The Link Between Psychosocial Factors and Coronary Heart Disease: A Possible Neuroendocrine Mechanism

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THE LINK BETWEEN PSYCHOSOCIAL FACTORS AND CORONARY HEART DISEASE: A POSSIBLE NEUROENDOCRINE MECHANISM

by

Sheila Wang

A Dissertation
Submitted to the Faculty of The Graduate College in partial fulfillment of the requirements for the Degree of Doctor of Philosophy Department of Psychology

Western Michigan University
Kalamazoo, Michigan December 1993

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Coronary heart disease continues to be the leading cause of death in the affluent world. Despite identification of several risk factors for coronary heart disease (age, sex, lipoprotein profile, hypertension, diabetes, cigarette smoking, obesity), a significant amount of variability associated with the incidence of coronary heart disease cannot be explained solely on the basis of these risk factors. The contribution of psychosocial factors to the development of coronary heart disease (type A behavior, social isolation, traumatic events, unstable social conditions) continues to be a promising area of investigation. However, a biochemical pathway linking psychosocial factors to coronary heart disease remains unclear.

The present study investigates the extent to which hormones (epinephrine, norepinephrine and cortisol) released during emotional arousal contribute to atherogenesis by enhancing the activity of an enzyme, acylCoA: cholesterol acyltransferase (ACAT), which esterifies cholesterol in the artery wall. Rat hepatoma cells (Fu5AH) were incubated with [1-\textsuperscript{14}C]oleate in the presence of epinephrine, norepinephrine and cortisol in three serum conditions (hyperlipemic, normolipemic and serum-free medium). Lipid was extracted from the cells and separated. The radioactivity of the lipid fractions is a measure of the incorporation of [1-\textsuperscript{14}C]oleate into phospholipids, triglycerides and cholesteryl ester. A slight increase in cholesteryl ester synthesis (ACAT activity) was observed across all serum conditions in cells.
incubated with epinephrine compared to controls. Cells incubated with cortisol showed a decrease in ACAT activity compared to controls.

Despite constancy of well known risk factors, the persistent variability of the incidence and severity of atherosclerosis needs to be explained. The present finding that ACAT activity is enhanced by epinephrine, provides a possible mechanism linking psychosocial factors and coronary heart disease.
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The link between psychosocial factors and coronary heart disease: A possible neuroendocrine mechanism

Wang, Sheila, Ph.D.
Western Michigan University, 1993
ACKNOWLEDGEMENTS

I would like to thank Dr. Frank Bell of The Upjohn Company for his help in the conceptualization of this project, for assistance regarding technical issues, for agreeing to be the fourth member of my committee, and for his solid support. I am grateful to Dr. J.P. Henry for offering to be an outside reader and providing consistent inspiration and encouragement during this work. I am indebted to Dr. John Mason for his visionary perspective regarding the interconnections between psychological and physiological processes which provided a foundation for my work.

I would like to thank several people who made it possible for me on a very practical level to transform an idea into an experimental project. Dr. Sue Stapleton from the Chemistry Department gave me access to her lab and provided guidance and assistance throughout the experimental work. I am very grateful to Mark Nickel who provided important technical assistance in the lab. His support in the lab and in many other ways was critical to my completion of this project. Dr. Brad Huitema, my advisor suffered through every detail of this very complex project and provided much needed guidance and support. For financial support I am grateful to the Graduate College Student Research Fund, the American Psychological Association Dissertation Science Directorate Award Committee and President Heinicke.

I want to thank my children, Paul, May Lin, and John, for their patience and their impatience during this long process. And finally, I am very grateful to my father for believing in me and supporting me in more ways than I can express.

Sheila Wang
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CHAPTER I

INTRODUCTION

The observation that one's response to significant life events, psychological trauma and chronic stress affects the body's ability to adapt and regulate itself effectively to maintain health, is not new. There is no question that we are profoundly affected by psychosocial events. The question is, how are responses to external events mediated and translated into metabolic events?

The answer lies in the integrative machinery of the central nervous system. Stimuli are perceived through the body's receptors which transform stimulation into electrochemical events travelling to the CNS via afferent neurons. Well studied and mysterious processes, outside the scope of this discussion, occur in the CNS which dictate efferent outputs. We are endowed with three modes of response: (1) skeletal muscular nervous system, (2) autonomic nervous system and, (3) endocrine system, (Figure 1).

The skeletal muscular nervous system controls voluntary muscle actions, including speech, and enables us to operate in our external environment. It is the most easily observable and measurable response mode and accordingly has been the most extensively studied.

The constancy of the internal environment is managed by the autonomic nervous system and the endocrine system. The autonomic system controls cardiac, smooth muscle and exocrine glands providing moment to moment regulation of heart rate, blood flow and visceral activity. The endocrine system regulates cell metabolism and links the brain with every cell in the body through the bloodstream.
Mason (1970) noted that some basic general similarities exist in the principles underlying the functional organization of these three systems responsible for adaptation.

1. They all appear to be organized on the principle of balance of opposing forces. Skeletal muscular action is executed by neural coordination of opposing muscles acting on a given bony lever. The net effects of the autonomic nervous system result from the coordination of opposing sympathetic and parasympathetic systems. The metabolic effects of the endocrine system also appear to result from similar coordination of secretion of opposing hormones (Mason, 1968).

2. The central nervous system appears to exert continuous tonicity on these effector systems which can be increased or decreased by psychologic as well as other

Figure 1. Three Effector Systems Mediating Integrative Functions of the Brain (Mason, 1970). Reprinted by permission of Author.
mechanisms.

How does disease relate to adaptation and regulation of bodily processes? It is well established that foreign agents such as bacteria, viruses or toxins can enter the body, disrupt its machinery and produce disease, however, the vast majority of current health problems, including coronary heart disease (CHD) are not the result of foreign agents. Could it be that the delicate integrative mechanisms responsible for regulation and coordination of vast numbers of separate physiological events are vulnerable to psychosocial stimuli? Mason (1975c) states

In about 20 years we have moved from a view of endocrine systems as controlled largely by humoral self-regulatory mechanisms to the view that a wide range of psychologic influences can profoundly affect hormonal balance on both a short- and long-term basis (p. 14).

Psychosomatic medicine is concerned with the role of adaptive factors in health and disease, recognizing that psychological and social factors profoundly influence the integrative mechanisms of the body. In the Presidential Address to the Psychosomatic Society in 1970, Mason introduced a diagram (Figure 2) outlining the various disciplines which must be drawn together effectively in a well coordinated effort to achieve success in psychosomatic research. His emphasis was on the interdependence of many diverse approaches, including (a) the social sciences to provide approaches to the study of epidemiologic and group factors; (b) psychiatry, clinical and experimental psychology to analyze environmental and psychologic factors; (c) neuroanatomy, neurophysiology and neurochemistry to study the neural substratum underlying emotional and related psychologic processes; (d) physiology and biochemistry to analyze endocrine and autonomic mechanisms; and (e) biochemistry, pathology, internal medicine and other clinical specialties to analyze, observe and manage clinical manifestations of disease.

It is in the spirit of the interdisciplinary approach that this review on

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psychosocial factors and coronary heart disease is written. Mason's diagram (Fig. 2) will serve as a guide for the organization of the data presented as I attempt to establish (a) an overall relationship between life situations/coping/defenses as independent variables and CHD as a dependent variable, (b) connections between life situations/coping/defenses as independent variables and cardiovascular and hormonal changes as dependent variables and finally, (c) possibilities of cardiovascular and hormonal patterns as independent variables related to changes potentially leading to CHD as dependent variables.

Figure 2. Disciplines Which Must Be Integrated in Psychosomatic Research (Mason, 1970). Reprinted by permission of Author.
Atherosclerosis is a pathologic process of arterial metabolism which gradually leads to vascular obstruction and which can precipitate coronary heart disease (including angina pectoris, myocardial infarction, and sudden death). Atherosclerotic cardiovascular disease continues to be the leading cause of death in the affluent world (Kannel and Thom, 1979; Levi, 1981) despite the decline in the incidence of the disease since 1968 (Kannel and Sytowski, 1987).

Despite identification of several "primary" risk factors: (a) sex, (b) age, (c) plasma lipoprotein profile, (d) hypertension, (e) diabetes mellitus, (f) obesity, (g) sedentary lifestyle and, (h) cigarette smoking (Kannel, 1966; Neaton and Wentworth, 1992; Menotti et al., 1992), a substantial amount of the variability in the incidence of coronary artery disease cannot be accounted for on the basis of these risk factors alone (Buell and Eliot, 1979; Patel, 1983).

In fact, Kottke et al. (1988) point out, using data from over 5000 men in the U. S. and Finland that the majority of non-fatal myocardial infarctions and the majority of deaths from coronary heart disease occur outside of the population clusters at highest risk.

Although total cholesterol/high-density lipoprotein cholesterol ratios and low-density lipoprotein cholesterol levels are reported by some investigators to be more accurate predictors of CHD than total serum cholesterol (Gordon et al., 1977; Castelli et al., 1986), many of the most recent epidemiologic studies still find total serum cholesterol a significant predictor variable for CHD (Neaton and Wentworth, 1992; Lei, 1992; Menotti et al., 1992).

There are several possible sources of the unexplained variability affecting the incidence and the severity of atherosclerosis. One area of focus has been the
relationship between the effects of stress (psychosocial factors) and the progression of coronary artery disease. Some researchers have found that type A behavior patterns, including an exaggerated sense of time urgency and competitiveness have been associated with an elevated risk of coronary artery disease (Rosenman et al., 1964; Haynes, Feinlieb & Kannel 1980).

Lifestyle changes from more traditional ways to modern ways have also been correlated with higher blood pressure (Henry and Cassel, 1969) and increased incidence of coronary heart disease. Japanese Americans who eat an American diet and follow an American lifestyle have more coronary heart disease than Japanese Americans who eat an American diet but follow a Japanese lifestyle (Marmot and Syme, 1976). Human traumatic life events, life dissatisfaction, social rank, anxiety, neuroticism, social mobility, and lack of social support have all been examined as making possible contributions to the etiology of coronary heart disease and playing a role in risk factor modification (Jenkins, 1976; Lebovitz et al., 1967; Friedman et al., 1974; Seeman and Syme, 1987). However, a biochemical pathway which links stressful life events and other psychosocial factors to CHD is yet to be discovered.

The most intensively studied psychosocial factor relating to CHD in humans is the Type A Behavior Pattern (TABP) which will be reviewed in the next section.

Type A Behavior and Coronary Heart Disease

Friedman and Rosenman (1959) formulated a specific overt behavior pattern (pattern A) which is characterized by excessive drive and competitiveness, aggressiveness and an enhanced sense of time urgency. They believed that this pattern, later called Type A Behavior Pattern (TAPB), was related to clinical coronary disease. In 1960, data from over 3,000 middle aged men regarding TAPB, blood lipids and coagulation, body measurements, socioeconomic factors, individual
habits and cardiovascular status began to be collected annually in the Western Collaborative Group Study to examine the relationship between TABP and CHD. By 1964, preliminary evidence indicated that TABP was related to CHD (Rosenman et al. 1964). In 1976, Rosenman et al. reported that individuals exhibiting TABP experienced about twice as many clinical CHD events over an eight and one half year follow up in comparison to those who were assessed as Type B's. The association between TABP and increased CHD rates was confirmed in the Framingham study (Haynes, Feinlieb & Kannel, 1980).

The Structured Interview (SI) and the Jenkins Activity Survey (JAS) are the two most widely used assessments of TABP. In the SI, individuals are asked about their usual responses to situations that potentially elicit impatience, competitiveness and hostility. The interviewer attends not only to the content of verbal responses but also to the expressive style, i.e., vocal speed, explosiveness and volume as well as response to challenge (Rosenman, 1978). In contrast, the JAS is a paper and pencil self report questionnaire with four standard subscales: (1) Type A, (2) speed and impatience, (3) job involvement and, (4) hard driving competitiveness (Jenkins et al., 1971).

Several researchers have investigated the relationship between TABP (assessed by both SI and JAS) and coronary artery atherosclerosis (CAA) among patients referred for diagnostic coronary angiography. Although most studies reported a positive relationship between level of CAA and TABP (Blumenthal et al., 1978; Frank et al., 1978 and Krantz et al., 1979) other studies reported no relationship (Dimsdale et al., 1979). In 1981, data from epidemiologic studies was reviewed by a National Heart Lung and Blood Institute Scientific panel and concluded that the TABP was a significant and independent risk factor for CHD. However, since that time, several investigators have challenged the strength and
reliability of the association between TABP and CHD (Cohen and Reed, 1985; Ragland and Brand, 1988). Further, data from recent epidemiologic studies such as the Multiple Risk Factor Intervention Trial (MRFIT, Shekelle et al., 1985) indicated that TABP was not positively associated with incidence of CHD.

To clarify some of the confusion, meta analytic techniques were used to evaluate the type A-CHD relationship (Booth-Kewley and Friedman, 1987; Lang and Shedler, 1987). They indicated that the assessment tool used to measure TABP altered the significance of the association between TABP and CHD. TABP assessed by the SI was found to be significantly related to initial CHD events and the type A score derived from the JAS was not a significant predictor. TABP assessed by the SI was a significant predictor only for initial CHD events; it was not a predictor for subsequent CHD events in post myocardial infarction patients.

Recent epidemiologic studies from around the world confirm that TABP, assessed by the SI and the JAS, is a significant risk factor for CHD (Maeda and Ito, 1990; Sprafka et al. 1990;)

Hostility and its relationship to CHD has also been investigated (Booth-Kewley at al., 1987; Matthews, 1988). Williams et al. (1980) in a study of 424 patients who underwent diagnostic coronary arteriography for suspected heart disease, found that hostility scores explained a significant amount of the variability in the extent of CAA in the sample. Hostility was independently and more strongly related to CHD than TABP. In an analysis of SI data from the MRFTT study, Dembroski et al. (1989) observed that though the type A scores failed to predict increased CHD risk, the hostility component of TABP when isolated and analyzed, became a significant predictor.

A depressive component of TABP has emerged as a potentially important factor related to CHD (Booth-Kewley et al., 1987). Thomas et al. (1975) noted that
medical students who developed CHD 20-30 years later were from a subgroup vulnerable to depression. Jenkins et al. (1979) observed anxiety and depression in coronary prone individuals. In a study from Japan, Fukunishi et al. (1992) reports that Japanese CHD patients with TABP are more likely to have a depression prone personality.

Summary

Type A behavior pattern, characterized by aggressiveness, competitiveness, hostility, time urgency and perhaps vulnerability to depression is associated with CHD. Assessment of TABP by the Structured Interview method appears to be more reliable than the JAS. TABP is a significant predictor for initial CHD events only.

Experimental investigations examining psychosocial influences on coronary artery atherosclerosis (CAA) and CHD in animal models will be described in the next chapter.
CHAPTER II

REVIEW OF THE RELEVANT LITERATURE

Animal Models of Psychosocial Influences on Coronary Heart Disease

Introduction

Epidemiologic studies are useful for identifying social and behavioral markers for increased risk of coronary heart disease. However, they are limited in their usefulness in identifying functional relationships between psychosocial factors and the progression of atherosclerosis and coronary heart disease. To answer questions related to more specific effects, it is necessary to assess coronary artery atherosclerosis (CAA) at specified time points in relation to psychosocial manipulations.

Using human subjects for investigation of the effects of psychosocial factors on CAA is almost impossible for at least four reasons. First, for ethical reasons, invasive techniques such as coronary artery arteriography cannot be performed on asymptomatic people. New non-invasive techniques are being developed and tested such as an ultrafast x-ray cine computed tomographic scanner which allows visualization of the coronary arteries without injection of contrast material (Coin, 1993). At this point, however, these procedures are not widely available. Second, long term psychosocial manipulations with strict dietary, personal and physical guidelines would be very difficult to conduct with human beings and probably ethically prohibited. Third, the wide variation of early social experience is a confounding factor. Fourth, atherosclerosis takes years to develop to the point where
clinical signs and symptoms suggest a diagnosis of CHD (Strong et al., 1972). A prospective study would require a series of observations over 40 years or more and even then, without the use of invasive assessment techniques, only the percentage of subjects manifesting clinical symptoms of CHD could be studied more thoroughly.

