THE INFLUENCE OF DIETARY FATTY ACIDS ON BLOOD PRESSURE REACTIVITY

by

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A THESIS

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ABSTRACT

Dietary fatty acids may be involved in the regulation of blood pressure. Several studies have reported that diets high in polyunsaturated fatty acids reduce blood pressure. However, other studies have cast doubt on this association by finding little or no change in blood pressure following dietary treatment with polyunsaturated fats. The purpose of the present study was to clarify the impact of several dietary fatty acids on blood pressure regulation in a carefully controlled experimental design.

Male volunteers were fed diets differing only in the following sources of dietary fat: fish oil, butterfat or safflower oil. The study employed a repeated measures, crossover design in which each subject received each dietary treatment for a six week period. Subjects received all food items from the Clinical Research Center and were instructed to eat only the food prepared for them during the experimental dietary phases. Experimental phases were separated by six week washout periods where subjects resumed their normal diet. Measurements of basal blood pressure and blood pressure reactivity were taken during a baseline period and repeated during the last week of each diet and washout period. Blood pressure reactivity was tested using a mental arithmetic challenge, the Stroop color-word task and a isometric handgrip challenge. Cortisol responses to a

PacMan video game were measured at the end of each dietary phase. It was predicted that diets rich in fish oil and safflower oil would lower basal blood pressure and blood pressure reactivity to the largest extent while a diet comprised of butterfat would increase blood pressure and blood pressure responses to stress.

The safflower oil diet produced the lowest basal blood pressure. In addition, blood pressure responses to the Stroop color-word task were significantly lower following treatment with the safflower oil diet in comparison to reactivity determined before dietary intervention. No differences in basal blood pressure and blood pressure reactivity were observed following dietary treatment with fish oil or butterfat. Subjects grouped as either hypertensive or normotensive did not differ in their responses to the diets. Cortisol responses were small and did not appear to be influenced by dietary fat intake.

Taken together, the results suggest that certain polyunsaturated fats may have a favorable impact on blood pressure regulation. Saturated and polyunsaturated fats do not appear to act as homogenous groups with regard to their impact on blood pressure regulation. Rather, the specific biochemical structure such as the number and positioning of carbon bonds seem to determine the ability of fatty acids to modify cardiovascular variables.

INTRODUCTION

Psychological stressors have been implicated as important precursors in the development of essential hypertension. Numerous studies have correlated the incidence of hypertension with the exposure to a wide range of stressful circumstances including job demand in air traffic controllers, westernization of rural cultures or residence in high stress areas where unemployment, crime and crowded conditions are prevalent (Mustacchi, 1990). Other research has attempted to directly associate stress with long term alterations in cardiovascular function (Forsyth, 1971; Henry, Stephens, and Santisteban, 1975). Although stressors are capable of evoking substantial responses in the cardiovascular and neuroendocrine systems, these studies concluded that stress-induced alterations alone are not sufficient to produce sustained primary hypertension. Rather, a combination of precipitating factors such as diet or genetic predisposition is necessary to impact the development and progression of hypertension (Brody et al., 1987; Folkow, 1982; Matthews et al., 1986). The goal of this thesis was to examine the interactions and associations among dietary fat, behavioral stressors and blood pressure.

Hypertension

Essential hypertension is defined as an elevation of systolic blood pressure and/or diastolic blood pressure in

excess of 140 mmHg and 90 mmHg respectively (Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure [JNC], 1993). High blood pressure is associated with an increased risk of developing coronary heart disease, congestive heart failure, stroke and renal insufficiency. The risks of morbidity and mortality from cardiovascular or renal diseases progressively increase with higher levels of both systolic and diastolic blood pressure. An estimated 15% of American adults are diagnosed with essential hypertension making it a prevalent health concern (Ritchie, 1990). Unlike secondary hypertension where high blood pressure can be attributed to renal or neuroendocrine disorders, the origin of essential hypertension is unknown.

Arterial blood pressure is determined by the cardiac output of the heart and the peripheral resistance of the arterioles. The hallmark of essential hypertension is an elevation in total peripheral resistance with a normal cardiac output. Given the importance of resistance in determining blood pressure, several theories have sought to explain the development of elevated peripheral resistance and its relation to sustained high blood pressure. First, the failure of the kidney to adequately regulate blood pressure is believed to be a critical factor in the establishment of primary hypertension. This view is based on the kidney's ability to adjust fluid volume by selectively excreting sodium and water. Under normal

conditions of long term blood pressure regulation, elevated blood pressure associated with increased blood volume stimulates an increase in kidney output of salt and water. The extracellular fluid volume and circulating blood volume are thus lowered and as a result blood pressure is attenuated. In this scheme, hypertension is thought to result from impaired renal function such that higher pressures are required to facilitate excretion of salt and water (Guyton, 1991). Consequently, sodium and water are retained until fluid volume is sufficiently increased to facilitate pressure natriuresis. However, because the cardiac output is also elevated, the tissues are perfused with more blood than is metabolically required. To return flow to normal levels, local arterioles constrict, thereby increasing peripheral resistance.

Other research has investigated cellular mechanisms that could be responsible for the raised peripheral resistance commonly found in hypertensive individuals. According to Folkow (1982), structural adaptation of the vasculature is the prominent determinant of elevated resistance. Greater vascular wall thickness is a common modification seen in the vasculature of hypertensive individuals, primarily within the small arteries and arterioles of the renal and mesenteric vascular beds (Mulvany, 1987; Sivertsson, 1984). The increase in the mass of the vessel wall encroaches upon the lumen raising the

peripheral resistance. The alteration in the wall to lumen ratio is thought to be mediated by hypertrophy and/or hyperplasia of the smooth muscle cell. A number of factors have been implicated in contributing to the growth of the cell. For example, platelet-derived growth factor (PDGF) has been shown to exert a trophic influence on blood vessels independent of blood pressure elevation (Bevan, 1984; Mulvany, 1987). PDGF has been shown to bind to receptors on smooth muscle cells stimulating cell division and migration (Ross, 1985). Other evidence has demonstrated that sympathetic activity may influence the growth of the cell. Infusion of epinephrine and norepinephrine induces polyploidy of smooth muscle cells in the spontaneously hypertensive rat (SHR) aorta (Yamori, Mano, Nara and Horie, 1987). Conversely, peripheral neonatal sympathectomy eliminated hyperplastic changes in the cells of both muscular arteries and resistance vessels (Lee, Triggle, Cheung and Coughlin, 1987).

Peripheral vascular resistance can also be raised by vasocontrictor agents. For example, norepinephrine and angiotensin II stimulate the inositol phosphate second messenger system initiating contraction of smooth muscle cells (Bohr and Webb, 1984). Other factors such as prostaglandin I2 or endothelium-derived relaxing factor produce compensatory vasodilation that offsets increased contractile responsiveness of smooth muscle cells.

Therefore, a reduction in vasodilator activity or an increase in vasoconstrictor activity could contribute to elevated peripheral resistance. In addition, the responsiveness of the cell to both vasoconstrictor and vasodilator agents may also determine the manifestation of elevated peripheral resistance. Impaired vascular smooth muscle function could influence the ability of vascular smooth muscle to respond to either type of factor and thereby modify the resistance of the vessel.

In summary, a number of renal and cellular disturbances are thought to contribute to the etiology of primary hypertension. In this regard, essential hypertension is generally not treated as a single disease. Rather, the pathophysiology as well as multiple risk factors including weight, diet and lifestyle require that essential hypertension be considered a multifactorial process.

Stress

Psychological stressors are among the risk factors commonly listed for hypertension. In general terms, psychological stress is usually thought of as an aversive threat or challenge confronting an individual. In this instance, stress is viewed as an environmental stimulus. Other definitions of stress have emphasized the adaptive response of the individual to external events or threats and rely on indices of behavioral or physiological changes as

measurements of 'stress'. However, the more recent and widely used formulation of stress has described stress as a process by which environmental events threaten or challenge the individual and thereby demand adaptation by the individual (Field, McCabe, and Schneiderman, 1985; Gatchel and Baum, 1983). This definition minimizes the confusing usage of 'stress' as both the stimulus and the response by using the term 'stressor' to indicate the environmental threat while allowing for measurement of stress through physiological responses. For purposes of clarity, this thesis will adopt the latter definition of the term.

Physiological Models of Stress

Environmental stressors are thought to contribute to the development of hypertension by increasing sympathetic outflow. Stressors typically evoke a defense reaction mediated by the lateral and posterior hypothalamus in which sympathetic activity to the heart, kidney, splanchic region and the skin is increased while vasodilation occurs in the skeletal muscle vascular beds. The net result is an increase in the rate and force of myocardial contraction, venoconstriction, splanchnic and renal vasoconstriction as well as decreased renal excretion of sodium (Herd, 1991). Secretion of cortisol and adrenal catecholamines is also stimulated.

Several plausible models have related such increases in

sympathetic activity associated with stressors to the induction and maintenance of primary hypertension. First, by activating the sympathetic nervous system, the stress response may potentiate the structural adaptation of the vasculature. Folkow (1982) contends that individuals genetically predisposed to hypertension exhibit sympathetic hyperreactivity to environmental stimuli. The frequent pressor responses caused by stress-induced sympathetic activity are postulated to increase the average pressure load and stimulate structural adaptation of the arterioles as a part of an adaptive mechanism to reduce excessive strain on the vessel.

Second, Obrist (1981) argues that beta adrenergic activity associated with stress raises cardiac output. As mentioned above, this elevation in blood flow overperfuses the tissue and thereby stimulates local arterial constriction. As a result, cardiac output is reduced within normal limits while peripheral resistance remains high. This autoregulation model is consistent with data obtained from animal and human studies. Forsyth (1971) exposed rhesus monkeys to a continous, 72 hour shock avoidance task. Sustained elevated blood pressure occurred throughout the session. The elevations were primarily due to increased cardiac output during the initial part of the procedure. As the session progressed, cardiac output returned to normal levels while elevated peripheral resistance maintained the

high blood pressure. More recently, Miller and Ditto (1988) exposed male volunteers to an extended aversive video task for one hour. They report that elevated blood pressure was significantly related to heart rate responses in the first 15 minutes but as the session progressed high blood pressure was more significantly related to elevated peripheral resistance as measured by digital blood volume pulse. These data suggest autoregulation may contribute to stress-induced changes in cardiac function. However, these studies do not address the possibility that neurohumoral factors may be acting independently of autoregulatory contol.

An alternative model suggests kidney regulation of blood volume and blood pressure may be altered during periods of stress. The stress response may influence kidney function through sympathetic activation. For example, it has been observed that the spontaneously hypertensive rat strain (SHR) shows an increase in sodium retention despite increases in blood pressure during air jet stress relative to a normotensive strain (Lundin and Thoren, 1982). The authors explain these results by the increased renal sympathetic activity in the SHR. Sympathetic activation acts by increasing renal arteriole resistance and tubular restriction thereby reducing glomerular filtration rate. The interpretation is reinforced by results demonstrating that renal dennervation reduces sodium retention (Winternitz, Katholi, and Oparil, 1980). Light, Koepke,

Obrist and Willis (1983) reported similar results in which a competitive challenge elicited decreased sodium and fluid excretion in normotensive men with a family history of hypertension. The authors suggest the concurrent increases in heart rate are indicative of sympathetic mediation.

In summary, psychological stressors have been proposed to influence regulation of blood pressure by increasing sympathetic nervous system activity which in turn may modify renal function, disrupt autoregulatory processes and induce vascular structural adaptation.

Cardiovascular Reactivity

These models provide the basis for the assertion that excessive physiologic responses to behavioral stressors may contribute to the development of primary hypertension (Matthews et al, 1986). Numerous retrospective studies have reported that hypertensive individuals display greater heart rate and pressor responses to a variety of standard laboratory stimuli in comparison to their normotensive counterparts (Krantz & Manuck, 1984). However, it has been argued that the exaggerated reactivity does not precede the development of hypertension, but is a consequence of the hypertensive condition. Apparent heightened responses in hypertensive individuals may be caused by the greater narrowing of the lumen in the hypertrophied resistance vessels. According to Poiseuille's law, resistance and

blood flow are amplified to the fourth power of the lumen radius (Folkow, 1982). Therefore, any blood pressure changes that occur during stress-induced vasoconstriction may be magnified in the narrow vessel.

Alternatively, other research suggests hyperreactivity may be characteristic of the pre-hypertensive state. Borderline hypertension is thought to represent an early stage in the development of established hypertension. Borderline hypertensive individuals do not display the secondary adjustments of established hypertension such as structural changes in the resistance vessels or elevated total peripheral resistance. However, individuals with borderline hypertension show similar pronounced responses to laboratory stressors (Light, 1989). For example, Falkner, Onesti and Hamstra (1981) reported that in a sample of borderline hypertensive adolescents, the degree of systolic blood pressure reactivity to a mental arithmetic challenge was predictive of hypertension development five years later.

A related literature provides evidence of a genetic component in reactivity. It has been documented that normotensive individuals with a family history of essential hypertension are more likely to develop hypertension relative to individuals without parental history of hypertension (Light, 1989; Matthews et al. 1986). Furthermore, reliable associations exist between familial history of hypertension and cardiovascular hyperresponsivity

to behavioral stressors. Accordingly, Manuck and Proietti (1982) reported that normotensive offspring of hypertensive parents displayed greater heart rate and systolic blood pressure responses during two cognitive tasks and a sustained handgrip challenge in comparison to subjects without parental history of hypertension.

Possible Mechanisms of Elevated Reactivity in Hypertensives

The enhanced cardiovascular reactivity found in hypertensive subjects or subjects genetically susceptible to hypertension as compared to normotensive individuals is thought to be the result of excessive sympathetic outflow in response to psychological stressors. Hypertensive patients have been shown to have a larger increment in total body spillover of norepinephrine during a mental challenge relative to age-matched normotensive subjects (Goldstein et al., 1989). Similarly, increased sympathetic outflow to the kidney has been reported in SHR but not in WKY (Koepke and DiBona, 1985). Such increases in sympathetic outflow appears to exist prior to the onset of sustained hypertension. Falkner et al. (1979) reported elevated plasma catecholamine levels after mental stressors in children with hypertensive parents. These findings of elevated sympathetic activity are consistent with Folkow's hypothesis of an exaggerated defense response in those genetically susceptible to hypertension.

Alternatively, other research suggests hypertensive subjects display heightened reactivity of the smooth muscle cells lining the vasculature (Bohr and Webb, 1984; Reid, 1988; Webb and Bohr, 1981). Mulvany, Aakljaer and Christensen (1980) found that the vascular smooth muscle cells of adult SHR rats exhibited greater sensitivity to exogenous norepinephrine in comparison to the WKY strain. Furthermore, the same norepinephrine sensivity was present in the non-hypertrophied smooth muscle cells of pre-hypertensive 6 week old rats. In human subjects, an early study by Doyle and Fraser (1961) found children of hypertensive families displayed greater responses to norepinephrine, further suggesting that heightened reactivity may precede the development of sustained hypertension. Likewise, researchers have reported diminished relaxation reponses of aortic strips in hypertensive rats following isoproterenol treatment (Cohen and Berkowitz, 1976).

These studies imply that smooth muscle cells of hypertensive individuals are in some way altered and subsequently function abnormally to external stimuli. In general, it appears that the vascular membrane processes are the principal determinants of elevated responsiveness. Alterations in second messenger relay systems, receptor number and affinity, electrogenic pumps or membrane permeability to ion flux are conceivable mediators of

augmented cellular excitability. The diversity of these membrane proteins suggests a general dysfunction of the membrane lipid bilayer in which they function. In fact, altered vascular membrane phospholipids have been reported in hypertension and are linked to increased membrane viscosity believed to influence membrane protein function (Dominiczak, Lazar, Das, and Bohr, 1991).

Dietary Lipids and Blood Pressure

Dietary fat intake is among the life-style modifications suggested for the prevention or reduction of high blood pressure. Several clinical studies have demonstrated that dietary enrichment of polyunsaturated fatty acids with concomitant reduction in saturated fatty acids exert hypotensive effects on blood pressure in both hypertensive and normotensive subjects (Heagerty et al., 1986; Puska et al., 1983; Rao, Rao and Srikantia, 1981). In particular, diets rich in fish oil have been shown to consistently reduce blood pressure (Bond et al. 1989; Karanja, Phanouvong & McCarron, 1989; Knapp and Fitzgerald, 1989; Rogers, James, Butland, Etherington, O'Brien and Jones, 1987; Singer et al., 1985). However, other studies have provided conflicting data where diets with high polyunsaturated-to-saturated ratios had little or no effect on blood pressure (Brussaard, van Raaij, Stasse-Wolthuis, Katan and Hautvast, 1981; Margetts et al., 1985). In

addition, diets containing high amounts of saturated fat such as butterfat have not been shown to increase blood pressure relative to unsaturated diets in hypertensive subjects (Sacks, Rouse, Stampfer, Bishop, Lenherr and Walther, 1987). Moreover, blood pressures of SHRs fed a butterfat diet were significantly lower compared to animals given a corn oil diet (Karanja et al., 1987).

Taken together, the role of the polyunsaturated-to-saturated fat ratio on blood pressure remains unclear. It has been suggested that this ratio alone may not define the action of dietary fats on blood pressure. Rather, the fatty acid structure such as carbon length and the positioning of the initial carbon bond may play a greater role in blood pressure regulation by dietary fats (Karanja et al., 1989).

