26 - 0.30	61
D2 - Amb.	61	0.34	0.16	×	×	×	31

Table 3.6: val05

Models	k	Coefficients		95% Confidence Intervals			V. acc.
		ACT	POST	K	ACT	POST	
D1 - Amb.	68	0.11	0.13	64 - 71	.08 - .13	.11 - .16	45
D2 - Amb.	61	.01	.25	×	×	×	38

Table 3.7: val06

Models	k	Coefficients		95% Confidence Intervals			V. acc.
		ACT	POST	k	ACT	POST	
D1 - Amb.	92	.28	.01	86 - 98	.25 - .32	.02 - .12	43
D2 - Amb.	51	.22	.40	×	×	×	64

Table 3.8: val07

Models	k	Coefficients		95% Confidence Intervals			V. acc.
		ACT	POST	k	ACT	POST	
D1 - Amb.	50	.33	.15	48 - 53	.30 - .36	.13 - .17	76
D2 - Amb.	55	.48	.12	×	×	×	53

Table 3.9: val08

Models	Coefficients			95% Confidence Intervals			V. acc.
	k	ACT	POST	k	ACT	POST	
D1 - Amb.	40	.21	.25	34 - 45	.17 - .25	.21 - .29	35
D2 - Amb.	60	.44	.01	×	×	×	33

Table 3.10: val10

Models	Coefficients			95% Confidence Intervals			V. acc.
	k	ACT	POST	k	ACT	POST	
D1 - Amb.	36	.34	.52	30 - 41	.31 - .37	.46 - .58	75
D2 - Amb.	68	.34	.14	×	✓	×	53

Table 3.11: val11

Models	Coefficients			95% Confidence Intervals			V. acc.
	k	ACT	POST	k	ACT	POST	
D1 - Amb.	42	.30	.01	40 - 47	.27 - .33	.02 - .08	62
D2 - Amb.	48	.39	.01	×	×	×	69

Table 3.12: val12

Models	Coefficients			95% Confidence Intervals			V. acc.
	k	ACT	POST	K	ACT	POST	
D1 - Amb.	78	.26	.37	75 - 80	.23 - .29	.33 - .42	65
D2 - Amb.	73	.25	.41	×	✓	✓	70

Table 3.13: val13

Models	k	Coefficients		95% Confidence Intervals			V _a acc.
		ACT	POST	K	ACT	POST	
D1 - Amb.	55	.23	.01	51 - 58	.20 - .25	.06 - .13	42
D2 - Amb.	54	.56	.11	✓	×	✓	52

3.2.4 Individual Diary Mood Scores

Interactive Prompts (IP) vs. Pre-set Prompts (PP)

Subjects who had more than 4 IPs and 4 PPs (on day 2) were selected for individual analysis of their responses to the diary mood scales (val05, val07, val10). Their responses were first categorised into IPs or PPs, then each diary page separated into the three dimensions and scored. Hence, a score was obtained for T, H and E for every prompt (i.e. diary page). An unpaired / independent t-test was then run, separately on the T, H and E scores for IP vs. PP. The results can be seen in the following tables:

Table 3.14a: Means for the Interactive prompts – val05

Mood	No.	Mean	Std.D
Tense	10	0.4	0.7
Hedonic	10	3.8	0.4
Energetic	10	3.9	0.3

Table 3.14b: Means for the Pre-set prompts – val05

Mood	No.	Mean	Std.D
Tense	7	0	0
Hedonic	7	3.7	0.49
Energetic	7	3.9	0.38

T - The Levene test for homogeneity of variance has a significant F value ($p < 0.05$) therefore the unequal variances results must be used for the t-test. There was no significant difference between the Tense arousal means of the IPs and PPs for this subject [$t(9) = 1.81, p = 0.104, 2$ -tailed sig.].

H - Levene test is not significant ($p > 0.05$) therefore the t-test is based on equal variances. There was no significant difference between the Hedonic tone means of the IPs and PPs for this subject [$t(15) = 0.39, p = 0.704$].

E - Levene test is not significant ($p > 0.05$) therefore the t-test is based on equal variances. There was no significant difference between the Energetic arousal means of the IPs and PPs for this subject [$t(15) = 0.25, p = 0.803$].

Table 3.15a: Means for the Interactive prompts – val07

Mood	No.	Mean	Std.D
Tense	6	0.17	0.41
Hedonic	6	3	1.26
Energetic	6	0.83	0.75

Table 3.15b: Means for the Pre-set prompts - val07

Mood	No.	Mean	Std.D
Tense	6	0	0
Hedonic	6	3.67	0.82
Energetic	6	2.33	1.21

T - The Levene test for homogeneity of variance has a significant F value ($p < 0.05$) therefore the unequal variances results must be used for the t-test. There was no significant difference between the Tense arousal means of the IPs and PPs for this subject [$t(5) = 1.00, p = 0.363, 2$ -tailed sig.].

H - Levene test is not significant ($p > 0.05$) therefore the t-test was based on equal variances. There was no significant difference between the Hedonic tone means of the IPs and PPs for this subject [$t(10) = -1.09, p = 0.304$].

