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Effects of Physical and Emotional Stress, Catecholamines and Naloxone on HDL and LDL Cholesterol Levels in Rats and Man

Andrew G. Goliszek
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EFFECTS OF PHYSICAL AND EMOTIONAL STRESS, CATECHOLAMINES AND NALOXONE ON HDL AND LDL CHOLESTEROL LEVELS IN RATS AND MAN

by

Andrew G. Goliszek

A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Biology

1983
To my father, John

Whose guidance and inspiration has given me the commitment and dedication to give of myself so that others may benefit and whose example has taught me what humanity and compassion are all about.
ACKNOWLEDGMENTS

This research was supported in part by research grants from Sigma Xi Research Society.

Special thanks go to Dr. LeGrande C. Ellis, my major professor, for his direction and guidance during this study. I would also like to recognize the members of my advisory committee and thank them for their help during the project: Dr. Jim Gessaman, Dr. Ray Sanders, Dr. Lanny Nalder, and Dr. Raghubir Sharma. My appreciation is also extended to the students who took the time to assist me with various aspects of my project (Bob McMullen, Jack Kirkley, Kerry Openshaw, and Lisa Shaw).

Finally, I would like to thank my wife, Kathy, and my children, Jennifer and David, for their patience, understanding and support during my project.

Andrew Goliszek
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ix</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>3</td>
</tr>
<tr>
<td>Lipoprotein-Cholesterol Pathway</td>
<td>3</td>
</tr>
<tr>
<td>Cholesterol, Lipoproteins and Coronary Heart Disease</td>
<td>7</td>
</tr>
<tr>
<td>Stress as a Factor in Altering Cholesterol Levels</td>
<td>9</td>
</tr>
<tr>
<td>Stress Hormones and Cholesterol Levels</td>
<td>13</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>16</td>
</tr>
<tr>
<td>Animals</td>
<td>16</td>
</tr>
<tr>
<td>Human Subjects</td>
<td>18</td>
</tr>
<tr>
<td>RESULTS</td>
<td>19</td>
</tr>
<tr>
<td>Effect of Physical Stress on Serum Total Cholesterol and Lipoprotein Fractions</td>
<td>19</td>
</tr>
<tr>
<td>Effect of Catecholamines on Serum Total Cholesterol and Lipoprotein Fractions</td>
<td>22</td>
</tr>
<tr>
<td>Effect of Naloxone and Dichloroisoproterenol on Total Cholesterol and Lipoprotein Levels</td>
<td>27</td>
</tr>
<tr>
<td>Effect of B-Endorphin Versus Epinephrine in Altering Serum Cholesterol and Lipoprotein Levels</td>
<td>27</td>
</tr>
<tr>
<td>Effect of Physical Stress on Serum Total Cholesterol and Lipoprotein Levels in Adrenalectomized Rats</td>
<td>32</td>
</tr>
<tr>
<td>Effects of Emotional Stress on Cholesterol and Lipoprotein Levels in Graduate Students During Comprehensive Exams or Dissertation Defense</td>
<td>38</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>43</td>
</tr>
</tbody>
</table>
Role of Long-Term Physical and Emotional Stress in the Etiology of CHD as a Result of Increased Cholesterol and LDL. 45
The Stress Response and its Link to Atherosclerosis Through the Action of Epinephrine and β-Endorphin. 50
Effect of Stress on Cholesterol and LDL Levels in Adrenalectomized Rats Given ACTH Injections. 58
SUMMARY AND CONCLUSIONS. 64
LITERATURE CITED 68
APPENDIX 78
Appendix A. 79
Appendix B. 80
Appendix C. 81
Appendix D. 82
VITA 84
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Some main pathways of adrenal steroids. Adapted from Turner and Baganara, 1976.</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Common pathways of Androgen and Esterogen biosynthesis. Adapted from Stryer, 1981.</td>
<td>5</td>
</tr>
<tr>
<td>4.</td>
<td>Effects of random daily shock on serum total cholesterol. Shock was administered beginning week 1 (A, P &lt; 0.05, B, P &lt; 0.05, and C, P &lt; 0.05 compared to control on weeks 3, 4, 7 and 8; ( \bar{x} \pm s_{\bar{x}} ) and C, P &lt; 0.05 compared to A and B on weeks 7 and 8; ( \bar{x} \pm s_{\bar{x}} ))</td>
<td>20</td>
</tr>
<tr>
<td>5.</td>
<td>Effects of random daily shock on serum total cholesterol. Shock was administered beginning week 1 (A, P &lt; 0.05 compared to control from week 5; ( \bar{x} \pm s_{\bar{x}} ), B, P &lt; 0.05 and C, P &lt; 0.05 compared to A and control from week 4; ( \bar{x} \pm s_{\bar{x}} ), C, P &lt; 0.05 compared to A and B on weeks 7 and 8; ( \bar{x} \pm s_{\bar{x}} ))</td>
<td>21</td>
</tr>
<tr>
<td>6.</td>
<td>Effects of random daily shock on HDL levels. Shock was administered beginning week 1 (A, P &lt; 0.05 compared to control on weeks 4, 5 and 6 and compared to B and C on week 4; ( \bar{x} \pm s_{\bar{x}} )).</td>
<td>23</td>
</tr>
<tr>
<td>7.</td>
<td>Effects of catecholamines on LDL levels. Daily injections began on week 1 and treatments were reversed on week 4 (*, P &lt; 0.05 compared to control; ( \bar{x} \pm s_{\bar{x}} ))</td>
<td>24</td>
</tr>
<tr>
<td>8.</td>
<td>Effects of catecholamines on LDL levels. Daily injections began on week 1 and treatments were reversed on week 4 (*, P &lt; 0.05 compared to control; ( \bar{x} \pm s_{\bar{x}} ))</td>
<td>25</td>
</tr>
<tr>
<td>9.</td>
<td>Lack of effects of catecholamines on HDL levels. Daily injections began on week 1 and treatments were reversed on week 4.</td>
<td>26</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>10. Effects of naloxone and dichloroisoproterenol on total cholesterol levels (a, P &lt; 0.05 compared to control; $\bar{x} \pm s_{\bar{x}}$ and a, P &lt; 0.05 compared to b and c; $\bar{x} \pm s_{\bar{x}}$)</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>11. Effects of naloxone and dichloroisoproterenol on LDL levels (a, P &lt; 0.05 compared to control; $\bar{x} \pm s_{\bar{x}}$ and a, P &lt; 0.05 compared to b and c; $\bar{x} \pm s_{\bar{x}}$)</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>12. Lack of effects of naloxone and dichloroisoproterenol on HDL levels</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>13. Influence of epinephrine on total cholesterol levels when injected together with naloxone (a, P &lt; 0.05 and b, P &lt; 0.05 compared to control; $\bar{x} \pm s_{\bar{x}}$)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>14. Influence of epinephrine on LDL levels when injected together with naloxone (a, P &lt; 0.05 and b, P &lt; 0.05 compared to control; $\bar{x} \pm s_{\bar{x}}$)</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>15. Lack of influence of epinephrine on HDL levels when injected together with naloxone</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>16. Lack of effect of adrenalectomy on total cholesterol levels following stress</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>17. Lack of effect of adrenalectomy on LDL levels following stress</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>18. Lack of effect of adrenalectomy on HDL levels following stress</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>19. Total cholesterol levels in graduate students during non-stress periods and during emotional stress (a, P &lt; 0.05 compared to control; $\bar{x} \pm s_{\bar{x}}$)</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>20. LDL levels on graduate students during non-stress periods and during emotional stress a, P &lt; 0.05 compared to control; $\bar{x} \pm s_{\bar{x}}$)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>21. LDL/HDL ratios in graduate students during non-stress periods and during emotional stress (a, P &lt; 0.05 compared to control; $\bar{x} \pm s_{\bar{x}}$)</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>22. HDL levels in graduate students during non-stress periods and during emotional stress.</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>23. Various factors which influence cholesterol and lipoprotein metabolism.</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>24. Hypothetical mechanisms by which epinephrine inhibits cellular uptake of LDL through receptor interference.</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>25. Possible pathways through which stress acts in altering cholesterol and LDL levels.</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>26. Multiple pathway that may be operating during a stress response. One or more pathways may be dominant with shifts occurring at various times.</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>
ABSTRACT