Dietary Lipids and Membrane Composition

Dietary lipids act at the cellular level by modifying the lipid composition of the cell membrane. The phospholipid bilayer forms the basic structure of all biological membranes in which fatty acid groups constitute the hydrophobic interior of the membrane while the polar phosphate groups line the surface of the membrane. Phospholipids are in a dynamic state of renewal where the fatty acid groups and polar phosphate groups turnover independently of each other. The fatty acid groups primarily arise from cellular synthesis and dietary lipid

intake (Wahle, 1983). The final membrane fatty acid composition is the net result of several factors, the most important being the competition for enzymes among members of different fatty acid families (Stubbs and Smith, 1984). For example, n-3 unsaturated fatty acids have precedence over the n-6 group which in turn have a higher enzyme affinity than n-9 fatty acids. Therefore, in the absence of n-3 fatty acids, n-6 fatty acids are more readily incorporated into the membrane. Dietary lipids contain different families of polyunsaturated fatty acids and thereby modify the membrane fatty acid composition with respect to the fatty acid content available in the diet. Diet-induced variations in membrane composition have the potential to differentially alter many integral activities of the membrane which may be associated with vascular smooth muscle reactivity.

Membrane Fluidity and Protein Function

The fluidity of the biomembrane is particularly sensitive to diet-induced modifications of fatty acid composition. In general, membrane fluidity has been shown to increase with a higher proportion of polyunsaturated fatty acids whereas saturated fatty acids tend to produce a less fluid membrane (Mead, Alfin-Slater, Howton and Popjak, 1986). In addition, several saturated fatty acids including lauric, myristic and palmitic acid are known to raise serum

cholesterol (Grundy, 1991). Cholesterol similarly reduces membrane fluidity (Yeagle, 1985).

Due to their intimate contact with the lipid membrane interior, many membrane bound transporters, receptors and enzymes are responsive to diet-induced alterations in the fluidity of the lipid bilayer. For example, an increase in activity of ion transporters such as the sodium-potassium pump have been observed following diets rich in oleic acid (Pagnan et al., 1989,) and linoleic acid (Heagerty, Ollerenshaw, Robertson, Bing and Swales, 1986; Kawahara et al., 1990). Because ion transportors are important regulators of ion flux and distribution in the cell, such diet-induced changes in the properties of ion transportors may alter the ionic distribution and in turn affect smooth muscle contractility. Likewise, the activity of adenylate cyclase, a membrane bound enzyme which catalyzes the production of the second messenger cyclic AMP, has been modified by various diets including menhaden oil (Alam and Alam, 1988) as well as linoleic and linolenic acid (Morson and Clandinin, 1986). In addition, other research has claimed that coconut and sunflower oil altered the function of the prejunctional alpha-adrenergic receptors (Semafuko, Rutledge and Dixon, 1989; Semafuko, Rutledge and Dixon, 1987).

Similar changes in membrane protein function have been observed with membranes enriched with cholesterol.

Cholesterol has been shown to elevate basal and some agonist stimulated calcium influx in rat vascular smooth muscle cells (Bialecki, Tulenko and Colucci, 1991). In addition, calcium channel antagonists inhibited the cholesterol-induced influx of calcium. In an earlier report, Broderick, Bialecki and Tulenko (1989) found an increase in norepinephrine sensitivity in rabbit arteries loaded with cholesterol. The observed reduction in the sodium-potassium pump and the dependence on extracellular calcium suggest that cholesterol altered the vascular smooth muscle cell rendering it more responsive to adrenergic stimulation. Other studies have reported that cholesterol modulates calcium-dependent pump activity in the sacroplasmic reticulum (Madden, Chapman and Quinn, 1979) and reduces vasodilator responses to acetylcholine, an agonist which is dependent on the endothelium-derived relaxing factor (Wright and Angus, 1986).

The lipoproteins, low density (LDL) and high density (HDL), are associated with cholesterol in its transport to and from the liver. In addition to its physiological role as a transporter, LDL has been reported to induce vascular contraction in rat aortic rings with concomitant increases in intracellular calcium (Sachinidis, Locher, Mengden, Steiner and Vetter, 1989) as well as to inhibit endothelium-dependent relaxation (Andrews, Bruckdorfer, Dunn and Jacobs, 1987). Conversely, HDL is inversely related to calcium

influx in human red blood cells (Stimpel, Neyses, Locher, Knorr and Vetter, 1985).

Collectively, these studies demonstrate the sensitivity of membrane proteins to alterations in lipid and cholesterol content associated with dietary intake. However, the precise mechanisms through which membrane lipids, proteins, and cholesterol interact are relatively unkown. One possiblity may be that surrounding lipids influence the conformational state of the proteins thereby enhancing or reducing the accessibility of binding sites. Or the inherent lateral mobility and molecular ordering of the proteins and lipids within the bilayer may be restricted or altered such that necessary membrane interactions do not occur (Spector and Yorek, 1985).

Regulation of Eicosanoid synthesis by n-3 & n-6 fatty acids

Changes in the bioavailability of membrane phospholipid precursors for eicosanoid (prostaglandins, thromboxanes and leukotrienes) formation is another important consequence of variation in dietary lipid intake. Eicosanoids are the end products of an enyzme cascade which begins in the phospholipid bilayer. Desaturation and elongation of dietary linoleic acid, an essential n-6 polyunsaturated fatty acid, gives rise to arachidonic acid. Agonist-stimulated release of arachidonic acid from the membrane phospholipids allows further enzyme conversion to yield

various prostaglandins and thromboxanes. Because linoleic acid serves as the primary precursor of arachidonic acid, dietary loading of linoleic acid understandably enhances eicosanoid synthesis, specifically the dienoic compounds such as prostaglandin I_2 (PGI_2) or thromboxane A_2 (TXA_2) (Mead, Alfin-Slater, Howton and Popjak, 1986). PGI_2 influences blood pressure regulation by causing vasodilation, diuresis and renin release as well as modifying norepinephrine output (Moore, 1985). The hypotensive effects of a linoleic acid enriched diet are thought to be mediated in part by PGI_2 function (Iacono et al., 1981). However, TXA_2, also an arachidonic acid-derived product, stimulates vasoconstriction and platelet aggregation (Moore, 1985).

N-3 fatty acid diets have been proposed to have beneficial effects on cardiac function by not only producing alternative and possibly weaker compounds of eicosaniods but also by inhibiting production or accumulation of arachidonic acid-derived eicosaniods (Lands, 1986). Diets rich in marine fish oil partly replace arachidonic acid in the membrane with n-3 eicosapentaenoic acid (EPA) promoting the production of trienoic eicosanoids such as prostaglandin I_3 (PGI_3) or thromboxane A_3 (TXA_3) (Fischer and Weber, 1984). With regard to function, PGI_3 has similar hypotensive properties of PGI_2 while TXA_3, in contrast to TXA_2, is only weakly vasoconstrictory and does not cause platelet

aggregation (Weber, Fischer, von Schacky, Lorenz and Strasser, 1986). As an example, Knapp and Fitzgerald (1989) found TXA_2 was reduced in men fed a fish oil diet while TXA_3 metabolites were detected. In addition, PGI_3 increased initially on the fish oil diet, but did not remain elevated as blood pressure fell suggesting that eicosaniods were not the sole mediators of blood pressure reduction during the fish oil diet.

Psychological Stressors and Dietary Fat Intake

A modest literature has investigated the interaction between dietary fat intake and blood pressure responses to stressors. Several studies have shown that supplementing rats with dietary gamma-linolenic acid reversed blood pressure elevation caused by isolation stress. (Mills and Ward, 1986a; Mills, Summers and Ward, 1985). Similar reductions in blood pressure during isolation stress have been observed in rats supplied with EPA (Mills and Ward, 1986b). In a human study, a 28 day dietary supplementation of borage oil, an n-6 polyunsaturated fatty acid, attenuated heart rate and blood pressure reactivity to the Stroop Color-Word task (Mills, Prkachin, Harvey and Ward, 1989).

Thus, it appears that cardiovascular responses to various stressors may be influenced by dietary lipids. By modifying the lipid composition of cellular membranes, dietary fatty acids may alter the response of the

vasculature to vasoactive stimuli associated with the stress response. Several studies have reported reductions in vascular reactivity to norepinephrine and angiotensin II following a diet supplemented with n-6 or n-3 polyunsaturated fatty acids in rats (Mills and Ward, 1986a; Mills, Summers and Ward, 1985; Yin, Chu and Beilin, 1991). More recently, vascular reactivity to norepinephrine and angiotensin II as measured in forearm resistance arteries of human subjects was shown to be markedly attenuated following dietary supplemetation of EPA (Chin, Gust, Nestel and Dart, 1993). The specific mechanisms of reduced vascular reactivity following dietary intervention are rather speculative. As mentioned previously, several conceivable mediators include modulation of receptors or second messenger signalling as well as alterations in vasoactive factors involved in the tone of the membrane such as various prostaglandins or endothelium-dependent relaxing factor.

RATIONALE

Dietary lipids have been proposed to influence blood pressure regulation. A number of studies suggest that diets rich in polyunsaturated fats are capable of reducing basal blood pressure as well as blood pressure responses to stressors. However, other studies have failed to show a significant relationship between the two variables. The inconsistencies in previous work have been attributed to flaws in the study design such that the conclusions reached are often open to alternative explanations. Therefore, the present study was conducted to clarify the effects of diets differing in fatty acid composition on blood pressure regulation in a tightly controlled design.

Aside from differences in the source of dietary fat (fish oil, butterfat and safflower oil), all diets were equivalent in the total amount of protein, carbohydrate, fat and electrolytes. Thus, the differential pressor effects of each diet were thought to reflect the actions of the particular fatty acid groups available in the diet. With regard to the diet rich in fish oil, the n-3 fatty acid groups commonly found in fish oil appear to exert a hypotensive effect on blood pressure by increasing membrane fluidity and producing vasodilatory eicosanoids. Similar findings have been reported with linoleic acid, an n-6 fatty acid which comprised approximately 70% of the

polyunsaturated fat in the safflower oil diet. Theoretically, both diet groups should favor a reduction in vascular reactivity. The butterfat diet, on the other hand, contained palmitic acid, a saturated fat which could potentially raise serum cholesterol levels. Elevated serum cholesterol may rigidify cellular membranes as well as alter calcium influx and vasodilatory responses of the cell. Taken together, these factors may foster heightened vascular reactivity.

In light of these findings, the following study was designed to test the hypothesis that diets rich in fish oil and safflower oil would lower basal blood pressure, while an increase in blood pressure would be observed as a consequence of the butterfat diet. Furthermore, blood pressure reactivity was expected to parallel any diet-induced changes in basal blood pressure. Several studies have reported alterations in vascular reactivity to exogenous norepinephrine and angiotensin II following manipulations in dietary fat intake without any apparent changes in resting blood pressure. Consequently, blood pressure reactivity may be a more sensitive index of the impact of dietary lipids on blood pressure regulation in comparison to basal blood pressure.

Blood pressure changes associated with dietary fat intake were evaluated in both normotensive and hypertensive male volunteers between the ages of 30 and 60. The study

utilized a within-subjects, experimenter blind, repeated measures design where each subject served as his own control. By using this design, the differential effects of each diet on blood pressure were observed in each subject, thereby reducing the variability caused by response differences between subjects. The order of diet presentation was counterbalanced for groups of subjects to control for possible confounding factors that could emerge with the passage of time. The present study maintained subjects on each diet for six weeks. The duration of exposure was chosen based on previous research which demonstrated the development of significant blood pressure responses within six weeks of diet administration (Puska et al. 1983). In addition, it has been shown that elevated serum levels of n-3 fatty acids associated with fish oil supplementation return to baseline levels within 2 to 3 weeks of diet completion (Knapp and Fitzgerald, 1989). Therefore, the diets were separated by six week washout periods to ensure sufficient elimination of possible carry-over effects from the preceding diet.

The within-subjects design of the study required the evaluation of the stability of individual responses upon repeated administration of reactivity testing. Of concern is habituation of blood pressure responses across successive testing due to factors such as task familiarity, skill acquisition or reduced motivation. Habituation may reduce

the magnitude of test-retest correlations and/or confound any diet effects on blood pressure reactivity. Therefore, measures of performance and subject ratings of tension and challenge to each task were obtained as indices of habituation.

In order to adequately identify the characteristic blood pressure responses of a given individual, a battery of standard test stimuli was used including the Stroop color-word interference task, mental arithmetic and an isometric handgrip challenge. Multiple tasks within a reactivity testing session were employed based on previous findings that cardiovascular reactivity may depend on the particular stressor presented. In this regard, it is thought that a single stressor may not identify the typical cardiovascular response for any particular individual. Both the color-word task and mental arithmetic task represent mental active coping stressors, while the isometric handgrip challenge was used to identify blood pressure responses elicited during exercise. All three tasks have been used extensively in previous research to evoke substantial blood pressure responses (Matthews, 1986; Lenders, Houben, van Valderen, Willemsen and Thien, 1988; Rozanski, 1988).

The initial baseline blood pressure of the subjects may influence the basal blood pressure sensitivity to dietary manipulations as well as blood pressure responses to stressors. A number of studies have reported heightened

reactivity in hypertensive subjects relative to their normotensive counterparts. In addition, other studies suggest that hypotensive effects of dietary fats such as fish oil are present only in subjects with high blood pressure. Thus, possible differences in outcome as a result of initial blood pressure status were examined.

To provide a neuroendocrine measure of reactivity, plasma cortisol was determined before and during the mental challenge of a video game. Due to the logistics of blood sampling, plasma cortisol could not be collected at the same time as blood pressure reactivity testing. Therefore, a different challenge was selected to alleviate further repetition of task performance. The video game also represents a mental, active coping task and like the other three tasks elicits a sufficient physiological response (Turner, 1989; Steptoe, Millville and Ross, 1984).

METHODS

The present study was designed to utilize the subjects and dietary protocol of an established, ongoing study examining the influence of dietary fats on blood pressure regulation and cholesterol metabolism, directed by Njeri Karanja, Ph.D. and David McCarron, M.D. in the Department of Medicine, Division of Hypertension and Nephrology at the Oregon Health Sciences University. The description of methods is therefore divided into two sections. The first section describes the protocol established and performed by Dr. Karanja and Dr. McCarron while the second section presents the methods of the current ancillary study.

Section I

Subjects

Twenty-one male volunteers between the ages of 30 and 60 years were recruited through local newspaper and radio advertisements. Individuals who responded to the advertisements were sent a letter describing the methods of the study as well as a questionnaire regarding medical history and dietary preferences (see Appendix A). The completed questionnaires were used to screen for obvious exclusion criteria such as special dietary needs and medical complications. Individuals with any of the following medical conditions were excluded: obesity (body weight > 50% of the ideal value), diabetes mellitus, hyperlipidemia

(cholesterol or triglycerides in the top 5% of Lipid Research Clinic values), end organ damage (overt cerebrovascular or coronary vessel disease), abnormal serum creatinine, peripheral artery disease, and alcoholism. Also, subjects taking medications that affect lipid metabolism such as hypolipidemic drugs and cyclooxygenase inhibitors were not enrolled in the study. A follow up interview with potential subjects served as a second screening procedure to verify the subjects' willingness and ability to commit to the long term study. Upon thorough explanation of the method and objectives of the study, informed consent was obtained. The study was approved by the Oregon Health Sciences University Institutional Review Board, Committee of Human Research.

Experimental Design

Baseline. The experimental design is depicted in table 1. Those subjects with hypertension were tapered off their antihypertensive medications over a period of 1-4 weeks under the supervision of Dr. McCarron. Blood pressure was monitored weekly by a nurse practitioner hired specifically for the study. If mean arterial pressure rose over 120 mmHg during this time, medication was resumed and the patient was referred back to his primary physician. Those who remained below this limit were entered into the four week baseline phase. During baseline, subjects visited the Clinical

Table 1. Experimental design and diet rotation of the overall study.

	BASELINE	DIET1	WASHOUT	DIET2	WASHOUT	DIET3
Week	1 2 3 4	123456	123456	123456	123456	123456
BP, heart rate	X X X X	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
physical exam	X					X
EKG	X					X
SMAC, CBC	X					X
Blood draw	X	XX	X	XX	X	XX
7-day food record	X		X		X	X
24-hour urine	X	X		X		X

Group A (n=4)	Butterfat	Fish oil	Safflower
Group B (n=4)	Safflower	Butterfat	Fish oil
Group C (n=3)	Fish oil	Butterfat	Safflower
Group D (n=5)	Fish oil	Safflower	Butterfat
Group E (n=3)	Safflower	Butterfat	Fish oil
Group F (n=2)	Fish oil	Safflower	Butterfat

Research Center (CRC) once a week for blood pressure measurement. Measurements were obtained by the nurse practitioner using a standard sphygmomanometer and a Hawksley random zero manometer in both the supine and sitting positions. If mean arterial pressure (MAP) exceeded 105 mmHg for four consecutive weekly measurements, subjects were considered hypertensive while those with MAP below 105 mmHg were considered normotensive.

During the final week of baseline, a 24 hour urine sample and a blood sample were obtained for the determination of biochemical parameters including; plasma total cholesterol, triglycerides, and phospholipids, LDL and HDL receptor activity, apolipoprotein E phenotyping, calcium transport and intracellular calcium concentrations. In addition, a detailed 7 day food dairy was kept by the patients during this final week of the baseline phase to gain insight into the eating habits of the group.

Experimental Period. Following the baseline phase, subjects rotated through the three dietary experimental periods in groups of four. All subjects received all three dietary treatments. Thus, each subject served as his own control. The order of treatment was presented randomly to each group (S-Plus software). As noted in table 1, several subjects did not complete the study, reducing the group size. In all cases, attrition was due to conflicts with work schedules. When possible, additional subjects were

entered into the diet sequence. Subjects were asked to eat only the food provided by the CRC during the three experimental periods. Alcohol consumption was not permitted throughout the experimental periods. To maintain constant contact with the subjects, they were asked to eat at least one meal per day at the CRC, but were permitted to take pre-packaged meals home. Subjects weighed daily with CRC nursing staff supervision. Resting blood pressure measurements were obtained three times a week by the nurse practitioner. At the end of each dietary period, a fasting blood sample and a 24 hour urine collection were obtained to again assess biochemical parameters. Dietary compliance was estimated by a combination of assessments that include weight, meal attendance and urinary sodium and potassium.