E - Levene test is not significant ($p > 0.05$) therefore the t-test was based on equal variances. There was a significant difference between the Energetic arousal means of the IPs and PPs for this subject [$t(10) = -2.58$, $p < 0.05$]. This difference was in the predicted direction.

Table 3.16a: Means for the Interactive prompts - val10

Mood	No.	Mean	Std.D
Tense	6	1.33	0.82
Hedonic	6	1.33	0.52
Energetic	6	1.67	1.21

Table 3.16b: Means for the Pre-set prompts – val10

Mood	No.	Mean	Std.D
Tense	5	1.4	0.55
Hedonic	5	1.6	0.55
Energetic	5	1	1

T - The Levene test for homogeneity of variance was not significant ($p < 0.05$) therefore the variances can be assumed to be homogeneous. There was no significant difference between the Tense arousal means of the IPs and PPs for this subject [$t(9) = -0.16$, $p = 0.88$, 2-tailed sig.].

H - Levene test was not significant ($p > 0.05$) therefore the t-test was based on equal variances. There was no significant difference between the Hedonic tone means of the IPs and PPs for this subject [$t(9) = -0.83$, $p = 0.428$].

E - Levene test was not significant ($p > 0.05$) therefore the t-test was based on equal variances. There was no significant difference between the Energetic arousal means of the IPs and PPs for this subject [$t(9) = 0.98$, $p = 0.352$].

These results suggest that individually the subjects were not reporting differences in mood between IPs and PPs, with the exception of subject val07 who showed reduced 'energetic arousal' during IPs suggesting a possible trend in the predicted direction.

3.2.5 Individual Interactive Monitoring

Each subject was individually checked (with the exception of val11) and all IP values of HR, activity and posture fell within the specified parameters. Several IPs correctly inhibited PPs, and only one PP failed to signal.

3.2.6 Challenge Appraisals

Contrary to the anticipated there was no pattern of responding with an appraisal of challenge during the IPs.

3.2.7 Idiosyncratic Stressors

Only 3 subjects of the 9 were able to provide an idiosyncratic stressor when asked, the rest professed to having none. Those who admitted to an idiosyncratic stressor did not have occasion to press their marker button during the two days, therefore no information can be gained concerning the effects of idiosyncratic stressors.

3.2.8 Group Analysis of Heart Rate Models

Regression of the coefficients

The purpose of this analysis was to determine whether or not there was any relationship between the subjects day 1 models and their day 2 models. Ideally a significant relationship between the two models is required, as it is the first days HR model which we use to program the interactive monitor. If the two HR models differ then the interactive monitor will respond incorrectly due to its attempt to fit its parameters to a different model on the second day.

We treated the coefficients as variables and performed a linear regression. We wanted to ask the question of whether or not the two lines (regression equations) were significantly similar. Therefore, a separate regression was performed on each pair of coefficients and the constant. Ideally we want the constant of the regression to be non-significant (i.e. not different from zero) and the slope to be significant (i.e. significantly different from zero). Additionally when considering the confidence intervals (CI's) we require the constant to contain zero (expect B to be positive) and the slope to contain 1 (ideally Beta to be 1).

The results are as follows:

Table 3.17: Regression results for the k coefficient

k	B	Std. E	Beta	t	Sig.	Confidence Inter.	
constant	57.16	54.79		1.04	0.33	-72.40	186.72
Day 2 k	-2.3E-02	0.92	-0.01	-0.03	0.98	-2.20	2.16

k - We found the overall regression to be non-significant [$F(1,7)=0.001$, $p=0.980$] with k to be unrelated between days. There is support for the constant being zero ($p=0.33$) but the slope also appears to support zero ($p=0.98$), showing the slope to be unrelated between day 1 and day 2 for k. The CI of the constant contains 1 and B is

positive as expected (57.16) but resembling the non-significance factor for the slope, Beta is not 1 (-0.01).

Table 3.18: Regression results for the ACT coefficient

ACT	B	Std. E	Beta	t	Sig.	Confidence Inter.	
constant	0.18	0.05		3.53	0.01	0.06	0.30
D2ACT	0.22	0.14	0.52	1.60	0.15	-0.11	0.55

ACT (α) - We found the overall regression to be non-significant [$F(1,7)=2.57$, $p=0.153$] with the coefficient of activity unrelated between days: There was no support for the constant of activity being zero as it was significantly different from zero ($p<0.01$). Likewise the slope was not significantly different from zero ($p=0.153$) in contrast to the predicted. The CI's follow suit in that the constant does not contain zero ($B=0.1$) and the Beta of the slope is not 1 (0.52).

Table 3.19: Regression results for the POST coefficient

POST	B	Std. E	Beta	t	Sig.	Confidence Inter.	
constant	0.17	0.10		1.68	0.14	-0.07	0.42
D2 POST	0.10	0.46	0.09	0.23	0.83	-0.97	1.18

POST (β) - Again the overall regression was found to be non-significant [$F(1,7)=0.051$, $p=0.827$] with the coefficient of posture unrelated between days: The constant was not significantly different from zero ($p=0.136$) as predicted however, the slope was also not significantly different from zero ($p=0.827$) showing the slope to be unrelated over days. The CI's show the constant as being improbable of being zero ($b=0.17$) and the Beta of the slope as being distinct from 1 (0.09).