Given these constraints, investigators interested in psychosocial factors and CHD have sought suitable animal models. In reviewing the animal research investigating the role of behavioral/social factors in atherosclerosis, it is important to describe the differences between atherosclerosis and other cardiovascular pathologies.

**Atherosclerosis**

The artery wall consists of (a) the adventitia, the outer layer of connective tissue; (b) the media, which contains smooth muscle cells embedded in a matrix of collagen, elastin and proteoglycans and; (c) the intima, the innermost layer directly adjacent to the flowing blood composed of endothelium at the luminal surface, subendothelial extracellular matrix and in some vessels, one or more layers of smooth muscle cells (White, 1989). Atherosclerosis is a process affecting the intimal layer of arteries (McGill, 1968). Smooth muscle cell proliferation occurs in the intima, along with extracellular and intracellular accumulation of lipids and the migration of monocytes and macrophages into the intima from the blood.

Proposed initiating factors in formation of atherosclerotic lesions are (a) "response to injury" which means that some form of injury to the endothelium leads to platelet adhesion and/or monocyte(macrophage migration, and growth factor release which stimulates smooth muscle cell proliferation (Ross, 1986); (b) smooth muscle cell proliferation (McMillan and Duff, 1948); (c) monocyte-macrophage migration (Leary, 1941) and, (d) hyperlipidemia (Solberg and Strong, 1983).
Lesions progress from early fatty streaks (characterized by the intracellular and extracellular lipid accumulation) to fibrous plaques. Some fibrous plaques progress to complicated atheromatous plaques which are characterized by rupture of the fibrous cap, tissue necrosis, mineralization, hemorrhage and thrombosis. The atherosclerotic plaque may reduce the lumen of the artery and ultimately compromise blood flow to tissues supported by the affected vessel; the greater danger, however, is occlusion of the vessel by a superimposed thrombus which forms at the surface of a complicated atheromatous plaque. Sections of the plaque may also break off and form emboli which can compromise blood flow (McGill, 1984). Coronary artery atherosclerosis accounts for the majority of clinical coronary heart disease cases.

Arteriosclerosis and Other Cardiovascular Pathologies

Several animal studies report arterial changes and lesions which cannot be defined as atherosclerosis. Indeed some species do not develop atherosclerosis. Arteriosclerosis is any fibrous thickening of the arteries and may include lesions characterized by the collection of connective tissue proteins and less often, mineral deposition between the elastic layers of the arterial media (Manuck, 1986). Arteriosclerotic changes do not include lipid deposits, intimal cell proliferation and other specific characteristics of atherosclerotic lesions.

Cardiomyopathy, which results from increased arousal of the sympathoadrenal medullary system can be observed by changes in staining characteristics, separation of myocardial fibers, fragmentation, fibrosis and necrosis of cardiac tissue (Henry, 1982). Other dependent variables used to assess cardiovascular pathology in animals are heart enlargement, cardiac arrhythmias, other electrocardiogram abnormalities and sudden death.
Choosing an Animal Model of Atherosclerosis

The choice of an appropriate animal model depends on its similarities to human beings in regard to the morphologic and physiologic characteristics of the development of atherosclerotic lesions and to the presence of environment/behavior interactions analogous to those speculated to be important in the pathogenesis of CAA in human beings (Manuck et al., 1987). The social behavior of animals can be broadly characterized into two categories: (1) territorial in which animals protect a certain location with variable behavior toward others or, (2) hierarchical in which a stable dominance hierarchy is formed, social alliances form, and aggression becomes ritualized and predictable such that overt fighting is minimized (Collias, 1944).


Tissue Preparation Methods

Results from these studies are difficult to compare because the methods used to prepare coronary arteries and quantify CAA. In general, after the animals are sacrificed, the heart and coronary arteries are removed and fixed (with 10% neutral buffered formalin). Cross sections of the arteries are usually stained with hematoxylin and eosin examined. When the heart is removed from the animal, the coronary arteries may collapse, so that when they are fixed, it is difficult to make accurate measures of lesion size in relation to the size of the arterial lumen.
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<td>increased frequency of CAA among birds and mammals at autopsy: 1916-1931: 2% 1931-1946: 18% 1946-1956: 22%</td>
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<td>Ratcliffe et al., 1960</td>
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<tr>
<td>Lang, 1967</td>
<td>squirrel monkeys n=18</td>
<td>8% fat, 0.5% chol increased to 14% fat, 1% chol at 20 months</td>
<td>25 months</td>
<td>Levels of stress: 1) psychic stress (avoidance training in Skinner box, 1 hr/day, 5 days/wk; exposure to shock), 2) control stress (no shock or avoidance, but in Skinner box same freq as above) 3) cage controls</td>
<td>presence/absence of coronary artery atherosclerosis</td>
<td>1) psychic stress: CAA present in 5 of 6 2) control stress: CAA present in 4 of 6 3) cage controls: CAA present 0 of 6</td>
</tr>
<tr>
<td>AUTHOR</td>
<td>SUBJECT</td>
<td>DIET</td>
<td>LENGTH OF STUDY</td>
<td>PSYCHOSOCIAL INDEPENDENT VARIABLE</td>
<td>MEASURE OF ATHEROSCLEROSIS DEPENDENT VARIABLE</td>
<td>FINDINGS</td>
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<tr>
<td>Ratcliffe et al., 1969</td>
<td>pigs n=37</td>
<td>whole milk=50% of cat; whole corn and protein/mineral concentrate</td>
<td>approx, 1 year</td>
<td>Social environment: 1) isolated males and females 2) male/female pairs 3) groups (8 males, 4 females)</td>
<td>&quot;heart scores&quot;= combination of frequency and grades of stenosis of coronary arteries</td>
<td>1) isolated animals &gt; pairs and groups 2) pairs &gt; groups (P&lt;.05)</td>
</tr>
<tr>
<td>Henry et al., 1971</td>
<td>mice n=40</td>
<td>lab chow</td>
<td>6 months</td>
<td>Social environment: special population cages (socially stimulated) vs. control cages</td>
<td>1) degree of stenosis of intramural coronary vessels 2) aortic arteriosclerosis score</td>
<td>1) socially stimulated &gt; controls 2) socially stimulated &gt; controls</td>
</tr>
<tr>
<td>Ely et al., 1981</td>
<td>mice n=51</td>
<td>lab chow</td>
<td>4 months</td>
<td>Classification by status dominant vs. submissive (housed in special population cages)</td>
<td>aortic arteriosclerosis score</td>
<td>dominant &gt; submissives (P&lt;0.05)</td>
</tr>
<tr>
<td>Nerem et al., 1980</td>
<td>rabbits</td>
<td>2% cholesterol</td>
<td>6 weeks</td>
<td>Social environment: special attention vs. normal lab care</td>
<td>% aortic surface exhibiting lipid stain</td>
<td>control &gt; special attention (P=0.015)</td>
</tr>
<tr>
<td>Gow et al., 1982</td>
<td>rabbits n=18</td>
<td>2% cholesterol</td>
<td>5 weeks</td>
<td>Social environment: special attention vs. normal lab care</td>
<td>% aortic surface exhibiting lipid stain</td>
<td>no significant difference</td>
</tr>
<tr>
<td>AUTHOR</td>
<td>SUBJECT</td>
<td>DIET</td>
<td>LENGTH OF STUDY</td>
<td>PSYCHOSOCIAL INDEPENDENT VARIABLE</td>
<td>MEASURE OF Atherosclerosis DEPENDENT VARIABLE</td>
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<tr>
<td>Kaplan et al., 1983</td>
<td>cynomolgus monkeys n=30</td>
<td>43% fat 0.34mg cholesterol/Cal</td>
<td>22 months</td>
<td>1) Social environment: unstable vs. stable 2) Social status: dominant vs. subordinate</td>
<td>1) mean intimal area of coronary arteries 2) lumen stenosis of coronary arteries</td>
<td>1) dominant/unstable &gt; all other groups (p=0.034) 2) dominant/unstable &gt; all other groups (p=0.003)</td>
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<tr>
<td>Kaplan et al., 1983</td>
<td>cynomolgus monkeys n=30</td>
<td>low fat low cholesterol</td>
<td>21 months</td>
<td>Social environment: unstable vs. stable</td>
<td>1) mean intimal area 2) intimal thickness 3) grade of arterial lesion (0-2)</td>
<td>1) unstable &gt; stable (p&lt;0.002) 2) unstable &gt; stable (p&lt;0.02) 3) unstable &gt; stable (p&lt;0.05)</td>
</tr>
<tr>
<td>Hamm et al., 1984</td>
<td>cynomolgus monkeys n=32</td>
<td>45% fat 0.56mg cholesterol/Cal</td>
<td>16 months</td>
<td>1) Social status: dominant vs. subordinate 2) Gender: male vs. female</td>
<td>1) coronary artery lumen stenosis 2) % aortic surface occupied by lesion</td>
<td>1) males &gt; females (p=0.008) sub &gt; dom (p=0.001) 2) sub &gt; dom (p=0.001) thoracic aorta</td>
</tr>
<tr>
<td>Kaplan et al., 1984</td>
<td>cynomolgus monkeys n=42</td>
<td>44% fat .043 mg cholesterol/Cal</td>
<td>30 months</td>
<td>1) Social environment: stable vs. unstable 2) Social status: dominant vs. subordinate 3) Gender: male vs. female</td>
<td>1) mean intimal area 2) grade of arterial lesion (0-3)</td>
<td>1) all males &gt; all dominant females (p=0.021) 2) all sub females &gt; all dom females (p=0.007)</td>
</tr>
</tbody>
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or to quantitate the percentage of stenosis. Few studies have reported pressure perfusion of coronary arteries with a fixative at physiological pressure in order to prevent arterial collapse. Aortic tissue samples are typically prepared by taking longitudinal sections and staining them for lipids. Psychosocial manipulations are not easily quantified across studies or across species and are therefore also difficult to compare. A more detailed discussion of animal models and descriptions of studies investigating psychosocial influences on atherosclerosis follows.

**Birds**

Among birds, pigeons have been used most extensively in atherosclerosis research. In fact, different strains of pigeons have been developed for that purpose.

Pigeons offer the advantage of being easy to manipulate, inexpensive to maintain and live 15-20 years under lab conditions.

The arterial lesions found in pigeons are similar to those found in human beings and tend to become complicated with deposits of calcium, hemorrhage, vascularization, elastic fiber degeneration with thickening of media, adventitial collections of lymphocytes, and ulceration with thrombus formation (Prichard et al., 1964). Show Racers are available as hypo- or hyperresponsive to the development of hypercholesterolemia in response to dietary cholesterol, and for exhibiting extensive coronary artery atherosclerosis and minimal aortic atherosclerosis (Clarkson et al., 1965). The White Cameau breed develops clinically significant atherosclerosis while maintained on its naturally preferred diet of grain (Clarkson et al., 1963) and is a good model for studying the effects of many experimental manipulations on lesion development.

Pick et al. (1963) found that when cockerels were fed an atherogenic diet while isolated in single cages, a highly unnatural environmental situation for
cockerels, atherosclerosis development was increased independent of plasma lipoprotein levels compared to group housed birds. Disturbing the "pecking order" had no effect the development of atherosclerosis.

Other birds develop atherosclerosis in their natural state as well as in captivity. Ratcliffe et al. (1958) reviewed the autopsy records of 65 families of birds at the Philadelphia Zoological Garden. They analyzed incidence of coronary artery atherosclerosis across time. During the period from 1916-1931 only 2% of birds at autopsy had developed arteriosclerosis, defined as intimal thickening with or without lipid accumulation. In 1935, the diets of the birds were upgraded and controlled; this resulted in longer life spans and an increase in arteriosclerosis found upon autopsy. From 1946 to 1956, after diets had been stabilized for over 10 years, population density had increased and mean ages had decreased to the levels found before 1931. Also, the rate of arteriosclerosis had increased by 20-fold. These increases in arteriosclerosis were independent of age and diet but were associated with the increases in population densities, suggesting that "social pressure" had become a major factor in the progression of arteriosclerosis.

In a later paper, Ratcliffe et al. (1960) reported that the character and location of arterial lesions in the birds had also changed. In the first decade after the improved diet, large atheromata of the proximal aorta were replaced by smaller more compact lesions, usually of the abdominal aorta. In the second decade of improved nutrition, arteriosclerosis became relatively common, developing in distal, intramural segments of coronary arteries as intimal thickening and occlusion which can lead to myocardial infarctions. Coronary arteriosclerosis was virtually absent for about a decade after the diet was changed, so its frequency did not appear to be related to diet. Ratcliffe concludes that CHD represents a response of adequately nourished animals to increased population densities.
Flock dwelling birds are socially organized in linear hierarchies. Because birds mature rapidly, the pecking order behavior found in bird hierarchies tends to be in large part innate with little influence of social learning on experience (Etkin, 1964). Social cooperation and complex behavioral interactions play a small part in the hierarchies formed. Therefore, studying the effects of psychosocial manipulations in birds which are relevant to human social environments and behavior may be unproductive.

Rodents

Mice and rats generally do not develop atherosclerotic lesions. Morrisett et al. (1982), reported as an exception, the finding that the C57BR/cdj strain of mice are susceptible to atherosclerosis under extreme dietary conditions. CBA/j mice are resistant to dietary induced atherosclerosis but do develop arteriosclerosis and have been used in studies investigating effects of social stimulation.

A creative psychosocial manipulation was designed by Henry et al. (1967). It involved housing CBA mice for 6-17 months in special population cages with narrow interconnecting tubes and a single central feeding and watering area. This housing arrangement (the "socially stimulated" condition) forced social interaction among the animals in order to obtain food, water and access to a mate.

Mice from three rearing conditions were housed in the "socially stimulated" environment in order to expose them to prolonged stressful social interactions (Henry et al., 1971). One group was reared in isolation in glass jars, another group was previously reared together as siblings in stock boxes and the third group was previously reared in the socially stimulated cages. The inadequate social experience of formerly isolated animals affected their ability to cope with the repeated social confrontation evidenced by increased injuries from bites and scratches. It appears
that the forced social confrontations inhibited the development of social stability in the colony.

All of the socially stimulated mice were compared with controls housed in standard boxes or maintained in isolation jars for their entire lives. The socially stimulated animals showed significantly greater luminal reduction in coronary arteries compared to controls as well as more severe myocardial fibrosis. Henry et al. (1971) points out that population density was not a contributing factor in this study because the socially stimulated animals were provided with as much or more space than the control groups.

Rodents are basically territorial (Etkin, 1974), although some species (e.g., the CBA strain employed in Henry's studies) form stable hierarchies. The social complexity of dominants tolerating submissives and cooperating with them in affiliative interactions is rare (Brown, 1966). The submissive-dominant behavior dimension has been found to be associated with changes in both blood pressure and coronary arteriosclerosis. Ely (1981) showed that dominant CBA/j mice housed in "population cages" for four months showed significant rises in blood pressure and increased aortic arteriosclerosis compared to subordinate and control animals.

Rabbits

As early as 1908, rabbits have been used as models for atherosclerosis. Ignatowsky produced intimal lesions resembling human atherosclerosis by feeding rabbits diets of milk, meat and eggs (Ignatowsky, 1908). It was first concluded that the high level of animal protein in the rabbit's diet was responsible for the lesions. Later, it was suggested that the fat portion of the diet was contributing to atherogenesis and then finally cholesterol was identified as the causative factor. Interestingly, rabbits fed a diet with added cholesterol but without added fat
sometimes develop more severe atherosclerosis than do others fed cholesterol with added fat (Kritchevsky, 1970). This may be due to the mobilization of endogenous fat stores which are more highly saturated than the usual dietary fats. Therefore the degree of saturation of dietary fat used to induce atherosclerosis in the rabbit is an important consideration.