Washout Period. Each experimental phase was followed by a six week washout period during which the subjects consumed their normal diets. Subjects were required to return to the CRC once each week for blood pressure measurement during this period. At the end of the each washout period, fasting blood samples and 24 hour urine collections were obtained to assess the battery of biochemical parameters. To estimate the extent of the washout, plasma free fatty acid concentration was measured. In addition, subjects were required to keep a 7 day food diary during the final week of washout.

Diet Composition

The composition of the three diets based on a 3400 kilocalorie diet is shown in table 2. Subjects were fed three diets differing only in the source of dietary fat (butterfat, safflower oil, and fish oil). With regard to the specific fatty acid content of the fat, all diets contained 15% of the total fat as monounsaturated fatty acids with the major difference occurring in the distribution of saturated and unsaturated fats.

Butterfat Diet. The butterfat diet consisted of a diet modeled along the typical American diet except that approximately 50% of all the dietary fat came from dairy products. The differences in the fatty acid content of the butterfat diet was achieved by providing butter in the place of margarine, whole as opposed to skim milk and baking with butter in the place of margarine. Other than these manuevers, all the foods in the butterfat diet were identical to those in the fish oil and safflower oil diets. The intake of both the monounsaturated and saturated fat was 15% of the calories and PUFA intake was maintained at 7%. To achieve the goal of comparing the three different types of fats, this diet provided 30g of butter plus approximately 27g of milk fat coming from dairy products (whole milk, cheese, yogurt) for a total intake of approximately 57g of butterfat per day. This quantity of dairy fat accounted for approximately 50% of all the fat in the diet. This level of

Table 2. Diet composition of the safflower oil, butterfat and fish oil diets.

Nutrient g/d (%kcal)	Dietary Phase		
	Butterfat	Fish Oil	Safflower Oil
Fat	139.78 (37)	139.78 (37)	139.78 (37)
Saturated	56.67 (15)	41.46 (11)	26.44 (7)
Polyunsaturated	26.44 (7)	41.56 (11)	56.57 (15)
Monounsaturated	56.67 (15)	56.67 (15)	56.67 (15)
P/S ratio	.47	1.00	2.14
Protein	127.50 (15)	127.50 (15)	127.50 (15)
Carbohydrate	408.00 (48)	408.00 (48)	408.00 (48)
Sodium (mg)	1720.00	1611.00	1381.00
Potassium (mg)	2301.00	2659.00	2617.00
Calcium (mg)	1395.00	1391.00	1391.00
Cholesterol (mg)	275.60	276.20	278.00

intake approximated dairy industry market projections of what butter intake would be if Americans substituted butter for margarine, plus the amount of dairy fat that would be consumed from other milk products. The polyunsaturated to saturated fat ratio of the diet was approximately 0.47.

Safflower Oil. The safflower oil diet was the same basal diet as the butterfat and fish oil diets except that 50% of the total fat came from polyunsaturated fats of vegetable origin supplied as margarine (Saffola). The intake of monounsaturated fat was 15% of the energy. Unlike the butterfat diet, however, this diet contained only half the saturated fat (7% of energy) content and double (15% of the energy) the PUFA content. The diet supplied 57g of fat as polyunsaturated fat from margarine (Saffola) and safflower oil. The polyunsaturated to saturated ratio was 2.14.

Fish Oil. The fish oil diet resembled the other two diets but had lower quantities of vegetable oil (21%) and milkfat (10%) and contained approximately 25% fish oil supplied in the form of fish oil supplements. To achieve 25% of the fat as fish oil, 27 capsules per 3000 kcalories were administered each day. Again, the intake of monounsaturated fat was 15% of the energy. However, the diet contained 11% of the energy as saturated fat and 11% of the energy as PUFA. The polyunsaturated to saturated fat ratio was 1.0.

Besides these differences in the fat composition and source, the three diets were comparable for any one day with regard to the total amount of nutrients. Protein, carbohydrates and fat contributed 15%, 48% and 37% of the energy respectively. In addition, no significant differences among vitamin or mineral composition existed between the three diets. As an example, the electrolyte content (calcium, potassium, and sodium) of all the three diets were comparable. The 7-day average intake for calcium was 1395, 1391, and 1391 mg per day for butterfat, safflower, and fish oil-based diets respectively. Comparable values for sodium were 1720, 1611, and 1381 mg for butterfat, safflower and fish oil diets respectively. The 7-day average potassium content was 2301, 2659, and 2617 mg for butterfat, safflower and fish oil diets respectively.

To ensure that subjects maintained the same weight, the caloric content of the diet was tailored to each participant by using a specialized computer program that extrapolates the caloric content upward or downward such that there is an across the board adjustment in all foods. This adjustment was necessary to guard against nutrient dilution.

Section II

Subjects

Subjects enrolled in the baseline phase of the foregoing study were recruited for the ancillary study

designed to measure cardiovascular reactivity. Specific objectives and requirements of the study were explained to each subject and written consent was obtained (Appendix B). One subject did not agree to participate in this portion of the study because of the additional time committment. All procedures and written consent forms for the study were approved by the Oregon Health Sciences University Institutional Review Board, Committee on Human Research.

Experimental Design

Table 3 depicts the experimental design for the ancillary study. The experimenter was blind to the diet condition of the subjects. However, the subjects were aware of the particular dietary fat source they were eating. Blood pressure reactivity was measured during the final week of each dietary phase including the baseline and washout periods. The daily schedule of each subject dictated the time of day in which testing occured. However, this time (morning or afternoon) was consistent throughout the six phases for any one subject. Reactivity was measured following meal consumption in the CRC.

During the last week of each dietary period, fasting blood samples were collected to assess plasma cortisol levels at rest and following a mental challenge. All blood samples were obtained in the morning. The exact time depended on the schedule of each subject, however, the hour

Table 3. Experimental design of the ancillary study.

	BASELINE	DIET1	WASHOUT	DIET2	WASHOUT	DIET3
Week	**1 2 3 4**	**123456**	**123456**	**123456**	**123456**	**123456**
BP Reactivity	X	X	X	X	X	X
Cortisol Reactivity		X		X		X

Group A (n=4)	Butterfat	Fish oil	Safflower
Group B (n=4)	Safflower	Butterfat	Fish oil
Group C (n=3)	Fish oil	Butterfat	Safflower
Group D (n=5)	Fish oil	Safflower	Butterfat
Group E (n=3)	Safflower	Butterfat	Fish oil
Group F (n=1)	Fish oil	Safflower	Butterfat

did not vary for each particular subject across the three dietary periods.

Blood Pressure Measurement

During testing, blood pressure was measured using an automated blood pressure device consisting of a sphygmomanometer and a physiograph recorder (Narco Biosystems P300). The instrument employs a standard occluding cuff with a small microphone which is positioned over the brachial artery and secured beneath the cuff. The sphygmomanometer automatically inflates and deflates the cuff at constant rates and time intervals. The pen on the physiograph records an inverted "V" slope. As the blood pressure cuff inflates, the pen ascends while the downslope of the pen reflects the deflation of the cuff. Vertical slash marks or lines along the downslope of the inverted "V" denotes the systolic blood pressure as perceived by the microphone.

Tasks

Stroop color-word task. Each subject was shown a rapidly changing series of 80 color slides for 3 minutes. Each slide was displayed for approximately one second with the interval between slides being one to three seconds. The slides were shown on a programable sound slide projector with a self-contained screen (Belle Howel Ringmaster II,

USA). The slides displayed the names of a primary color (e.g., blue) each written in letters of nonmatching color (e.g., green). The subject was required to state aloud the color used for the letters of the word rather than the word itself. In addition, to provide another means of interference, a tape was played of a male voice stating colors (red, blue, yellow and green) which might or might not have agreed with the particular slide being shown. A listing of the stimuli is presented in Appendix C. Performance was measured as the number of incorrect responses in stating the color of each slide.

Mental arithmetic. The subject was instructed to serially subtract a one digit number from a three digit number for 3 minutes as quickly and accurately as possible. The subject was asked to report each answer orally. The number of incorrect subtractions, total number of serial substractions and the percent correct were used as measures of performance.

Isometric hand grip. Using an adjustable hand dynamometer (Asimow Engineering Co., Los Angeles), subjects were asked to maintain a handgrip equal to 30% of their maximum voluntary force for a period of 3 minutes.

Video Game. A hand-held, PacMan video game (Nintendo Game Boy, 1991) was used. Subjects were given basic instructions on how to play the game and were allowed to practice for approximately 3 minutes prior to venipuncture.

During testing, subjects were instructed to play as many games as 5 minutes would permit while attempting to reach a high score of 3500 points. The score of each game was recorded as a measure of performance.

Protocol

Blood Pressure Reactivity. Reactivity testing was conducted in the outpatient clinic. The subjects were seated in a comfortable chair in a quiet room. A blood pressure cuff was placed on the nondominant arm and subjects were asked to sit quietly for 5 minutes. At the end of that time, a series of 3 systolic blood pressure measurements was taken over a period of 3 minutes. Following the baseline measures, subjects were informed about the succession of test phases. The tasks were presented in the following sequence: Stroop color word task, hand grip challenge, and mental arithmetic. The nature of each task was explained just prior to administration and all instructions were standardized (Appendix D). Each task was 3 minutes in length and separated by a 3 minute baseline period. The cycling interval of the sphygmomanometer was adjusted to allow for 30 sec inflation of the cuff and 30 sec for deflation. Thus, blood pressure was recorded every minute throughout the task and baseline periods. At the end of each task, subjects were asked to rate their feelings of tension or challenge throughout the particular task on a 1-

10 Leikert scale (Appendix E).

Cortisol Reactivity. Fasting blood samples were collected at the CRC. Subjects were asked to lie down or sit quietly for several minutes before blood sampling. Venipuncture was performed by the CRC nursing staff, using a 21-gauge butterfly needle. After blood samples (50 ml) were drawn for the biochemical parameters of Dr. Karanja's study, two additional blood samples were taken. The first, 10 ml sample, was drawn into a heparinized vacutainer during resting conditions to provide a measure of resting cortisol levels. The catheter was flushed with 1 ml of saline and secured to the arm with tape. Sujects were then instructed to play a hand-held PacMan video game for 5 minutes. During the fifth minute of the game, a CRC nurse collected 1 ml of blood into a syringe and discarded it. This was followed immediately by a second 10 ml blood sample collected in heparinized tube. This sample provided a measure of cortisol levels during the mental challenge. Subjects were asked to rate their level of tension and challenge during the game (Appendix E). Blood samples were immediately spun in a refrigerated centrifuge (Beckman Instruments, Inc.) at 3200 RPM for 10 minutes. The plasma was removed with a pipet and placed into small microfuge tubes. Samples were stored at -20°C until cortisol assays were performed.

Cortisol Assay

Plasma cortisol was assayed using radioimmunoassay. Plasma samples were thawed and immersed in boiling water to denature corticosteriod binding globulin, a molecule which actively competes with the antibody binding of cortisol (Murphy, 1963). Duplicate standard solutions were made containing 0, 10, 20, 50, 100, 200, 500, 1,000, 2,000, 5,000 and 10,000 pg cortisol in 100 ul buffer. In addition, tubes were also prepared to estimate the total binding capacity as well as the nonspecific binding of the assay. One hundred μl (equal to 10,000 counts per minute) [^{125}I]-corticosteriod (ICN) and 100 μl corticosteriod antibody (Ventrex), titrated to bind approximately 40% of the total [^{125}I]-corticosteriod, were added to both the samples and standards. The tubes were vortexed and subsequently incubated at approximately 4°C. Twenty-four hours later, 500 μl dextran-coated charcoal at 5°C was added to each tube. The tubes were again incubated for an additional 10 min and then centrifuged to separate free from antibody-bound cortisol. The supernatant was decanted into new test tubes and counted to a 2% error (Micromedic Automatic Gamma Counter).

Counts per minute were normalized and fit to a least squares fit regression equation produced by log-logit transformation standards. Sensitivity of the assay ranged from 1.81×10^{-3} to 3.26×10^{-3} μg/dl. The cross-reactivity to other endogenous and synthetic glucocorticoids in this

procedure was less than 1%.

Data Analysis

Blood pressure responses to the tasks were calculated as change scores where pre-task baseline measurements were subtracted from blood pressure values recorded during the task. Differences in systolic basal blood pressure and blood pressure reactivity across the six phases of the study were analyzed using an overall repeated measures analysis of variance (MANOVA) with repeated measures. Cortisol reactivity across the dietary phases was analyzed using a Phase (dietary phase) by Period (pre-task, task) MANOVA with both Phase and Period assessed as repeated measures. Pairwise comparisons using a modified Scheffe's test based on Hoetelling's T^2 (Hochberg and Tamhane, 1987) were performed to determine the origin of significant effects. The alpha level for all comparisons was set at 0.05.

In order to examine the stability of blood pressure responses across time, the data were collapsed across dietary treatment and analyzed according to the order of presentation. A repeated measures MANOVA was used to assess whether blood pressure responses to the tasks and performance changed as a function of time. Subsequent pairwise comparisons with a modified Scheffe's test were performed to determine the source of the effect of time. Self-ratings of challenge and tension to the tasks were also

collapsed across dietary treatment and analyzed using Friedman's rank order nonparametric test. Additionally, this nonparametric test was used to analyze the performance during the PacMan video game because the distribution of scores was not normal.

To assess differences in outcome as a result of the initial blood pressure status of the subjects, subjects were grouped as either hypertensive or normotensive according to their mean arterial pressure assessed at baseline. A Diet (6) by Task (3) Blood Pressure Status (2) repeated measures MANOVA was performed. Blood Pressure Status was treated as a between subjects factor.

The primary aim of this study was to identify possible alterations in blood pressure reactivity resulting from changes in dietary fat intake. Therefore, an a priori decision was made to perform separate analyses involving only the three dietary conditions during which fat intake was controlled, in addition to the overall analyses comprised of all six phases.

Assuming a type I error of 0.05 and an estimated power of .80, a post hoc power analysis (Keppel, 1991) determined that the current study was able to detect a difference of 5 mmHg in blood pressure and blood pressure reactivity across the dietary periods.

RESULTS

Population Characteristics

The twenty subjects had a mean age of 45.55 (SEM±1.98) years (range, 30-60 years), a mean weight of 94.86 (SEM±3.30) kg and a mean height of 179.33 (SEM±1.63) cm.

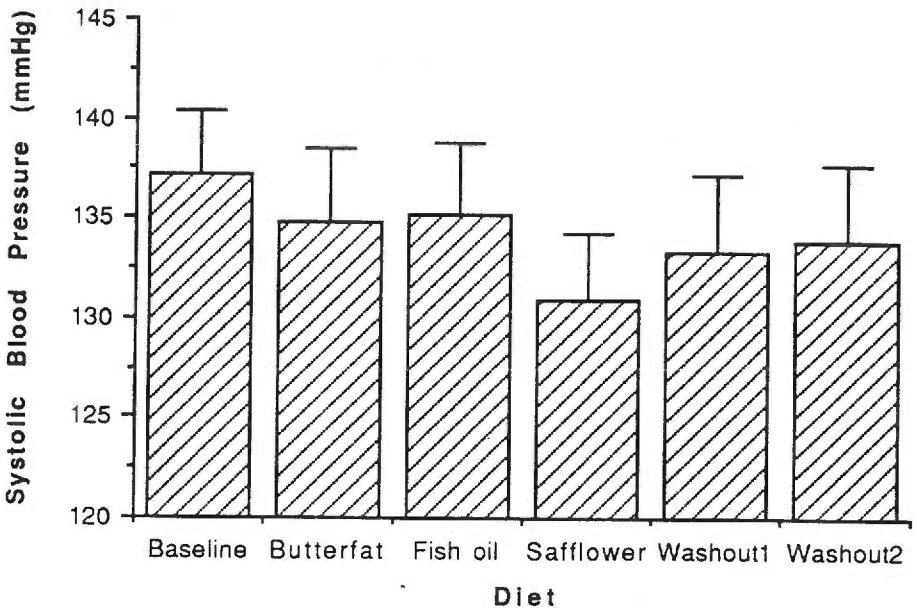
Basal Blood Pressure

Systolic blood pressure determined during resting conditions before and after stressor presentation within a session were averaged together to provide a single basal blood pressure value. The basal blood pressure obtained for each phase of the study is shown in Figure 1. An overall repeated measures MANOVA revealed that blood pressure differed significantly across the six phases of the study ($F\{5,95\}=2.32$ $p=.049$). A modified Scheffe's post hoc comparison of group means demonstrated that the significant difference was due to a reduction in blood pressure following safflower oil treatment in comparison to the blood pressure measurements collected during the initial baseline phase ($p<.05$). A separate repeated measures MANOVA involving just the three dietary treatments did not reveal any significant differences ($F\{2,38\}=2.67$, $p=.082$).

Blood Pressure Responses to Stressors

Systolic blood pressure values measured during the 3

Figure 1. Basal systolic blood pressure across the 6 phases of observation. Order of diet presentation is not shown. Subject n was 20 for each phase. Data are expressed as group means ± SEM.


Systolic Blood Pressure (mmHg)
145
140
135
130
125
120
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Diet

minutes immediately prior to task administration were averaged together to provide a pre-task baseline blood pressure value. Change scores were calculated by subtracting baseline values from the pressor response obtained during the three minute task. An overall Diet (6) by Task (3) MANOVA was performed to examine whether reactivity to the tasks differed throughout the six phases of the study. The analysis revealed a significant main effect for Task ($F\{2,38\}=35.51$, $p<.001$). As depicted in figure 2, the main effect was due to the larger blood pressure responses to the isometric handgrip challenge in comparison to the responses elicited by the two mental tasks. There was no main effect of diet. However, a significant Diet by Task interaction emerged ($F\{10,190\}=3.18$, $p=.001$), indicating that the blood pressure responses to the tasks varied across diet conditions. To assess the interaction, separate repeated measures MANOVAs were performed for each task across the six dietary phases.