Effects of Physical and Emotional Stress, Catecholamines and Naloxone on HDL and LDL Cholesterol Levels in Rats and Man

by

Andrew G. Goliszek, Doctor of Philosophy
Utah State University, 1983

Major Professor: Dr. LeGrande C. Ellis
Department: Biology

A series of investigations were undertaken to determine whether physical or emotional stress, catecholamines or naloxone (B-endorphin blocker) would increase serum total cholesterol and LDL and HDL levels. Physical stress given daily over a period of time caused a steady increase in serum total cholesterol and LDL without a significantly altering high density lipoproteins (HDL) or serum triglycerides. Daily injections of epinephrine in oil caused an increase in both serum total cholesterol and LDL levels while daily injections of norepinephrine did not. Reversal of the treatments caused a reversed response in both groups of rats. Similar increases in both total cholesterol and LDL levels occurred in graduate students during preparation for their comprehensive written or oral thesis/dissertation defense.
Injection of either dichloroisoproterenol (M.W. = 248) or naloxone (M.W. = 346) in rats prior to stress inhibited the increase in total cholesterol and LDL levels, although naloxone at the dosage given was more effective, possibly due to its larger molecular weight. When naloxone plus epinephrine was injected into non-stressed rats, there was a significant increase in total cholesterol and LDL levels, but the increase was not as great as that of groups injected with epinephrine only. Stressed, adrenalectomized rats exhibited higher cholesterol and LDL levels than the normal reported range for rats of their age and weight, but their levels did not differ from those of stressed, sham-operated rats indicating that the adrenals per se are not needed for stress-induced elevation of blood LDL levels.
INTRODUCTION

Beginning in the early twentieth century, cholesterol was suspected as being a contributor to atherosclerosis and coronary heart disease (Sabine, 1977). Thus, an excess of plasma cholesterol is thought to contribute to deposition of plaques that eventually leads to coronary ischemia and finally to myocardial infarction. These initial studies focused exclusively on serum total cholesterol. It was not until the last few decades that researchers recognized the importance of lipoproteins as the carriers of cholesterol throughout the circulatory system (Barr et al., 1951; Levy, 1981).

The bulk of studies done on the accumulation of cholesterol deposits in blood vessels has been done with respect to diet, exercise, and lifestyle. Several studies have been undertaken over the years to ascertain if stress increases plasma cholesterol levels. Most of the latter studies, however, have concentrated on the measurement of total cholesterol levels and not on the individual lipoprotein fractions which are now considered to be the critical factors in potentiating atherosclerosis. Furthermore, almost all studies to date have been on human subjects. This type of research has limitations since manipulations on human subjects is subject to scrutiny and data that could otherwise be collected are not. By utilizing laboratory
animals, a more lucid picture can be drawn concerning stress-related lipoprotein levels and the results can be applied to a better understanding of atherosclerosis in human patients.

The last decade has brought us closer to unravelling the pathogenic mechanism of coronary artery disease and today, more than ever, increasing our knowledge is vital in order to curb the threat of a disease which generally manifests itself only after years or even decades. With all the cholesterol studies done over the years, no thorough or systematic research attempted has been undertaken to evaluate the effects on specific lipoprotein levels. Therefore, the purpose of this study was to ascertain how cholesterol and lipoprotein fractions are altered during stressful situations by utilizing laboratory animals as well as human subjects. Understanding the results of this type of study may give us a better insight into how stress in today's society plays a major role in the increasing rate of heart disease.
Lipoprotein - Cholesterol Pathway

Cholesterol is a necessary component of all eucaryotic plasma membranes and is also the precursor of steroid hormones such as progesterone, estradiol, testosterone, and cortisol (Figs. 1 and 2). Too much cholesterol can be lethal, however, and the main concern of many studies has been on the elucidation of the pathway by which cholesterol is transported.

Cholesterol does not exist in free solution (Gofman et al., 1966; Levy, 1981) but enters and leaves the plasma bound to specific proteins. The four major classes of plasma lipoproteins are Chylomicra, Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), and High Density Lipoproteins (HDL). Cells outside the liver and intestine obtain cholesterol from the plasma rather than synthesizing it de novo (Stryer, 1981). Depending on whether the lipoprotein is either high density or low density, cholesterol is either transported away from the peripheral tissues to the liver or is transported toward the peripheral tissues from the liver. The steps in cholesterol uptake by peripheral tissues is as follows (Fig. 3):
Fig. 1. Some main pathways of adrenal steroids. Adapted from Turner and Bagnara, 1976.
Fig. 2. Some common pathways of Androgen and Estrogen biosynthesis. Adapted from Stryer, 1981.
Fig. 3. Mechanism of LDL uptake through cell receptors. Adapted from Goldstein and Brown, Ann. Rev. Biochem. (1977).
1. LDL binds to specific receptors on the cell surface of non-hepatic cells and is then internalized by endocytosis.

2. The protein component of the LDL is hydrolyzed to free amino acids by lysosomes and the cholesterol esters in the LDL are hydrolyzed by lysosomal cholesterol esterase.

3. The unesterified cholesterol is either used for membrane and steroid biosynthesis or is reesterified for storage within the cell.

Rate of cholesterol formation in the liver is dependent on the formation of 3-hydroxy-3-methyl glutaryl CoA reductase, which is the first stage in cholesterol synthesis, and by LDL receptor feedback regulation (Brown and Goldstein, 1976). When cholesterol is abundant within the cell, new receptors are not synthesized and, therefore, there is no consequent cellular uptake of cholesterol (Brown, 1983; Eisenberg and Levy, 1976; Montgomery et al., 1980). When plasma concentrations of LDL are high, cholesterol is deposited in the peripheral tissues inducing atherosclerosis.

**Cholesterol, Lipoproteins and Coronary Heart Disease**

Studies of cardiovascular disease in human populations have for many years emphasized the importance of serum total cholesterol as a critical factor in coronary heart disease.
(Davis et al., 1937; Lerman and White, 1946; Steiner and Domanski, 1943). During the last few decades, however, researchers have focused on the partition of cholesterol into various lipoprotein fractions (Goldstein and Brown, 1977; Levy, 1981; Morrisett et al., 1975). Furthermore, the lipoprotein fractions have been examined as to their role in atherosclerosis (Barr, 1953; Barr et al., 1951; Gofman et al., 1956, 1966; Gordon et al., 1977; Noma et al., 1979; Oliver and Boyd, 1955) as well as to how they themselves are affected by variables such as exercise (Clarkson et al., 1981; Hartung et al., 1980), diet (Brown, 1983; Connor et al., 1961; Flaim et al., 1981; Oliver, 1981), and lifestyle (Keys, 1955; Stamler, 1978). According to these and numerous other studies, the following generalizations can be made regarding the relationship between cholesterol, lipoproteins, and cardiovascular disease:

1. An increase in cholesterol concentrations, specifically the LDL-cholesterol, causes an increase in the risk of cardiovascular disease.
2. Risk of cardiovascular disease is inversely related to HDL-cholesterol concentrations.
3. Physical activity may cause an increase in the HDL fraction or a decrease in the LDL fraction and thus reduce the risk of heart disease.