Rabbits exhibit coronary atherosclerosis after approximately one month of consuming a high cholesterol (1-3%) diet alone or consuming a combination of high cholesterol and high fat diet (5-15%). The distribution of coronary lesions is reported to be different from that observed in man. In rabbits, small intramyocardial branches are affected primarily; lesions in large, proximal coronary arteries are more rare (Prior et al., 1961). Coronary artery lesions similar to those in humans were produced by Wilson et al. (1982) after five years of feeding rabbits a diet comparable to that consumed by humans in North America. Rabbits appear to be a very useful animal model for atherogenesis, particularly for studying the relationships between hyperlipoproteinemic and immune complex damage to the endothelium and to atherogenesis (Kaplan et al., 1985).

Nerem et al. (1980) reported an interesting finding regarding the effects of the social environment on atherogenesis which has not been confirmed independently. Cholesterol-fed rabbits provided extra daily attention and physical coddling by a familiar investigator developed less extensive aortic atherosclerotic lesions than rabbits given no individual treatment or attention. Gow et al. (1982), could not replicate this finding.

Rabbits are basically territorial. Rabbit warrens are communal in nature, however, males typically maintain exclusive access to the nesting areas of one or more breeding females. Few investigations of the effects of psychosocial stimulation on atherosclerosis in rabbits have been reported.
Pigs

Pigs have been regarded as a preferred model for atherosclerosis because of the similarity of their plasma lipoproteins and the anatomic distribution and character of their atherosclerotic lesions to those seen in humans (Kaplan et al., 1985). Pigs also readily accept the kinds of diets consumed by human beings. Because of their size, pigs can be difficult to work with to obtain blood samples, etc. Despite this problem, extensive data have been published on many aspects of atherosclerosis in pigs (Skold & Getty, 1961; Ratcliffe & Luginbuhl, 1971; Gerrity et al., 1979; Gerrity, 1981). Both domestic and miniature pigs develop atherosclerosis naturally with increasing age. The addition of fat and cholesterol to the diets induces a modest hyperlipoproteinemia which hastens atherogenesis. A combination of arterial injury and atherogenic diet produces a more rapid production of large lesions as well as lesions at particular sites (Fritz et al., 1980). Regression studies using this combination technique have shown that plaques are reduced in size following withdrawal from an experimental atherogenic diet and that unesterified cholesterol leaves atheromatous plaques more readily than does cholesteryl esters (Daoud et al., 1981).

Ratcliffe et al. (1969), prompted by observations at the Philadelphia Zoo that increased arteriosclerosis in birds and mammals correlated with "social pressures", studied the effects of social manipulations on arteriosclerosis in swine for approximately one year. The animals were divided into four different housing groups: (1) individually housed male pigs, (2) individually housed female pigs, (3) paired male and female pigs and, (4) two groups of 12 pigs, 8 males and 4 females. "Heart scores" consisting of grades of stenosis and number of arteries affected were calculated for each animal. Coronary heart scores were lowest for pigs housed in...
groups, intermediate for pairs and highest for separated animals, especially separated females. The frequency and severity of coronary heart disease was not related to population density. It appears that the social situations most clearly associated with CHD in animals of the Philadelphia Zoo and in this study are ones which prevent development and expressions of characteristic species specific behavior patterns.

Pigs establish stable dominance hierarchies usually through aggressive interaction, however, once the order is established, pushing, butting or occasional biting replaces overt fighting. Dominance orders are mutually recognized by olfaction and regulate access to resources such as food, space and sexual partners. Subordinates are tolerated by dominants and are allowed to retaliate when attacked (Dantzer and Mormede, 1986).

The pathologic similarities of atherosclerosis, the sensitivity to psychosocial manipulations, the economic considerations (compared to primates) and the convenience of their reduced size makes the miniature pig a very attractive model for studying psychosocial influences on atherogenesis.

**Monkeys**

In an overview of animal models of behavioral influences on atherogenesis, Kaplan et al. (1985) consider non-human primates as the most appropriate for atherosclerosis research. The pathologic characteristics of the arterial lesions developed in some non-human primates with dietary manipulations result in a pattern of atherosclerosis closely related to that in human beings. Selective breeding programs are in progress to enhance atherosclerosis characteristics similar to those in humans and to repress those not found in humans.

Squirrel monkeys and rhesus monkeys have been extensively studied in atherosclerosis research (Taylor, 1962 and Clarkson et al., 1976), and well studied in
psychosocial research. Studies regarding the effects of psychosocial interventions on atherosclerosis, however, have been rare.

Lang (1967) investigated the effects of "psychic stress" on the development of atherosclerosis in squirrel monkeys. The monkeys were fed a mildly atherogenic diet (8% fat and 0.5% cholesterol) for 20 months at which time none of the animals showed sustained hypercholesterolemia. Consequently, the dietary fat and cholesterol were increased to 14% and 1.0% respectively. Eighteen animals were randomly divided into three groups: (1) a psychic stress group, (2) a box control group and, (3) a cage control group. Each animal in the first two groups was placed in an operant chamber for 1 hour per week, 5 days/week. During this time the monkeys in group 1 were exposed to the Sidman avoidance procedure in which the animal had to press a lever on a certain time schedule to avoid a two volt shock. Group 2 animals were not exposed to shock or the avoidance procedure but were exposed to daily capture and handling as well as restraint in the operant chamber. Group 3 animals were left in their cages for the 25 months of the experiment. Coronary atherosclerosis was exhibited in five of six monkeys in group 1 (psychic stress) and four of six monkeys in group 2 (restraint controls) while it was not detected in any of the six monkeys in group 3 (normally caged controls).

Pigtail macacques have been used more extensively in research on neurophysiologic control of cardiovascular function than as a model for atherosclerosis, yet some interesting findings have been reported on ethanol ingestion in relation to levels of plasma lipoproteins and extent of atherosclerosis. In 1978, Leathers et al. reported that high-density lipoprotein concentration increased and the molecular weight of low-density lipoproteins decreased in pigtail macacques after ingestion of ethanol. Later, in 1981, Rudel et al. further reported that these ethanol induced changes in plasma lipoproteins were correlated with significant
decreases in coronary artery atherosclerosis.

Cynomolgus macaques exhibit naturally occurring atherosclerosis. They accept experimental diets well and develop atherosclerotic lesions with similar pathologic characteristics to man. These monkeys have a relatively high frequency of myocardial infarction, exhibit differences in susceptibility to CAA between males and females, and are a conveniently sized animal to work with.

Behaviorally, monkeys offer many characteristics that are attractive to psychosocial researchers: (a) they live much longer (often to 20-30 years) than rodents, birds, rabbits and swine; (b) they have a long juvenile period during which much social learning and training takes place and; (c) they typically dwell in complex, hierarchically organized social groups involving patterns of affiliation based on generational kinship and peer associations (Kaplan et al., 1985); (d) unlike many birds and rodents, relatively few young are produced but the offspring are well nurtured and trained; (e) monkeys exhibit anxiety when exposed to appropriate social manipulations, (f) monkeys show significant plasticity in behavior, (e.g., to the point of establishing different behavioral traditions among the same species).

Dominance hierarchies in monkeys are more complex than those of other animals and display certain similarities to human hierarchies. An important commonality is the "ability of both human beings and some species of non-human primates to establish, accept and utilize hierarchical relationships among individuals" (Kaplan et al., 1985, p. 137). Monkeys tend to survive well despite repeated conflicts over food, space and access to mates because of their ability to recognize dominance relationships and to display aggression, alliance and submission in reference to those relationships. Maintenance of the social system is mediated by well developed behavioral sequences including ritualization of fighting via an elaborate repertoire of motor patterns and facial expressions as well as complex
coalitions for defense enabling subordinate animals to live successfully in the presence of dominant animals (Kaplan et al., 1985).

The most and extensive work in biobehavioral investigations of atherogenesis have been done using cynomolgous macaques at the Arteriosclerosis Research Center of Bowman Gray School of Medicine in North Carolina. Macaques develop complicated atherosclerotic lesions in the main branch coronary arteries, the aorta, and carotid arteries similar to human beings when fed a diet approximating that consumed by North Americans. They live successfully in large groups and establish hierarchies of dominance with networks of affiliation and coalition which serve to minimize overt aggression.

The psychosocial intervention used by the Bowman Gray group is based upon the observation that the introduction of unfamiliar monkeys into an already established social group is perceived as a threat to existing associations and intense fighting occurs in an attempt to reestablish generalized hierarchical relationships and affiliative coalitions (Bernstein, Rose & Gordon, 1974). It is hypothesized that the social disruption accompanying a deliberate introduction of strangers into established primate groups enhances the development of atherosclerosis and, further, that the severity of atherogenesis varies among individuals as a function of their social status in the group.

Two experiments were designed to investigate this speculation (Kaplan et al., 1982 and Kaplan et al., 1983). In both studies, monkeys were assigned to unstable (stressed) and stable (control) social conditions for a period of 21-22 months. All animals were housed in identical pens with outdoor exposure. The unstable condition involved reorganization by redistribution of animals among three groups of five monkeys every 12 weeks during the first year and once every four weeks thereafter. An ovarectomized, estrogen-primed female was also introduced to the
unstable group for half of each four week period in the final ten months to enhance social uncertainty among the animals. The stable condition animals, in contrast, were housed in groups of fixed membership with no exposure to females or unfamiliar animals. Behavioral ratings of dominance were collected periodically using standard focal sampling techniques to establish dominant and submissive status and to record affiliative and aggressive behavior. Each animal was observed for approximately 50 hours. Winners and losers of confrontations were recorded as well as rates of affiliative acts, aggression, grooming, etc.

In the first study, monkeys consumed a diet similar to that of an urban North American male (43% fat and 0.34% chol/Cal). In the second study, monkeys were fed a low fat and cholesterol diet.

After necropsy, the heart was removed and the coronary arteries were perfused with a fixative at 100mg Hg and then stained for lipids. Mean intimal area was calculated from 15 sections of coronary artery, five from the left anterior descending, five from the left circumflex and five from the right coronary arteries. Intimal area included intima and/or intimal lesion defined as the area between the internal elastic lamina and the lumen of the artery.

In Experiment 1 the monkeys were fed a moderately atherogenic diet and housed in stable vs unstable social environments. Results indicated that dominant monkeys from unstable social groups exhibited substantially greater CAA than all other monkeys, i.e., subordinate monkeys in the unstable social group, and dominant and submissive monkeys in the stable group.

The purpose of Experiment 2 was to determine whether the atherogenic changes as a result of social manipulations in the first experiment would be present in monkeys fed a low fat, low cholesterol diet. In contrast to Experiment 1, ratings of dominant and subordinate monkeys in the unstable group were not consistent over

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time so comparisons were made solely on the basis of stable vs unstable social conditions. In general, the intimal lesions observed in this study were much less extensive and therefore in addition to intimal area, two more indices of CAA were used: (1) intimal thickness, defined as the maximum distance at any one point between the internal elastic lamina and the lumen of the artery and, (2) a three-point visual grading of the severity of arterial lesions judged by investigators blind to the conditions of the experiment. All three measures of CAA revealed significantly greater coronary artery atherosclerosis in monkeys housed in the unstable group relative to the stable group. The extent of atherosclerosis in both studies was independent of the monkeys' blood pressure or lipoprotein profile.

The effects of dominant status and social stability among female monkeys were studied by Hamm et al. (1983). They reported that subordinate female cynomolgous monkeys fed a high cholesterol diet developed more severe CAA than dominant females in both stable and unstable groups.

Summary

The evidence presented thus far supports the notion that psychosocial factors contribute to CHD. It is clear that in many species, psychosocial factors (e.g., isolation and unstable social environments), especially those that disturb species specific instinctual behavior patterns, are correlated with cardiovascular pathology. The most convincing evidence for increased atherogenesis as a result of social disruption is found among the studies using an animal model most similar to humans, the non-human primate. The presence of increased dietary fat and cholesterol appears to enhance psychosocial effects on CAA. What is not so clear is the mechanism by which these effects are mediated.
Physiologic Changes as a Result of Psychosocial Stimulation

Referring back to Figure 1, psychosocial stimuli are processed by the CNS where arousal and anti-arousal forces (coping, defenses) result in output to three effector systems; the musculoskeletal system which mediates change in the external environment and the autonomic and endocrine systems which mediate change in the internal environment. Therefore, the autonomic nervous system and the endocrine system have been the foci for researchers investigating the mechanisms by which psychosocial factors affect the development of coronary heart disease.

The peripheral autonomic nervous system is made of parasympathetic and sympathetic neurons which innervate cardiac smooth muscle and exocrine glands. The sympathetic nervous system is activated in response to, or, in anticipation of situations evoking fear, anger, uncertainty or demanding physical exertion. The parasympathetic nervous system governs the vegetative aspects of day to day living.

Cannon (1914) established the association between sympathetic nervous system activity and emotional states. In several experiments with cats, he, de la Paz and others found that a cat placed in a holder, physically safe but frightened by a barking dog secreted "adrenalin" measured by an intestinal biostrip assay (Cannon, 1911). His observations led to the notion that psychological stimuli evoking anticipation of fear, rage or pain elicit a "fight or flight" response which results in increases in heart rate, blood pressure, cardiac output, redistribution of blood flow from splanchnic beds to skeletal muscles, myocardium and brain, lipid mobilization, renin secretion and secretion of norepinephrine and epinephrine from the adrenal medulla. Cannon deduced that activation of the sympathetic adrenal medullary system serves an "emergency function" because many of its physiologic and metabolic consequences are "directly serviceable in making an organism more
effective in the struggle in which fear or rage or pain may be involved" (Cannon, 1914, p. 372).

Cannon conceptualized the amazing consistency of the internal milieu of the body as a dynamic homeostasis with second to second adjustments to stimuli. He realized that emotions exerted powerful effects on physiology. In his words,

"Probably before many years have passed we shall have satisfactory tests for internal secretions, and then shall know better the total expression of an emotional storm in our own bodies" (Cannon, 1922, p. 28).

The endocrine system regulates cell metabolism. Unlike the autonomic nervous system which affects smooth and cardiac muscle and exocrine glands, hormones exert their effects on every cell in the body. Central nervous system connections to the endocrine system are illustrated in Figure 3.

![Figure 3. Points of Contact Between the Central Nervous System and the Endocrine System (Mason, 1974). Reprinted by permission of Author.](image)

The endocrine system functionally links central nervous system activity with every body cell. Mason (1975c) states:
The superimposition of such complex and idiosyncratic psychological factors as emotion, defensive style, and neurotic processes on virtually all bodily functions via the neuroendocrine machinery deserves prime suspicion in our search for fallibility or proneness to disease (p.14).

Hundreds of studies have demonstrated the responsiveness of the pituitary-adrenal cortical system (resulting in increased circulation and excretion of cortisol) to a variety of naturalistic and laboratory situations, including arithmetic tasks, stressful and other kinds of films, underwater diving, pilot and paratrooper training, stressful interviews, avoidance conditioning and anticipation of extreme exercise and surgery (Price et al., 1957; Mason, 1968; Rubin et al., 1969; DeWied et al., 1972; Frankenhauser, 1975; Henry et al., 1981).

It is evident that the pituitary-adrenal cortical system can be powerfully affected by psychosocial influences, especially in situations which involve novelty, uncertainty, unpredictability (Mason, 1968), ineffective coping and loss of control (Henry, 1983).

Even subtle changes in environmental conditions produce alterations in adrenal cortical responses. Mason (1975) showed that in monkeys, urinary corticoids reflected the day to day activities in the lab where they were housed. On Monday, corticoids were the highest, fairly stable from Tuesday to Friday, then decreased by 30% on weekends when people were absent. Under some circumstances suppression of the adrenal cortical system can also occur such as when certain psychological defenses are used effectively to counter arousal (Wolff et al., 1964).