Stroop Color-Word Interference Task. Pressor responses to the Stroop color-word task are shown in figure 3. A repeated measures MANOVA revealed a significant difference among change scores throughout the six phases of the study ($F\{5,95\}=6.63$, $p<.001$). Scheffe's modified post hoc comparison of group means demonstrated that the significant difference was due to lower blood pressure reactivity following the safflower oil diet ($p<.05$) and the final

Figure 2. Change in systolic blood pressure during each of the three tasks across the six phases of observation, n=20. Order of diet administration is not shown. Data are expressed as group means ± SEM.

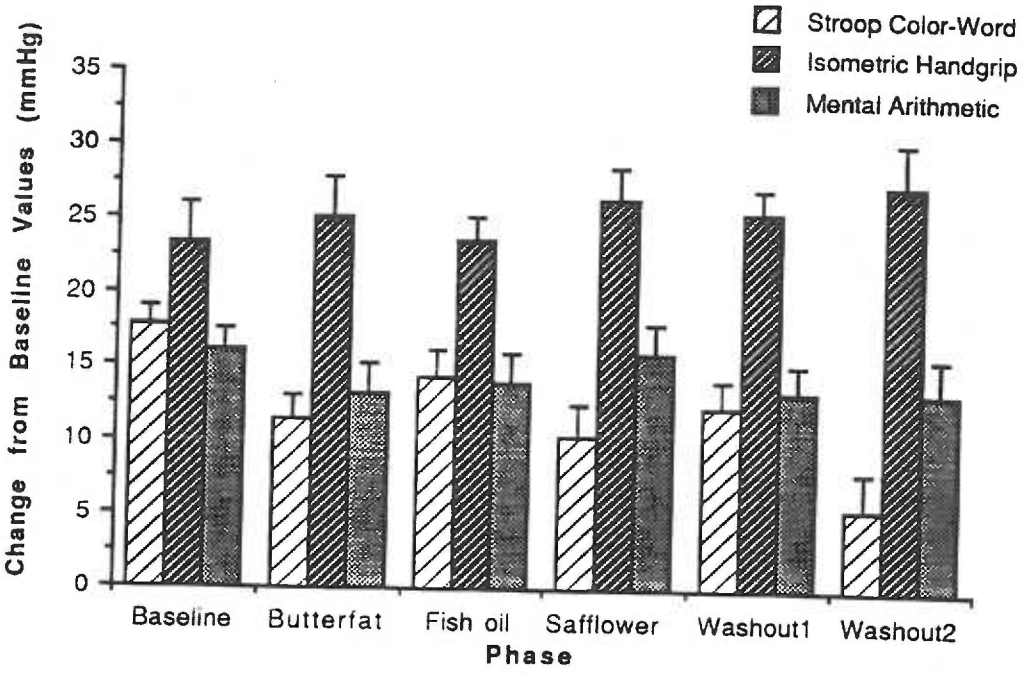
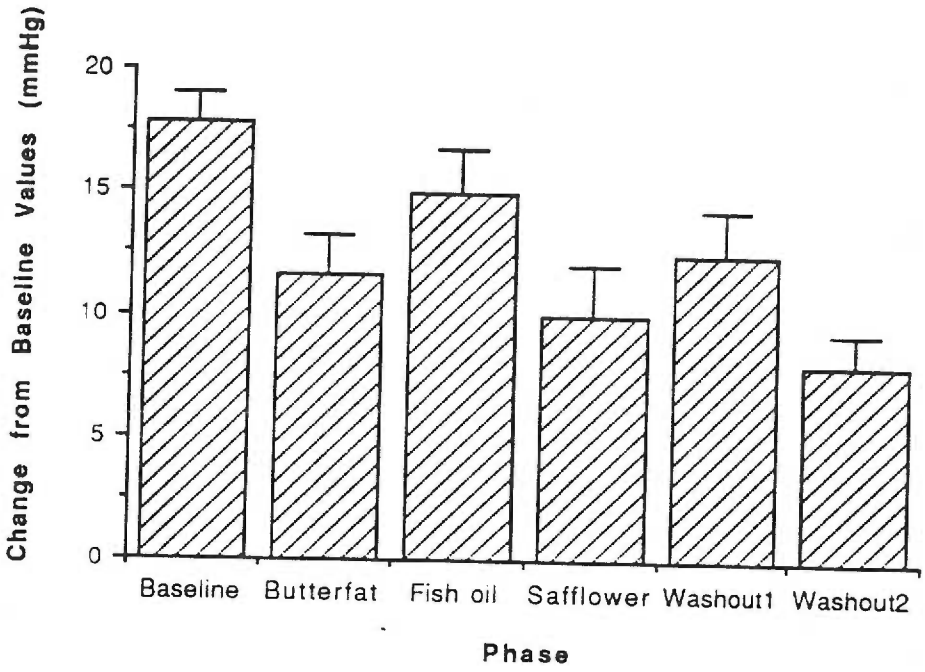

Stroop Color-Word
Isometric Handgrip
Mental Arithmetic
Change from Baseline Values (mmHg)
35
30
25
20
15
10
5
0
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Phase

Figure 3. Change in systolic blood pressure during the Stroop color-word task across the 6 phases of observation, n=20. Order of diet administration is not shown. Data are expressed as group means ± SEM.


Change from Baseline Values (mmHg)
20
15
10
5
0
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Phase

washout period ($p<.05$) in comparison to responses measured during the baseline phase before dietary intervention. A separate MANOVA involving the three dietary periods did not reveal any significant differences in the change scores ($F\{2,28\}=2.58$, $p=.089$).

Handgrip Challenge. The handgrip challenge elicited substantial increases in blood pressure over the pre-task baseline measures as seen in figure 4. However, a nonsignificant MANOVA indicated that blood pressure responses to the handgrip challenge were not influenced by dietary fat intake.

Mental Arithmetic Task. Figure 5 depicts the blood pressure responses to the mental arithmetic task. As with the other tasks, blood pressure was increased during the mental arithmetic task as compared to the pre-task baseline values. These pressor responses to the task did not significantly differ across the six phases of the study.

Initial Blood Pressure Status

Blood Pressure Reactivity. To examine the influence of initial blood pressure status on reactivity, subjects were classified as either normotensive or hypertensive according to mean arterial pressure values determined by Dr. Karanja during the baseline phase. An overall Diet (6) by Task (3) by Blood Pressure Status (2) MANOVA assessed whether the two groups differed in their blood pressure responses to stress

Figure 4. Change in systolic blood pressure during the isometric handgrip challenge across the 6 phases of observation, n=20. Order of diet administration is not shown. Data are expressed as group means ± SEM.

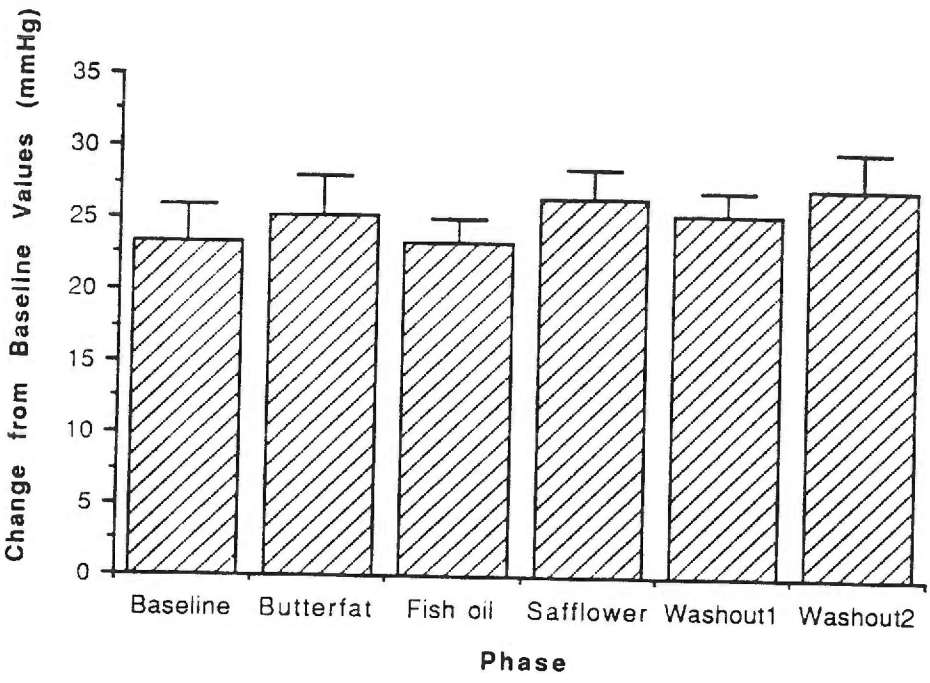
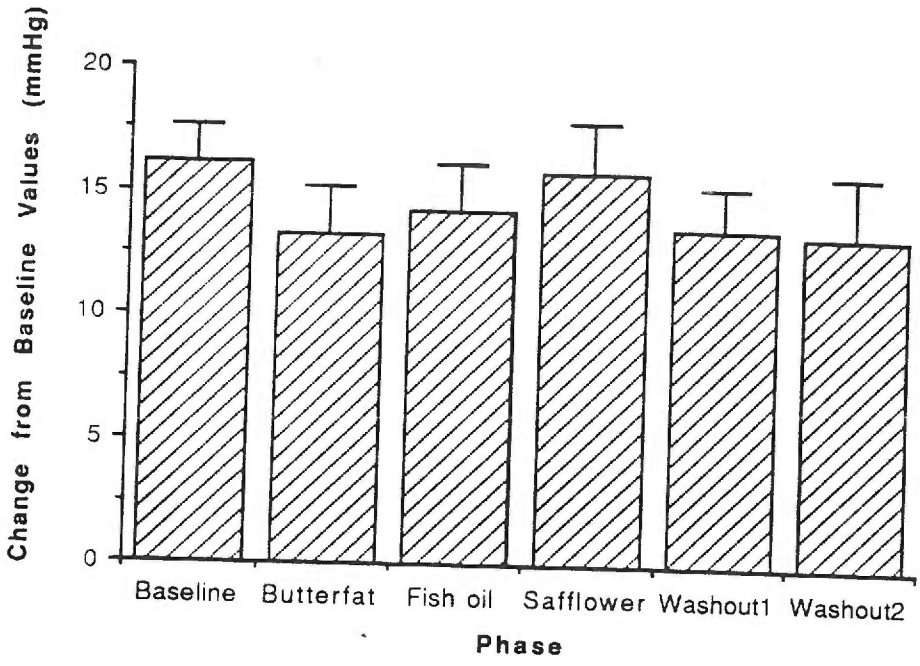

Change from Baseline Values (mmHg)
35
30
25
20
15
10
5
0
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Phase

Figure 5. Change in systolic blood pressure during the mental arithmetic challenge across the 6 phases of observation, n=20. Order of diet administration is not shown. Data are expressed as group means ± SEM.


Change from Baseline Values (mmHg)
20
15
10
5
0
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Phase

throughout the six phases of the study. Blood Pressure Status was treated as a between subjects factor while Diet and Task were assessed as within subjects factors. The analysis did not reveal any significant effects involving Blood Pressure Status, suggesting that normotensive and hypertensive subjects responded similarly to the tasks within each dietary phase.

When the three dietary phases were examined alone with a separate Diet (3) by Task (3) by Blood Pressure Status (2) MANOVA, a significant Blood Pressure Status by Task interaction ($F\{2,36\}=3.37$, $p=.046$) emerged, indicating that normotensives and hypertensives responded differently to the tasks when diet was controlled. Simple main effects of blood pressure status within each task indicated that the interaction was due to the significantly higher reactivity of hypertensive subjects during the Stroop color-word task ($F\{1,18\}=9.42$, $p=<.01$) as shown in figure 6. Hypertensive and normotensive subjects did not differ in reactivity to either the isometric handgrip challenge or the mental arithmetic task. These results are depicted in figures 7 and 8.

Basal Blood Pressure. To assess whether the basal blood pressure of hypertensive subjects was more responsive to manipulations of dietary fat intake in comparison to subjects with normal blood pressure, a repeated measures Diet (3) by Blood Pressure Status (2) MANOVA involving only

Figure 6. Change in systolic blood pressure during the Stroop color-word task across the 6 phases of observation in subjects classified as hypertensive and normotensive. Subject n was 10 and 10 for hypertensive and normotensive groups respectively. Data are expressed as group means ± SEM.

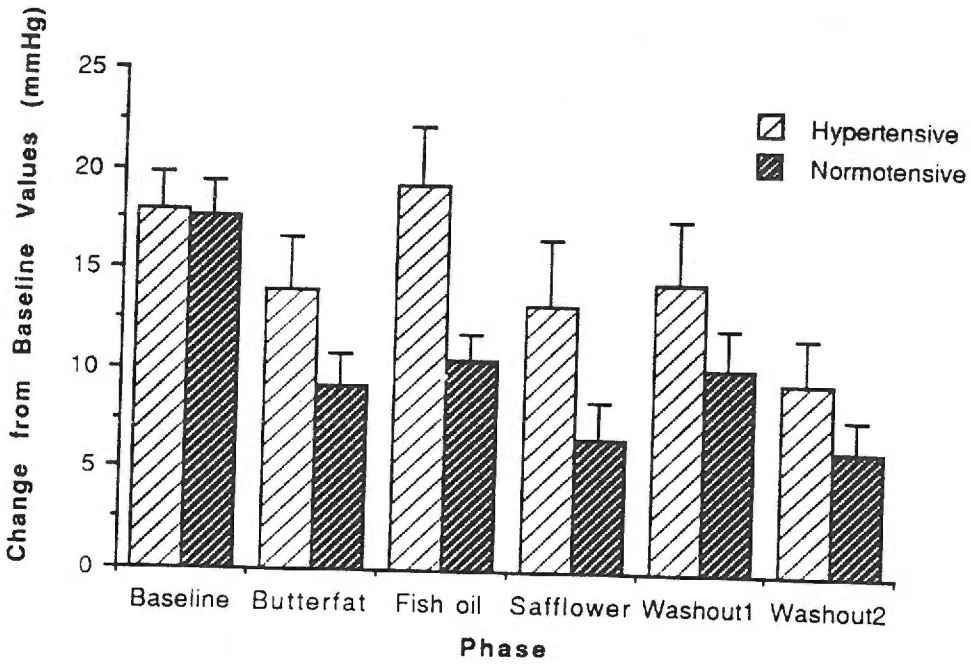

Change from Baseline Values (mmHg)
25
20
15
10
5
0
Hypertensive
Normotensive
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Phase

Figure 7. Change in systolic blood pressure during the isometric handgrip challenge across the 6 phases of observation in subjects classified as hypertensive and normotensive. Subject n was 10 and 10 for hypertensive and normotensive groups respectively. Data are expressed as group means ± SEM.

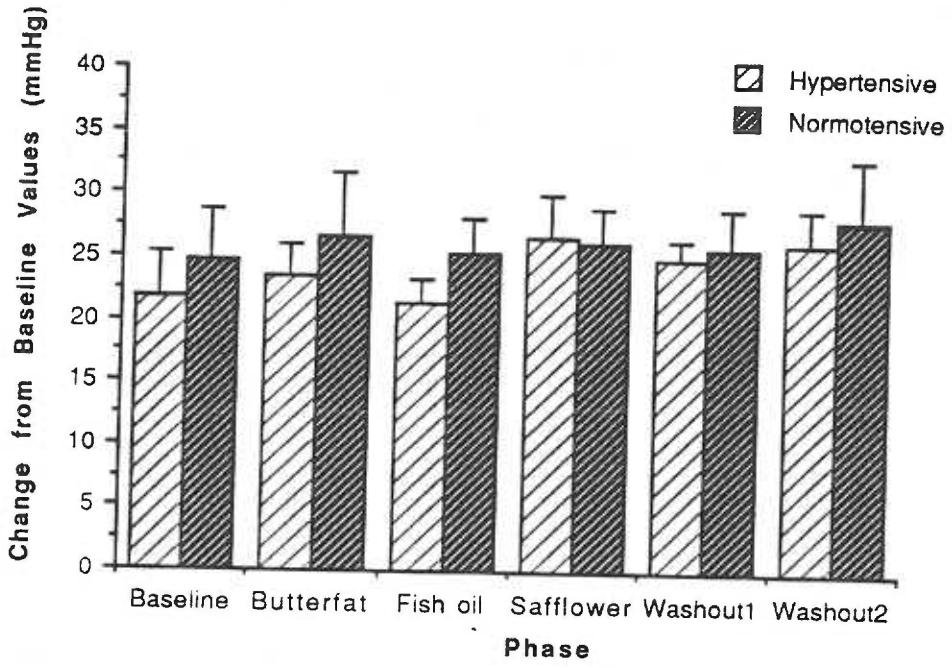
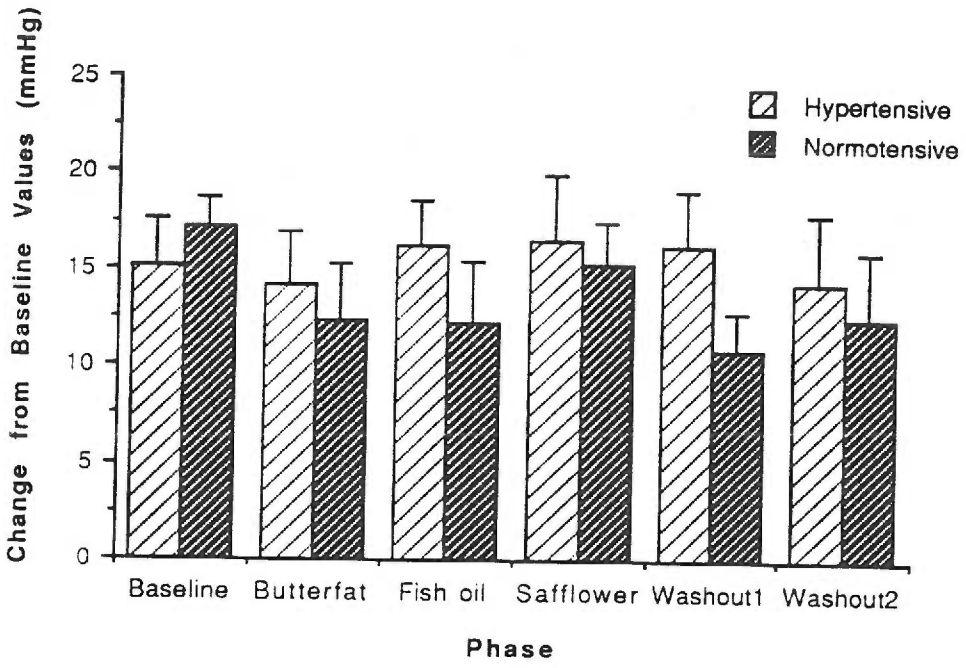

Change from Baseline Values (mmHg)
40
35
30
25
20
15
10
5
0
Hypertensive
Normotensive
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Phase

Figure 8. Change in systolic blood pressure during the mental arithmetic task across the 6 phases of observation in subjects classified as hypertensive and normotensive. Subject n was 10 and 10 for hypertensive and normotensive groups respectively. Data are expressed as group means ± SEM.