Removal of outlier - Subject val11 day 1 data was found to contain an artefact HR of 199bpm. This was removed and his data was refitted to the day 1 HR model. This

produced only a slight difference in the k coefficient and no difference in the ACT and POST coefficients. For this reason only the regression of the k coefficient was rerun. As can be expected this slight change made very little difference.

Table 3.20: Regression results for the k coefficient

k	B	Std. E	Beta	t	Sig.	Confidence Inter.	
constant	55.78	55.03		1.01	0.35	-74.35	185.91
Day 2 k	-2.0E-03	0.93	-0.001	-0.002	0.99	-2.19	2.19

The overall regression was still found to be non-significant [$F(1,7)=0.00$, $p=0.998$] with k being unrelated over the two days: The constant was still non-significant ($p=0.345$) as predicted, with the B value as positive (55.78). However, as before the slope was also not significantly different from zero ($p=0.998$), and Beta was very different from the ideal value of 1 (-0.001).

3.2.9 Variance Accounted For

Of interest however is the amount of variance each HR model accounted for. The variance accounted for on day 1 ranged from 35% - 76% with a mean of 56%. The variance accounted for on day 2 ranged from 31% - 70% with a mean of 51.44%. A t-test was performed to check for differences in variance accounted for over the two days, where ideally we would like to find no significant differences. However, since the regression results suggest no relationship between the coefficients then it is more probable that differences will be found. Nonetheless the t-test showed there to be no significant difference [$t(8)=0.79$, $p=0.45$] between the variance accounted for each day.

3.2.10 Analysis of Prompts

Descriptives

The pre-set prompts (PPs) were defined by a random numbers table between 0 and 90, to simulate an average of one pre-set prompt every hour and a half on a random basis. They can however be inhibited by an interactive prompt occurring up to 20 minutes before the time of the said pre-set prompt.

Subject val11 was removed from any prompt or diary analysis due to the fact that the subject had completed diary pages where there were no prompts and vice versa, consequently the diary was indecipherable.

The number of PPs which occurred on day 2 ranged from 1 to 8 with a mean of 5 (Std.D=2.51). The number of interactive prompts (IPs) which occurred on day 2 ranged from 0 to 25 with a mean of 7.63 (Std.D=8.19).

3.2.11 t-testing: IPs vs. PPs

Physiological Measures

Heart Rate

An IP should occur when HR has gone beyond the set parameter (of $1.5 \times \text{SD}$ of residual HR) therefore indicating a significantly high HR. Hence it is predicted that HR during IPs will be significantly higher than HR during PPs.

Subject val08 was removed from the analysis due to having had no IPs occur. Mean HR during the IPs was 92.81bpm (Std.D=16.8) while mean HR during the PPs was 79.92bpm (Std.D=13.23). A paired samples t-test showed there to be a significant difference between HR during IPs and PPs [$t(6)=5.57, p<0.001$].

Activity Levels

Since HR is known to increase directly with increasing activity, the IPs were programmed to occur only when the level of activity was below the 90% level, whereas the PPs were random and irrespective of activity level. Therefore there is no set prediction here although a desirable result to confirm that the programming worked, would be a lower level of activity during IPs but not necessarily a significant difference. More specifically to be able to say that significantly high HRs were not due to metabolic activity (activity and posture) it is predicted that there should be no significant difference in activity between IPs and PPs.

Subject val08 was removed from the analysis due to having had no IPs occur. The mean activity level during the IPs was 7.84 (Std.D=6.65), while the mean activity level during the PPs was 11.16 (Std.D=12.11). The paired samples t-test revealed no significant difference in activity levels between the IPs and the PPs [$t(6)=-1.18$, $p=0.282$].

Posture Values

The literature on postural effects during stress is insufficient to lend itself to a confident prediction. Although one of the many failings of laboratory studies in representing real-life is the lack of postural change occurring, it is safe to say that stress and resulting high HRs occur in varied postures in real-life. In other words high HRs should be due to factors other than posture (metabolic). Therefore it is predicted that no significant difference should occur in posture values between IPs and PPs.

Subject val08 was removed from the analysis due to having had no IPs occur. The mean posture value during the IPs was 96.45 while it was 95.34 during the PPs. The paired samples t-test confirmed the prediction and showed no significant difference in posture values between the IPs and the PPs [$t(6)=0.15$, $p=0.887$] suggesting that posture is not a significant factor in significantly high HRs.

3.2.12 Diary Mood Scales

Interactive Prompts (IPs) vs. Pre-set Prompts (PPs)

Subjects' diary pages from day 2 ambulation were first categorised into IPs or PPs, then each page was separated into the three mood variables and scored.

The separate scores for T, H and E were each averaged. A paired samples t-test was then performed, between the IPs and the PPs averaged scores for each variable. Again subject val08 was removed from the analysis due to having had no IPs occur.