The sympathetic adrenal medullary system which primarily secretes epinephrine and lesser amounts of norepinephrine, is also quite responsive to psychosocial stimuli. Norepinephrine has a dual role as a neurotransmitter for
adrenergic neurons of the sympathetic nervous system and as a hormone. Since the physiological aspects of circulating norepinephrine relates to its role as a hormone, it will be included in this discussion of the sympathetic adrenal medullary system.

Investigations measuring catecholamine responses have been similar to those described for cortisol including the effects of aircraft flight, paratroop training, watching different types of films, physical tasks, mental tasks, exams, hospitalizations and public speaking (Elmadjian et al., 1957; Mason, 1968; Taggart et al., 1973; Frankenhauser, 1975; Engel et al., 1980; Ward et al., 1983).

Dissociation between levels of norepinephrine and epinephrine in relation to psychosocial stimulation has been reported by several investigators (Funkenstein, 1956; Elmadjian et al., 1957; Mason, 1961; De Boer, 1990). Selective epinephrine secretion appears to occur in situations that (a) arouse anxiety or fear, or (b) include novelty or uncertainty. Selective norepinephrine secretion appears to occur in response to familiar but unpleasant tasks, anger and aggressiveness or any physical exertion.

Elmadjian et al. (1957) provided an interesting example of the dissociation of catecholamines. He measured catecholamine excretion in hockey players, some of whom played the game while others watched. The results showed significant elevations in norepinephrine (NE) and smaller increases in epinephrine (E) excretion during play (larger NE/E ratio) as compared with significant elevation of epinephrine excretion only in the physically passive condition (smaller NE/E ratio). Discussion of active and passive responses to psychosocial stimuli will be expanded in a later section.

**Summary**

The autonomic nervous system and the endocrine system demonstrate
sensitivity and responsiveness to psychosocial stimuli and represent the two pathways by which psychosocial factors can influence the development of CHD.

Mechanisms by Which Psychosocial Factors May Contribute to Coronary Heart Disease

Review of the research investigating mechanisms by which psychosocial factors may influence CHD will be divided into two sections: (1) research relating psychosocial factors to increased CHD mediated by an increase in risk factors, i.e., increased cholesterol levels and increased blood pressure and; (2) research relating psychosocial factors to increased CHD not mediated by risk factors (Figure 4).

Figure 4. Psychosocial Factors May Lead to Increased Coronary Heart Disease Through Enhancement of Risk Factors or Through Other Mechanisms.
Possible Mechanisms by Which Psychosocial Factors Contribute to CHD Mediated by Increased Risk Factors

It has been proposed that psychosocial factors contribute to atherogenesis and CHD by increasing established risk factors such as cholesterol level and blood pressure.

Psychosocial Factors and Increased Cholesterol

Many investigators have reported relationships between psychosocial stress and serum or plasma cholesterol. Friedman, Rosenman and Carroll (1958) studied the effects of cyclic occupational stress upon serum cholesterol and blood clotting time in male accountants while meeting tax deadlines and at less stressful times. They found that each subject’s highest serum cholesterol level occurred during severe occupational or other stress and his lowest at times of minimal stress. Blood clotting times also were markedly accelerated during times of maximum occupational stress as compared to normal blood clotting during baseline occupational stress. The results occurred independent of changes in diet, exercise or diet. In 1959, Grundy and Griffin studied medical students during academic final examinations and reported a significant increase in the mean total serum cholesterol levels during examination periods as compared to control periods of relaxation. Peterson et al. (1962) reported hourly variation in serum cholesterol concentration and that significant changes in serum cholesterol may relate to the anticipation of stressful events as well as to the event itself. In a longitudinal study, Rahe et al. (1971), reported significant positive correlations between serum cholesterol and reported feelings of depression, anger and fear in naval trainees.

In a review of over 60 studies investigating plasma lipid responses to emotional arousal, Dimsdale and Herd (1982) concluded that "in the astonishingly
different situations that have been examined, the studies yield similar findings, suggesting a robustness to the association between emotional arousal and plasma lipids" (p. 229). Their summary included the following findings: (a) free fatty acids almost invariably increased in response to stressful events, (b) there was no consistent pattern of triglyceride response to emotional arousal and, (c) though cholesterol levels may be highly variable, most studies found cholesterol increases from 8-65% above baseline under stressful conditions. Thomas et al., (1985) reported that among 256 healthy elderly adults, individuals with good social support systems determined by a social interaction scale tended to have lower serum cholesterol and uric acid levels and higher indices of immune function measured by total lymphocyte count. These relationships were independent of age, body mass, tobacco use, alcohol intake and degree of perceived psychological distress. Weidner et al., (1987) assessed hostility, Type A behavior and plasma lipids in 282 women and men and reported that individuals scoring high on Type A behavior as well as hostility had an elevation of total and low-density lipoprotein cholesterol after controlling for age and body mass index. These findings were replicated in a one year followup.

Relationships between cholesterol levels and individual behavioral traits have also been examined. In 1959, Friedman and Rosenman observed that extreme Type A men had higher cholesterol levels than Type B males. Most studies confirm this finding (Friedman et al., 1960; Komitzer et al., 1977), however, some studies found no A-B difference (Keith et al., 1965 and Friedman et al., 1968).

van Doorman (1980) suggests that the coronary prone behavior pattern includes not only Type A behavior but also a depression/neuroticism component. In 1987, van Doornen and Blokland reported that cholesterol levels of 29 male and 23 female students were higher on an examination day compared to a control day. In
regard to males, 62% of the variance of the baseline cholesterol level and 40% of the stress induced (from examination day) cholesterol rise were explained by psychological variables measured. Achievement, motivation and depression were important in both predictions. No significant predictions could be made in the female group.

The mechanism by which response to stress elicits an increase in serum cholesterol is not known. One speculation is that increases in plasma epinephrine levels caused by heightened sympathetic adrenal medullary activation mediates the altered lipoprotein response. Plasma epinephrine levels are significantly increased under moderately stressful circumstances in humans, such as public speaking. Most people triple their baseline epinephrine levels and some individuals can show a ten fold increase (Dimsdale & Moss, 1980a, 1980b).

Dimsdale et al., (1983) studied the effect of physiological elevations of plasma epinephrine levels on plasma cholesterol levels in cynomolgus monkeys. The amount of epinephrine administered to each monkey was chosen to produce an elevation in plasma epinephrine similar to that encountered during emotional arousal. Plasma epinephrine levels were sustained at the increased level for a two week period, five days per week, six hours per day. Control monkeys were treated with saline injections. After two weeks, the cholesterol levels of the epinephrine treated monkeys increased on the average of 15mg%. Control monkeys showed no increase in cholesterol levels.

Psychosocial Factors and Hypertension

The most recent epidemiologic evidence confirms elevated blood pressure as a significant risk factor for CHD (Lei et al., 1992; Nedeljkovic et al., 1993).

The cardiovascular response of increased heart rate and blood pressure via

Though there are differing theories on the cause of primary hypertension related to sodium intake, genetic factors and environmental influences, one conclusion is firm: primary hypertension involves a disturbance of central regulation (Weiner, 1976). Strains of rats bred to develop hypertension spontaneously, or upon increased salt intake, both have changes in CNS control of blood pressure. Folkow (1982) showed that the magnitude of the hypertensive responses in these genetically vulnerable rats are not determined by genetics alone. Manipulation of psychosocial arousal (increasing or decreasing stimulation) resulted in increases and decreases in blood pressure in spontaneously hypertensive rats.


Henry and Stephens (1975, 1977) have done extensive work investigating the effects of psychosocial stimulation on hypertension in CBA mice. As described previously, they designed special "population cages" which forced continuous confrontation among the animals as they attempted to gain access to centrally located food and water. The cage design and nutrient location were altered to produce differing levels of social disorder, from stable and peacable to maximal stress and confrontation. Psychosocial hypertension was reliably induced in mice by the social environment.
Evidence of increased levels of the biosynthetic enzymes tyrosinehydroxylase (the rate limiting enzyme in catecholamine synthesis) andphenylethanolamine-N-methyltransferase (the enzyme that converts norepinephrine to epinephrine) in psychosocially stimulated hypertensive mice suggests thatincreases of blood pressure are mediated by sympathetic nervous system activity(Henry et al., 1971). Folkow and Rubenstein (1966) and Zanchetti (1976) showedthat chronic and direct stimulation of the defence area of the hypothalamus in ratsleads to hypertension. Katholi (1977) found that infusions of norepinephrine couldinitiate hypertension.

Goldstein (1983) in a review of 32 papers on primary hypertension concludedthat most of the evidence points to an increase in sympathetic nervous system activityand increase plasma catecholamines in early primary hypertension. Since then,several other investigators have confirmed this observation (Tuck, 1986; Bohm et al.,1987; Egan et al., 1987).

Dickinson (1991) notes the methodological difficulties in accuratelymeasuring sympathetic nervous system activity. He suggests that if basal bloodpressure is stabilized long term by the brain, then in early primary hypertension,plasma norepinephrine levels present during sleep (2AM) might give the bestindication of basal sympathetic tone. Measurement of plasma norepinephrine at this
time may facilitate the discrimination between hypertensive and normal subjects.Tuck et al., (1985) found that during sleep, hypertensive individuals had increasedvalues of plasma norepinephrine compared to age matched controls. Dickinson(1991) concluded that "when the confounding effects of physical and emotionalactivity are absent, during sleep, sympathetic nervous activity from a central controlsystem should stand revealed as the main determinant of basal blood pressure" (p.133).
Folkow (1987) described the rapid adaptation of arterial vessels to altered functional demand leading to wall hypertrophy when any sustained arterial pressure elevation occurs. The structural reduction of the inner radius, as a result of the wall hypertrophy, leads to an upward resetting of systemic resistance to flow such that increased systemic resistance can be maintained at normal vascular smooth muscle activity. He maintains that although functional increases in blood pressure are reversible initially, any sustained elevations lead to structural adaptation of blood vessels and increased systemic resistance to flow.

Henry and Grim (1990) describe the chain of events leading to hypertension when an organism is challenged beyond its control:

As the threat persists from day to day in an unstable disordered society, subtle pathophysiological changes gradually develop which can affect the blood pressure control system and lead to hypertension. Thus repeated arousal of the defence alarm, or fight or flight response, leads to vascular changes and geometrically (structurally) based hyperactivity. These are reversible initially but eventually become fixed (p.786).

Type A Behavior and Cardiovascular Responsivity

Similar to other areas in which Type A Behavior Pattern (TABP) is assessed, the relationship between cardiovascular reactivity and TABP is mixed because of methodological issues described earlier. Harbin (1989) employed a meta-analysis in an attempt to quantitatively evaluate the link between TABP and physiologic reactivity. The analysis indicated that individuals with TABP are more responsive to cognitive and psychomotor stimulus situations in terms of heart rate and blood pressure. The structured interview (SI) was a much more effective instrument in this context. Both effect sizes and statistical significance were greater when the SI was administered compared to the Jenkins Activity Survey (JAS). TABP was found to be more prevalent in untreated mildly hypertensive employed individuals than
occupationally matched normotensive subjects (Irwin et al., 1991). Frederickson and Blumenthal (1992) reported that blood pressure correlated positively with total cholesterol levels in type A men.

Summary

Emotional arousal can alter blood lipid/lipoprotein levels. The elevation of lipoproteins may be mediated by adrenal medullary secretion of epinephrine. Psychosocial factors can increase sympathetic nervous system activity and lead to hypertension in animals, presumably including man. The cardiovascular system of individuals exhibiting TABP are more responsive to challenging situations, responding with increased heart rate and systolic blood pressure compared to controls.

Ample evidence has been presented demonstrating that psychosocial factors can increase established risk factors such as elevated total cholesterol levels and blood pressure, hence contributing to increased risk of CHD. However, two important points must be kept in mind when considering the evidence: (1) established risk factors do not explain a significant amount of the variability in the incidence of CHD and, (2) the most compelling evidence from animal models linking psychosocial factors and atherosclerosis points to increased severity of atherosclerosis independent of serum lipoprotein level or blood pressure. In the following section, alternative mechanisms will be discussed.

Possible Mechanisms by Which Psychosocial Factors Contribute to CHD Not Mediated by Risk Factors

A task force on "Biobehavioral Mechanisms in Coronary Artery Disease" (Manuck et al., 1987) proposed two general hypotheses for mechanisms by which
psychosocial factors exert significant influence on atherogenesis independent of established risk factors: (1) hemodynamic responses that accompany sympathetic nervous system responses to behavioral stimuli and, (2) neuroendocrine responses to stress.

**Hemodynamic Responses to Stress**

One hypothesis proposed is that the cardiovascular adjustments elicited by significant psychosocial stressors and mediated by sympathetic nervous system activation, promote atherogenesis by injuring arterial endothelium through hemodynamic disturbances such as turbulence, shear stress (Manuck et al., 1989), and disruptions in laminar flow resulting from dynamic changes in blood velocity (Spence, 1987). Intact normal endothelium provides a permeability barrier for the artery against intrusion by macromolecules such as lipoproteins and presents an antithrombotic surface to the flowing blood. Endothelial damage can lead to intimal accumulation of lipoproteins, adherence of platelets and thrombus development, release of mitogenic substances from regenerating endothelial cells and intimal smooth muscle cell proliferation (Davies, 1986). Endothelial injury is thought to be an initial step in atherosclerotic lesion development (Ross, 1981). Behavioral stressors such as physical restraint and tail shock have been shown to induce such structural changes in the arterial endothelium and intima (Gordon et al., 1981; Hirsch et al., 1984).

**Summary**

Hemodynamic responses to stress are postulated to initiate endothelial injury, making the vessel vulnerable to intrusion by lipoproteins and increasing the likelihood of platelet deposition and cell proliferation, all of which are related to...
lesion development.

**Neuroendocrine Responses and CHD**

After some early work by Raab (1943) and others, neuroendocrine responses and their relationship to CHD have not been aggressively investigated. This fact is somewhat puzzling because hormones affect every body cell and exert powerful influences on cell metabolism. Herd (1983) states:

Behavioral physiologists have given the most attention to the short term physiological correlates of behavioral phenomena. The most attention of all has been given to the short term cardiovascular correlates. Although marked changes in heart rate and blood pressure can occur, we must not ignore the neuroendocrine responses and metabolic concomitants of behavioral phenomena (p. 131).

Weinstein and Stemerman (1981) note that in rats, hypophysectomy (removal of the pituitary gland) can inhibit proliferation of arterial smooth muscle cells that occurs in response to aortic de-endothelialization (mechanically removing the endothelium) indicating that pituitary derived hormones may be significant in the regulation of arterial smooth muscle migration and proliferation in vivo.

Several hormonal systems may affect the development of CHD, including strong evidence for the protective effects of estrogens in females (Kaplan et al., 1991), however, this discussion will be limited to the hormones most highly correlated with psychosocial factors, i.e., epinephrine (also adrenaline), norepinephrine (also noradrenalin) and cortisol (a corticosteroid). Norepinephrine and epinephrine are catecholamines associated with increased sympathetic nervous system and sympatho-adrenal medullary activity and cortisol is associated with pituitary adrenal cortical activity.

**Catecholamines and CHD.** As part of the fight or flight response, catecholamines are catabolic hormones which greatly enhance fuel utilization...
including lipid mobilization (Landsberg and Young, 1992) in preparation for muscular exertion.

Evidence of increased sympathetic nervous system activity in hypertension and in individuals with coronary prone behavior patterns suggests that catecholamines may play a role in the pathology of CHD. Additional support comes from observations of patients suffering from pheochromocytomas, which are catecholamine secreting tumors of the adrenal medulla. Coronary sclerosis and myocardial infarction have been observed in pheochromocytoma patients as young as 10 years of age and are commonly found in adults (Raab, 1943). Hauss et al., 1990, conclude that "adrenaline and/or noradrenalin exhibit a strong atherogenic potency and thus may well contribute to the development of arteriosclerotic vascular lesions" (p. 92).