Change from Baseline Values (mmHg)
25
20
15
10
5
0
Hypertensive
Normotensive
Baseline
Butterfat
Fish oil
Safflower
Washout1
Washout2
Phase

the three experimental diets was performed. Blood Pressure Status was treated as a between subjects factor. Although basal blood pressure was higher in the subjects classified as hypertensive as evidenced by a main effect for Blood Pressure Status ($F\{1,18\}=5.95$, $p<.05$), the lack of a significant Blood Pressure Status by Diet interaction indicated that the two groups did not differ in their blood pressure responses to the diets. These results are depicted in figure 9.

Repeated Administration of Reactivity Testing

To assess whether blood pressure responses to the stressors were diminished as a consequence of repeated testing, the data were collapsed across dietary phase and analyzed according to the order of presentation. Again, change scores were used to evaluate the pressor responses stimulated by the three tasks. A repeated measures MANOVA with change scores as the dependent variable was performed for each task. As depicted in figure 10, the analysis revealed a significant effect of order presentation with the Stroop color-word task ($F\{5,95\}=6.43$, $p<.001$). Scheffe's modified post hoc comparison of group means indicated that the effect was due to a reduction in the magnitude of the pressor response during the fifth ($p<.05$) and sixth ($p=.05$) testing session in comparison to baseline values. However, separate MANOVAs demonstrated that neither the handgrip

Figure 9. Systolic blood pressure of normotensive and hypertensive subjects across the 3 diet phases. Order of diet administration is not reflected. Subject n was 10 and 10 for hypertensive and normotensive groups respectively. Data are expressed as group means ± SEM.

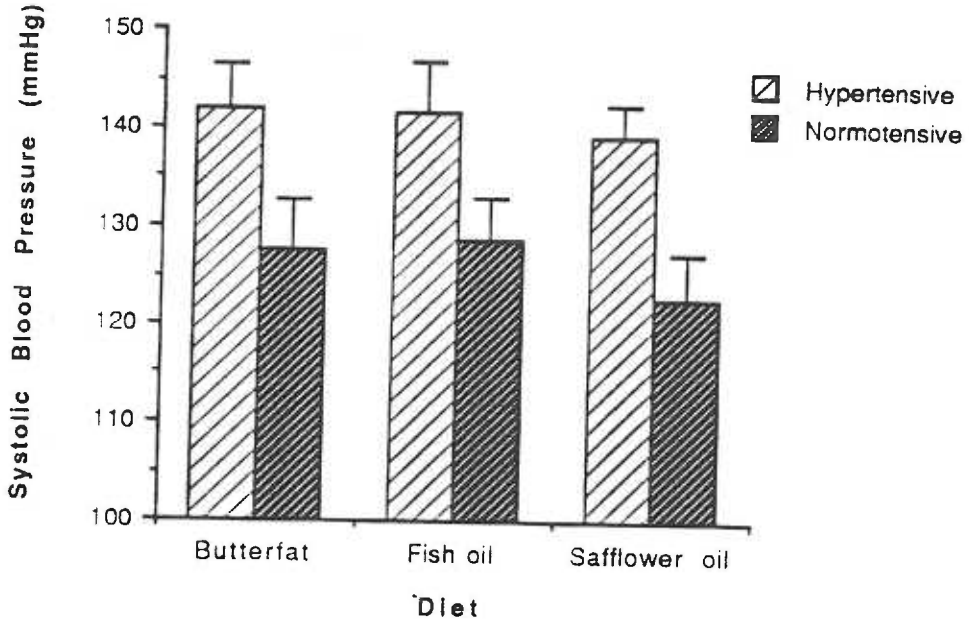
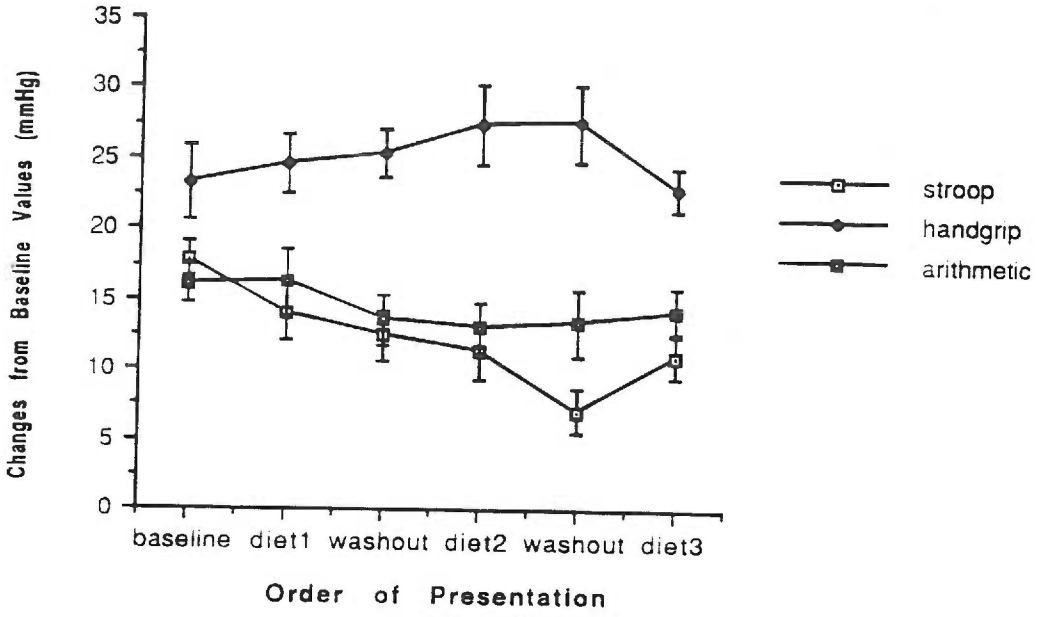

Systolic Blood Pressure (mmHg)
150
140
130
120
110
100
Butterfat
Fish oil
Safflower oil
Diet
Hypertensive
Normotensive

Figure 10. Systolic blood pressure responses to the three tasks collapsed across diet and presented in the order of administration. Subject n was 20 for each point. Data are expressed as group means ± SEM.


35
30
25
20
15
10
5
0
Changes from Baseline Values (mmHg)
baseline diet1 washout diet2 washout diet3
Order of Presentation
stroop
handgrip
arithmetic

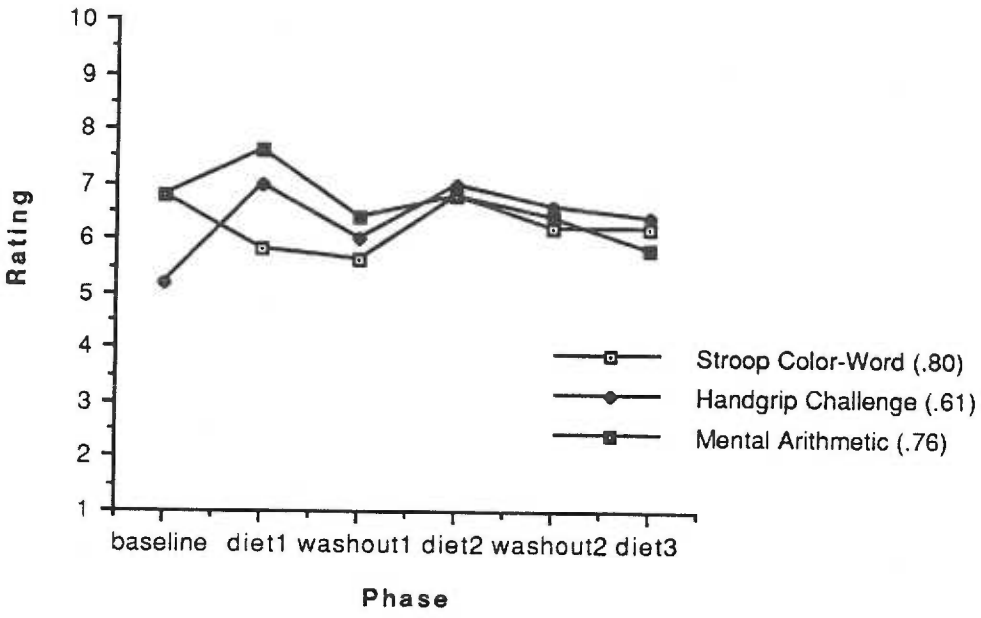
challenge or the mental arithmetic task were susceptible to the habituation effects of repeated testing. Results for these tasks are also shown in figure 10.

As another estimate of habituation, five subjects were asked to rate the tension and challenge of each task on a Likert scale ranging from one to ten. Friedman's two-way analysis of variance by ranks was performed for each task to measure the consistency of ratings across repeated testing. Again, data were collapsed across dietary intake. As shown in figure 11, the analysis failed to reveal any significant differences, indicating that there was considerable agreement in the ratings of challenge and tension during each repeated measure of a task.

The effect of repeated reactivity testing was also examined using measures of performance to determine whether subjects improved in their task-taking ability over time. For the Stroop color-word task, the number of errors was used as a measure of performance. However, analyses could not be performed due to the lack of error during the task. Performance on the mental arithmetic task was measured using the number of errors and the number of serial subtractions completed in the 3 minute period. These measures were further used to calculate the percentage of correct responding. Separate overall repeated measures MANOVAs collapsed across diet were computed for each of these measures. As shown in figures 12 and 13, analyses

Figure 11. Self-ratings of tension (A) and challenge (B) during the three tasks. Data are collapsed across diet and presented in the order of administration. Subject n was 5 for each data point. Data are expressed in group means. Numbers in parentheses in the legend indicate the mean SEM across the 6 phases for that particular task.

(A)


10
9
8
7
6
5
4
3
2
1
Rating
baseline diet1 washout1 diet2 washout2 diet3
Phase
Stroop Color-Word (.80)
Handgrip Challenge (.61)
Mental Arithmetic (.76)

(B)

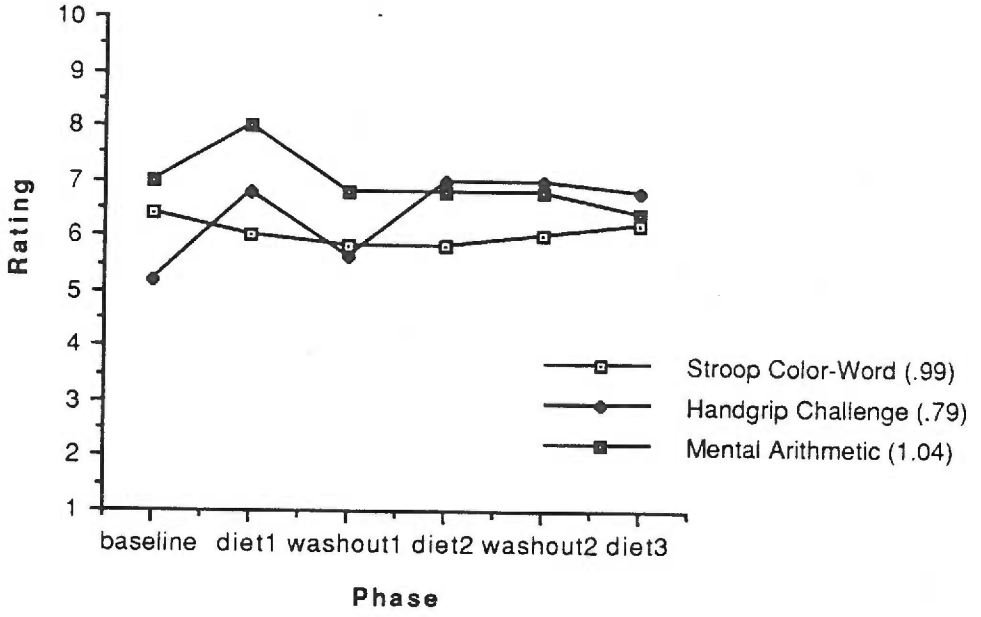
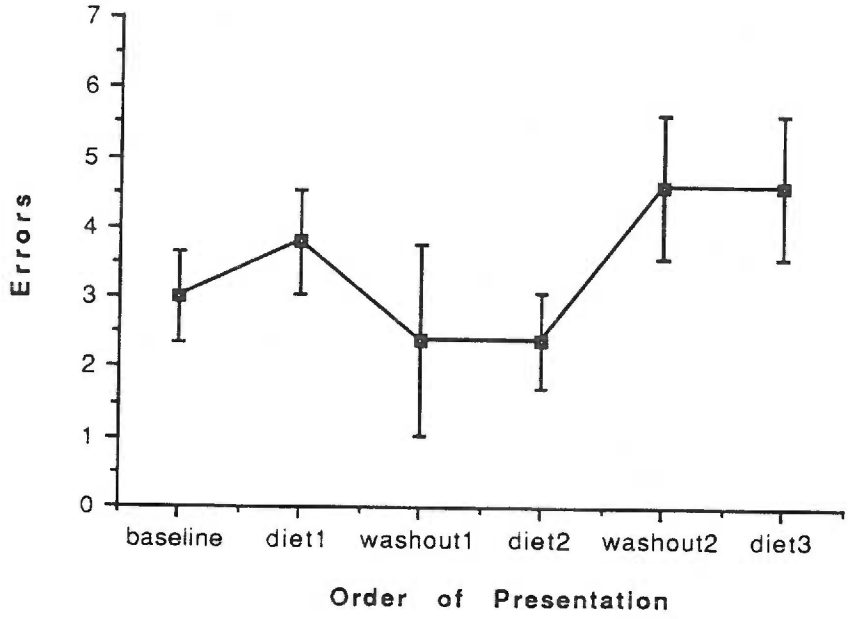

10
9
8
7
6
5
4
3
2
1
Rating
baseline diet1 washout1 diet2 washout2 diet3
Phase
Stroop Color-Word (.99)
Handgrip Challenge (.79)
Mental Arithmetic (1.04)

Figure 12. Performance on the mental arithmetic challenge collapsed across diet and presented in the order of administration. (A) Number of errors during the 3 minute task. (B) Number of serial subtractions completed. Subject n was 5 for each data point. Data are expressed in group means ± SEM.

(A)


Errors
7
6
5
4
3
2
1
0
baseline
diet1
washout1
diet2
washout2
diet3
Order of Presentation

(B)

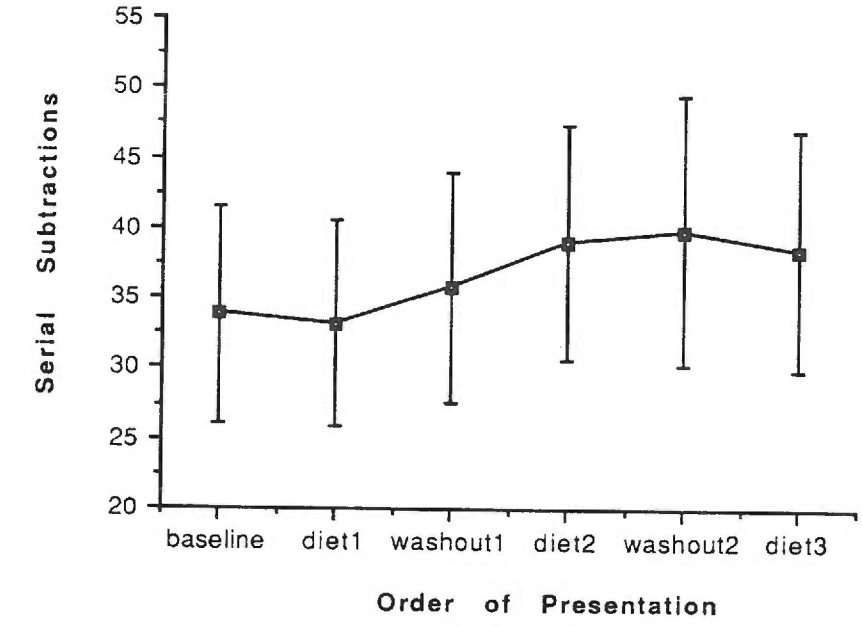
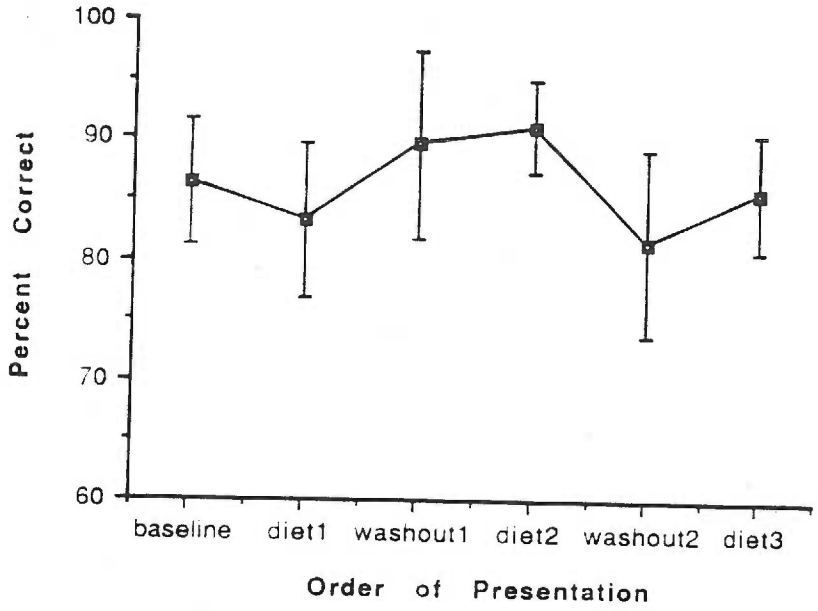

Serial Subtractions
55
50
45
40
35
30
25
20
baseline
diet1
washout1
diet2
washout2
diet3
Order of Presentation

Figure 13. Performance on the mental arithmetic challenge expressed as the percent correct. Data were collapsed across diet and presented in the order of administration. Subject n was 5 for each data point. Data are expressed as group means ± SEM.