T - The mean score for tense arousal during the IPs was 0.89 (Std.D=0.67) while the mean score was 0.42 (Std.D=0.64) during the PPs. The t-test showed no significant difference between T scores during the IPs and the PPs [$t(6)=1.62$, $p=0.16$].

H - The mean score for hedonic tone during the IPs was 2.95 (Std.D=0.98) while the mean score was 3.07 (Std.D=0.93) during the PPs. The t-test showed no significant difference between H scores during the IPS and the PPs [$t(6)=-0.72$, $p=0.50$].

E - The mean score for energetic arousal during the IPs was 2.60 (Std.D=1.31) while the mean score was 2.13 (Std.D=1.20) during the PPs. The t-test showed no significant difference between E scores during the IPS and the PPs [$t(6)=1.06$, $p=0.33$].

3.2.13 P.A.S.A.T.

Descriptives

Of the 9 subjects 4 had an IP during or directly after the P.A.S.A.T. stressor and a fifth subject's physiological response was in line with the IP parameters but was inhibited by an earlier IP. Performance was not analysed as the P.A.S.A.T. was used to attempt to induce a stress response in the subjects.

Heart rate averages and residual heart rate averages were calculated for during the 3 minutes baseline prior to the P.A.S.A.T., during the P.A.S.A.T., and for the 8-minute control period of vocalisation after the P.A.S.A.T. It must be noted that the baseline period occurred while seated non-cycling on a static cycle machine, therefore the HR may be slightly higher than an actual baseline. Additionally the vocalisation period was not counterbalanced, as it always followed the P.A.S.A.T. Therefore again the HR and residuals may be slightly higher than expected.

Table 3.21: Averages of heart rate and residual heart rates during task epochs

	No.	Min.	Max.	Mean	Std. Dev
P.A.S.A.T.	9	56.80	106.70	78.22	16.82
Baseline	9	56.00	104.67	73.04	17.01
Vocalisation	9	50.38	98.88	68.75	16.43
P.a.s.a.t Resh.	9	0.60	12.20	7.19	4.11
Baseline Resh.	9	0.00	10.55	1.74	3.41
Vocal. Resh.	9	0.00	7.00	1.45	2.31

It is predicted that the baseline period heart rate should be significantly lower than the P.A.S.A.T. HR; that the P.A.S.A.T. HR should be significantly higher than the vocalisation HR; that the P.A.S.A.T. residual HR should be significantly higher than the baseline residual and that the P.A.S.A.T. residual HR should also be higher than the vocalisation residual.

P.A.S.A.T. HR vs. Baseline period HR - A paired samples t-test showed there to be a significant difference between the two HRs [$t(8)=4.57, p<0.01$].

P.A.S.A.T. HR vs. Vocalisation HR - A paired samples t-test showed there to be a significant difference between the two HRs [$t(8)=12.97, p<0.001$].

P.A.S.A.T. residual HR vs. Baseline residual HR - A paired samples t-test showed there to be a significant difference between the two HRs [$t(8)=3.57, p<0.01$].

P.A.S.A.T. residual HR vs. Vocalisation residual HR - A paired samples t-test showed there to be a significant difference between the two HRs [$t(8)=4.52$, $p<0.01$].

Since the P.A.S.A.T. was used as a stressor it is pertinent to compare the HRs which occur before the IP. It was predicted that if the P.A.S.A.T. was working as a stressor then there should be no significant differences in HR between the P.A.S.A.T. and the IPs, while a significant difference should occur between the PPs and the P.A.S.A.T. HRs. The IP and the PP average HR was calculated from the four minutes prior to the IP and / or the PP.

Subjects val08 and val11 were again removed from analysis due to having no IPs and an incomprehensible diary respectively. Therefore the P.A.S.A.T. HR now as a mean of 82.59bpm.

P.A.S.A.T. HR vs. IP HR - A paired samples t-test showed there to be a significant difference between the two HRs [$t(6)=3.69$, $p<0.01$]. The means show the IP HR ($m=92.81$ bpm, $Std.D=16.8$) to be higher than that for the P.A.S.A.T. ($m=82.59$ bpm, $Std.D=16.26$).

P.A.S.A.T. HR vs. PP HR - A paired samples t-test showed there to be no significant difference between the two HRs [$t(6)=0.98$, $p=0.36$]. The means show the PP HR ($m=79.92$ bpm, $Std.D=13.29$) to be lower than that for the P.A.S.A.T. ($m=82.59$ bpm, $Std.D=16.26$).

Further, there was no significant differences in the number of IPs which occurred during ambulation between the subjects who did and did not have an IP during the P.A.S.A.T. [$t(6)=1.15$, $p=0.29$] (Levene not significant [$F(6)=1.79$, $p=0.229$]). This suggests that the P.A.S.A.T. is not a predictor of whether or not someone is a high reactor. Additionally there was no significant difference in the amount of variance accounted for by the HR models, of neither day 1 nor day 2 between those subjects who did and did not react to the P.A.S.A.T. ($t=-0.17$, $p=0.87$ and $t=-0.51$, $p=0.63$).