The value of lipoprotein profiles as predictors of CHD has recently been questioned (Keys, 1955) because of the short follow-up period in the studies conducted and the lack
of attention paid to mortality. Despite this controversy, researchers have clearly demonstrated that there is a correlation between low HDL levels and the pathogenesis of coronary heart disease (Berge et al., 1982; Brook et al., 1982; Castelli et al., 1977; Gordon et al., 1977; Kannel et al., 1979; Levy and Rifkind, 1980; Noma et al., 1979; Tisi et al., 1981). Currently, investigations are being undertaken to explain the means by which HDL exerts its apparent protective effect and what are the genetic and environmental elements that modulate plasma HDL levels (Levy and Rifkind, 1980). Particular attention is being given to LDL and its role as the principle factor in potentiating atherosclerosis (Castelli et al., 1977; Levy, 1981).

**Stress as a Factor in Altering Cholesterol Levels**

Mann and White (1953) were among the first researchers to study the effects of stress on cholesterol levels. They proposed that the physiological response to stress is a decrease in the total serum cholesterol, but they based their conclusions on a series of experiments in which the stress was feed deprivation. Other researchers have since refuted their conclusions and have shown that cholesterol can increase significantly during stressful conditions. For example, when hospitalized patients were maintained on balanced diets, and exercise was carefully regulated, serum
cholesterol increased significantly with occurrence of emotionally stressful situations (Wolf et al., 1962). Furthermore, it has been shown that serum total cholesterol can increase during stressful situations by as much as 35% despite constant diet and exercise (McCabe et al., 1959). Moreover, significant deviations in total cholesterol levels may occur within hours in certain individuals during short-term stress (Peterson et al., 1960, 1962). Thomas and Eisenberg (1957) and Wertlake and Wilcox (1959) observed striking variations in day to day serum cholesterol with serum cholesterol increasing significantly one day and becoming stable the next. Changes in serum cholesterol have also been observed in men undergoing job loss (Kasl et al., 1968) and in professionals such as accountants during periods of occupational stress (Friedman et al., 1958). A long-term study by Uhley and Friedman (1959) has shown that rats exposed to intermittent stress over a 10-month period had a 47% greater plasma cholesterol concentration than controls and concluded that stress may accelerate atherosclerosis.

Beginning in the late 50's, a series of observations were made on changes in serum cholesterol levels of students during stressful periods. Thomas and Murphy (1958), Wertlake et al. (1958), Grundy and Griffin (1959), and Wertlake and Wilcox (1959) all found statistically significant increases in serum cholesterol under mental and emotional
stress of examinations. Sloane et al. (1961) and Sloane et al. (1962) described the "high cholesterol type student" in which there were elevations in cholesterol levels in students described as excessively ambitious, hostile, and restlessly tense.

Other types of stressful situations have been observed in which the stress of physical activity or the anticipation of a demanding situation affected cholesterol levels. Cholesterol levels increased with physical activity (Naughton and McCoy, 1966) and with high energy output, more drive, and more concern with meeting deadlines (Sletten et al., 1964). Rahe and Arthur (1967) further showed that cholesterol levels increased during periods of stress even though the physical activity of the subjects was at a maximum before and during the time of increased cholesterol levels. Later studies demonstrated that increased cholesterol was correlated with having failed and with a feeling of being overburdened (Rahe et al., 1968) or with feelings of anger, fear, and lethargy (Rahe et al., 1971). In occupational studies, elevated serum cholesterol was correlated with self-criticism and dependability (Jenkins et al., 1968), cyclic job stress (Friedman et al., 1958), and extreme drive and competitiveness (Friedman and Rosenman, 1959, 1960). Kasl et al. (1968) reported that anticipation of job loss did not increase cholesterol levels, but it did increase serum uric acid levels. Groen et al. (1952)
observed fluctuations of cholesterol levels in response to stress independent of the type of diet and suggested that the fluctuations resulted from infection, exertion or emotional tension.

Most of the studies mentioned thus far have been concerned with measurement of serum total cholesterol without regard to the lipoprotein fractions carrying the cholesterol. A paucity of information, therefore, exists in this area of investigation. Dreyfus and Czaczkes (1959) found that HDL was higher than LDL during stressful examination periods. Jenkins et al. (1966) and Francis (1979), on the other hand, found that it was the LDL-cholesterol that increased significantly during stressful periods while Harlan et al. (1967) found VLDL to be associated with unrestrained aggressiveness. In reviewing the studies associated with plasma lipid variability in response to emotional arousal,Dimsdale and Herd (1982) concluded that, with rare exceptions, stressful events can significantly increase cholesterol by as much as 65% even though dietary intake or physical activity are rigidly controlled. These studies illustrate the need to examine non-dietary lipid metabolism and to elucidate the relationship between lipoprotein fractions and stress.
Stress Hormones and Cholesterol Levels

It has been well established that a response to stress involves the release of several "stress hormones" including adrenal cortical steroids and the catecholamines epinephrine and norepinephrine. Epinephrine produces a hyperglycemic effect which augments energy reserves and enables an organism to cope with stress very quickly (Hole, 1981). It has produced myocardial necroses when administered extrinsically or when the animal underwent experimental stimulation or lesions of the brainstem (Raab et al., 1964). Friedman et al. (1960) showed that stress discharged catecholamines into the circulatory system during emotional stress. Thus, epinephrine release is necessary for short-term survival, but can be harmful when relied upon for long-term survival during physical or emotional stress.

Cortisol in man and corticosterone in rodents mobilizes amino acids from muscle tissue and fatty acids from adipose tissue (Guyton, 1981). This mechanism makes amino acids available for new protein synthesis or gluconeogenesis and fats available for energy during starvation or stress.

Since cholesterol is a precursor molecule for steroid hormones (Turner and Bagnara, 1976), an increase in serum cholesterol during stress may be a mechanism whereby synthesis of stress hormones is maximized. The rise in cholesterol levels during stress could be intensified as a
result of CNS stimulation or as a result of feedback caused by a decreased level of steroid hormones. Gunn et al. (1960) concluded that a CNS mechanism exists which under certain conditions is capable of significantly influencing arterial atherogenesis. When rabbits were fed a diet high in cholesterol along with chronic hypothalamic stimulation, the animals exhibited a greater degree of aortic and coronary atherosclerosis than did the non-stimulated controls fed the same diet. Similarly, stimulation of the hypothalamus and thalamus evoked a serum turbidity response thought to be associated with an increase in the total lipid concentration (Correll, 1969) in which chronic hypercholesterolemia resulted from bilateral hypothalamic injury involving the ventral medial nuclei, the fornices, and the medial portion of the lateral hypothalamus (Friedman and Byers, 1972).

Current investigations have centered around B-endorphinergic systems and their involvement in stress reactions. In particular, B-endorphin has been found to contain opiate-like properties. They also serve as tranquilizing agents and analgesic regulators and are called upon as a reserve mechanism in emergency situations (Emrich and Millan, 1982). In humans, electrical stimulation of the periaqueductal gray and other centers of the brainstem induces a release of B-endorphin (Hosobuchi et al., 1977). Furthermore, endorphin
blockers such as naloxone have been shown to decrease the pain threshold of acupuncture (Mayer et al., 1977), thus demonstrating the analgesic effect of endorphin.

More recent investigations have shown that β-endorphin increases plasma concentrations of epinephrine, norepinephrine, and dopamine in rats (Van Loon and Appel, 1980; Van Loon et al., 1981) and that psychosomatic disorders may be related to a dysfunction of endorphinergic reactions during stress (Emrich and Millan, 1982). Since catecholamines are important in stress responses and endorphins play a role in catecholamine release, both endorphins and catecholamines may represent an integral part of the overall stress mechanism. Other variables, such as diet and heredity, may act synergistically along with stress to compound its effects and cause an increase in cholesterol levels.
MATERIALS AND METHODS

Animals

Male Wistar rats weighing 300-400 grams at the start of the experiments were maintained in an animal care facility at Utah State University on a 14:10 hr (L:D) cycle and were given feed (Wayne Lab-Blox) ad libitum except when noted otherwise. All rats were housed in groups and adapted to handling by being handled daily for 3 weeks prior to the start of the experimental period.