100
90
80
70
60
Percent Correct
baseline
diet1
washout1
diet2
washout2
diet3
Order of Presentation

demonstrated a significant effect of serial subtractions ($F\{5,20\}=5.21$, $p<.01$) indicating that the total number of serial subtractions performed in 3 minutes was increased. However, the number of errors and the percent correct did not change over time.

Plasma Cortisol Responses

As an estimate of neurohormonal activity during stressors, plasma cortisol levels were measured in ten subjects prior to and during a video game mental challenge. A Diet by Period (pre-task, task) MANOVA was performed to assess any changes in the plasma cortisol response during the three diets. The analyses showed nonsignificant main effects for both Diet ($F\{2,18\}=3.15$, $p=.067$) and Period ($F\{1,9\}=1.79$, $p=.215$). In addition, a significant Diet by Period interaction did not emerge ($F\{2,18\}=.97$, $p=.389$). These results depicted in figure 14, suggest that cortisol levels were not increased during the task nor were the levels influenced by dietary intake.

To further examine the effectiveness of the task as a stressor, subjects were asked to rate their feelings of tension and challenge during the video game on a Likert scale ranging from one to ten. In addition, performance was measured as the highest score obtained during the 5 minute task. Friedman's two-way analysis of variance by ranks did not reveal any signifcant differences among the ratings or performance as shown in figures 15 and 16.

Figure 14. Plasma cortisol response before task presentation (Pre-Task) and during the PacMan video game (Task) across the 3 dietary periods. Order of diet administration is not reflected. Subject n was 10 for each data point. Data are expressed as group means ± SEM.

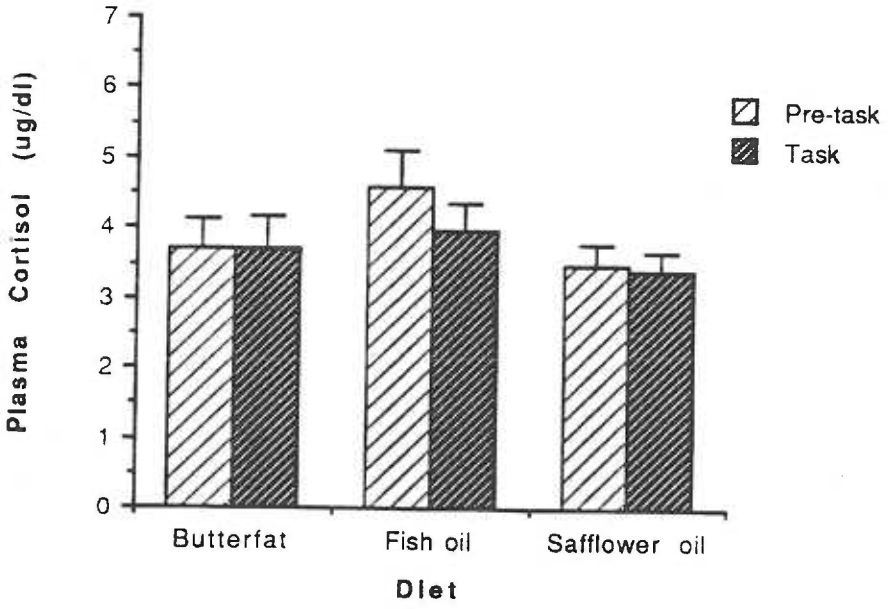

Plasma Cortisol (ug/dl)
7
6
5
4
3
2
1
0
Pre-task
Task
Butterfat
Fish oil
Safflower oil
Diet

Figure 15. Self ratings of tension and challenge during the PacMan video game. Data are collapsed across diet and presented in the order of administration. Subject n was 5 for each data point. Data are expressed as group means ± SEM.

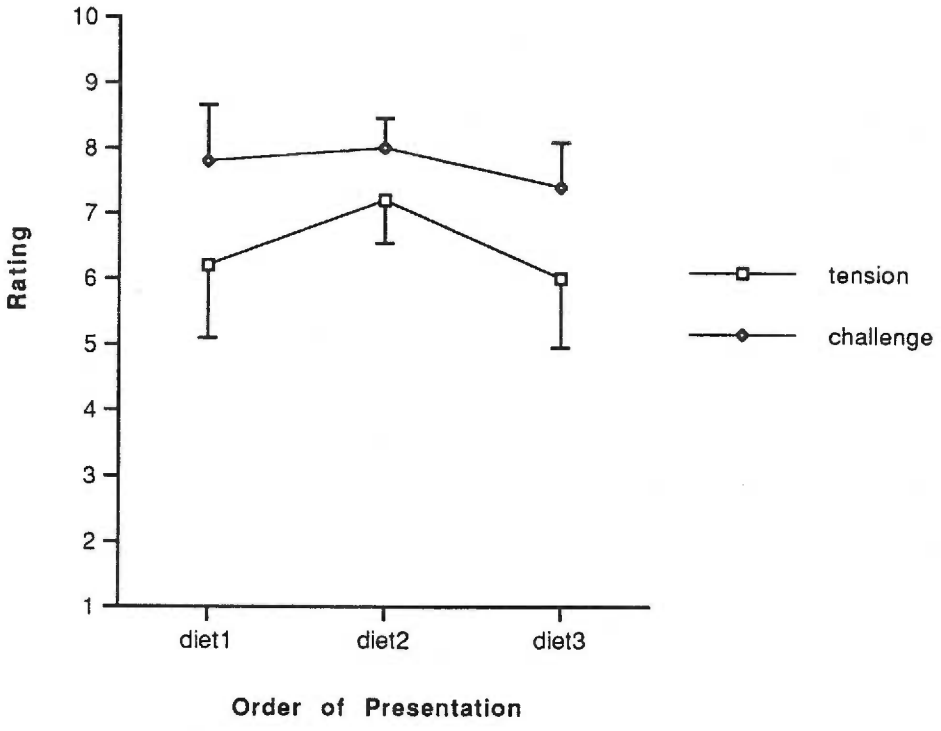
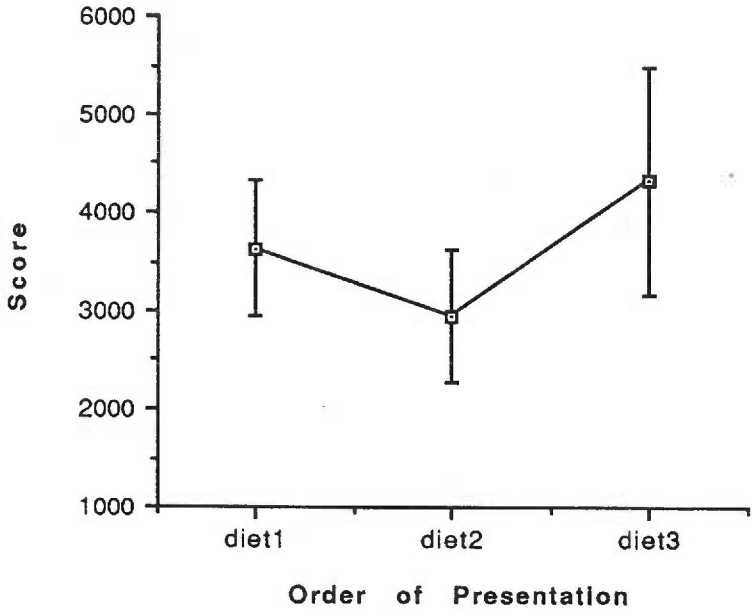

10
9
8
7
6
5
4
3
2
1
Rating
diet1
diet2
diet3
Order of Presentation
tension
challenge

Figure 16. Performance on the PacMan video game expressed as the high score obtained during the 5 minute task. Data are collapsed across diet and presented in the order of administration. Subject n was 5 for each data point. Data are expressed as group means ± SEM.


6000
5000
4000
3000
2000
1000
Score
diet1
diet2
diet3
Order of Presentation

DISCUSSION

Basal Blood Pressure

Relative to blood pressure values determined at baseline, six weeks of exposure to the safflower oil diet significantly reduced blood pressure. When comparing the three diets alone, no differences in blood pressure were found. However, blood pressure tended to be lower following safflower oil in comparison to the other two diets. Taken together, the results suggest a trend for an attenuation in systolic blood pressure following dietary treatment with safflower oil while both the fish oil and butterfat diets appeared to have no effect on basal blood pressure.

The apparent hypotensive effect of polyunsaturated n-6 fatty acids which constitute the safflower oil diet is consistent with several previous studies. Four weeks of dietary supplementation with linoleic acid reduced blood pressure in both normotensive (Heagerty et al, 1986) and mildly hypertensive subjects (Comberg Heyden, Hames, Vergroesen, and Fleischman, 1978). Similarly, Rao, Rao and Srikantia (1981) noted lower diastolic blood pressure following diets rich in oleic and linoleic acid relative to a placebo control. Other studies interested in the polyunsaturated to saturated fat ratio (P/S ratio) of particular diets have demonstrated a reduction in blood pressure as a result of a lower total fat intake and/or a higher intake of polyunsaturated fatty acids in both

normotensive and hypertensive subjects (Puska et al., 1983; Iacono et al., 1983 & Iacono et al., 1981).

However, such results have not been consistently observed. In separate reports, Brussaard et al (1981) and Margetts et al (1985) compared low P/S ratio diets with diets consisting of a high P/S ratio and found no alteration in blood pressure as a consequence of dietary treatment. Furthermore, more recent studies examining the influence of specific dietary fatty acids on blood pressure do not support the contention that n-6 fatty acids in vegetable oils lower blood pressure in normotensive or hypertensive subjects. Sacks et al. (1987b) supplemented subjects with 23 g/day of linoleic acid or oleic acid for four weeks in a double-blind crossover study. Blood pressure was not altered following administration of either dietary treatment. In another randomized, crossover study, Lofgren, Wilt, Nichol, Crespin, Pluhar and Eckfeldt (1993) found no significant differences in blood pressure from pretreatment values in subjects fed either 15.2 g/day linoleic acid or 6 g/day n-3 fatty acids for 12 weeks.

The disparity in outcome of these reports has been attributed to differences in methodology. The study design in many of the early studies has been criticized for the absence of a true control group as well as observer and subject blindness of diet condition. Given that measurement of blood pressure is highly variable and subject to placebo

effects and observer influences, blood pressure changes due to dietary manipulation may be confounded by these errors in design. In addition, early studies which reported an inverse relationship between blood pressure and polyunsaturated diets increased the intake of other nutrients such as carbohydrate, fiber and vitamins A and C in addition to altering the polyunsaturated fat content. Therefore, it cannot be readily concluded that alterations in dietary fat alone accounted for the reported fall in blood pressure. In contrast, more recent studies which argue against any beneficial effect of polyunsaturated fats on blood pressure have been more tightly controlled using randomized, double-blind crossover designs to eliminate possible extraneous variables (Sacks et al., 1987b; Lofgren et al., 1993).

Although the present study employed a similar single-blind, crossover design, other differences may explain the modest hypotensive effect of n-6 fatty acids in the current study as compared to the lack of such findings in other recent reports. First, the present study differs considerably from most studies by placing subjects on a controlled diet rather than allowing subjects to consume monitored yet self-selected diets. Using a controlled diet, the complete nutrient intake is known and any problems involving the validation of nutrient intake from free diets are avoided. Consequently, any blood pressure changes that

occur during the diet cannot be explained by variations in nutrients other than dietary fat.

Second, the diet used in the present study differs from previous studies in regard to the method of administration and the amount of safflower oil administered. The length of treatment is comparable to other studies. Throughout the safflower oil diet, safflower oil was used in the baking and preparation of the food. The amount of safflower oil administered was adjusted according to the daily calories alloted for each subject. For a subject fed 3400 kilocalories per day, 40 grams of linoleic acid were incorporated into the menu each day. In contrast, other studies provided linoleic acid in the form of capsules which amounted to 23 g/day (Sacks et al., 1987b) and 15.2 g/day (Lofgren et al., 1993) regardless of the total daily calories. Therefore, in some cases, the subjects in the present study received a larger amount of linoleic acid per day when compared to these other studies.

The larger amount of safflower oil may lead to a greater incorporation of n-6 fatty acids in the cell membrane which in turn could influence prostaglandin production and other membrane processes to a larger extent. Although a dose-response study involving n-6 fatty acids has not been performed, a study examining n-3 fatty acids noted a dose-proportionate incorporation of n-3 fatty acids in erythrocyte membranes such that a higher dose of fish oil

resulted in an increase in the percent of n-3 fatty acids found in the membrane (Knapp and Fitzgerald, 1989). Thus, the modest decline in blood pressure found in the present study could be due to the relatively large dose of safflower oil administered over the six week period.

The inability to demonstrate a hypotensive response following dietary treatment with fish oil was not expected. The n-3 fatty acids found in fish oil are presumed to alter vascular reactivity and thus attenuate blood pressure by changing the prostaglandin profile in both the vasculature and the kidney as well as reducing vascular responsiveness to vasoconstrictors. However, an overall impression of the previous literature suggests that fish consumption exerts only a slight blood pressure lowering effect in subjects with mild hypertension with little or no effect in normotensive subjects as reviewed by Singer (1991). A meta analysis of initial randomized trials supported this interpretation of the literature in failing to show any antihypertensive effect of fish oil diets (Radack and Deck, 1989). The recent studies of Lofgren et al. (1993), Morris, Taylor, Stampfer, Rosner and Sacks (1993) and the TOHP Collaborative Research Group (1992) further demonstrated that moderate doses of fish oil did not have a substantial effect on blood pressure.

The finding that blood pressure was not modified by butterfat consumption questions the literature which

suggests that diets with a low P/S ratio are associated with higher blood pressure in humans. The results of the present study are in agreement with the only previous study which specifically investigated blood pressure responses to dietary butterfat intake in humans. Sacks et al. (1987a) compared a diet supplemented with 81 g/day of cream with diets rich in fish oil or carbohydrate. Blood pressure was stable across all dietary treatments despite differences in the saturated fat content.

In summary, dietary treatment with fatty acids, saturated or unsaturated, did not produce any dramatic alterations in resting systolic blood pressure. A slight reduction in blood pressure was observed following a diet rich in n-6 polyunsaturated fats. These results refute the premise that dietary polyunsaturated fats alone are capable of substantially reducing blood pressure. It has been proposed that other dietary constituents such as fiber or vitamins act in combination with polyunsaturated fats to favor the lower blood pressure found in previous dietary studies as well as in populations consuming vegetarian-like diets (Beilin, Rouse, Armstrong, Margetts and Vandongen, 1988).

Blood Pressure Reactivity

The trend for lower resting blood pressure following the safflower oil diet was supported during measurement of

blood pressure reactivity. The blood pressure response to the Stroop color-word task was significantly reduced following the six week administration of safflower oil relative to responses measured before dietary intervention. However, safflower oil intake did not influence reactivity to the mental arithmetic challenge or the isometic handgrip challenge. Much like the data regarding basal blood presssure, neither the butterfat or fish oil diets significantly altered blood pressure responses to any of the stressors.

Previous research examining the impact of dietary fatty acids on cardiovascular reactivity have reported similar findings. Straznicky, Louis, McGrade and Howes (1993) reported that a low-fat, polyunsaturated diet was associated with a reduction in cardiovascular reactivity to exogenous norepinephrine administration and the cold pressor task when compared to a high-fat saturated diet. Such results imply that any diet rich in polyunsaturated fats should have a favorable influence on blood pressure reactivity. However, in reviewing the literature, the effect appears to be contingent on the specific polyunsaturated fatty acids present in the diet. Human studies have shown that diets with a high percentage of n-6 fatty acids are consistently effective in lowering cardiovascular reactivity whereas diets comprised of n-3 fatty acids have little or no effect on cardiovascular responses to stress (Mills, Prkachin,

Harvey and Ward, 1989; Hughes, Ringer, Francom, Caswell, DeLoof and Spillers, 1991; Mills, 1991). The results of the present study support this contention.

The differences in the ability of polyunsaturated n-3 and n-6 fatty acids to influence cardiovascular reactivity are not easily explained. Separate reports of n-3 and n-6 fatty acids have projected that both groups modify the various biochemical and physiological components involved in blood pressure regulation via similiar mechanisms. For example both n-6 and n-3 fatty acids appear to influence the adrenergic receptor system. Animal studies have provided reports of reduced chronotropic and inotropic responsiveness to adrenergic agents in isolated rat heart following diets rich in n-6 fatty acids (Hoffman, Mest, Krause and Will-Shahab, 1988) and n-3 fatty acids (Reibel, Holahan and Hock, 1988). It could be hypothesized that such reductions in adrenergic responsiveness may underlie the lower blood pressure reactivity associated with dietary fats, given the importance of the sympathetic nervous system in mediating cardiovascular responses to stressors. Accordingly, both n-3 and n-6 fatty acid should be equipotent in modifying cardiovascular reactivity.