4 Discussion

4.1 Technical Problems

With the nature of psychophysiology, this is a brief overview of what could actually be a very lengthy section. Over the course of set up and piloting there were multiple equipment failures:

1. The software appears to have problems coping with programming commands, refuses to use the clock function correctly on screen and often crashes or cannot cope with running several functions simultaneously, which it was designed to. However, of more concern, but probably of little overall effect, is the fact that on an irregular occasion for no apparent reason the PP does not activate. This leads to the question of what IPs does it not activate and would we ever know without analysing the data minute by minute, which is undesirable for ambulatory monitoring. Additional to this we have a subject with prompts and no diary pages and vice versa who's diary data was unusable - could this be the same problem? Has there been a corruption of the PPs and the IPs, or has the subject just failed to complete the diary pages at the appropriate times? Another concern is that the software is having problems handling low HRs. HRs around 40bpm are being treated as errors and the machine is filling in the missing points, whereas an error HR should be <30bpm as many people have HRs around and about 40bpm but few at 30bpm. This provides very inaccurate HR data which is concerning.
2. The activity devices are fragile piezo-sensor shock detectors which are prone to breaking suddenly whilst on a subject, as well as decreasing in sensitivity with age. Several failures meant inconsistent results at the set up stage and heroic attempts at calibrating the devices to the same sensitivity. In spite of this there are still occasions where there appears to be a shift in gain (amplitude of activity registered) from one day to the next with the same device. The shift is not large and hence often difficult to notice, but it may on occasion be enough to alter the weighting of the activity coefficient in the HR model.

3. The posture device has caused extensive problems. Breakage on a subject is bad enough and acceptable due to its fragile connections but there appears to be a problem of recording drift. This is a particularly pervasive problem, which returned even after the transducer was replaced with a new one. The device acts normally at set up, reading as it should however, very soon after this the reading on the posture device begins to drift upwards, and on occasion temporarily off the scale. This fault was never noticed on the second day as yet again at the start it recalibrated as it should. The recording usually drifts upwards and levels off, but we cannot be positive that the device drifts consistently over the two days. Therefore this may account for the subjects who have had reasonable posture coefficients on one day yet on the other have tiny values which are input as the minimum of 0.01. Subjects val01, 05, 07, 11 all wore the drifting device.

4.2 *Discussing the Data*

4.2.1 Reflecting on the Study

The physiological measures used appeared highly reliable. Both the activity levels and the posture values show good correlations suggesting highly reliable devices yet they were both significantly different over the two days. This could be due to one of two things: faulty / inconsistent devices; the subjects participating in two days each with different behaviours. The positive correlations suggest the latter is the reason (although there has been some problems with the posture device) where the subjects have engaged in two entirely different days behaviour.

When observing the individual heart rate models, the coefficients from the second days HR model fail to fall completely within the CIs of the day 1 model. There is no subject with all 3 day 2 coefficients within the 95% CI of the day 1 model. Of the 9 subjects 6 have day 2 coefficients which all lie out-with the CI s of their day 1 models.

Of the remaining 3 subjects: 1 has only the ACT coefficient within the CI; the second has ACT and POST with the CI; and the third has HR and POST within the CI.

Individually, the data looks terrible but subjects have been plagued with equipment errors - for example, drifting POST - however, the more likely reason is that the subjects participated in radically different behaviours on both days.

Individual analysis of the diary mood scores suggest that the subjects were not reporting differences in mood between IPs and PPs, except subject val07 who showed reduced Energetic arousal during IPs suggesting a possible trend in the predicted direction.

Regression of the coefficients confirmed the disappointing picture portrayed by the individual CIs. By treating the coefficients as variables and performing a regression on them we can see that there was no significant relationship between either the day 1 values of k, ACT and POST and the day 2 values of k, ACT and POST. In other words there was no relationship between the two days HR models. Had there been any relationship between the HR models then a t-test would have been performed to check for size consistency of the coefficients. The inconsistency of HR models has implications for the operation of the interactive monitor. The monitor was attempting to fit day 1 HR models to different day 2 models therefore the validity of the IPs is called into question.

When the amount of variance accounted for by the heart rate models is considered it suggests that, as a group, although the 2 days models differed significantly the variance accounted for was just as good from one day to the next. However there are 3 distinct outliers for which this does not appear to be the case. These 3 subjects had distinctly different variances over the 2 days. One had a day 1 variance accounted for of 61%, while for day 2 it almost halved to 31%. The second subject had a good model with 76% variance accounted for on day 1 that dropped to 53% on day 2.

Similarly the third subject had 75% variance accounted for on day 1 with only 53% on day 2. Speculatively, these subjects, having such a variance in their variance accounted for, may have covered up any actual differences in the amount of variance accounted for in the models of the other subjects. The differences in these three subject's variances over the two days may be a factor of equipment problems or more likely due to the subjects engaging in two days with completely different behaviours.