Rats were divided into groups of 6 according to the amount and type of stress that was administered or groups of 8 according to the type of catecholamine or receptor blocker that was injected. Stress was always administered in the form of electrical tail shock (150 Volts; 2 shocks per second) for a period of 30 seconds (Appendix A). The groups were divided as follows:

A. Shocked randomly once a day.
B. Shocked randomly 8 times per day.
C. Same as B except that shock was preceded by a buzzer. When rats learned to associate the buzzer with shock, the buzzer was set off at different times of the day and night in addition to the times of shock.
D. Injected Sub Q daily with epinephrine (1 mg/kg body wt. in corn oil).

E. Injected Sub Q daily with norepinephrine (1 mg/kg body wt. in corn oil).

F. Injected with dichloroisoproterenol (1 mg/kg body wt. in saline) and stressed 30 minutes following injection.

G. Injected with naloxone (1 mg/kg body wt. in saline) and stressed 30 minutes following injection.

H. Injected with naloxone plus epinephrine.

I. Adrenalectomized and stressed.

Blood samples from groups A through E were collected weekly while blood samples from Groups F through I were collected 2 hours following stress or following injection. All samples were collected approximately the same time between 9:00 and 10:00 a.m. Rats in group I were adrenalectomized and then allowed 10 days to recover from surgery before stress was administered. Corticosterone (5 mgs in oil 3 days prior to stress) was administered in order to replace that lost by removed adrenals.

Rats were fasted for 10 hours prior to collecting blood (3 ml) which was done by cardiac puncture after ether anesthesia. Serum was separated from the erythrocytes after refrigeration at 8°C within 2 hours by centrifugation at 5000 X g in a refrigerated centrifuge for 30 minutes. All
samples were analyzed colorimetrically within 24 hours for total cholesterol, HDL, and triglycerides. Total cholesterol was determined using a total cholesterol reagent kit (Fisher Scientific); HDL was determined using a magnesium-phosphotungstate reagent kit (Fisher Scientific); serum triglyceride was determined using the ESC triglyceride non-enzymatic procedure (Stanbio Diagnostics); LDL was calculated using the formula by Friedwald et al. (1972). Comparison between experimental and control groups was determined by Student's t-test and .05 was considered to be significant.

**Human Subjects**

Eleven volunteer graduate students were also used to study the effects of stress on cholesterol and lipoprotein levels. Blood was taken from the vein during a period of non-stress following a 10 hour fast and then again just prior to comprehensive exams or dissertation defense. Serum was separated and analyzed as previously stated. All the volunteers were carefully screened so that errors due to other factors were avoided. A questionnaire (Appendix B) was filled out by each student regarding diet, activity, and lifestyle in order to interpret results more accurately.
RESULTS

Effect of Physical Stress on Serum Total Cholesterol and Lipoprotein Fractions

Serum total cholesterol increased during the first 6 weeks from about 40 to 55 mg/dl and then rose to a maximum of 80 mg/dl following the introduction of a randomly timed buzzer as a second form of stress (Fig. 4). The values for all groups were significantly increased above control (p < 0.05) on weeks 3, 4, 7 and 8. There was no depletion of red blood cells after spinning of blood during the 8 week experimental period.

Electrical shock stress over a 6-week period (Fig. 5) increased LDL levels from about 5 to a maximum of 17 mg/dl. A sharp rise in value to about 33 mg/dl resulted from introduction of the buzzer. Group C, which was conditioned to associate the buzzer with electric shock, experienced the greatest increase in both serum total cholesterol and LDL levels. Increases in LDL levels were significantly elevated (p < 0.05) over controls in group A from week 5, in group B from week 4, and in group C from week 4. The sharp increase in both total cholesterol and LDL in groups A and B following introduction of the buzzer may have been due to the additional stress caused by the noise or by pheromones.
Fig. 4. Effects of random daily shock on serum total cholesterol. Shock was administered beginning week 1 (A, p < 0.05, B, p < 0.05, and C, p < 0.05 compared to control on weeks 3, 4, 7 and 8; \( x \pm s \)) and C, p < 0.05 compared to A and B on weeks 7 and 8; \( x \pm s \)).
Fig. 5. Effects of random daily shock on LDL levels. Shock was administered beginning week 1 (A, $p < 0.05$ compared to control from week 5; $x \pm s$, B, $p < 0.05$ and C, $p < 0.05$ compared to A and control from week 4; $x \pm s$, C, $p < 0.05$ compared to A and B on weeks 7 and 8; $x \pm s$).
released from group C rats which associated the buzzer with electric shock. HDL levels fluctuated somewhat during the first 6 weeks, but then increased following introduction of the buzzer (Fig. 6). This slight increase was associated with the increase in total cholesterol. Since triglyceride levels did not change between groups, they were not considered to be a factor that contributed to a rise in cholesterol levels. Average cholesterol values for some laboratory animals are shown in Appendix C.

Effect of Catecholamines on Serum Total Cholesterol and Lipoprotein Fractions

Daily subcutaneous injections of epinephrine produced a significant increase ($P < 0.05$) in serum total cholesterol from about 48 to 70 mg/dl (Fig. 7). Injections of norepinephrine produced a somewhat smaller increase from about 48 to 53 mg/dl which was not significant compared with controls ($p > 0.05$). When the treatments were reversed after the fourth week, the group response was also reversed.

There was a significant increase in LDL levels with epinephrine injections ($P < 0.05$ (Fig. 8), but not with norepinephrine injections ($p > 0.05$). When the two groups were reversed, the response also reversed. Despite a rise on week 2, HDL levels were not significantly affected ($p > 0.05$) by either treatment during the entire experimental period (Fig. 9). Thus, the effects of stress were
Fig. 6. Effects of random daily shock on HDL levels. Shock was administered beginning week 1 (A, p < 0.05 compared to control on weeks 4, 5 and 6 and compared to B and C on week 4; x ± s).
Fig. 7. Effects of catecholamines on serum total cholesterol. Daily injections began on week 1 and treatments were reversed on week 4 (*, P<0.05 compared to control; \( x \pm s_x \)).
Fig. 8. Effects of catecholamines on LDL levels. Daily injections began on week 1 and treatments were reversed on week 4. (*, *P* < 0.05 compared to control; x ± s).
Fig. 9. Lack of effects of catecholamines on HDL levels. Daily injections began on week 1 and treatments were reversed on week 4.
primarily evidenced by a rise in the serum LDL. Triglyceride levels did not increase during the experimental period and were not a factor in cholesterol increase.

**Effect of Naloxone and Dichloroisoproterenol on Total Cholesterol and Lipoprotein Levels**

Injection with naloxone or dichloroisoproterenol inhibited the increase of serum total cholesterol (Fig. 10). Stressed animals, which were not given either blocker, exhibited significantly higher serum total cholesterol levels ($p < 0.05$) than stressed animals which were treated with one or the other drug prior to stress. An increase in LDL levels was also inhibited significantly ($p < 0.05$) in treated animals (Fig. 11). As predicted from previous experiments, HDL levels did not fluctuate much (Fig. 12), and therefore, the increase in total cholesterol was associated with the increase in the LDL fraction. Triglyceride levels were unaffected by the treatments.