Nonetheless, few studies have directly compared the two groups in regard to their biochemical actions or to any subsequent changes in vascular reactivity. Often studies differ in the dosage of fatty acids, length of treatment and

the species used making it difficult to fully evaluate differences and similarities among the fatty acids groups. The minor structural differences in the fatty acid profile such as carbon chain length or the number and position of double bonds may differentially influence biochemical properties enough to translate into alterations in vascular reactivity and hence blood pressure. As an example, Engelmann, Duhm, Schonthier, Streich, Op den Kamp and Roelofsen (1993) reported that the ability of fatty acids to activate a cation transporter was altered simply by the addition of double bonds and the shortening of the carbon chain length. Furthermore, fatty acids are converted into different prostaglandins depending on their biochemical structure. Prostaglandins have a number of effects on the cardiovascular system, though their influences on cardiovascular responses to stress are not well characterized.

The results of the current study suggest that alterations in reactivity associated with dietary treatment may depend on the particular type of stressor presented. Only blood pressure responses to the Stroop color-word task appeared to be susceptible to dietary manipulation. It could be speculated that the physiological responses to each of the respective laboratory tasks differs in such a way as to afford differential sensitivity to dietary fatty acids. The specific hemodynamic pattern of responding to each of

the tasks could not be identified in the present study due to the limited number of variables measured. However, previous research has characterized the typical responses evoked by the stressors.

The isometric handgrip challenge is inherently different from the two mental tasks because it represents a physical stressor. In this case, mean arterial pressure rises in an attempt to overcome the reduced perfusion pressures occuring in the region of muscle contraction rather than as a result of behavioral activation of the sympathetic system. Increases in sympathetic tone producing an elevation in left ventricular contractility and cardiac output with little change in peripheral resistance are the components responsible for the increased blood pressure. Dietary fatty acids appear to have limited effects on cardiovascular responses to exercise as shown in this study and others (Straznicky, Louis, McGrade and Howes, 1993).

With regard to the mental challenges, previous research has shown that the rise in blood pressure during both the Stroop color-word task and mental arithmetic appears to be caused by increases in cardiac output and a reduction in systemic peripheral resistance with increases in skeletal muscle vasodilation (Hjemdahl, Freyschuss, Juhlin-Dannfelt and Linde, 1984; Allen, Obrist, Sherwood and Crowell, 1987). Thus, both mental tasks elicit similar hemodynamic responses which resemble the typical defense, 'flight or fight'

reaction.

However, minor variations in task characteristics or task instructions can elicit considerably different hemodynamic response patterns (Matthews et al, 1986). Laboratory tasks have been classified along a number of different dimensions including active versus passive coping or sensory intake versus sensory rejection. While tasks involving sensory rejection such as mental arithmetic have been shown to produce a defense reaction, physiological responses to tasks requiring sensory intake typically include a reduction in cardiac output and heart rate with vasoconstriction of the skeletal muscle (Williams, Bittker, Buchsbaum and Wynne, 1975). It has been suggested that tasks which involve both sensory intake as well as problem solving such as the Stroop color-word task produce a mixed pattern of cardiac or vascular reactions (Krantz, Manuck and Wing, 1986).

Furthermore, subtle variations in the instructions of the tasks may have conferred differences in task difficulty and hence hemodynamic responding. During the mental arithmetic challenge, subjects were permitted to subtract at their own pace. In contrast, the Stroop color-word task imposed time pressure effects by requiring subjects to keep pace with the slide presentation. Thus, differences in the physiological response to the mental tasks in the current study as a consequence of the task characteristics could

contribute to the apparent differential effect of dietary fatty acids.

Alternatively, the reduction in the blood pressure response to the Stroop color word task following the safflower oil diet could be partially due to habituation and/or practice effects. Blood pressure responses to the Stroop color-word task were significantly higher during the baseline phase in comparison to subsequent measurements. These results typify the phenomena of habituation to the testing environment and/or the task itself. In addition, blood pressure responses to the Stroop color-word task were more likely to diminish simply as a function of repeated testing in comparison to the other two tasks, probably reflecting the influence of practice effects.

The variability in reactivity to the task is compounded by the fact that the counterbalancing of the diet rotation is not complete as noted in figure 1. Each diet was not represented in each experimental phase an equal number of times. Such incomplete counterbalancing does not guard against possible practice effects. In this case, lower reactivity to the Stroop color-word task following the safflower oil diet could be due to the frequency in which the safflower oil diet was administered as the last dietary treatment. Fortunately, in the present study, safflower oil was administered first and last an equal number of times such that the influence of repeated testing may have been

minimal. Furthermore, both the consistent self-ratings of tension and challenge as well as the rather minor differences in task performance across repeated testing to each of the tasks support the notion that practice effects contributed little to the observed results.

In summary, even though resting blood pressure was only slightly influenced by dietary fatty acids, the beneficial effects of n-6 polyunsaturated were evident when the cardiovascular system was challenged. These results extend the findings of vascular reactivity studies which have shown lower vascular responses to exogenous norepinephrine and angiotensin II following dietary treatment without any apparent changes in basal blood pressure. As proposed by Mills (1989), the interaction among diet and stress may provide a means of primary intervention for cardiovascular disease. It appears as though dietary fatty acids may be effective in minimizing the physiological responses to stressors believed to increase the risk of developing hypertension.

Initial Blood Pressure Status

It is of interest to examine whether hypertensive and normotensive subjects respond differently to dietary treatment. It has been suggested that hypertensive subjects are more responsive to manipulations in dietary fat in comparison to normotensive subjects. In the accumulated

evidence to date, any modest delcine in blood pressure associated with dietary fat treatment has generally been found in hypertensive subjects. In the present study, baseline blood pressure, measured before dietary intervention, did not appear to influence blood pressure responses to dietary treatment.

With regard to blood pressure reactivity, the reactivity hypothesis proposes that borderline hypertensive and hypertensive subjects are generally more physiologically reactive to stressors and such exaggerated responses contribute to the development of hypertension. In the present study, the acute rise in blood pressure measured during the tasks was somewhat influenced by the initial blood pressure status of the subjects at baseline. Hypertensive subjects displayed greater reactivity to the Stroop color-word task in comparison to normotensives during the dietary conditions. Dietary fat intake did not differentially influence blood pressure reactivity among subjects classified as either hypertensive or normotensive.

Neurohormonal Activity

Plasma cortisol was measured as an index of neurohormonal activity during a stressor. Levels of plasma cortisol were not altered during the Pac Man video game nor were they influenced by dietary fatty acids. The lack of a cortisol response during the task could be due to the time

at which blood was sampled in relation to task administration. The response of the hypothalamo-pituitary-adrenal axis (HPA) is somewhat sluggish compared to sympathetic nervous system activation because of the various steps involved in the stimulation and synthesis of cortisol (Orth et al., 1983). An elevation in cortisol usually occurs within 3-5 minutes of stressor presentation and values tend to peak around 15 to 20 minutes (Kuhn, 1989). In the present study, blood samples were drawn during the fifth minute of the task when levels were presumably rising. A later sampling may have demonstrated a significant response of cortisol.

Alternatively, it could be argued that the Pac Man video game as presented in the current study was not stressful enough to activate the HPA axis. It appears to be the case that the intensity of the stimulus required to evoke measurable increases in cortisol is quite high when compared to the intensity necessary to elicit changes in heart rate, blood pressure and catecholamines (Costa, Martignoni, Blanidin, Petraglia, Genazzani and Nappi, 1993; Kuhn, 1989). Chronic stress, novelty of the stressor or very intense stressors are characteristics which are most likely to elicit a substantial response of the HPA axis (Herd, 1986) whereas acute mental challenges used in the laboratory have not been shown to consistently elevate plasma cortisol levels (Costa et al, 1993; Jorgensen et al,

1990). Studies which have required subjects to perform laboratory tasks for longer periods of time tend to report increases in cortisol (Bohnen, Nicholson, Sulon and Jolles, 1991; Fredrikson, Tuomisto, Bergman-Losman, 1991). It should be noted that the Pac Man video game did evoke relatively high feelings of stress as evidenced by self-ratings of tension and challenge. It is likely that the stressor stimulated sympathetic pathways within the shorter time frame and measurement of catecholamines may have provided a more appropriate index of the neurohormonal response.

Conclusions

The results of this study indicate that n-6 fatty acids may have beneficial effects on blood pressure regulation as reflected in the observed reduction in blood pressure reactivity in combination with the slightly lower resting blood pressure following dietary treatment with safflower oil. The modest decline in blood pressure observed in this study may seem minimal compared to the 10-30 mmHg reduction acheived by antihypertensive agents. However, small decrements in blood pressure, as little as 2-5 mmHg, have the potential to produce a large reduction in cardiovascular risk by shifting the diastolic blood pressure distribution of the population (JNC, 1993). Moreover, primary intervention may act synergistically with drug therapy to

reduce drug dosages and its associated costs. Thus, the results of the present study suggest that dietary fat modification may have important ramifications for the treatment and prevention of hypertension.

APPENDIX A

Recruitment letter, medical and dietary questionnaire and the informed consent for the overall study.

Dear Mr. ,

Thank you for your interest in volunteering for our research studies. This study represents our ongoing effort to develop ways of managing high blood pressure other than with drugs. It is a nutritional study that will evaluate the role of dietary fat on blood pressure and cholesterol.

We know from previous studies that fats such as fish oil lower blood pressure and blood cholesterol on some people. In this new study, we will try to determine why this occurs by comparing the effects of fish oil with those of butter and safflower oil on several biological mechanisms that regulate blood pressure and blood cholesterol. In addition, we would like to know whether there is a genetic basis for why blood cholesterol goes up when some people eat certain types of fat, while it does not change in others.

The time commitments for this study are considerable. If you chose to participate in this study, you would be asked to eat only food that is provided by us (a specific amount to maintain your weight) and nothing else, including alcoholic beverages. There will be three dietary periods, each lasting six weeks. The three periods would be separated by six weeks during which you can eat whatever you wish. During the six weeks that you eat foods provided by us, you would be asked to eat at least one meal a day on week days and one meal on weekends in the Clinical Research Center at OHSU. The other meals would be prepackaged for you to take home.

Periodically, we would ask you to give blood samples for cholesterol measurements. We would follow your blood pressure closely, measuring it at least three times a week when you come in for your meals, and at least once every week when you are not eating the meals we provide.

If you are currently taking medications to lower your blood pressure, you would be asked to stop taking them. This would be done by providing you with a schedule to taper off your medication gradually, while following your blood pressure closely to make sure it does not rise significantly. If during the time you are reducing medications your blood pressure rises, we would restart your medications and exclude you from the study.

The benefits to you include free meals, at least two physical exams, laboratory (blood) tests as well as nutritional assessment. A monetary compensation of $600.00 will be paid to you after you complete all three phases.

You would not qualify to be in the study if you have, or have had any of the following conditions: Abnormal blood sugar, diabetes, heart attack, kidney disease, TB, thyroid problems, osteoporosis, or a stroke.

If you are interested and feel that you have the time to give, please fill out the enclosed medical questionaire and return to us in the self addressed stamped envelope. We will then telephone you to set up an orientation appointment and further explain the research protocol to you.

Thank you for your interest, and we look forward to hearing from you.

Sincerely,

Dr. Njeri Karanja
Principal Investigator

Peggy Cook
Study Coordinator

THE OREGON
HEALTH SCIENCES UNIVERSITY
3181 S.W. Sam Jackson Park Road, OP11, Portland, Oregon 97201
(503) 279-7593

School of Medicine
General Clinical Research Center

The study you have volunteered to participate in is designed to provide information on the relationship of dietary factors and blood pressure. Participation in the study requires a considerable commitment on your part, both in terms of time and adherence to the study requirements. However, we will work with you and do whatever we can to make this a pleasant experience for you.

Since this is a research diet study, you will be required to eat only the food provided by the Clinical Research Center (CRC) metabolic kitchen, with the exception of coffee, tea, sugar-free carbonated beverages, sugarless gum and water. While on the study, you should eat all of the food provided to you, if at all possible. Any deviation (some of the research diet not eaten or additional "outside" foods eaten) should be reported to the dietary staff. Alcoholic beverages are not allowed during the course of the study.

The research diet is calculated to provide an exact amount of carbohydrate, protein and fat and to furnish energy (calories) in the amount needed for weight maintenance. If you lose or gain weight during the study your calories will need to be adjusted. Please try to keep your physical activity as constant from day to day as is possible, to minimize weight fluctuations resulting from changes in activity levels. We will ask you to keep a record of your physical activity.

Each day when you come in we will ask you to record your food and beverage intake of the previous day in the "Volunteer Intake Records Book" located at the kitchen door.

We will attempt to plan the menus, whenever possible, according to your food preferences. Please let someone on the dietary staff know if there are items on your menu that must be changed.

You will receive a copy of your study schedule which will indicate the days blood pressure will be taken and blood is to be drawn. It is important that you do not eat or drink anything (other than water) during the 12 hours prior to having your blood drawn. Although they are not available between 7:00-7:30 AM, CRC nurses are usually available at any other time to draw blood. Blood should drawn at approximately the same time of day throughout the study. You can determine the time that best fits your schedule.

The metabolic kitchen is staffed and able to serve meals from 6:15 AM-5:30 PM, seven days per week. We will check with you weekly to set up your meal schedule. If you need to make a change in your meal schedule, let us know as early as possible; our work with any given menu begins two days before the actual meal is served. If you will be late for a meal or have a last minute change of plans, please call us at 494-8265 (diet staff) or 494-7601 (nursing staff).

Always "check in" at the kitchen when you first arrive then sign in at the nurses station and stamp your parking validation card. A nurse will weigh you, and if scheduled, draw blood or take blood pressure. After these procedures have been done, please return to the kitchen and your meal will be served.

When you come to the CRC kitchen, please wait near the kitchen entryway. Although we appreciate your willingness to help, we ask that you not walk around the counter, use the microwave, or get anything out of the refrigerator. The CRC diet staff is there to prepare your meal and to serve you. Let us know if there is anything you need--we will be happy to help you. When you finish you meal, please return your tray to the kitchen.

Participants should not donate blood at any time from one month prior to beginning the study through one month following the completion of the study. Please check with the nursing or dietary staff before taking ANY medication, prescription or non-prescription, including vitamin supplements.

If you become ill or do not feel well during the study, notify us at once as this will affect the study. Call us at 494-8265 (diet staff) or 494-7601 (nursing staff).

Proper food handling and refrigeration is very important to insure you will be consuming safe and nutritious food. If you are unable to take you food directly home to refrigerate, let us know so we can put it in a cooler for you. We are also able to pack your soups and chili in a thermos to keep hot for a few hours if you are unable to heat your meal.

Protocol #345: Regulation of blood presssure and cholesterol metabolism by dietary fats.

Name: _____ Date: _____

Address: _____ Height: _____

_____ Weight: _____

Phone No. (work): _____ Birth Date: _____

(home): _____

How did you find out about the study clinic? _____

When did you find out you had high blood pressure? _____

What is the highest your blood pressure has been? _____

Do you have any symptoms you feel are related to your high blood pressure? _____

_____

What medicines have you taken in the past for your blood pressure? _____

_____

Any side effects from the medicines you have taken in the past for your blood pressure?

_____

Present medicine for your blood pressure: _____

Other medicine you are currently taking (including vitamins, antacids, cold medicine):

_____

Allergies (including medicines, food, hay fever): _____

Have you ever been told you had: (please circle)

Abnormal blood sugar	Arthritis	Hepatitis or jaundice	Inflammatory bowel disease
Diabetes	Ulcers	TB	Osteoporosis
Heart murmur	Kidney disease	Thyroid problems	Kidney stones in last 10 yrs
Heart attack	Cancer	Bone fracture in the past 6 months	Stroke

Hospitalizations: (indicate date and reason) _____

_____

_____

Name of current physician: _____

F-NUTR.cdm

CLINICAL RESEARCH CENTER

POTENTIAL VOLUNTEER INFORMATION FOR PROTOCOL #345:
Regulation of Blood Pressure and Cholesterol Metabolism by Dietary Fats

Date _____

Name _____
Last First MI

Birthdate_____ Age_____

Phone (home)_____ (work)_____ (message)_____

Address_____
Street City State Zip

OHSU Clinic Card #:_____Social Security #_____-____-_____

State/County of Birth_____Mother's Maiden Name _____

Marital Status _____

Do you have children? Yes____ No____ How many?_____ Ages_____

What is your occupation?_____

Typical work schedule? _____/_____
days hours

If you are eligible for a dietary research study, are there any times in the next ten months that you would <u>not</u> want to be involved in a study (vacations, holidays, out of town business)? If so, please indicate the approximate dates below:

_____

_____

Please check below any of the health problems experienced by you or a family member such as grandmother, grandfather, sister, brother, aunt or uncle:

	YOU	MOTHER	FATHER	OTHER FAMILY MEMBER (specify)
High Cholesterol				
High Triglyceride				
Stroke				
Heart Attack				
Angina				
Abnormal Blood Sugar				
Diabetes				
Heart Murmur				
Other Heart Problems (specify)				
Gall Bladder Problems				
Bone fracture (in past 6 months)				
Osteoporosis				
Liver disease				
Kidney stones (last 10 years)				
Kidney disease				
Inflammatory bowel disease				
Cancer				
Alcohol abuse				
Mildly overweight				
Extremely overweight				
Arthritis				
Ulcers				
Hepatitis or jaundice				
Tuberculosis (TB)				
Thyroid problems				

Please list any other health problems: (include major illnesses and/or surgeries along with approximate dates)

_____

_____

Is your mother living? Yes____ No____ If yes, present age_____

If no, age at death and cause of death_____

Is your father living? Yes____ No____ If yes, present age_____

If no, age at death and cause of death_____

Do you usually have your blood pressure checked by a health professional? If so, how often? (number of times per week, month, year, etc)

_____

What is your most recent blood pressure reading?