As early as 1904, Josue reported degenerative changes and scarring in the wall of the aorta after injections of adrenalin into rabbits. Anitschkow (1933) observed intensified lipid infiltration of the aortic intima in cholesterol fed rabbits after administration of epinephrine. A few years later, after several studies demonstrating the cardiotoxic effects of catecholamines, Raab (1943) stated that "adrenaline is unquestionably a most powerful biological substance regarding dynamic and destructive actions upon the cardiovascular system" (p. 189). In 1955, Friedman et al. observed proliferation of the intima after injections of epinephrine and norepinephrine. Raab (1961) and Constantinides (1969) reported that catecholamines foster arterial injury and increase the permeability of the vascular endothelium. Catecholamines administered by injection in rabbits induce atherosclerosis (Helin et al., 1970; Cavallero et al., 1973) and result in more severe aortic and coronary artery atherosclerosis in cholesterol-fed monkeys (Kukreja et al., 1981).
The seminal work of Raab started over fifty years ago and the contributions of the other investigators cited have not attracted much interest until recently. Hauss et al. (1990) postulated that if catecholamines act as chemical mediators in the pathogenesis of arteriosclerosis in man, they should possess the following qualities: (a) the ability to trigger metabolic dysfunctions in vascular wall cells and circulating blood cells involved in the pathogenesis of arteriosclerosis; (b) be associated with certain risk factors; (c) be correlated with different stages or activities of arteriosclerosis in humans; and (d) be associated with severe persisting arteriosclerotic vascular diseases like myocardial infarction and stroke.

They found that:

1. Cultured endothelial and smooth muscle cells from vessel walls (human umbilical veins) exhibited enhanced proliferation when exposed to epinephrine or norepinephrine which is a predominant characteristic change in early atherosclerosis.

2. Individuals with atherogenic risk factors of smoking, primary hypertension and mental stress show elevated catecholamines.

3. In dialysis patients suffering from arteriosclerosis, plasma catecholamines were positively correlated with severity of arteriosclerotic disease.

4. Surviving myocardial infarction and stroke patients had significantly higher plasma catecholamine levels as late as one year after the event.

These results suggest that catecholamines may act as chemical mediators during atherogenesis in man (Hauss, 1990).

This hypothesis is supported by the effects of norepinephrine on the repair mechanisms of vascular endothelium. Endothelial cells can repopulate small denuded areas in vitro by migration, which is considered to play an important role in the repair of vascular injuries (Sholley et al., 1976). Migration of endothelial cells is significantly reduced by norepinephrine (Bottaro et al., 1985), indicating that
norepinephrine could interfere with repair mechanisms of the vascular endothelium and thus might contribute to vascular injury and the formation of atherosclerotic lesions (Hauss, 1990).

Catecholamines have also been shown to affect platelet aggregability, another factor in lesion formation. Booyse et al. (1975) found that pre-exposure to epinephrine significantly increased platelet adherence to endothelial cells. Haft (1974) and Grant (1990) showed that elevations of plasma catecholamines cause an increase in platelet activation and circulating clotting factors.

Lipid mobilization and transport are also affected by catecholamines. Born et al. (1989) compared the uptake of intravenously injected radioactively labelled low-density lipoproteins (LDL) into the two carotid arteries of anesthetized rabbits. After two hours of norepinephrine infusion into one carotid and saline control into the other, they found that norepinephrine significantly increased uptake of LDL by arterial wall and suggest that this may provide some explanation for the accelerated atherosclerosis in conditions associated with elevated plasma norepinephrin.

In summary, evidence indicates that catecholamines may contribute to CHD and lesion development through (a) the enhancement of smooth muscle cell proliferation, (b) the inhibition of endothelial migration (and therefore vascular endothelial repair), (c) increased platelet aggregability and clotting factors and, (d) lipid mobilization and increased arterial uptake of LDL.

Cortisol and Coronary Heart Disease. Cortisol, cortisone and corticosterone are glucocorticoids which are catabolic hormones that increase glucose production, lipid mobilization and potentiate epinephrine-induced elevations in blood lipids (Orth et al., 1992).

Chronic administration of corticosterone for conditions such as rheumatoid
Arthritis is associated with increased atherogenesis (Kalbak, 1972) and hypercoagulation of blood (Cosgriff et al., 1950). Autopsy studies conducted on patients with systemic lupus erythematosus revealed that 29 of 36 subjects treated with corticosteroids for more than 12 months exhibited significant coronary artery narrowing. In contrast, 20 similar patients studied before steroids became available showed no coronary artery narrowing (Bulkley and Roberts, 1975). Accelerated atherosclerosis was found in a series of 50 patients with Cushing's syndrome, a condition marked by chronically elevated plasma cortisol (Soffer et al., 1961).

Increased plasma cortisol has been associated with CHD. Although most epidemiologic studies do not include neuroendocrine measures, Lei (1992) reported cortisol level among the risk factors for CHD among 743 Chinese office workers. Troxler et al. (1977) showed a significant correlation between elevated serial morning plasma cortisol levels and moderate to severe atherosclerosis (assessed by angiography) in air force pilots. In a later study, the same group (Sprague, Troxler et al., 1980) investigated the effects of exogenous cortisol administered orally on the severity of atherosclerosis in monkeys on a control diet and a 0.25% cholesterol-containing diet. After one year, the percentage of aortic intimal surface involved with atherosclerotic lesions was significantly higher in the cortisol-high cholesterol diet group (71%) compared to the high cholesterol diet only (37%). Cortisol did not significantly increase lesion development in normocholesterolemic monkeys. The enhanced lipid deposition in the aortas of hypercholesterolemic monkeys that received cortisol occurred independently of any effect of cortisol on serum lipoprotein cholesterol concentration.

Results of studies on the effects of corticosteroids on experimental atherosclerosis have been mixed (Stout, 1982). Cortisone increased arterial lesions in cockerels (Stamler et al., 1954), inhibited the development of atheromatous
lesions in cholesterol fed rabbits (Oppenheim et al., 1952; Stumpf et al., 1954; Gordon et al., 1954; Friedman et al., 1964) and had little effect on spontaneous lesions in chickens (Malinow et al., 1965).


In summary, extensive investigations linking cortisol and CHD are lacking. Results from animal studies are mixed. However, preliminary evidence from epidemiologic, angiographic, experimental and biochemical studies warrants further research.

Coping Styles and Loss of Control: Possible Interactive Effects of Catecholamines and Cortisol on CHD

Selye's (1936) notion of a nonspecific physiological response to a variety of stressors, i.e., the "general adaptation syndrome", marked by increased adrenal cortical activity has been challenged by Mason (1975) and others (Frankenhauser, 1975; Koolhaus et al., 1983; Henry, 1986).

Evidence from animal and human studies supports the concept of specificity of neuroendocrine responses to stressful stimuli based on (a) the predictability or sense of control over the environmental stimulus and, (b) whether active or passive coping mechanisms are used.

Mason et al. (1961) identified two distinct patterns of hormonal responses in monkeys when presented with two specific stimulus situations. When the animals were presented with a stimulus signalling a familiar though aversive task,
norepinephrine levels increased consistently with varying degrees of cortisol elevation. Situations in which there was a high degree of unpredictability or uncertainty were associated with elevated norepinephrine, epinephrine, and cortisol; this study established that norepinephrine and epinephrine were independently regulated. Frankenhauser and Rissler (1970) confirmed Mason's finding, reporting that in humans, epinephrine output was inversely related to the degree of control and predictability of electric shocks. Norepinephrine was not affected by control, but remained elevated as long as the subject was engaged in attention demanding activity.

Weiss (1972) showed that rats able to avoid a certain percentage of shocks by appropriate responses developed less severe stomach ulcers than yoked controls receiving the same number of shocks but were not able to perform coping responses. Inescapable shock, compared to controlled shock, is associated with increased plasma corticosterone (Dess-Beech et al., 1983), reduction in food intake and enhanced tumor development (Visintainer et al., 1982).

Henry and Stephens (1977) studied dominant and submissive mice and suggested that the sympathetic adrenal medullary system is preferentially activated when animals display active responding in order to escape from or confront an environmental challenge i.e., the fight or flight response. The pituitary adrenocortical axis is preferentially activated when loss of control is perceived and passive coping behavior is exhibited. Koolhaus et al. (1983) reported contrasting neuroendocrine responses in winners and losers among rats. Shively and Kaplan (1984) found differential arousal in dominant and submissive monkeys. Increased adrenal cortical activity in free ranging subordinate baboons was reported by Sapolsky (1990).

DeBoer (1990) performed a series of carefully designed studies to investigate
neuroendocrine responses and coping responses to (a) aversive environmental stimuli and, (b) reinforced and extinguished operant behavior in rats. Canulas were inserted into the vena cava so that blood sampling was accomplished without disturbing the animal. Baseline measures were collected showing that habituation to the process of blood sampling tubing did not disturb the animal, behaviorally or physiologically. The experiments were based on the observation that rats have a strong innate tendency to bury a localized source of noxious stimulus, such as an electrified prod from which they have received a shock (active coping). If there is no bedding material available, rats cannot perform that coping response and therefore display a passive coping response, i.e., freezing in locations away from the prod. These contrasting behavioral responses to the same shock were associated with distinct neuroendocrine patterns. Burying the shock prod (the active coping response) is accompanied by increased norepinephrine (NE) and epinephrine (E) levels, with an increased NE/E ratio. Freezing (passive coping) is accompanied by increased cortisol, norepinephrine and epinephrine with a decreased NE/E ratio.

Loss of behavioral control can be observed in operant conditioning experiments. DeBoer (1990) reported that during food reinforced lever pressing, norepinephrine increases markedly, epinephrine is stable and cortisol declined. Extinction (i.e., loss of behavioral control) is accompanied by a decrease in norepinephrine, transient elevation of epinephrine and an increase in cortisol.

DeBoer (1990) concludes that

Our findings in rats together with those in human subjects lead to the view that sympathetic nervous system activity as indicated by plasma norepinephrine is especially related to conditions involving actual skeletal muscle exertion regardless of emotional connotations of the challenge whereas adrenomedullary (epinephrine) and adrenocortical (cortisol) stimulation occurs primarily during emotional stress, fear or anxiety provoking situations, characterized by limited, or abolished coping capabilities (p. 141).
It is important to note that many of the cardiovascular responses to exercise and stress are similar, e.g., increased heart rate and blood pressure. However, the neuroendocrine responses differ. During exercise, NE increases, E and cortisol are fairly stable (Connell et al., 1958). Under challenge conditions in which a loss of control is perceived, norepinephrine, epinephrine and cortisol are elevated.

The cynomolgus monkey studies in which social environments were manipulated are relevant here (Kaplan et al., 1983). Dominant monkeys in the unstable social group were exposed to frequent challenge conditions, potential loss of control and prolonged social disruption through periodic rotation of group members. On the other hand, dominant monkeys in the stable social group were not exposed to social disruption and frequent challenges. The unstable/dominants developed significantly more coronary artery atherosclerosis than stable/dominants.

Henry and Meehan (1981) single out "the attempt to achieve or maintain control when challenged" and "the perception of control thus far achieved being tenuous" (p. 309) as significant aspects of the coronary prone personality. They cite Glass (1977) in an analysis of the behavioral and hormonal aspects of the challenge to control:

Once an individual perceives a threat to his sense of environmental control, he struggles to reestablish and maintain better control. During this period, we may expect active coping efforts and concomitant elevations in circulating norepinephrine. As long as there is not fear, adrenaline should remain unchanged, or, perhaps even show a decline. In appraising the situation thus far, any pathophysiological changes would be explained by activation of the sympathetic adrenal axis. Glass (1977), however, makes the crucial suggestion that at intervals the type A struggling in a competitive milieu will sense a threat to his control, and with this realization, he will become passive. His noradrenalin is likely to decline and 'central cholinergic dominance may prevail'. He now posits that the resulting alternation of control efforts followed by giving up is repeated over and over again during the lifetime of an individual. He finds it "Not unreasonable to suggest that the more frequently this cycle occurs, the more the coronary arteries are likely to be affected by atherosclerotic disease." Thus he sees atherosclerosis as requiring the influence of both adrenal medullary and adrenal cortical response patterns (p. 310).
The observation that there may be a significant depressive component in coronary prone individuals (Thomas et al., 1975; Booth-Kewley et al., 1987; Fukunishi et al., 1992) could contribute to the neuroendocrine profile. Depression is associated with increased excretion of urinary cortisol (Sachar, 1967; Carroll and Curtis, 1976; Rosenbaum and Maruta, 1983; Kathol et al., 1989). Hostility, competitiveness and aggressive behavior are associated with increased plasma catecholamines (Frankenhauser, 1975; Henry, 1981). Williams et al. (1991) reports strong evidence for chronic increases in both sympathetic nervous system and adrenal-cortical axis activity among middle aged Type A men. Suarez (1992) found that among Type A individuals, elevation of total serum cholesterol was associated with catecholamine and cortisol responses to the stress of mental arithmetic. Perhaps as Henry suggests, the aggressive (when challenged) and depressive (threat of loss of control) characteristics of coronary prone individuals correlate with increased sympato-adrenal-mediullary (catecholamine) activity and increased adrenal cortical (cortisol) and the combination of increased catecholamines and cortisol elicits pathological changes.

Some early work supports the notion that cortisol and catecholamines can, in combination, produce greater pathology. Balasz et al. (1962) and Hatch et al. (1963) report that catecholamine cardiotoxicity is increased up to 16 fold in the presence of adrenal cortical hypertrophy such as in long isolated animals. Raab (1966) states:

From the point of view of cardiac pathogenicity, it is of interest that both emotional stresses and physical exercise cause sympathetic stimulation and catecholamine discharges, but that only the former are associated with a simultaneous liberation of catecholamine-toxifying adrenal corticoids, whereas exercise does not elicit this potentially detrimental combination (p. 550).

Shafrir (1960) established that cortisone considerably potentiates the plasma cholesterol, free fatty acid, and phospholipid response to epinephrine in dogs. He
suggests that the hypercholesterolemia of stress may in part be due to the accompanying overactivity of the adrenal medulla and the adrenal cortex, simultaneously producing epinephrine and corticosteroids.

Summary

Coping styles, the predicability and controllability of the environment and social status all appear to influence neuroendocrine responses to a psychosocial challenge. Although many cardiovascular changes in stress and exercise are similar, stress increases the risk of CHD and regular exercise has a protective effect against it. The combination or cycling of adrenal medullary and adrenal cortical activation, present in certain individuals' response to challenge but absent in moderate exercise, may underlie the pathological effects of stress.

Summary of the Review

In reviewing the literature on psychosocial factors and CHD, I have attempted to adhere to Mason's notion of interdisciplinary effort. My first goal was to articulate connections between life situations/emotions/coping as independent variables and (a) CHD; and (b) physiologic changes (cardiovascular and hormonal), as dependent variables. My second goal was to establish connections between the physiologic changes as independent variables and pathologic changes as dependent variables. I will briefly trace these steps linking psychosocial factors and coronary heart disease.