**Effect of B-Endorphin Versus Epinephrine in Altering Serum Cholesterol and Lipoprotein Levels**

Rats injected with either epinephrine or epinephrine plus naloxone had significantly higher serum total cholesterol levels ($p < 0.05$) than control animals (Fig. 13). The animals injected with only epinephrine had significantly
Fig. 10. Effects of naloxone and dichloroisoproterenol on total cholesterol levels (a, $P<0.05$ compared to control; $\bar{x} \pm s_\bar{x}$ and a, $P<0.05$ compared to b and c; $\bar{x} \pm s_\bar{x}$).
Fig. 11. Effects of naloxone and dichloroisoproterenol on LDL levels (a, $P<0.05$ compared to control; $x \pm \bar{s}_X$ and a, $P<0.05$ compared to b and c; $x \pm \bar{s}_X$).
Fig. 12. Lack of effects of naloxone and dichloroisoproterenol on HDL levels.
Fig. 13. Influence of epinephrine on total cholesterol levels when injected together with naloxone (a, $P < 0.05$ and $b$, $P < 0.05$ compared to control; $\bar{x} \pm s_x$).
higher total cholesterol levels ($p < 0.05$) than both the control group and the group injected with naloxone plus epinephrine. LDL levels increased in a similar way (Fig. 14) and were significantly higher in both injected groups when compared to their controls ($p < 0.05$). LDL was highest in the epinephrine treated group and was significantly higher ($p < 0.05$) than the group injected with naloxone plus epinephrine. HDL did not vary much between groups (Fig. 15) and the levels were not significantly different ($p < 0.05$). Triglyceride concentrations were not altered during the treatments.

**Effects of Physical Stress on Serum Total Cholesterol and Lipoprotein Levels in Adrenalectomized Rats**

After a recovery period of 10 days following surgery, there was no significant difference ($p < 0.05$) in cholesterol and lipoprotein levels between adrenalectomized and sham operated rats 2 hours after stress (Figs. 16, 17, and 18). Both groups had higher than normal cholesterol and LDL levels (normal range for total cholesterol is 40-50 mg/dl and for LDL is 3-10 mg/dl, but similar HDL levels to normal non-stressed rats (normal range for HDL is 25-35 mg/dl).
Fig. 14. Influence of epinephrine on LDL levels when injected together with naloxone (a, \( P < 0.05 \) and b, \( P < 0.05 \) compared to control; \( x \pm s_x \)).
Fig. 15. Lack of influence of epinephrine on HDL levels when injected together with naloxone.
Fig. 16. Lack of effect of adrenalectomy on total cholesterol levels following stress.
Fig. 17. Lack of effect of adrenalectomy on LDL levels following stress.
Fig. 18. Lack of effect of adrenalectomy on HDL levels following stress.
Graduate students subjected to the stress of comprehensive examinations of dissertation defense had significantly higher (p < 0.05) total cholesterol levels, LDL levels, and LDL/HDL ratios than they did during periods of non-stress (Figs. 19, 20, and 21). HDL levels did not change significantly (p > 0.05) indicating that most of the change in total cholesterol was due to the LDL fraction (Fig. 22). There was a wide range of variability in both serum total cholesterol (164-221 mg/dl during non-stress; 183-264 mg/dl during stress) and LDL levels (97-152 mg/dl during non-stress; 119-174 mg/dl during stress) suggesting that individual cholesterol and lipoprotein levels during non-stress may have been influenced by factors such as diet, heredity, or lifestyle. However, since every student's cholesterol and LDL levels increased prior to stress despite the fact that none of the students reported any changes in lifestyle, diet or exercise, the increase was assumed to be the direct result of stress. Appendix D shows average cholesterol and lipoprotein values for human populations.
Fig. 19. Total cholesterol levels in graduate students during non-stress periods and during emotional stress.

\( a, p < 0.05 \) compared to control; \( \bar{x} \pm s_x \).
Fig. 20. LDL levels in graduate students during non-stress periods and during emotional stress (a, p < 0.05 compared to control; x ± s_x).
Fig. 21. LDL/HDL ratios in graduate students during non-stress periods and during emotional stress (a, $p < 0.05$ compared to control; $x \pm s_x$).
Fig. 22. HDL levels in graduate students during non-stress periods and during emotional stress.
DISCUSSION

Although much is known about the relationship between cholesterol and cardiovascular disease, only recently has a correlation been found between lipoprotein fractions and atherosclerosis. The major focus of studies in the past has been on the effect of diet and exercise on serum total cholesterol, but there have been a few studies which emphasized the importance of stress on altering cholesterol levels. Only a very small part of stress-related cholesterol research, however, has dealt with lipoprotein fractions (most notably, LDL) as important contributors to stress-induced cardiovascular disease.

As early as 1950, stress was implicated as a main contributing factor in increasing the risk for coronary heart disease (Stewart, 1950). Some of the earlier investigations showed clearly than the etiology of coronary heart disease was a consequence of various factors with stress being one of the more important factors (Russek, 1965, 1967; Russek and Zohman, 1958). Additional studies have also shown that the stress of working long hours is positively correlated with onset of cardiovascular disease (Ereslow and Buell, 1960) as are the effects of social and cultural factors (Cassel and Tyroler, 1961; Syme et al., 1964). These studies demonstrated that factors other than
than diet and heredity may be contributing significantly to the development of heart disease and they focused attention to the effects of stress in influencing homeostatic mechanisms.

Rosenman et al. (1966) found that the significant prognostic factors in the development of heart disease were abnormalities in the lipoprotein profiles, hypertension, and the exhibition of a specific overt behavior pattern called "Type A" (excessive drive, aggressiveness, and ambition). Further evidence by Eastwood and Trevelyan (1971), Groen et al. (1972), Bengtsson et al. (1973), and Wardwell and Bahnson (1973) linked coronary heart disease with emotional stress, depression, and anxiety. These complex psychological patterns are often related to social stress and may play a key role in triggering LDL synthesis which in turn contributed to onset of atherosclerosis. Moreover, the strongest evidence for a link to cardiovascular disease exists for the Type A behavior pattern and incidence of coronary heart disease (Brand et al., 1976; Harlan, 1980).

Since the Type A individual responds to stress with greater secretion of catecholamines than the Type B individual (Friedman et al., 1975; Williams et al., 1982), the hyperresponsivity to stress in terms of catecholamine release may represent a mechanism for the expression of excess coronary events and atherosclerosis among Type A persons. Indeed, several past studies have demonstrated
the cardiotoxic effects that catecholamines have on heart tissue (Gazes et al., 1959; Raab, 1943, 1956; Raab et al., 1962; Szakacs et al., 1959) as well as their increased discharge during emotional stress (Elmadjian et al., 1957). The relationship between emotional stress or trauma and increased levels of cholesterol and LDL may be more significant in light of the necrotic effect of catecholamines on heart muscle and would explain one's increased risk for coronary heart disease and atherosclerosis under those conditions.

Role of Long-Term Physical and Emotional Stress in the Etiology of CHD as a Result of Increased Cholesterol and LDL

The fact that serum cholesterol in general and LDL in particular contribute to the onset of atherosclerosis has been debated by researchers during the past two decades. Most of the controversy stems from the argument over whether single or multiple factors are responsible for atherosclerotic mechanisms and over which factor plays the leading role. If a synergistic effect occurs over a long-term period, then a single factor could invariably be intensifying the effect of the other factors and hence play a leading role.

In this study, stress was the single variable that proved to cause increases in both serum total cholesterol and LDL levels without significantly altering HDL levels.
Daily, random stress, both in the form of physical stress (shock) and emotional stress (anticipation of shock), produced this effect. Even though a rise in cholesterol and LDL was noted within 2 hours following stress in rats, daily stress over a long-term period (2 months) produced a greater and continual rise in cholesterol and LDL levels and is, therefore, a more pathogenic situation than short-term stress. Furthermore, the group that was subjected to the emotional stress was affected the most in terms of overall increases in total cholesterol and LDL levels.

Human subjects in this study had undergone only emotional stress of comprehensive exams or dissertation defense and therefore physical stress was not involved. Every student had an increase in total cholesterol and LDL a few hours prior to the anticipated stressful situation demonstrating that emotional stress is sufficient to cause a significant change. One student, who had taken his exams prior to the Thanksgiving holiday and who had an elevated cholesterol level, actually had a decrease in total cholesterol and LDL levels immediately after Thanksgiving despite the enormous amount of rich foods than he reported eating. Although this is only one example, it demonstrates that stress may be a more significant factor in altering the normal levels of cholesterol and lipoproteins than diet.