As predicted there was a significant difference between heart rate during IPs and PPs, with the higher HRs occurring during the IPs. This confirms that the interactive monitor operated accordingly. No significant differences were found between IP and PP activity levels. This follows the vague prediction that although the difference was not significant the activity level had a trend towards being lower during the IPs, confirming the programming. More importantly the non-significant difference suggests that high HRs may be due to something other than activity, such as stress. There were also no differences in posture values between the different prompts (IPs and PPs) suggesting that posture is not a significant factor in significantly high HRs.

The activity and posture results combined indicate that high HR recordings above that predicted by the model can be accepted as related to a stress response and not metabolic activity. In other words when a high HR is not due to large amounts of physical activity (where an IP was programmed to be inhibited) it can be said to be due to stress.

Although the diary mood scale means do suggest general trends in the correct directions, the results are most disappointing. The results show no differences in mood responses during the different prompts. The pilot subjects also showed no significant differences in the mood scores although they like the 3 subjects analysed individually showed a general trend towards increased T and reduced E during IPs. It could be argued that the reason the 3 subjects were selected (more than 4 IPs and 4 PPs) is the same reason that they did not report mood differences - their numbers of IPs reflects their variability of mood, hence any PPs were just as likely to occur during

this variability. Another explanation is that the terms selected to reflect Matthews' UMACL scale failed to do that, either by being simply the wrong terms or by there being too few terms in the scale to capture the mood. However the terms chosen were the ones most heavily weighted for the mood variable in question. Therefore a more reasonable explanation is that in this study subjectively reported mood did not vary with heart rate.

The results of the P.A.S.A.T. compared to baseline and vocalisation period HR and residual HR are all significant, while the means show the differences to be in the predicted direction. This suggests that in comparison to sitting on a cycle machine and verbalising a list of numbers the P.A.S.A.T. produced a significantly higher HR and residual HR.

These results of the P.A.S.A.T. compared to IP and PP heart rate suggest that although the P.A.S.A.T. had some effect on HR compared to the random PP average HR, it was not similar to that of a naturally occurring IP. This could be for one of two reasons either: the P.A.S.A.T. is not a true stressor akin to that found in real-life; or that the parameters for the IPs were not set sensitive enough i.e. perhaps the definition for a significantly high HR should be less than the $1.5 \times \text{Std.D.}$ of the residual HR it is currently set at. However, it should be noted that a standard deviation of 1.5 can occur by chance more than 5% of the time. A standard deviation of 1.645 would be necessary for the chance of high heart rates to be reduced to 5%. Therefore, it is unlikely that the IP parameters were a factor in the P.A.S.A.T. not evoking a response like that found in real-life. This also has implication for how we originally defined an IP and the corresponding high heart rate.

Furthermore, returning to the issue of the P.A.S.A.T., the occurrence of an IP to the P.A.S.A.T. was not predictive of the number of IPs a subject would have during ambulation. Neither did the difference in the amount of variance accounted for by each days heart rate model predict those subjects who would react to the P.A.S.A.T. In other words those who did not react did not have better or worse HR models than

those who did. These facts coupled with the significant differences found for a baseline and vocalisation period suggests that although the P.A.S.A.T. can increase HR in the laboratory it does not act like a stressor found in real-life.

4.2.2 Research Questions Answered

The research questions were:

1. Does the regression model of heart rate on activity and posture predict ambulatory heart rate?

Imposing the rules of linear regression modelled ambulatory heart rate very well, with variance accounted for ranging from 31% - 76% with a day 1 mean of 56% and a day 2 mean of 51.44%. These are more than satisfactory results. However, we were unable to use one days model to predict another days heart rate.

2. Can we generate a model of heart rate from the 'mimicking' tasks, which will correlate to a model from the ambulatory data?

No the pilot data failed to show any similarity between mimic heart rate models and ambulatory heart rate models. This suggested that attempting to model heart rate, that is similar to ambulatory heart rate, from a set of mimicking tasks needs far greater preparation, planning and comparisons.

3. Does the day 1 model of ambulatory heart rate predict the day 2 ambulatory heart rate model?

No, the consistency of the modelling technique has to be pulled into question when the discrepancies between the two days heart rate models are considered. However, the supporting data points to the fact that the subjects behaviour differed over the two days rather than the problem being due to the regression. Additionally, technical problems have hampered the stability of the heart rate models.

4. Can heart rate recordings above that predicted, by the ambulatory heart rate model, be reliably accepted as related to stress and not metabolic activity?

It would appear from this study that yes we can accept heart rates beyond that predicted not to be a factor of metabolic activity. Activity and posture were found not to differ between IPs and PPs. However this result must be treated with caution when the difference in the two days heart rate models are considered. The IPs were calculated from the day 1 ambulatory heart rate model while they operated on the completely different day 2 ambulatory heart rate model. Furthermore the value of 1.5SD of the residual is open to the factor of more than 5% chance, indicating that this may be an unreliable method for defining and predicting IPs.

5. When heart rate is high does it relate to differences in mood state?

The subjective diary mood scale failed to account for any of the variability or increases in heart rate. Therefore this study could not conclude subjective mood to be a factor of high heart rate.