In this review, I have described evidence to suggest that (a) various psychosocial factors influence the progression of CHD; (b) individual behavioral characteristics in humans, especially hostility, aggressiveness and competitiveness (when challenged) and perhaps vulnerability to depression (Type A Behavior...
Pattern) appear to be related to CHD; (c) physiological responses to psychosocial factors are mediated by the autonomic nervous system (primarily cardiovascular changes via the sympathetic nervous system) and the neuroendocrine system (primarily increased plasma norepinephrine, epinephrine and cortisol via the sympatho-adrenal medullary and pituitary adrenal cortical systems); (d) individuals exhibiting Type A behavior pattern are more responsive physiologically to challenges; (e) cardiovascular and hormonal changes may influence the progression of CHD by increasing established risk factors such as elevated serum cholesterol and blood pressure or through independent mechanisms such as vascular endothelial injury, smooth muscle cell proliferation and increased platelet aggregation which are significant processes in atherosclerotic lesion development; (f) neurohormonal response to challenge depends on coping style, predictability and controllability of the environment, and may include combinations of hormonal responses which are pathological; and (g) further research investigating the metabolic effects of norepinephrine, epinephrine and cortisol on processes in the progression of atherosclerosis is needed to help uncover mechanisms relating psychosocial factors and CHD. The focus of this dissertation is the effects of these hormones on lipid metabolism.
CHAPTER III

PURPOSE OF THE PRESENT WORK

The purpose of the research reported in this dissertation is to identify the effects of norepinephrine, epinephrine and cortisol on cholesterol ester synthesis. An understanding of these effects is important in specifying key aspects in the progression of lesion formation in atherosclerosis. Future work on the effects of psychosocial factors on the accumulation of cholesterol and cholesteryl esters in the artery may be facilitated by knowledge of these mechanisms.

The ACAT Hypothesis

ACAT and Its Role in the Development of Atherosclerosis

AcylCoA: cholesterol acyl transferase; E.C.2.3.2.26 (ACAT) is an arterial enzyme that esterifies cholesterol in the vessel wall (St. Clair, 1976). It is found in the endoplasmic reticulum (membrane-bound) and is isolated in the microsomal fraction of cells. Cholesteryl esters account for a significant amount of the cholesterol which accumulates in atheromatous lesions and are the major components of lipid droplets which are found in fat- filled arterial cells. Once deposited within the arterial cells, cholesteryl esters are difficult to hydrolyze; for this reason, cholesteryl ester deposition is progressive and unchecked as atherogenesis proceeds. In effect, the ACAT reaction can be conceptualized as a biochemical trapping mechanism for cholesterol entering the artery from the plasma (Bell, 1986). ACAT activity, relatively low in normal arterial tissue, is significantly increased in atheromatous tissue (St. Clair, 1976; Breecher et al., 1980) which can be
measured in rabbits in as few as three days of feeding a cholesterol containing atherogenic diet (Day and Proudlock, 1974). Since ACAT activity affects the amount of cholesteryl ester deposited in the artery, its activity should be reflected in the amount of cholesteryl ester found in arterial lesions.

Bell (1986) postulated a direct role of ACAT in the atherogenic process based on (a) the relationship between increased arterial ACAT activity and the progressive accumulation of arterial cholesteryl esters \textit{in vitro}; (b) the observation that several compounds, e.g., propranolol, diazepam and chlorpromazine, reported to reduce the severity of atherosclerosis in animal models were later found to be ACAT inhibitors; (c) direct experimental evidence: ACAT activity was reduced 40\% in aortic tissue from rabbits fed the an atherogenic diet and receiving chlorpromazine (an ACAT inhibitor) orally compared to rabbits fed the atherogenic diet only. The ACAT activity reduction was correlated with significantly decreased cholesterol and cholesteryl ester accumulation in the chlorpromazine exposed arteries. The reduction of arterial cholesterol and cholesteryl ester occurred independent of serum lipoprotein levels.

Hepatic ACAT activity is also related to the progression of coronary artery atherosclerosis (CAA). Carr et al. (1992) found that in African green monkeys fed a high fat/high cholesterol diet for three to six years, hepatic ACAT activity was highly positively correlated with the extent of CAA.

\textbf{Summary}

The accumulation of lipids, particularly cholesterol and cholesteryl ester in the artery wall is well established as a pathological process in the progression of coronary artery atherosclerosis. Arterial cholesterol stored as cholesteryl ester accounts for a significant amount of cholesterol in atheromatous lesions. The major
enzyme responsible for the esterification of cholesterol in the artery wall is acylCoA: cholesterol acyl transferase (ACAT). It is suggested that ACAT activity may have a direct role in the atherogenic process.

**Inhibition of ACAT**

Research directed toward identifying and developing ACAT inhibitors has increased dramatically in the past few years (Bocan et al., 1991; Tomoda et al., 1991; Naganuma et al., 1992; Bell et al., 1992) One reason for the heightened interest is that none of the five classes of lipid-lowering drugs approved by the FDA has an antiatherosclerotic indication (Gotto, 1993), whereas some ACAT inhibitors have demonstrated lipid lowering effects (DeVries et al., 1986; Heiden, 1986) and anti-atherosclerotic activity (Bocan et al., 1991).

The therapeutic potential of ACAT inhibitors is promising given the evidence that they appear to reduce intestinal absorption of cholesterol and/or directly affect the accumulation of cholesteryl ester in arterial lesions (Sliskovic & White, 1991).

Various types of compounds have ACAT inhibiting properties such as local anesthetics (Bell & Hubert, 1980), tranquilizers (Bell, 1983 and 1984), antihypertensives (Bell, 1985) and penicillin derivatives (Naganuma et al., 1992; Tomoda et al., 1991).

**Possible Enhancement of ACAT Activity by Hormones Released During a Stress Response: Norepinephrine, Epinephrine and Cortisol**

If ACAT inhibition does in fact reduce the severity of atherosclerosis, then it follows that an increase in ACAT activity may enhance atherosclerosis. What substances would be likely to enhance ACAT activity? A clue may be found in...
describing the properties of specific ACAT inhibitors.

Three specific compounds, propranolol, chlorpromazine and diazepam not only inhibit ACAT activity but also reduce the severity of experimental atherosclerosis in animals (Wilens et al., 1956; Patel et al., 1982; Whittington-Coleman et al., 1973). All three compounds are behaviorally active and appear to alter stress induced hormonal responses. Chlorpromazine, a tranquilizer, and diazepam, a sedative, both interfere with the pituitary-adrenocortical response to stress (Rose, 1985; Roy-Byrne et al, 1988) and appear to suppress subcortical reflex stimulation of the sympathoadrenergic system, (Gunn et al., 1955). Propranolol blocks beta-adrenergic receptors and decreases sympathetic activity. Clinically, chlorpromazine and diazepam are used widely, often to control agitated behavior in patients. Propranolol is mainly prescribed as an anti-hypertensive, but is also used to treat panic disorder and anxiety. All three drugs appear to counteract arousal of the sympathetic nervous system and perhaps alter the regulation of catecholamines. Chlorpromazine and diazepam appear to interfere with release of ACTH and cortisol.

Manuck et al. (1991) reported that propranolol attenuated the psychosocial exacerbation of atherosclerosis in cynomolgus monkeys. Cholesterol fed dominant monkeys housed in unstable social conditions receiving propranolol developed less than half the coronary artery atherosclerosis of cholesterol-fed untreated dominants also housed in unstable conditions.

If diazepam, propranolol and chlorpromazine inhibit ACAT and inhibit some aspect of catcholamine and cortisol activity, then perhaps increased levels of catecholamines and cortisol, present during a stress response, enhance ACAT activity. Increased ACAT activity enhances cholesteryl ester synthesis in the artery and thereby contributes to the progression of atherosclerosis. This proposed mechanism linking hormonal responses to ACAT activity and atherosclerosis could
theoretically increase the severity of CAA independent of lipoprotein and blood pressure elevations.

To investigate this hypothesis, the effect of norepinephrine, epinephrine and cortisol on cholesteryl ester accumulation (ACAT activity) was examined in Fu5AH rat hepatoma cells.
CHAPTER IV

METHODS

Introduction

Liver cells in vitro mimic the lipid metabolism of arterial cells in vivo; this model has been used to study several aspects of lipid metabolism (Bell, 1980; Ross et al., 1984; Faust et al., 1989) including cholesteryl ester metabolism (Suckling, 1982; Bell et al., 1982; Bell et al., 1992). Specifically, Fu5AH rat hepatoma cells have been used to study cholesterol esterification (Rothblat, 1977). The Fu5AH cell line was derived from the Reuber H-35 rat hepatoma (Reuber, 1961).

ACAT Activity and Cholesteryl Ester Accumulation

Plasma lipids are carried by plasma lipoproteins. There is an exchange of lipids between lipoproteins and the plasma membrane of the cell. Lipid in the cell surface can cross the membrane and move into the cell by exchange with intracellular organelles such as mitochondria, endoplasmic reticulum, golgi apparatus, nuclear membrane, and lysosomes. This bidirectional exchange process is illustrated in Figure 5. The exchange process may be disturbed and net transfer of lipid into the cell could occur when a non-exchangeable form of lipid is synthesized. This could occur, for example, when cholesterol is esterified to cholesteryl ester by ACAT (Bell, 1978) shown in Figure 6. If ACAT activity is increased, greater accumulation of cholesteryl ester is likely to occur reducing the amount of exchangeable intracellular cholesterol and allowing more free cholesterol to enter the cell from the plasma. If a substance inhibits ACAT activity, cholesteryl ester
accumulation and therefore net transfer of cholesterol into the cell is likely to be decreased. If a substance enhances ACAT activity, cholesteryl ester accumulation and therefore net transfer of cholesterol into the cell would be increased.

Overview of the Procedures

Rat hepatoma cells (Fu5AH) were incubated (a) in the presence of hyperlipemic or normolipemic rabbit serum or in a serum free medium and, (b) in the presence of epinephrine, norepinephrine, cortisol or the combination of epinephrine/cortisol, or a control condition which was free of additional hormones. \(^{[1-14C]}\)Oleate was added as a substrate to the cells and was incorporated into cholesteryl ester, phospholipid, diglyceride and triglyceride as the fatty acid component of these molecules. Esterification of cholesterol is most rapid with oleate and cholesteryl-oleate is the most common cholesteryl ester in atheromas (Morin et al., 1987). Figure 7 shows the molecular structure of oleate, cholesteryl ester, a phospholipid, a diglyceride and a triglyceride.

The lipids were extracted and separated. The resulting radioactivity of the lipid fractions was measured and taken as the index of lipid synthesis. Specifically, the cholesteryl ester fraction reflected ACAT activity. A diagram of operations is shown in Figure 8.

Procedure

Maintenance of Stock Cultures

The cells were maintained in Dulbecco’s Modified Eagle’s Medium (Gibco) supplemented with 5% bovine calf serum and 50mg/ml gentamicin sulfate (Gibco).
Figure 5. Lipid Exchange and Transfer Between Biological Lipid Protein Structures. L=lipoprotein, M=mitochondria, ER=endoplasmic reticulum, G=Golgi apparatus, NM=nuclear membrane, LYS=lysosomes. Reprinted from Bell (1978) with permission of Author.

Figure 6. Esterification of Cholesterol by ACAT

\[ \text{ACAT} \]
\[ \text{Cholesterol} + \text{fatty acyl-CoA} \longrightarrow \text{Cholesteryl ester} + \text{CoA} \]
Figure 7. The Molecular Structure of Oleate, Cholesteryl Ester, a Triglyceride, a Diglyceride and a Phospholipid. Oleate is incorporated as one of the O-C-R groups found on the other lipids.
Cultured cells Fu5AH

incubated for 18 hours in

serum free media (SF)  normolipemic serum (NRS)  hyperlipemic serum (HRS)

epinephrine, norepinephrine, cortisol and epinephrine/cortisol added

incubation for 22 hours

[1-\text{\textsuperscript{14}}\text{C}]oleate added

incubation for 2 hours

Extraction and separation of lipids

Determination of [1-\text{\textsuperscript{14}}\text{C}]oleate incorporation into cholesteryl ester, phospholipid, triglycerides by liquid scintillation counting

INDEPENDENT VARIABLE 1

serum condition

INDEPENDENT VARIABLE 2

hormone condition

DEPENDENT VARIABLES

1) total incorporation of [14C]oleate into lipids
2) cholesteryl ester synthesis
3) phospholipid synthesis
4) triglyceride synthesis

Figure 8. Diagram of Operations of the Study. Synthesis of lipid fractions is expressed as percentage of total incorporation of [1-\text{\textsuperscript{14}}\text{C}]oleate into all lipids.

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Incubation was at 36°C in an atmosphere of 5% CO$_2$ in air. Cells from stock cultures, grown to confluency in 25cm flasks (Falcon) containing 10ml of medium, were detached with 0.25% trypsin diluted 1:10 with medium and mixed by agitation through a pipette which serves to disrupt cellular clumps. Stock cultures were replenished with fresh medium at 3 to 4 day intervals.

**Preparation of Cultures and Culture Medium for Assay**

250μl aliquots of the diluted stock suspension described above were transferred to 22mm dishes (Falcon) containing 1 ml of medium and the seeded cells were allowed to grow to near confluence (2 days). The medium was aspirated and the cells were washed once with fresh medium. One ml of medium supplemented with 5% hyperlipemic rabbit serum (HRS$^1$), 5% normolipemic rabbit serum (NRS) or serum-free (SF) medium was added to the dishes as the experimental medium. The cultures were incubated for 18 hours at which time epinephrine, norepinephrine, cortisol, epinephrine/cortisol or control vehicle were added to the dishes. Final concentrations of hormones were: (-)-epinephrine 10$^{-6}$M, (-)-norepinephrine 10$^{-6}$M, and cortisol 10$^{-6}$M (in the form of hydrocortisone 21-hemisuccinate). All hormones were purchased from Sigma Chemical Co. After 22 hours of incubation with hormones, 1μCi of [1-1$^{14}$C]oleate$^2$ was added for an additional 2 hours. The culture medium was removed by aspiration and the cells were washed three times

---

1 HRS was obtained from The Upjohn Co. from male New Zealand rabbits fed Purina Chow supplemented with 1% cholesterol and 3% peanut oil (w/w/w) for 2-3 weeks. Pooled sera from two or more rabbits was filtered through a .45μm filter (Nalgene) and stored frozen at -20°C in convenient aliquots until used.

2 [1,1$^{14}$C]-oleic acid was obtained in ethanol from NEN Dupont, Boston, MA, and was converted to its sodium salt by evaporation under N$_2$ and addition of 0.05N NaOH. The [1-1$^{14}$C]oleate solution was warmed to 35°C, vortexed until foam formed on its surface and then left for several hours until the foam disappeared. Small aliquots were frozen and stored in 2ml plastic stoppered vials, covered with aluminum foil.
Preliminary experiments using hormonal concentrations of $10^{-10}$M, $10^{-8}$M, and $10^{-6}$M and incubation times of 2, 6, 12, and 24 hours were performed to determine the most suitable conditions for this experiment. Cells incubated at all time points with final hormonal concentrations less than $10^{-6}$M showed no consistent differences in [1-14C]oleate incorporation compared to controls with the exception of cortisol which had some activity at $10^{-8}$M. Hormonal concentrations of $10^{-6}$M and 24 hour incubations with hormones were chosen because both cortisol and epinephrine showed activity under these conditions. Alternate choices of hormone concentration and incubation times may yield more information about hormonal influences on lipid synthesis.

**Analysis of Cultures**

Following the last wash above, 1ml of hexane/isopropanol (3:2 vol/vol) was added to the culture dishes and the lipids were permitted to extract for 15 minutes at room temperature. The extracts were pipetted into test tubes and evaporated under nitrogen gas. The lipid residue was dissolved in 250μl of chloroform/methanol (2:1 vol/vol). A 125μl aliquot of each extract was separated by thin-layer chromatography using silica gel G-coated (250μm) glass plates (Whatman). The samples were applied to the plates and overlaid with lipid standard. The plates were placed in a glass chromatography chamber (solvent system, n-hexane: diethyl ether: glacial acetic acid, 146:50:4 v/v/v) and removed when the solvent front reached 1.5 inches from the top of the plate. The lipid bands were visualized by spraying the plates with Rhodamine 6G (0.05% in ethanol) and scraped into vials containing 10ml Redisafe scintillation fluid (Beckman) for liquid scintillation counting. The cellular residues left in the culture dishes after lipid extraction were soaked in 0.5ml
phosphate buffered saline for 24 hours at 4°C, and scraped with a vinyl scraper. The cell residues in solution were centrifuged at 5000 rpm for 5 minutes after dispersion in a pipette. The supernatant was analyzed for protein by the Lowry et al. (1961) method using a Beckman spectrophotometer set at 500nm. Sample protein values were determined from a standard curve produced by assaying solutions containing known quantities of bovine serum albumin. Values for a new standard curve were determined each time proteins were analyzed.