There have been several studies which failed to produce any changes in cholesterol levels when cholesterol was added (in the form of eggs) to free-living diets already
containing moderate amounts of cholesterol (Flynn et al., 1979; Kummerow and Kim, 1977; Porter et al., 1977; Slater et al., 1976). Furthermore, some foods (such as dairy products) which were once thought to increase cholesterol levels, have recently been shown to have a hypocholesteremic effect (Mann, 1977; Mann and Spoerry, 1974; Richardson, 1978). This evidence suggests that diet alone may account for only a small percentage of mortality due to CHD.

If cholesterol and LDL are important in the pathogenesis of coronary heart disease, then the present study demonstrates that stress is not only an indirect variable but an actual catalyst that mediates the stress response. Because of its ability to increase the levels of serum LDL, stress may thus be considered one of the principle contributors to the atherosclerotic mechanism. Other factors such as diet and heredity may be secondary contributors augmenting the initial reactions caused by stress. In this manner, the risk for CHD would depend on the multiplicative effect of various factors with one factor, stress, initiating or at the very least intensifying the process.

The human liver produces about 2 grams of cholesterol per day (Kritchevsky, 1958) while the average American diet consists of 300-600 mg of cholesterol daily (Connor, 1958; Connor and Connor, 1983). As the dietary consumption of cholesterol increases, the endogenous synthesis of
cholesterol decreases accordingly (Bhattathiary and Siperstein, 1963; Frantz et al., 1954; Langdon and Bloch, 1953; Taylor et al., 1960), and unless the daily intake of cholesterol is very high, normal cholesterol levels are maintained. This fact raises the question of why so many individuals who maintain a proper diet still succumb to coronary heart disease. The answer may be that diet is a factor that is important in the case of some individuals but, in general, is not significant without the concomitant effects of various other factors.

Fig. 23 shows a hypothetical scheme in which the different factors influence the risk of CHD both directly and indirectly. Together with diet and heredity, stress (especially daily stress over a long period of time) no doubt insures that cholesterol levels will rise above normal when an organism encounters any stressful situation. It is yet unknown why or how stress produces a rise in cholesterol levels although speculation would suggest that the body is preparing for trauma by producing the precursor molecule for steroid hormones. This ensures short-term survival at the expense of long-term health and could be the result of an evolutionary process that developed when long-term survivability was not an issue.

The rats than were subjected to both physical and emotional stress exhibited abnormal behavior such as tail biting and anxiety as well as physiological abnormalities
Fig. 23. Various factors which influence cholesterol and lipoprotein metabolism.
such as diarrhea and high cholesterol and LDL levels. All these effects were more pronounced in this group (group C) than the other groups which experienced only physical stress. Thus, emotional stress may be more important in terms of CHD pathogenesis than physical stress or it may simply have an additive effect that compounds an already existing condition. Further studies are needed in this particular area in order to delineate between the effects of psychological versus physical stress on cholesterol and lipoprotein levels.

The Stress Response and its Link to Atherosclerosis Through the Action of Epinephrine and Endorphin

According to Selye's "General Adaptation Syndrome" (1959), the adaptive mechanisms that are called into operation during exposure to stress are detrimental to certain physiological processes and may induce a variety of pathologic changes such as hypertension, arthritis, ulcers, ateriosclerosis, nephroscclerosis, and many others. Although not enough direct evidence is available, it is possible that these diseases may be produced by hormonal excess or imbalances.

In the present study, rats injected daily with epinephrine (in oil) exhibited an increase in both total cholesterol and LDL levels. When a B-blocker such as dichloroisoproterenol was injected prior to stress, increase
in cholesterol and LDL levels was inhibited. The mechanism responsible for the increase in cholesterol during stress is unknown, but the fact that epinephrine is involved in the general stress response and also stimulates an increase in serum cholesterol and LDL levels makes possible some theories involving the cellular uptake of LDL and release of epinephrine.

It has been well established that new LDL receptors (called "coated pits") will not be synthesized when the cellular content of cholesterol is at a maximum (Brown and Goldstein, 1976). When this occurs, excess cholesterol is unable to enter into the cell and is subsequently deposited in the surrounding tissue in the form of plaques which eventually harden and cause arteriosclerosis. Proper functioning of LDL receptors is critical because any abnormality will produce hyperlipoproteinemia and hence a very high risk of coronary heart disease. Epinephrine may interfere with cellular uptake of LDL in several ways (Fig. 24):

1. There may be an actual physical interference whereby epinephrine binds to the cell membrane and discourages proper functioning of LDL receptors.

2. There may be indirect interference in that epinephrine activates adenyl cyclase which converts ATP to cAMP and the cAMP in turn alters the permeability of the cell membrane. The cellular change may be sufficient enough to inhibit the entrance of at least some of the LDL molecules.
Fig. 24. Hypothetical mechanisms by which epinephrine inhibits cellular uptake of LDL through receptor interference.
3. Glycogenolysis, stimulated by epinephrine, may cause an inhibition of LDL receptor function if the cell is unable to efficiently take up both the large amount of glucose and the LDL molecules. These hypothetical schemes are possible if high levels of epinephrine can interfere with or inhibit receptors. Since the half life of epinephrine is roughly 30 minutes (Turner and Bagnara, 1976), short-term stress that causes release of catecholamines over a short time period may not have the same effect as long-term stress which causes continued release of catecholamines over a long time period. In the present study, daily injected epinephrine in oil represented a longer continual release and was a more realistic test of long-term catecholamine effect. The deleterious effects of epinephrine are no doubt intensified when the catecholamine is present for a prolonged time period. Thus emotional stress, which has been implicated with coronary heart disease more so than his physical stress, and which usually manifests itself over a much longer period of time, is probably the form of stress that is involved most intimately with the epinephrine-cholesterol mechanism.

Recent investigations have shown that physical activity such as daily exercise, increases HDL levels (Huttunen et al., 1979; Streja and Mymin, 1979) or decreases LDL
levels (Altekruse and Wilmore, 1973; Lopez et al., 1974). Since physical activity also increases epinephrine release, there appears to be an inconsistency with a theory which correlates a rise in epinephrine with an increase in cholesterol and LDL levels. However, this inconsistency may be explained by a recent finding at the University of Utah Artificial Organs Division where it was discovered that a steady exercise regime prior to surgery actually depressed the rise in epinephrine associated with the trauma of surgery (Olsen, personal communication). This adaptation to continuous physical activity could explain why epinephrine is not a significant factor in cholesterol and LDL increase in individuals who exercise regularly. Furthermore, epinephrine that is released as a result of stress may not be subject to inhibition by any adaptive mechanism and would, therefore, be released even in physically active individuals whenever they encounter stressful situations.

The present study has also demonstrated that the B-endorphin blocker, naloxone, is able to inhibit a rise in serum total cholesterol and LDL levels following stress (electric shock) and that this inhibition is even stronger than it is in the case of rats injected with the B-adrenergic blocker dichloroisoproterenol. Since both naloxone and dichloroisoproterenol inhibit (albeit not to the same extent) cholesterol and LDL increases, a mechanism involving both epinephrine and B-endorphin is likely to be
operating during stress. B-endorphins, which are naturally occurring opiate-like substances found in the brain, are secreted into the bloodstream during stress and stimulate catecholamine release thus increasing plasma levels of epinephrine, norepinephrine and dopamine (Van Loon and Appel, 1980). The rise in epinephrine during a stressful situation can therefore result from both the regular pathway via hypothalamic stimulation and from the mechanism which mediates B-endorphin-induced increases in plasma catecholamines. This dual pathway would not only enhance an organism's ability to cope with immediate life threatening situations but would also enhance an organism's risk of atherosclerosis by increasing the amount of epinephrine in the plasma (Fig. 25).