6. Does the ambulatory heart rate model predict heart rate response to a known stressor?

No, there was no relation between the variance accounted for by the heart rate models and subjects reactions to the P.A.S.A.T. Nor were the subjects who had an IP during

the P.A.S.A.T. anymore or less reactive during ambulation than those subjects who did not react to the P.A.S.A.T. Not all subjects found the P.A.S.A.T. to be stressful (as indicated by their lack of IP) however, the P.A.S.A.T. has to be called into question on its ability to elicit stress, due to the heart rate differences between it and IPs and the tendency of the subjects to give up on the task.

4.2.3 Drawing Conclusions

These entire results must be treated with caution as they include problematic equipment and, unfortunately an extremely small sample, therefore I would hesitate before using it as the basis of a validation study. However, the ambulatory heart rate models developed demonstrated a high reliability of measures despite the regression of the coefficients revealing differences in the subject's behaviour over the two days. Additionally, we can conclude that the interactive heart rate monitor operated as programmed, with no differences in activity and posture and significantly higher heart rates during IPs than PPs, indicating periods of non-metabolic related cardiovascular reactivity. Using the P.A.S.A.T as a stressor resulted in heart rate increases but failed to produce laboratory results similar to those elicited by real-life stressors, further highlighting the problem with attempting to generalise laboratory results to the real world. Additionally, the diary mood scale results were disappointing, and suggest that subjective mood, does not relate significantly to the changes occurring in ambulatory heart rate. However, the overshadowing cloud was the selection of 1.5SD of the residual heart rate as an indicator of interestingly high heart rates. Perhaps had we been more conservative when defining a 'high heart rate' and used a value of 1.75SD, for example, we may have seen very different results. For future investigation into the validity of this monitor I would suggest the use of a tri-axial accelerometer to measure activity and posture and investigation into the value of residual heart rate employed to determine an interestingly high heart rate.

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6 Appendices

• Appendix One:

PILOT PROTOCOL

DAY 1

Meet subject.

- subject complete vol., info. and consent forms
- explain what is being measured and what will be expected of the subject this morning
- attach ECG, ACT and POST
- check performance and movement interference set gain, Zero POST.

When satisfied start recording

- subject needs to sit, stand and lie down for 1 minute each.
- record POST and ACT values

Let subject sit quietly, for 5 mins, permitting HR to lower

Return to subject.

- subject fills out Spielberg Trait (day 1 only) and State questionnaire

'Mimics'

- explain some locomotion round dept. is required now
- subject walks, on one level, with experimenter
 - 2 mins at normal pace
 - 2 mins at quick pace
- subject climbs stairs for 4 mins, returning to lab where
 - subject permitted a rest for 5 mins
- music
 - subject sits while listening to 2 mins of peaceful music
 - subject stands while listening to 2 mins of agitative music
 - subject completes rating scale
 - subject stands while listening to 2 mins of peaceful music
 - subject sits while listening to 2 mins of agitative music
 - rest period of 3 mins

Ambulatory Instructions

- inform subject about the need for normal activity during the day
- confirm the recording process and tone sounding
- reiterate need for honest and complete diary
- possible show example of completed diary

Ask for an individual stressor

- make note of

- instruct subject to fill out appropriate diary page if this occurs

Subject leaves

DAY 2

Subject returns in the morning

- download data
- replace batteries
- reprogram INT
- Apply HR Limit, ACT Limit, new preset prompts

Start Recording

- Sit, Stand, Lie - 1 min each.
- 5 min seated rest period
- subject completes Spielberger's State questionnaire

'Mimics'

'Stressor'

- explain the P.A.S.A.T. and verbal repetition to subject.
 - subject performs P.A.S.A.T. while seated for 8 mins
 - rest 3 mins
 - subject repeats aloud P.A.S.A.T. numbers while seated for 8 mins

Subject leaves, after experimenter answers any more queries on ambulation

DAY 3

Subject returns in the morning

- subject completes Spielberger's State questionnaire

'Mimics'

Removing the equipment

- download data
- remove ECG, POST and ACT monitors from subject

Subject leaves

- pay subject
- thank
- debrief

Day 1: 1hr 15mins

Day 2: 1hr 30mins

Day 3: 1hr 00mins

• **Appendix Two:**

VALIDATION PROTOCOL

DAY 1

Meet subject.

- subject complete vol., info. and consent forms
- explain what is being measured and what will be expected of the subject this morning
- attach ECG, ACT and POST
- check performance and movement interference set gain, Zero POST.

When satisfied start recording

- subject needs to sit, stand and lie down for 1 minute each.
- record POST and ACT values

Let subject sit quietly, for 5 mins, permitting HR to lower

Return to subject.

- subject fills out Spielberger Trait (day 1 only) and State questionnaire

seated static on cycle machine - 3mins

Subject cycles exerting effort - 2mins with 1kg weight

- 2mins with 1.5kg weight

- 5 mins rest

Ambulatory Instructions

- inform subject about the need for normal activity during the day
- confirm the recording process and tone sounding
- reiterate need for honest and complete diary
- possible show example of completed diary

Ask for an individual stressor

- make note of
- instruct subject to fill out appropriate diary page if this occurs

Subject leaves

DAY 2

Subject returns in the morning

- download data
- replace batteries
- reprogram INT
- Apply HR Limit, ACT Limit, new pre-set prompts

Start Recording

- Sit, Stand, Lie - 1 min each.