Expression of Data

Radioactive counts from total incorporation of [1-14C]oleate were expressed as disintegrations per minute/μg protein (dpm/μg). Lipid synthesis in lipid fractions was expressed as the percentage distribution of [1-14C]oleate incorporated into phospholipids, triglycerides and cholesteryl ester. Diglycerides accounted for approximately 2-3% of total incorporation and were not analyzed separately.

Data Analysis

A two-factor analysis of variance was used to test for main effects and interaction on mean [1-14C]oleate incorporation into lipids of (a) serum condition (HRS, NRS, SF) and (b) hormones (epinephrine, norepinephrine, cortisol, epinephrine/cortisol and controls). Fisher's Protected LSD was used for multiple comparisons of individual marginal means associated with the three serum conditions (serum free, normolipemic and hyperlipemic). Dunnett's Test (two tailed) (Kirk, 1968) was used for comparisons of individual marginal means associated with hormonal and control conditions.

In the case of significant serum X hormone interactions, simple effects tests and Dunnett's Test were carried out to evaluate simple effects of hormones at each
individual serum condition.

Results

The results will be described with respect to each dependent variable separately. Experimental hormonal effects on lipid synthesis compared to control conditions are of primary interest, so although the analysis of variance is sensitive to significant differences among all means, tests on comparisons were performed only on contrasts between experimental hormonal and control conditions.

Total Lipid Synthesis

The analysis of variance summary table, cell means and marginal means, and differences between control and hormone condition means (at each serum condition) for measures of total lipid synthesis are shown in Table 2. Figure 9 summarizes the main effects on total lipid synthesis of each serum condition averaged across all hormone conditions and each hormone condition averaged across all serum conditions. Figure 10 shows the serum x hormone interaction.

Main Effects

Mean total lipid synthesis was significantly increased (p<0.05) in the presence of hyperlipemic serum compared to SF or NRS conditions. The marginal mean differences between any hormonal condition and the control condition were not statistically significant (p>0.05) on measures of total lipid synthesis (Figure 9).

Interaction Effects

The effects of hormones on total lipid synthesis were inconsistent across serum conditions (Figure 10). For example, the cortisol condition yielded the highest
### Table 2.

Summary of Results for Measures of Total Lipid Synthesis

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>dF</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Conventional p</th>
<th>Bonferroni p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Condition</td>
<td>48,103,146</td>
<td>2</td>
<td>2,401,573</td>
<td>3.72</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Hormone Condition</td>
<td>69,850,136</td>
<td>4</td>
<td>17,462,534</td>
<td>2.70</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>at SF condition only</td>
<td>98,140,774</td>
<td>4</td>
<td>24,535,193</td>
<td>3.80</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>at NRS condition only</td>
<td>13,280,748</td>
<td>4</td>
<td>3,320,187</td>
<td>0.51</td>
<td>0.73</td>
<td>1.00</td>
</tr>
<tr>
<td>at HRS condition only</td>
<td>81,306,725</td>
<td>4</td>
<td>20,325,581</td>
<td>3.15</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Interaction</td>
<td>122,878,113</td>
<td>8</td>
<td>15,359,764</td>
<td>2.38</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>387,560,926</td>
<td>60</td>
<td>6,459,349</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>628,392,321</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2-Continued

**Cell Means (std error) and Marginal Means (std error) for Measures of Total Lipid Synthesis**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Epinephrine</th>
<th>Cortisol</th>
<th>Norepinephrine</th>
<th>Epinephrine/Cortisol</th>
<th>Marginal Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Free</td>
<td>8952(587)</td>
<td>12548(1869)</td>
<td>13648(1929)</td>
<td>9849(962)</td>
<td>8762(671)</td>
<td>10752(679)*</td>
</tr>
<tr>
<td>Normolipemic</td>
<td>11771(1494)</td>
<td>11376(917)</td>
<td>9824(635)</td>
<td>10994(787)</td>
<td>10171(568)</td>
<td>10827(412)*</td>
</tr>
<tr>
<td>Hyperlipemic</td>
<td>14807(1509)</td>
<td>14569(1184)</td>
<td>10790(840)</td>
<td>11077(1048)</td>
<td>11192(736)</td>
<td>12487(580)*</td>
</tr>
<tr>
<td>Marginal Mean</td>
<td>11843(933)</td>
<td>12831(819)</td>
<td>11420(805)</td>
<td>10640(523)</td>
<td>10042(442)</td>
<td></td>
</tr>
</tbody>
</table>

**Cell Mean Differences Between Control and Each Hormone Condition**

(Hormone condition-Control condition)

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine</th>
<th>Cortisol</th>
<th>Norepinephrine</th>
<th>Epinephrine/Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Free</td>
<td>3596</td>
<td>4696*</td>
<td>897</td>
<td>-190</td>
</tr>
<tr>
<td>Normolipemic</td>
<td>-395</td>
<td>-1947</td>
<td>-777</td>
<td>-1600</td>
</tr>
<tr>
<td>Hyperlipemic</td>
<td>-238</td>
<td>-4017</td>
<td>-3730</td>
<td>-3615</td>
</tr>
</tbody>
</table>

*Significant Fisher's Protected LSD or Dunnett's Test (p<0.05)
Figure 9. Main effects of Experimental Serum and Hormonal Conditions on Total Lipid Synthesis. SF=serum free, NRS=normolipemic, HRS=hyperlipemic, EPI=epinephrine, NOREPI=norepinephrine, EPI/CORT=epinephrine and cortisol. Hormone Concentration=$10^{-6}$M.

Figure 10. Serum x Hormone Interaction for Total Lipid Synthesis.
mean under serum-free conditions but the lowest mean under the NRS and HRS conditions.

**Simple Effects Tests**

Under serum-free conditions, total lipid synthesis was increased (p<0.05) in the presence of cortisol compared to control conditions.

**Cholesteryl Ester Synthesis**

The analysis of variance summary table, cell means and marginal means, and differences between control and hormone condition means (at each serum condition) for cholesteryl ester synthesis are shown in Table 3. Figure 11 summarizes the main effects on cholesteryl ester synthesis of each serum condition averaged across all hormonal conditions and each hormonal condition averaged across each serum condition. Figure 12 shows the serum x hormone interaction.

**Main Effects**

Mean cholesteryl ester synthesis, expressed as percentage of total [1-14C]oleate incorporation was dramatically increased under hyperlipemic serum conditions compared to normolipemic conditions and serum free conditions. Mean cholesteryl ester synthesis was significantly increased in normolipemic conditions compared to serum free conditions. Mean cholesteryl ester synthesis was increased (p<0.05) in the presence of epinephrine and decreased (p<0.05) in the presence of cortisol compared to controls (Figure 11).
Table 3
Summary of Results for Measures of Cholesteryl Ester Synthesis

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>dF</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Conventional p</th>
<th>Bonferroni p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Condition</td>
<td>84.45391</td>
<td>2</td>
<td>42.22695</td>
<td>31057.45</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Hormone Condition</td>
<td>0.12998</td>
<td>4</td>
<td>0.03250</td>
<td>23.90</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00037</td>
<td>4</td>
<td>0.00009</td>
<td>0.07</td>
<td>0.99</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>0.01230</td>
<td>4</td>
<td>0.00307</td>
<td>2.26</td>
<td>0.08</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>0.27732</td>
<td>4</td>
<td>0.06933</td>
<td>51.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.16000</td>
<td>8</td>
<td>0.02000</td>
<td>14.71</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>0.08158</td>
<td>60</td>
<td>0.00136</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>84.82547</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3-Continued

Cell Means (std error) and Marginal Means (std error) for Measures of Cholesteryl Ester Synthesis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Epinephrine</th>
<th>Cortisol</th>
<th>Norepinephrine</th>
<th>Epinephrine/Cortisol</th>
<th>Marginal Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Free</td>
<td>0.872(.02)</td>
<td>0.898(.04)</td>
<td>0.93(.02)</td>
<td>0.952(.03)</td>
<td>0.847(.05)</td>
<td>0.899(.02)*</td>
</tr>
<tr>
<td>Normolipemic</td>
<td>1.918(.09)</td>
<td>2.072(.14)</td>
<td>1.44(.05)</td>
<td>1.984(.04)</td>
<td>1.940(.09)</td>
<td>1.87(.06)*</td>
</tr>
<tr>
<td>Hyperlipemic</td>
<td>24.4(.25)</td>
<td>25.3(.27)</td>
<td>22.1(.08)</td>
<td>23.9(.31)</td>
<td>23.7(.33)</td>
<td>23.9(.24)*</td>
</tr>
<tr>
<td>Marginal Mean</td>
<td>9.06(2.9)</td>
<td>9.42(3.0)*</td>
<td>8.15(2.6)*</td>
<td>8.94(2.7)</td>
<td>8.84(2.8)</td>
<td></td>
</tr>
</tbody>
</table>

Cell Mean Differences Between Control and Each Hormone Condition

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine</th>
<th>Cortisol</th>
<th>Norepinephrine</th>
<th>Epinephrine/Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Free</td>
<td>0.026</td>
<td>0.058</td>
<td>0.08</td>
<td>-0.025</td>
</tr>
<tr>
<td>Normolipemic</td>
<td>0.154</td>
<td>-1.87</td>
<td>0.066</td>
<td>0.022</td>
</tr>
<tr>
<td>Hyperlipemic</td>
<td>0.9</td>
<td>-2.32</td>
<td>-0.52</td>
<td>-0.66</td>
</tr>
</tbody>
</table>

*Significant Fisher’s Protected LSD or Dunnett’s Test (p<0.05)
Figure 11. Main Effects of Experimental Serum and Hormone Conditions on Cholesteryl Ester (CE) Synthesis. SF=serum free, NRS=normolipemic, HRS=hyperlipemic, EPI=epinephrine, NOREPI=norepinephrine, EPI/CORT=epinephrine and cortisol. Hormone Concentration=10^{-6}M.

Figure 12. Serum x Hormone Interaction for Cholesteryl Ester Synthesis.
**Interaction Effects**

The effects of hormones on cholesteryl ester synthesis were inconsistent (p=0.00) across serum conditions (Figure 12). Under serum free-conditions, incubations in the presence of cortisol showed the highest mean cholesteryl ester synthesis while under hyperlipemic conditions, incubations in the presence of cortisol showed the lowest mean cholesteryl ester synthesis.

**Simple Effects Tests**

Under normolipemic and hyperlipemic conditions, mean cholesteryl ester synthesis was decreased (p<0.05) in samples incubated in the presence of cortisol compared to controls. Under hyperlipemic conditions, mean cholesteryl ester synthesis was increased (borderline significance p=0.06) in the presence of epinephrine compared to the control condition.

**Phospholipid Synthesis**

The analysis of variance summary table, cell means and marginal means, and differences between control and hormone condition means (at each serum condition) for phospholipid synthesis are shown in Table 4. Figure 13 summarizes the main effects on phospholipid synthesis of each serum condition averaged across all hormonal conditions and each hormonal condition averaged across each serum condition. Figure 14 shows the serum x hormone interaction.

**Main Effects**

Mean phospholipid synthesis was significantly increased (p<0.05) under serum free conditions compared to normolipemic and hyperlipemic conditions.
Table 4

Summary of Results for Measures of Phospholipid Synthesis

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Convention. p</th>
<th>Bonferroni p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Condition</td>
<td>89.54747</td>
<td>2</td>
<td>44.77373</td>
<td>1128.75</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Hormone Condition</td>
<td>1.18488</td>
<td>4</td>
<td>0.46220</td>
<td>11.65</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>[at SF condition only]</td>
<td>2.72160</td>
<td>4</td>
<td>0.68040</td>
<td>17.16</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>[at NRS condition only]</td>
<td>0.13040</td>
<td>4</td>
<td>0.03260</td>
<td>0.82</td>
<td>0.52</td>
<td>1.00</td>
</tr>
<tr>
<td>[at HRS condition only]</td>
<td>0.46000</td>
<td>4</td>
<td>0.11500</td>
<td>2.89</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>Interaction</td>
<td>1.46320</td>
<td>8</td>
<td>0.18290</td>
<td>4.61</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>2.38000</td>
<td>60</td>
<td>0.03967</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>95.23947</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4-Continued

**Cell Means(std error) and Marginal Means(std error) for Measures of Phospholipid Synthesis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control</th>
<th>Epinephrine</th>
<th>Cortisol</th>
<th>Norepinephrine</th>
<th>Epinephrine/Cortisol</th>
<th>Marginal Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Free</td>
<td>70.4(.75)</td>
<td>74.4(1.63)</td>
<td>75.8(.97)</td>
<td>69.2(.73)</td>
<td>78.0(.63)</td>
<td>73.6(.79)*</td>
</tr>
<tr>
<td>Normolipemic</td>
<td>59.6(.93)</td>
<td>58.6(1.0)</td>
<td>60.6(1.5)</td>
<td>59.4(.51)</td>
<td>60.4(.93)</td>
<td>59.7(.44)*</td>
</tr>
<tr>
<td>Hyperlipemic</td>
<td>46.0(.32)</td>
<td>46.2(.86)</td>
<td>49.4(.40)</td>
<td>45.6(.51)</td>
<td>46.8(.58)</td>
<td>46.8(.36)*</td>
</tr>
<tr>
<td>Marginal Mean</td>
<td>58.7(2.7)</td>
<td>59.7(3.2)</td>
<td>61.9(2.9)*</td>
<td>58.1(2.6)</td>
<td>61.7(3.4)*</td>
<td></td>
</tr>
</tbody>
</table>

**Cell Mean Differences Between Control and Each Hormone Condition**

(Hormone condition-Control condition)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Epinephrine</th>
<th>Cortisol</th>
<th>Norepinephrine</th>
<th>Epinephrine/Cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Free</td>
<td>4*</td>
<td>5.4*</td>
<td>1.2</td>
<td>7.6*</td>
</tr>
<tr>
<td>Normolipemic</td>
<td>-1</td>
<td>1</td>
<td>-0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Hyperlipemic</td>
<td>0.2</td>
<td>3.4*</td>
<td>-0.4</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

*Significant Fisher's Protected LSD or Dunnett's Test (p<0.05)
Figure 13. Main Effects of Experimental Serum and Hormone Conditions on Phospholipid (PL) Synthesis. SF=serum free, NRS=normolipemic, HRS=hyperlipemic, EPI=epinephrine, NOREPI=norepinephrine, EPI/CORT=epinephrine and cortisol. Hormone Concentration=10^{-6}M.

Figure 14. Serum x Hormone Interaction for Phospholipid Synthesis.

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Mean phospholipid synthesis under normolipemic conditions was increased (p<0.05) compared to hyperlipemic conditions. The marginal mean differences in phospholipid synthesis between each hormonal condition and the control condition were not statistically significant (p>0.05) (Figure 13).

**Interaction Effects**

The effects of hormones on phospholipid synthesis were inconsistent (p=0.00) across serum conditions (Figure 14). For example, under serum-free conditions, incubations in the presence of epinephrine showed increased (p<0.05) mean phospholipid synthesis compared to the control condition but under normolipemic serum conditions, incubations in the presence of epinephrine showed decreased (though not statistically significant, p>0.05) mean phospholipid synthesis compared to the control condition.

**Simple Effects**

Under serum free conditions, incubations in the presence of epinephrine, cortisol, and epinephrine and cortisol combined, showed increased (p<0.05) phospholipid synthesis. Under hyperlipemic conditions, incubations in the presence of cortisol showed increased (p<0.05) phospholipid synthesis.