When naloxone was administered prior to stress, there was virtually a complete inhibition of cholesterol and LDL synthesis. Yet, when naloxone plus epinephrine were administered, there was a rise in cholesterol and LDL levels although not as great as when epinephrine alone was administered. The inhibition of cholesterol due to naloxone demonstrates that the B-endorphin pathway is important and may be a key in understanding how cholesterol and LDL levels are being altered during stress. The inhibition of cholesterol and LDL with naloxone despite stress but the rise in cholesterol and LDL levels following injection with
Fig. 25. Possible pathways through which stress acts in altering cholesterol and LDL levels.
epinephrine despite administration of naloxone may be explained in two ways, respectively:

1. Short-term stress may not have produced enough epinephrine for a long enough period of time to cause an effect. The naloxone further decreased the amount of epinephrine that would normally have been present under a naturally occurring stress situation.

2. The large amount of exogenous epinephrine produced an increase in both cholesterol and LDL levels without the benefit of B-endorphin induced stimulation of sympathetic outflow of catecholamines. The reason for a smaller increase is that B-endorphins were not involved in the stress response.

The evidence seems to point to a mechanism involving the action of epinephrine and B-endorphin in which both substances contribute to the overall increase in serum cholesterol and LDL. Onset of stress triggers the release of epinephrine by stimulating the hypothalamus to secrete certain releasing factors which act on the adrenal medulla. B-endorphins come into play as a result of stimulation of various brain sites and increase existing plasma concentrations of epinephrine by also acting on the adrenal medulla. Since B-endorphins are not easily degraded (Emrich and Millan, 1982), they remain in the plasma and continue their stimulatory action on the adrenal gland. For this reason,
epinephrine may be present in the bloodstream for even longer periods following a stress response than one would expect without the effect of endorphins. Emotional stress, which is considered a longer lasting type of stress than physical stress, under most conditions, can be instrumental in increasing cholesterol levels because of its effect on continued secretion of B-endorphins. Physical stress, on the other hand, unless continued over long periods, may have only a temporary effect on B-endorphin secretion, and, therefore, would have a somewhat lesser effect on that particular pathway of catecholamine release. This would explain why rats subjected to both physical and emotional stress (group C) had the greatest overall increase in both total cholesterol and LDL levels. When both pathways are being used, for example, the additive effects would be significant. Epinephrine would have been released during stress, as well as between stresses because B-endorphin was present to insure continued epinephrine release. During emotional stress, this mechanism could be the method which keeps plasma epinephrine concentrations at high levels.

**Effect of Stress on Cholesterol and LDL Levels in Adrenalectomized Rats Given ACTH Injections**

Adlersberg and Schaefer (1950) observed hypercholesteremia of serum total cholesterol that exceeded 280 mg/dl
when either ACTH or cortisone was administered to human patients. As illustrated in Fig. 25, ACTH is released from the anterior pituitary gland and stimulates the adrenal cortex to release cortisol which has various effects including gluconeogenesis. The abnormal cholesterol elevations in the above case may have been due to receptor interference as mentioned previously or by another mechanism involving the increase in circulating amino acid concentration or the release of fatty acids from adipose tissue - two important consequences of ACTH release.

During the course of the study by Adlersberg and Schaefer (1950), it was found that fluctuations in cholesterol levels were, in general, related to changes in dosages of ACTH or cortisone, i.e., an increase in the dosage was often accompanied with the increase in serum cholesterol values. The change in cholesterol averaged +20% in the cortisone-treated group and +33% in the ACTH-treated group. These results are important in that ACTH and cortisone are linked to stress, and since the onset of stress produces an increase in cholesterol and LDL. From this study, it is reasonable to conclude that stress is a principle cause of atherosclerosis through cholesterol synthesis.

In the present study, adrenalectomized rats that were stressed were not expected to have any increase in either cholesterol or LDL levels since epinephrine and corticosterone were unavailable from the adrenal medulla and adrenal
cortex, respectively. This was not the case, however, in that the adrenalectomized rats had cholesterol and LDL levels as high as the control sham-operated rats which were also stressed. Both groups had levels which were above the normal range for rats of that age and weight. At first, it appears that a mechanism other than any of the ones already presented may be operating in this case such as a direct B-endorphin pathway not associated with catecholamine release. B-endorphin is a viable possibility since the present research has established it as a contributing factor in hypercholesteremia and LDL increases. Since epinephrine has been strongly implicated in the increase of cholesterol and LDL as well, some other possibilities may be equally as convincing. Some of the possibilities that may explain a cholesterol increase in adrenalectomized rats are as follows:

1. In the absence of epinephrine, B-endorphin release became the only stimulatory agent and somehow caused a rise in cholesterol and LDL levels without the involvement of epinephrine.

2. Since corticosterone was given to the adrenalectomized rats during the 10-day recovery period, corticosterone may have contributed to cholesterol and LDL increase via receptor interference caused by gluconeogenesis or by another mechanism yet unknown.
The fact that the adrenalectomized rats receiving corticosterone injections had higher levels of cholesterol and LDL levels following stress than the control group indicates that corticosterone may play a leading role in stress-induced cholesterol and LDL changes when epinephrine is not present. The combination of injected ACTH and physical stress may have caused a more pronounced response and thus more cholesterol and LDL synthesis occurred.

If we examine all the results of the present study, a more complete scheme can be hypothesized in which various pathways are involved in altered cholesterol and lipoprotein levels (Fig. 2b). This kind of scheme is probably more realistic than one in which a single variable or pathway is operating. During the course of a stressful episode, a shift may result from one pathway to another with one of the pathways being dominant at any given time depending on the particular individual or the circumstance. Individuals having certain psychological characteristics, which make them more prone to coronary heart disease, could have more than one dominant pathway and consequently would increase their risk over the long term. Coupled with diet, heredity and lifestyle, this mechanism, if activated over a long period of time, would certainly hasten the process of atherosclerosis and would explain the propensity of some individuals to contract this disease early in life.
Fig. 26. Multiple pathway that may be operating during a stress response. One or more pathways may be dominant with shifts occurring at various times.
Since Selye proposed his General Adaptation Syndrome nearly 50 years ago, researchers have been trying to discover the nature of the pathogenic mechanism leading to cardiovascular disease. This study has established that stress and various stress hormones are involved in the increased plasma concentrations of cholesterol and LDL, and that B-endorphin seems to be operating as well in intensifying the reactions involved during a stress situation. The evidence for a multiple pathway rather than a single one is strong, but this does not preclude the possibility of a dominant pathway which operates during the greater portion of an individual's life and is the main source of excess cholesterol and LDL. Until the actual mechanism is identified, research efforts must be intensified and focused on the possibility of the existence of more than one pathway. Hopefully, this study will increase our understanding of the atherosclerotic mechanism and ultimately contribute to the elimination of this disease within the next decade.
SUMMARY AND CONCLUSIONS

During the course of this investigation, an interesting pattern had emerged in which the onset of stress or the injection of stress hormones (epinephrine and corticosterone) increased serum cholesterol and LDL levels. Furthermore, it was observed that the stimulatory action of B-endorphin was diminished by injecting rats with naloxone. Both laboratory animals and human subjects had similar reactions to stressful situations, but it now appears that emotional stress had a more positive effect in altering cholesterol and lipoprotein levels than did physical stress alone. In summary:

1. Daily stress over a period of 2 months continually increased both serum total cholesterol and LDL levels in rats while not affecting HDL or triglyceride levels. The group that was subjected to both physical and emotional stress had the highest values of cholesterol and LDL. Students undergoing the stress of exams or dissertation defense had total cholesterol, LDL, and LDL/HDL levels higher than they did during periods of non-stress.

2. Rats that were injected daily with epinephrine in oil had much higher cholesterol and LDL levels but similar HDL levels than did rats injected with either norepinephrine or oil alone. When the
treatments were reversed, the effects were also reversed accordingly.