- 5 min seated rest period
- subject completes Spielberger's State questionnaire

'Stressor'

- explain the P.A.S.A.T. and verbal repetition to subject.
 - subject performs P.A.S.A.T. while seated for 8 mins
 - rest 3 mins
 - subject repeats aloud P.A.S.A.T. numbers while seated for 8 mins

Subject leaves, after experimenter answers any more queries on ambulation

DAY 3

Subject returns in the morning

- download data
- remove ECG, POST and ACT monitors from subject

Subject - paid - thanked- debriefed - leaves

Day 1: 1 hr 00 mins

Day 2: 1 hr 10 mins

Day 3: 0 hr 10 mins

- **Appendix Three:**

VOLUNTEER INFORMATION SHEET

School of Psychology

University of St Andrews

Project: *"Field and Laboratory, Validation Testing of an Interactive Heart Rate Monitor"*

Your heart rate, general posture and activity will be measured for a period of 48 hours using an ambulatory recorder. Heart rate will be measured via three electrodes attached to your chest, posture from a slim water filled tube which runs from your calf to your shoulder and activity from a small movement detector taped to your thigh. Only very mild discomfort could be expected. The data from these devices will be stored on a small computer, which is secured around your waist on a belt.

You will wear the ambulatory recorder for two days while you go about your normal activities (day 1 and day 2). Occasionally a tone will be emitted as a sign that you should complete one page of a diary indicating your activities and feelings over the previous five minutes. The tone will not sound more than twice in one hour and will not sound at night.

You will be required to return to the laboratory on the second morning for approximately 1 hour 10 mins. Where the physiological data will be downloaded from the recorder to a computer. The recorder will then be reprogrammed and the diary collected and replaced. On day one while you are visiting the laboratory you will be required to cycle for a few minutes and complete a mood questionnaire. This is simply to assess your heart rate and general mood. On day two you will also complete a mental arithmetic task. On the third morning the equipment will be removed. On completion of the experiment you will be paid £25.

While wearing the equipment, we regret, you cannot have a bath or shower.

If you have any queries, or feel you cannot take part in the experiment for any reason please discuss these with the experimenter.

• **Appendix Four:**

0-90 mins Random Preset-Prompts

Subject	Minutes past the hour (one per hour)								
VAL01A 09	10	11	12	13	14	15	16	17	18
VAL01B 19	20	21	44	25	37	58	27	43	27
VAL02A 09	01	03	26	56	01	09	26	20	09
VAL02B 24	24	51	26	21	07	08	21	27	12
VAL03A 48	16	14	14	01	58	29	26	21	23
VAL03B 57	23	04	06	59	32	44	05	16	04
VAL04A 24	55	05	29	09	38	08	10	42	14
VAL04B 17	23	10	58	28	54	28	14	13	22
VAL05A 07	08	51	01	18	17	43	13	24	15
VAL05B 50	16	09	45	20	25	50	17	09	24
VAL06A 15	29	05	47	17	05	23	27	44	01
VAL06B 05	34	49	31	06	25	27	10	21	36
VAL07B 00	03	23	46	59	05	08	25	15	23
VAL08A 02	10	46	06	50	40	11	30	24	50
VAL08B 58	21	31	26	40	36	56	30	57	15
VAL09A 54	21	34	51	17	08	43	18	19	17
VAL09B 02	29	21	01	54	43	45	30	43	15
VAL10A 08	06	28	38	12	21	49	25	00	03
VAL10B 06	11	48	38	25	16	20	24	28	14
VAL11A 12	31	35	33	21	10	31	16	13	18
VAL11B 56	07	33	35	42	00	02	11	24	49
VAL12A 49	16	47	01	43	51	41	26	07	11
VAL12B 21	16	45	33	53	27	04	39	24	42
VAL13A 00	07	00	19	22	17	06	59	18	11
VAL13B 23	24	44	00	16	29	29	46	55	01
VAL14A 04	10	50	28	39	22	14	41	16	04
VAL14B 52	40	55	30	25	27	16	19	22	53
VAL15A 34	06	16	32	40	16	13	36	28	56
VAL15B 01	13	07	23	20	16	05	24	55	15
VAL16A 03	06	55	19	01	46	20	55	25	22
VAL16B 38	07	18	13	56	04	46	37	57	30

day one (A)

day two (B)

- **Appendix Five:**

ETHICS APPROVAL FORM



**UNIVERSITY OF ST ANDREWS
SCHOOL OF PSYCHOLOGY ETHICS COMMITTEE**

18th February 1998

Lorraine Paterson
School of Psychology
University of St Andrews

Dear Lorraine

Re: Field and Laboratory, Validation Testing of an Interactive Heart Rate Monitor

Thank you for submitting the amended information sheet and advert that the Ethics Committee requested. This project has been approved.

If, during the course of the proposed research, any important condition were to alter, then the Committee would wish to be informed.

Yours sincerely

Dr Hugh Morris
Convener

Dictated but not read