_____ Date_____

Have you been told that your blood pressure is high? Yes_____ No_____

If your blood pressure is high when did you first know that it was high? (Approximate date, or number of years)_____

Do you have any symptoms you feel are related to your high blood pressure? (List)

_____

_____

Do any of your relatives have high blood pressure? List:

_____ _____

_____ _____

_____ _____

Are you currently taking medication to control your blood pressure? Yes_____ No_____

If yes, what are the medicines (names and doses) _____

_____

How long have your been taking these medications? (approximate date or number of years)_____

Are there side effects to the medicines you are now taking for your high blood pressure?_____

_____

Are you taking any other medications? Yes _____ No_____

If yes, please indicate below:

	NAME OF MEDICATION	DOSE	FOR HOW LONG?
Diuretic (water pill)			
Diabetes medication			
Medication to lower cholesterol and/or triglycerides			
Thyroid medication			
Hormones			
Aspirin or anti-inflammatory drugs			
Anticoagulates			
Others (identify)			
Vitamins (identify)			
Antacids (identify)			

Are you allergic to any medications? Yes_____ No_____

If yes, please list: _____

Please provide your physician's name and address:

Name _____

Address_____

_____

_____

May we contact him/her for information if you participate in the study?

Yes_____ No_____

Are you following any kind of special diet recommended by a physician or other health professional?

_____

Have you experienced recent weight changes: Current height:_____ wt:_____

Yes _____ No_____ Weight gain_____ Weight loss _____

If yes, please indicate over what time period _____

How long have you been at your current weight? _____

What was your weight in high school? _____

Typical meal times: Breakfast _____ (indicate meals skipped)

Lunch _____

Dinner _____

Do you typically snack? Yes_____ No_____ When?_____

Do you have a microwave at home? Yes_____ No_____

Do you have access to a microwave at work? _____

Do you have any known food allergies? _____

Do you have any difficulty chewing foods? Yes_____ No_____

If yes, please list foods: _____

_____

_____

EXERCISE REGIMEN

Type	Minutes/Session	Sessions/Week

Oregon Health Sciences University
Informed Consent

Regulation of blood pressure and cholesterol metabolism by dietary fats

This study is under the supervision of Dr. David McCarron, whose address is 3181 S.W. Sam Jackson Park Road, Portland, OR 97201.

You have been asked to read the following information regarding this study and its risks and benefits.

1. Purpose of the Study

You are invited to participate in a study to determine how different types of fat in the diet affect blood pressure and cholesterol metabolism. The overall goal is to see how three different types of fat regulate blood pressure, and influence the way in which the body uses cholesterol when they are incorporated into normal diets. You have been selected for the study because your blood pressure is either higher (hypertensive) than the limits defined as normal by health professionals, or because your blood pressure is at or below the normal limits (normotensive). If you are normotensive, you are volunteering as a control subject to provide normal comparison data.

2. Procedures

a. Your participation in this study will last for approximately 30 weeks or approximately eight months. You will be eating controlled diets provided by the Clinical Research Center (CRC) for three six-week blocks of time, each separated by six weeks during which you eat foods of your choice. To begin the study, you will be asked to visit the Research Center once a week for four consecutive weeks for measurement of blood pressure, heart rate and weight. If you are taking medications to lower your blood pressure, or certain drugs that change fat levels in your body, you will be asked to stop taking these drugs. To accomplish this, you will be given a schedule to taper your medication gradually. A physical examination will be performed during the third week of this initial phase. The physical examination will include a routine blood test and an EKG.

b. If your blood pressure is too high or too low during the initial phase, you will be excluded from the study and where necessary referred to your primary physician. If your blood pressure meets our criteria, you will be formally enrolled in the study.

You will be asked to come to the CRC at least once a day for six consecutive weeks to eat one meal and to collect food for the other meals that you may take home with you. At these times, your blood pressure will be checked by the CRC nursing staff. During the six weeks you are on the controlled diets, we will draw small amounts of blood (about 1/4 of a cup) once every two weeks for chemical analyses. At the end of every six weeks of a special diet we will draw slightly more blood (3/4 cup or half of what you would give for blood donation).

c. After the six weeks are over, you will be asked to revert back to your normal diet for six weeks. This time is to "wash out" the effects of the controlled diet that you had been eating the preceding six weeks. During these wash-out periods, we will ask that you visit the CRC once a week for blood pressure measurements. As before, we will ask that you donate a small amount of blood every two weeks during the wash-out period.

d. After the wash-out is over, you will be asked to return for a second period of six weeks, during which your diet will again be controlled. This second period of controlled diet will be much like your first controlled period except that you will be receiving a diet with a different type of fat. When this is over, you will then go through another six-week wash-out period, followed by a final six weeks of a controlled diet when we test the effect of a third type of oil on your blood pressure.

e. All medical procedures that are part of the study will be provided at no cost to you. In addition, the food during each of the six week blocks you are on controlled diets will be provided to you free of charge.

3. Risks and Inconveniences

a. For every six weeks that you are on the controlled diets, you will be required to come to the CRC daily to eat at least one meal and collect pre-packaged food for your other meals. During this time the staff will request that you not eat anything else but the food given to you at the CRC. During the wash-out periods, you may eat whatever you wish, but you will be asked to come to the CRC once a week for blood pressure measurements.

b. If you have high blood pressure, it will not be treated during your participation in the study. This should cause you no harm since blood pressure will be followed very carefully. If your pressure becomes markedly elevated at any time during the study, you will immediately be started on proven medications and/or referred to your primary physician.

c. Blood tests will be taken once every two weeks once you are formally enrolled in the study. You may experience minor pain or bruising at the site of blood drawing.

4. Possible Benefits

Your participation in this study will possibly help scientists understand how dietary fat manipulations may be used (or not used) to control high blood pressure. You will receive free physical examinations and laboratory work-ups. There is a possibility that some of the dietary maneuvers may lower your blood pressure as well.

5. Alternatives

If you are hypertensive, you should understand that there are many drugs available for the treatment of hypertension. Thus, it is not necessary for you to participate in this study if you do not wish to do so. If you decline the invitation to participate or if you decide to withdraw from the study, medical

treatment for your high blood pressure will be arranged.

6. Confidentiality

The data from this study will be kept confidential. Participants will be assigned a code number and will not be identified by name. Neither your name nor your identity will be used for publicity or publication purposes.

7. State's Liability

The Oregon Health Sciences University, as an Agency of the State, is covered by the State Liability Fund. If you suffer any injury from the research project, compensation would be available to you only if you establish that the injury occurred through the fault of the University, its officers or employees. If you have further questions, please call Dr. Michael Baird at (503) 225-8014.

8. Questions About the Study

You are free to ask questions concerning any aspect of the research study that you may have at any time. You should contact the doctor conducting this study or his representative regarding any questions, your rights as a participant, or any research-related injury.

Participant's Statement

Dr. McCarron and/or his associates have offered to answer any questions that I may have.

I understand that I may refuse to participate or withdraw from this study at any time without affecting my relationship with, or treatment at, the Oregon Health Sciences University.

MY SIGNATURE BELOW MEANS I HAVE READ THIS FORM, THAT I UNDERSTAND ITS CONTENTS AND HAVE HAD THE OPPORTUNITY TO ASK QUESTIONS, AND THAT I AGREE TO PARTICIPATE IN THE STUDY.

I have read the foregoing and agree to participate in this study.

Subject Signature	Date	Time
Address		
Witness Signature	Date	Time
Principal Investigator Signature	Date	Time

APPENDIX B

Informed consent forms for the ancillary study.

OREGON HEALTH
SCIENCES UNIVERSITY
3181 S.W. Sam Jackson Park Road, L470
Portland, Oregon 97201-3098 (503) 494-8464
School of Medicine, Department of Medical Psychology

1/5/93

INFORMED CONSENT

Regulation of Blood Pressure and Cholesterol Metabolism by Dietary Fats: Cardiovascular Reactivity and Affect

PURPOSE OF THE STUDY.

This study is being conducted by Daniel C. Hatton, Ph.D., Njeri Karanja, Ph.D., Jean-Baptiste Roullet, Ph.D.. David A. McCarron, M.D. is the responsible physician. You have been invited to participate in this study because of your current enrollment in the ongoing study concerning dietary fats and regulation of blood pressure and cholesterol metabolism. The principle aim of this ancillary study is to determine whether alterations in the dietary fat you will be consuming can alter mood and cardiovascular responses to mental challenges.

PROCEDURES.

As a part of this study, you will be asked to fill out two questionnaires each day during the last week of each diet rotation and 'washout' period. One questionnaire asks you to check adjectives describing your mood. The other questionnaire asks you to describe your general well being.

In addition, your blood pressure will be taken while you perform four short tasks. The tasks include mental arithmetic, a color-word task, public speaking and a hand grip task. Each task is designed to mildly elevate blood pressure and will last 3 minutes. There is no known physical risk for performing the tasks. The mental challenge may be somewhat discomforting to some people.

You will be asked to participate in this study a total of seven times. You will be tested once every six weeks at the end of each diet rotation and 'washout' period. For your convenience, we will try to schedule the testing according to your usual arrival for meals and meal pick-up at the Clinical Research Center. From the CRC, you will be directed to the outpatient clinic where the testing will occur. Total time requirement is estimated to be one hour per test session.

BENEFITS.

It is not known whether you will derive any direct personal benefit from this study. It is hoped that medical science in general will benefit by understanding more about the relationship between diet, blood pressure and sense of well being.

INFORMED CONSENT (p.2)

Regulation of Blood Pressure and Cholesterol Metabolism by Dietary Fats: Cardiovascular Reactivity and Affect

CONFIDENTIALITY.

The information obtained about you will be used for research purposes only and will be related to you only upon your specific request. Neither your name nor your identity will be used for publication or publicity purposes.

LIABILITY.

The Oregon Health Sciences University, as an Agency of the State, is covered by the State Liability Fund. If you suffer any injury from the research project, compensation would be available to you only if you establish that the injury occurred through the fault of the University, its officiers, or employees. If you have further questions, please call Dr. Michael Baird at (503)494-8014.

COSTS.

There will be no costs to you for blood pressure monitoring or filling out the questionnaires.

QUESTIONS ABOUT THE STUDY.

You are free to ask any questions concerning any aspect of the research study that you may have at any time. You should contact Daniel Hatton at 494-8464 regarding any questions, your rights as a participant, or any research-related injury.

You may refuse to participate or withdraw from this study at any time without affecting your relationship with, or treatment at, the Oregon Health Sciences University.

Your signature below indicates that you have read the foregoing and agree to participate in this study.

_____ _____

Signature of Subject Date

_____ _____

Signature of Witness Date

_____ _____

Signature of Principle Investigator Date

OREGON HEALTH
SCIENCES UNIVERSITY
3181 S.W. Sam Jackson Park Road, L470
Portland, Oregon 97201-3098 (503) 494-8464
School of Medicine, Department of Medical Psychology

1/5/93

INFORMED CONSENT

Regulation of Blood Pressure and Cholesterol Metabolism by Dietary Fats: Cardiovascular Reactivity and Affect

PURPOSE OF THE STUDY.
This study is being conducted by Daniel C. Hatton, Ph.D., Njeri Karanja, Ph.D., Jean-Baptiste Roullet, Ph.D.. David A. McCarron, M.D. is the responsible physician. You have been invited to participate in this study because of your current enrollment in the ongoing study concerning dietary fats and regulation of blood pressure and cholesterol metabolism. The principle aim of this ancillary study is to determine whether alterations in the dietary fat you will be consuming can alter neuroendocrine responses to mental challenges.

PROCEDURES.
During your routine blood test for measurement of lipoproteins and cholesterol, you will be asked to play a video game for 6 minutes which is designed to mildly elevate blood pressure and neuroendocrine release. The blood drawn during this normal blood test will be used for our assays. Thus, the neuroendocrine test will not require insertion of an additional catheter. There is no known risk for performing this task. You may experience minor pain or bruising at the site of blood drawing.

You will be asked to participate in this study a total of three times. You will be tested at the end of each diet rotation.

BENEFITS.
It is not known whether you will derive any direct personal benefit from this study. It is hoped that medical science in general will benefit by understanding more about the relationship between diet, blood pressure and sense of well being.

CONFIDENTIALITY.
The information obtained about you will be used for research purposes only and will be related to you only upon your specific request. Neither your name nor your identity will be used for publication or publicity purposes.

Regulation of Blood Pressure and Cholesterol Metabolism by Dietary Fats: Cardiovascular Reactivity and Affect

LIABILITY.

The Oregon Health Sciences University, as an Agency of the State, is covered by the State Liability Fund. If you suffer any injury from the research project, compensation would be available to you only if you establish that the injury occurred through the fault of the University, its officiers, or employees. If you have further questions, please call Dr. Michael Baird at (503)494-8014.

COSTS.

There will be no costs to you for blood pressure monitoring or filling out the questionnaires.

QUESTIONS ABOUT THE STUDY.

You are free to ask any questions concerning any aspect of the research study that you may have at any time. You should contact Daniel Hatton at 494-8464 regarding any questions, your rights as a participant, or any research-related injury.

You may refuse to participate or withdraw from this study at any time without affecting your relationship with, or treatment at, the Oregon Health Sciences University.

Your signature below indicates that you have read the foregoing and agree to participate in this study.

_____ _____

Signature of Subject Date

_____ _____

Signature of Witness Date

_____ _____

Signature of Principle Investigator Date

APPENDIX C

Stimuli presented during the Stroop color-word task.

	Word	Color of Print	Voice
1.	blue	red	green
2.	green	blue	yellow
3.	red	green	blue
4.	blue	yellow	green
5.	yellow	blue	red
6.	green	red	yellow
7.	red	yellow	blue
8.	yellow	green	red
9.	green	red	blue
10.	red	blue	green
11.	blue	yellow	red
12.	red	green	yellow
13.	yellow	blue	green
14.	yellow	red	blue
15.	red	yellow	yellow
16.	blue	red	blue
17.	yellow	green	red
18.	green	yellow	yellow
19.	blue	green	green
20.	yellow	red	yellow
21.	green	blue	green
22.	blue	yellow	blue
23.	yellow	red	green
24.	red	yellow	yellow
25.	red	blue	red
26.	green	yellow	yellow
27.	blue	green	blue
28.	green	red	red
29.	green	yellow	green
30.	yellow	red	red
31.	yellow	green	green
32.	blue	yellow	yellow
33.	red	green	red
34.	yellow	blue	yellow
35.	blue	green	blue
36.	green	yellow	red
37.	green	blue	blue
38.	yellow	green	green
39.	red	blue	yellow
40.	blue	red	red
41.	blue	green	blue
42.	green	red	yellow
43.	red	green	red
44.	yellow	blue	blue
45.	green	yellow	green
46.	blue	red	red
47.	red	yellow	yellow
48.	yellow	red	blue
49.	yellow	green	yellow
50.	green	blue	red
51.	red	green	yellow

	Word	Color of Print	Voice
52.	green	red	red
53.	blue	green	blue
54.	red	blue	green
55.	blue	yellow	red
56.	green	red	yellow
57.	yellow	blue	green
58.	blue	yellow	red
59.	blue	red	yellow
60.	red	yellow	red
61.	blue	green	green
62.	green	blue	blue
63.	yellow	green	green
64.	red	blue	yellow
65.	green	yellow	red
66.	yellow	red	blue
67.	red	green	green
68.	green	blue	yellow
70.	red	yellow	red
71.	green	red	green
72.	yellow	green	blue
73.	red	blue	red
74.	blue	yellow	yellow
75.	red	green	green
76.	green	yellow	yellow
77.	yellow	red	blue
78.	blue	red	green
79.	red	yellow	yellow
80.	blue	green	blue

APPENDIX D

Standardized instructions for the four tasks.

Stroop Color-Word Interference Task:

I would first like you to perform the Stroop color-word task. I am going to show you some slides of words of colors; red, blue, yellow and green. The words are printed in a different color than what they say. For instance, the word blue may be printed in the color green. I would you like you to tell me the color you see rather than the word itself. I'll show you the colors of the slides. This is red. This is blue. This is yellow. This is green. In addition, as you are viewing the slides, you will hear the voice of a man reciting colors which don't necessarily match the colors you see. I will take three measures of blood pressure while you perform this task. Remember state aloud the color you see. You may begin when you see the first slide.

Isometric Handgrip Challenge:

I am going to have you provide your maximal grip by squeezing the dynomanometer as hard as you can. From this number, I am going to set the red arrow at one-third of your maximal grip. With your arm resting on the chair, I would like you to hold the black arrow to the red arrow while I take three measures of blood pressure. Is your arm comfortable? I will tell you when to begin.

Mental Arithmetic:

The last test is mental arithmetic. I will give you a three digit number such as 756 and from that number, I'd like you to subtract 13 and say aloud the answer--743. From that number you substract 13 again and you get 730. Continue to subtract 13 from the previous answer until I tell you to stop. I will give you a number and you can begin.

APPENDIX E

Questionnaires used to assess challenge and tension for each of the tasks.

Please rate the PacMan video game on the following dimensions.

Tension:

1	2	3	4	5	6	7	8	9	10
relaxed									tense

Challenge:

1	2	3	4	5	6	7	8	9	10
not challenging									challenging

Please rate the Stroop color-word task on the following dimensions.

Tension:

1	2	3	4	5	6	7	8	9	10
relaxed									tense

Challenge:

1	2	3	4	5	6	7	8	9	10
not challenging									challenging

Please rate the handgrip challenge on the following dimensions.

Tension:

1	2	3	4	5	6	7	8	9	10
relaxed									tense

Challenge:

1	2	3	4	5	6	7	8	9	10
not challenging								challenging	

Please rate the mental arithmetic task on the following dimensions.

Tension:

1	2	3	4	5	6	7	8	9	10
relaxed									tense

Challenge:

1	2	3	4	5	6	7	8	9	10
not challenging								challenging	

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