3. Injection with naloxone (B-endorphin blocker) or dichloroisoproterenol (B-adrenergic blocker) caused an inhibition of plasma cholesterol and LDL concentrations. The group injected with naloxone had a much greater inhibition than the group injected with dichloroisoproterenol which was still significant compared with controls.

4. Injection with naloxone plus epinephrine produced an increase in cholesterol and LDL levels although not as great as when epinephrine alone was injected.

5. Adrenalectomized rats (given supplementary injections of corticosterone) had cholesterol and LDL levels as high as sham-operated rats when both groups were stressed following a 10-day recovery period.

This study has established that stress is a principle factor in altering cholesterol and LDL synthesis either through a direct pathway or an indirect pathway involving the action of B-endorphin. Since epinephrine is released during periods of stress, and B-endorphin enhances adrenal medullary secretion of epinephrine, a dual pathway may be involved in which the plasma concentration of epinephrine is increased. Moreover, because B-endorphins are not easily degraded, they would remain in the circulatory system longer
and continue their stimulatory action on the adrenal medulla even after the originally released epinephrine has been eliminated. In this manner, an individual undergoing stress over a long period of time would be subject to cholesterol and LDL increases due to being subjected to epinephrine for a longer period of time.

This study established that stressed rats, which were adrenalectomized and given supplementary injections of corticosterone, had increased levels of cholesterol and LDL as well. The data suggest that there was either a shift in the pathway with B-endorphin affecting cholesterol levels independently, or that corticosterone somehow caused an increase in cholesterol and LDL through a separate mechanism. In either case, there seems to be a multiple pathway rather than a single one that increases or intensifies cholesterol synthesis during a stress response.

Although we now know that stress and stress hormones affect cholesterol levels, we still do not know by what mechanism they mediate this action. I propose that the mode of action could be receptor interference in which cellular uptake of LDL is inhibited due to the presence of epinephrine. The interference may be the result of changes in membrane permeability caused by cAMP, direct interference caused by the epinephrine molecule itself, or indirect interference due to the glucagon-like effect of epinephrine in which a large volume of glucose is produced and causes
the cell to take up the glucose instead of the LDL molecules. A more detailed examination is needed in order to determine whether or not the LDL receptor is prone to interference activity or if the stress response produces so much additional endogenous LDL that cellular uptake is unable to keep up with the amount produced.

Without question, the stress response may be acting not only to insure the short-term survivability of an organism under severe conditions, but in the long-term may also be initiating a panhogenic response leading to atherosclerosis. Certainly other factors, such as diet and heredity, would contribute to the general susceptibility of an individual to this disease, but stress could be the link in discovering the main pathways and mechanisms involved. Thus far, the search for a cure has been illusive simply because the pathway through which the atherosclerotic mechanism operates has not been identified. Perhaps now is the time to turn our attention to behavior and psychological well-being in order to find an answer. Further investigations into the role of stress in altering cholesterol levels may very well carry us closer to that answer and bring to an end another chapter in man's long history of disease.
LITERATURE CITED


Appendix A

[Diagram showing a circuit with labels:
- 6V
- 6V
- 273-050 PRI 117V60CY
- SEC 6.3V 1.2 AMP
- To Tail
- To Cage]
Appendix B

QUESTIONNAIRE

Subject Name ____________________________
Address ________________________________
Tel. # ______________ Ext. ______
Age ______ Ht. ______ Wt. ________

1. What type of exercise program are you currently on? (Include the number of times you actually exercise) ______

2. Do you smoke? ______ How much? ______

3. Do you take medication for any reason? ________________

4. Do you consume alcohol? ______ How much? ________________

5. Do you have any illnesses at present? ________________

6. Will you be changing your lifestyle or exercise program between now and your next blood test? ________________

7. Other than your comprehensives or defense, will you be subject to any unusual stress? ________________

8. Does anyone in your family have:
   Heart Disease ______
   High Blood Pressure ______
### Appendix C

<table>
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<tr>
<th>Animal</th>
<th>Plasma Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baboon</td>
<td>116</td>
</tr>
<tr>
<td>Calf</td>
<td>80</td>
</tr>
<tr>
<td>Dog</td>
<td>123</td>
</tr>
<tr>
<td>Monkey</td>
<td></td>
</tr>
<tr>
<td>Rhesus</td>
<td>140</td>
</tr>
<tr>
<td>Squirrel</td>
<td>200</td>
</tr>
<tr>
<td>Pig</td>
<td>90</td>
</tr>
<tr>
<td>Rabbit</td>
<td>20</td>
</tr>
<tr>
<td>Rat</td>
<td>40</td>
</tr>
</tbody>
</table>

-Average cholesterol values for some select laboratory animals. Adapted from Sabine, Cholesterol. 1977.
### Appendix D

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Avg. Cholesterol (Males)</th>
<th>Avg. Cholesterol (Females)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>155±2</td>
<td>156±2</td>
</tr>
<tr>
<td>5-9</td>
<td>160±1</td>
<td>163±1</td>
</tr>
<tr>
<td>10-14</td>
<td>158±1</td>
<td>160±1</td>
</tr>
<tr>
<td>15-19</td>
<td>150±1</td>
<td>158±1</td>
</tr>
<tr>
<td>20-24</td>
<td>167±1</td>
<td>172±1</td>
</tr>
<tr>
<td>25-29</td>
<td>182±1</td>
<td>176±1</td>
</tr>
<tr>
<td>30-34</td>
<td>192±1</td>
<td>179±1</td>
</tr>
<tr>
<td>35-39</td>
<td>201±1</td>
<td>186±1</td>
</tr>
<tr>
<td>40-44</td>
<td>206±1</td>
<td>195±1</td>
</tr>
<tr>
<td>45-49</td>
<td>212±1</td>
<td>204±1</td>
</tr>
<tr>
<td>50-54</td>
<td>213±1</td>
<td>218±1</td>
</tr>
<tr>
<td>55-59</td>
<td>214±1</td>
<td>226±1</td>
</tr>
<tr>
<td>60-64</td>
<td>213±1</td>
<td>229±1</td>
</tr>
<tr>
<td>65-69</td>
<td>213±1</td>
<td>230±1</td>
</tr>
<tr>
<td>70+</td>
<td>207±1</td>
<td>226±1</td>
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</tbody>
</table>

Appendix D, continued

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Avg. LDL (Males)</th>
<th>Avg. LDL (Females)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td>91±3</td>
<td>100±2</td>
</tr>
<tr>
<td>10-14</td>
<td>93±4</td>
<td>97±3</td>
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<tr>
<td>15-19</td>
<td>94±3</td>
<td>94±4</td>
</tr>
<tr>
<td>20-24</td>
<td>105±4</td>
<td>103±4</td>
</tr>
<tr>
<td>25-29</td>
<td>115±4</td>
<td>114±4</td>
</tr>
<tr>
<td>30-34</td>
<td>124±5</td>
<td>114±5</td>
</tr>
<tr>
<td>35-39</td>
<td>132±5</td>
<td>118±5</td>
</tr>
<tr>
<td>40-44</td>
<td>137±4</td>
<td>123±6</td>
</tr>
<tr>
<td>45-49</td>
<td>145±5</td>
<td>129±4</td>
</tr>
<tr>
<td>50-54</td>
<td>146±4</td>
<td>138±6</td>
</tr>
<tr>
<td>55-59</td>
<td>149±6</td>
<td>147±5</td>
</tr>
<tr>
<td>60-64</td>
<td>152±6</td>
<td>154±6</td>
</tr>
<tr>
<td>65-69</td>
<td>150±5</td>
<td>157±7</td>
</tr>
<tr>
<td>70+</td>
<td>142±4</td>
<td>148±4</td>
</tr>
</tbody>
</table>

VITA

Andrew G. Goliszek
Candidate for the Degree of
Doctor of Philosophy

Dissertation: Effects of Physical and Emotional Stress, Catecholamines and Naloxone on HDL and LDL Cholesterol Levels in Rats and Man

Major Field: Biology

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