**Triglyceride Synthesis**

The analysis of variance summary table, cell means and marginal means, and differences between control and hormone condition means for triglyceride synthesis are shown in Table 5. Figure 15 summarizes the main effects on triglyceride synthesis of each serum condition averaged across all hormonal conditions and each hormonal condition averaged across each serum condition.
Table 5
Summary of Results for Measures of Triglyceride Synthesis

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>dF</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Conventional P</th>
<th>Bonferroni P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Condition</td>
<td>24.05247</td>
<td>2</td>
<td>12.02623</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Hormone Condition</td>
<td>0.37280</td>
<td>4</td>
<td>0.09320</td>
<td>2.02</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>at SF condition only</td>
<td>0.47760</td>
<td>4</td>
<td>0.11940</td>
<td>2.59</td>
<td>0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>at NRS condition only</td>
<td>0.08200</td>
<td>4</td>
<td>0.02050</td>
<td>0.44</td>
<td>0.78</td>
<td>1.00</td>
</tr>
<tr>
<td>at HRS condition only</td>
<td>0.04640</td>
<td>4</td>
<td>0.01160</td>
<td>0.25</td>
<td>0.91</td>
<td>1.00</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.23320</td>
<td>8</td>
<td>0.02915</td>
<td>0.63</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>2.77000</td>
<td>60</td>
<td>0.04617</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27.42847</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5-Continued

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Epinephrine</th>
<th>Cortisol</th>
<th>Norepinephrine</th>
<th>Epinephrine/Cortisol</th>
<th>Marginal Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Free</td>
<td>22.0(1.2)</td>
<td>22.2(1.6)</td>
<td>19.6(.93)</td>
<td>20.8(.86)</td>
<td>18.6(.68)</td>
<td>20.6(.53)*</td>
</tr>
<tr>
<td>Normolipemic</td>
<td>34.6(.51)</td>
<td>35.0(.71)</td>
<td>33.7(1.4)</td>
<td>35.2 (.86)</td>
<td>34.0(1.73)</td>
<td>34.5(.48)*</td>
</tr>
<tr>
<td>Hyperlipemic</td>
<td>27.4(.51)</td>
<td>27.6(.60)</td>
<td>26.4(.51)</td>
<td>26.8(.58)</td>
<td>27.2(.37)</td>
<td>27.1(.23)*</td>
</tr>
<tr>
<td>Marginal Mean</td>
<td>28.0(1.5)</td>
<td>28.3(1.5)</td>
<td>26.6(1.6)</td>
<td>26.6(1.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant Fisher's Protected LSD (p<0.05)
Main Effects

Mean triglyceride synthesis was significantly decreased (p<0.05) under hyperlipemic and serum free conditions compared to normolipemic conditions. Mean triglyceride synthesis was lowest (p<0.05) under serum free conditions (Figure 15). Marginal mean differences between each hormonal condition and the control condition were not statistically significant (p>0.05).

Interaction Effects

No significant interactions for triglyceride synthesis were detected (p=0.75).
Discussion

Serum Conditions

Total mean incorporation of [1-\textsuperscript{14}C]oleate was increased approximately 15% in the presence of hyperlipemic serum compared to normolipemic and serum free conditions (Figure 9). Mean cholesteryl ester (CE) synthesis, reflected by percent [1-\textsuperscript{14}C]oleate incorporation into CE, was most dramatically affected by serum condition, increasing over 25 fold under hyperlipemic conditions compared to serum free conditions (Figure 11).

Because excess lipid can be stored as CE in the cell, increased CE synthesis is one response to an overabundance of lipid entering the cell under hyperlipemic conditions (Rothblat, 1977).

In contrast, cellular CE synthesis is lower in the SF condition because there is little need for lipid storage. The cells in the SF condition are nutrient depleted which is reflected in an overall decrease in metabolic activity. Also, these cells are more likely to oxidize the added [1-\textsuperscript{14}C]oleate for fuel which would liberate the labelled carbon atom in the form of [\textsuperscript{14}CO\textsubscript{2}].

The major function of phospholipids is formation of bilayer structures in cell membranes. In a serum free environment, much of the available lipid would likely be incorporated into phospholipids to sustain the integrity of the cell membrane. In a hyperlipemic environment, cellular lipid storage becomes a primary activity and the relative synthesis of phospholipid decreases. The present findings follow this pattern. Mean percent [1-\textsuperscript{14}C]oleate incorporation into phospholipids was highest for cells exposed to the serum free condition followed by the NRS condition and the HRS condition (Figure 13).
Mean percent [1-\textsuperscript{14}C\textsubscript{1}]oleate incorporation into triglycerides in cells exposed to normolipemic rabbit serum (NRS) was highest followed by the HRS condition and the SF condition (Figure 15). Lipid storage in the form of triglycerides is another response to excess lipid entering the cell. The lower rate of triglyceride synthesis in the cells exposed to the SF condition is likely related to less need for lipid storage. Interestingly, mean triglyceride synthesis in the cells exposed to NRS was higher than in cells exposed to HRS. Logically, triglyceride synthesis would be expected to increase under hyperlipemic conditions. Two possible contributing factors are (1) the rate of hydrolysis of triglyceride is three to four times faster than that for CE in smooth muscle cells converted to foam cells (Minor et al., 1989); perhaps the turnover of [1-\textsuperscript{14}C\textsubscript{1}]oleate in triglycerides was greater in the hyperlipemic condition whereas the [1-\textsuperscript{14}C\textsubscript{1}]oleate turnover in CE was minimal; or (2) the increased CE synthesis competed for oleate with triglyceride and phospholipid synthesis.

Summary

Percent [1-\textsuperscript{14}C\textsubscript{1}]oleate incorporation into cholesteryl esters, triglycerides and phospholipids was significantly affected by serum conditions. These findings can be explained in terms of the needs of the cell given different extracellular environments. In a serum free environment, nutrients are unavailable. Any added lipid is likely to be used for repairing and maintaining cell membrane resulting in increased phospholipid synthesis, and for energy production. There is little need for lipid storage reflected in a decrease of cholesteryl ester synthesis and triglyceride synthesis. In a hyperlipemic environment, lipids are abundant, therefore lipid storage in the form of cholesteryl ester synthesis is dramatically increased. Phospholipids are decreased. The finding that triglyceride synthesis in HRS is less
than triglyceride (TG) synthesis in NRS may be due to the more rapid hydrolysis of TG and competition with cholesteryl ester synthesis for the labelled substrate.

**Hormones**

**Total Lipid Synthesis**

Total incorporation of [1-14C]oleate into all lipids was highest in cells in the presence of epinephrine (Figure 9), though not significantly higher than in cells under control conditions.

Simple effects tests on measures of total lipid synthesis indicated significantly increased total lipid synthesis under serum-free conditions in the presence of cortisol compared to control conditions.

Figure 10 shows the serum x hormone interaction for measures of total lipid synthesis. Under control conditions, total lipid synthesis in a serum-free environment (SF), starts at a low level then increases linearly from (SF), to normolipemic (NRS), to hyperlipemic (HRS) conditions. In contrast, total lipid synthesis in the presence of epinephrine alone and cortisol alone starts under SF conditions at a higher level, decreases from the SF to NRS condition, then increases from the NRS to HRS condition. The significance of the two patterns is not clear. Under hyperlipemic conditions, total lipid synthesis under control conditions and in the presence of epinephrine is high and nearly equal. The presence of norepinephrine and cortisol under hyperlipemic conditions appears to decrease total lipid synthesis.

**Cholesteryl Ester**

Overall mean percent cholesteryl ester synthesis for cells incubated with
epinephrine $10^{-6}$M was slightly higher (4.0%) than controls (Figure 11). The percentage difference is quite small but statistically significant. The slight increase in percent cholesteryl ester synthesis is most meaningful under hyperlipemic conditions in which total lipid synthesis is higher. Since ACAT is the primary cholesterol esterifying enzyme, the increase in cholesteryl ester synthesis represents enhanced ACAT activity. This finding is not consistent with previous work. Maziere et al. (1985) reported that epinephrine decreased cholesteryl ester synthesis in cultured human fibroblasts. Maximum suppression was 70% at epinephrine concentrations of $10^{-3}$M. Further investigation is necessary to clarify the effect of epinephrine on cholesteryl ester synthesis. If the present findings can be replicated and confirmed, in vascular tissue, increased cholesteryl ester synthesis as a result of elevated circulating epinephrine could be an important mechanistic link between psychosocial factors and coronary heart disease. The increase in the percentage of cholesteryl ester synthesis in the presence of epinephrine reported here is small (4.0%), however, over a long period of time, the cumulative effect of this increase could be substantial.

Mean percent [1-14C]oleate incorporation into cholesteryl ester in cells incubated with cortisol was decreased by 10.2% compared to controls (Figure 11). This finding is consistent with experimental work in rabbits showing that cortisone injections significantly inhibit lipid deposition in the aorta, despite hyperlipemic conditions (Oppenheim et al., 1952; Stumpf et al., 1954; Gordon et al., 1954). The evidence supporting a significant anti-atherogenic effect of glucocorticoids is inconsistent with the previously mentioned observation of coronary artery narrowing in lupus erythematosus patients treated with glucocorticoids (compared to those not treated with hormones), and other observations linking glucocorticoids to coronary heart disease. Brindley and Rolland (1989) propose that increased control
of metabolism by glucocorticoid hormones, relative to insulin, is a common feature of many physiologic changes associated with coronary heart disease such as insulin resistance, hyperglycemia, hypertriglyceridemia and hypercholesterolemia. The results of this study indicate that on a cellular level, cortisol appears to inhibit cholesteryl ester synthesis. Whether elevated plasma cortisol contributes to atherogenesis in humans through other mechanisms such as its permissive effect on the mobilization of lipids by catecholamines, remains to be determined.

Main effects of hormone conditions on CE synthesis must be interpreted cautiously because of the significant serum x hormone interaction. Simple effects tests, which examined the effects of hormones on CE synthesis under serum free, normolipemic and hyperlipemic conditions separately, indicated (a) significantly reduced CE synthesis in cells incubated with cortisol under normolipemic and hyperlipemic conditions and, (b) a borderline significant (p=0.06) increase in CE synthesis in cells incubated with epinephrine under hyperlipemic conditions. The results of the simple effects tests are consistent with the main effects reported above.

The serum x hormone interaction for measures of cholesteryl ester synthesis is shown in Figure 12. Each hormone condition, including controls showed the same pattern of incorporation in SF, NRS and HRS conditions. The pattern is characterized by a small increase in cholesteryl ester synthesis from SF to NRS conditions and a large increase from NRS to HRS conditions. These changes in cholesteryl ester synthesis are related to the need for lipid storage in the cell.

**Phospholipids**

Several investigators have reported norepinephrine and epinephrine stimulation of phospholipid hydrolysis (Fowler et al., 1986; Brindley et al., 1988;
Billah & Michell, 1979) and resynthesis (Hauser & Smith, 1981; Miller & Kowal, 1983). The stimulated incorporation of radiolabelled precursors ($^{32}$P) into phospholipids is a measure of its resynthesis after receptor mediated breakdown of certain phospholipids. A specific increase in the metabolism of phosphatidylinositol is a widespread cellular response to activation of certain cell-surface receptors for hormones and neurotransmitters (Bellah & Michell, 1979). Other investigators have reported no change in fatty acid esterification into phospholipids in the presence of norepinephrine (Oberhaensli et al., 1985) and a decrease in cellular phospholipid content in the presence of epinephrine (Brindley & Ontko, 1988).

In the present study, overall phospholipid synthesis was not significantly affected by the presence of norepinephrine or epinephrine alone, however, in cells incubated in the presence of cortisol alone and epinephrine/cortisol, mean percent [1-14C]oleate incorporation into phospholipids was increased (p<0.05) compared to the control condition (Figure 13). Oppenheim et al. (1952) reported increased serum phospholipids in rabbits in response to cortisone injections.

The significant serum x hormone interaction indicates the need to interpret main effects cautiously. Simple effects tests showed significantly increased phospholipid synthesis (a) in the presence of cortisol under serum-free and hyperlipemic conditions and, (b) in the presence of epinephrine and epinephrine/cortisol under serum free conditions. The simple effects test are consistent with the main effects reported above.

Figure 14 shows the serum x hormone interaction for measures of phospholipid synthesis. As mentioned earlier, phospholipids are a major component of cell membranes so an increase in percentage phospholipid synthesis to maintain integrity of the cell membrane is expected under serum-free conditions. In contrast, under hyperlipemic conditions (HRS), lipid storage is the priority; cholesteryl ester
synthesis increases dramatically which results in a decrease in percentage phospholipid synthesis.

Comments

Many factors affect the outcome of cell incubation studies investigating hormonal effects on lipid synthesis. This may account for conflicting results in the literature. There are species differences in lipid responses to hormones (Endo et al., 1991) as well as differing cellular responses to hormones in the same organism (Kigoshi et al., 1976). High concentrations of a hormone may have an opposite effect to that of lower concentrations of the same hormone (Palmer et al., 1981). Long term metabolic effects of hormones may be different from short term effects (Smith et al., 1976). Carefully designed studies are necessary to control for the complex relationship between variable affecting hormonal influences on lipid metabolism.

Summary

Rat hepatoma cells incubated with epinephrine (10^{-6}M) exhibited an 4.0% mean increase in percentage incorporation of [1-^{14}C]oleate into cholesteryl ester compared to controls, indicating slightly enhanced ACAT activity. The cumulative effect of a small increase in percentage CE synthesis could be meaningful over a long period of time and/or under hyperlipemic conditions in which total lipid synthesis is increased. This finding is not consistent with earlier work and needs to be confirmed. If confirmed, enhanced ACAT activity in response to epinephrine provides a possible mechanism directly linking behavior and coronary heart disease.

Incubations containing cortisol (10^{-6}M) exhibited a 10.2% mean decrease in percent cholesteryl ester synthesis which is consistent with observations that
glucocorticoids appear to have anti-atherogenic effects in rabbits. The suggested relationship between elevated glucocorticoids and coronary heart disease in humans is inconsistent with these findings. Further investigations may elucidate the validity of the association and the specific mechanisms by which glucocorticoids influence atherogenesis. Incubations containing cortisol also increased mean percent phospholipid synthesis compared to controls.
CHAPTER V

FINAL SUMMARY

Coronary heart disease (CHD) continues to be a major health problem in the Western world. Coronary artery atherosclerosis is responsible for most of the clinical cases of coronary heart disease. Several primary risk factors have been identified for CHD: age, sex, lipoprotein profile, hypertension, diabetes, cigarette smoking and obesity. Yet, a significant amount of variability associated with the incidence of CHD cannot be explained solely on the basis of these factors. Psychosocial factors, including Type A behavior, social isolation and unstable social environments, appear to contribute to CHD through enhancement of established risk factors and independent of them. Psychosocial stimuli are mediated through autonomic and neuroendocrine pathways which can produce profound physiological changes. The mechanisms which link physiological changes in response to psychosocial variables and atherogenic processes are not clear.

In this dissertation, literature describing the relationship between psychosocial factors and CHD, neuroendocrine and autonomic responses to psychosocial stimuli and potential atherogenic consequences of those responses in animal models and humans is reviewed. The contributory role of esterification of cholesterol in the development of atherosclerosis is discussed. Experimental work with Fu5AH rat hepatoma cells investigating the effects of hormones on the activity of the enzyme responsible for cholesterol esterification, acylCoA: cholesterol acyl transferase (ACAT), is presented. Incubation of cells in the presence of epinephrine

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very modestly, but significantly increased ACAT activity providing a possible mechanistic link between responses to psychosocial factors and coronary heart disease. Incubations containing cortisol significantly decreased ACAT activity indicating the need to investigate alternate mechanisms by which cortisol may contribute to atherogenesis.
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