Circulatory, Hormonal, and Metabolic Effects of
Arbutamine Compared to Exercise in Persons
With Known or Suspected Coronary Artery Disease

by

Karen Toft Dorn

Dissertation submitted to the faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
Educational Research and Evaluation

APPROVED:

William G. Herbert, Chair

Jimmie C. Fortune

F. C. Gwazdauskas

Marion F. Ehrich

J. Edwin Wilder

October, 1994

Blacksburg, VA

Circulatory, Hormonal, and Metabolic Effects of Arbutamine Compared to Exercise in Persons With Known or Suspected Coronary Artery Disease

by

Karen LaVonne Toft Dorn

Committee Chairman: William G. Herbert
Exercise Science

(ABSTRACT)

The purpose of this study was to test the hypothesis that arbutamine, a specific $B_1$-adrenergic agonist, will not cause different circulatory and physiologic effects than the less specific endogenous catecholamines released in response to an exercise stress test in persons with known or suspected coronary artery disease. Nine male subjects, mean age 66 years, completed symptom-limited arbutamine (ESA) and exercise (ETT) stress tests in a randomized cross-over study. The ESA delivery device controlled infusion rate to induce a graded heart rate increase of 8 $bt \cdot min^{-1}$. Heart rate, systolic blood pressure, diastolic blood pressure, rate pressure product, ST segment shift, and specimens for epinephrine, norepinephrine, dopamine, cortisol, insulin, glucagon, glucose, free fatty acids, glycerol, and lactate were collected at baseline, immediate post-stress, and 10, 30, and 60 minutes post-stress. The research hypothesis was rejected. Repeated measures analysis of variance for each measure demonstrated a significant ($p \leq .05$) time·treatment interaction in heart rate, systolic blood pressure, rate

pressure product, insulin, glucagon, glycerol, free fatty acid, and lactate responses and a significant time effect for cortisol response. Circulatory differences included higher systolic blood pressure and rate pressure product responses for ETT than ESA and a more rapid recovery of circulatory variables following ETT. Metabolic differences were due to higher free fatty acid and glycerol responses for ESA than ETT and a slower recovery of these metabolites and lactate following ESA. Hormonal differences included an earlier and greater magnitude rise in insulin response for ESA than ETT. There were no differences ($p \leq .05$) by treatment, time, or time·treatment interaction for diastolic blood pressure, ST segment shift, catecholamines, or glucose. In conclusion, arbutamine caused different circulatory and physiologic effects consistent with differences in adrenergic receptor activity. Arbutamine caused substantial $B_{1+2}$ agonist effects on hormonal and metabolic responses in cardiotonic doses employed clinically for diagnostic stress testing and may impact clinical interpretation of stress test results.

ACKNOWLEDGEMENTS

I thank the members of my committee for their guidance and support in my educational endeavor. I thank my advisor, Dr. William G. Herbert, for his academic advisement, his partnering with me in the Gensia project, and for his friendship. I thank my co-advisor, Dr. Jimmie Fortune for his expertise in statistics and research design and also for his encouragement beginning with the first course of my doctoral studies. I thank the members of my committee who contributed expert instruction and advisement in physiology, pharmacology, and cardiology. The members are: Drs. Frank Gwazdauskas, Marion Ehrich, and Edwin Wilder. I thank Dr. Doug Southard for his contribution to the dissertation defense and for his support thoughout my doctoral studies.

A special thank you to Laura Craft and Jeff Ocel for your assistance with data collection, for a home away from home, and most of all for listening. I thank Dr. Pete Brubaker for assistance with data collection at Wake Forest and Scott VanGeluwe for assistance at Virginia Tech.

To my husband, Paul. Together we share the joys, sorrows, and many lessons life has to offer.

To my children, Jesse, David, Tim, and Katie. I wish for you a spirit of inquiry.

TABLE OF TABLES

Table I. General Effects of Catecholamines on Tissue Receptors and Responses . . . . . 20

Table II. Chemical Structures of Catecholamines Employed Pharmacologically as Cardiotonic Agents . . . 22

Table III. Relative Adrenergic Receptor Binding Characteristics of Arbutamine, Dobutamine, and Isoproterenol . . . . . 25

TABLE OF FIGURES


Figure 1. Heart rate responses of arbutamine compared to exercise . . . . . 97

Figure 2. Systolic and diastolic blood pressure responses of arbutamine compared to exercise . . . . . 98

Figure 3. Rate pressure product responses of arbutamine compared to exercise . . . . . 99

Figure 4. Glucose responses of arbutamine compared to exercise . . . . . 100

Figure 5. Insulin responses of arbutamine compared to exercise . . . . . 101

Figure 6. Glucagon responses of arbutamine compared to exercise . . . . . 102

Figure 7. Free fatty acid responses of arbutamine compared to exercise . . . . 103

Figure 8. Glycerol responses of arbutamine compared to exercise . . . . . 104

Figure 9. Cortisol responses of arbutamine compared to exercise . . . . . 105

Figure 10. Epinephrine responses of arbutamine compared to exercise . . . . . 106

Figure 11. Norepinephrine responses of arbutamine compared to exercise . . . . . 107

Figure 12. Dopamine responses of arbutamine compared to exercise . . . . . 108

Figure 13. Lactate responses of arbutamine compared to exercise . . . . . 109

TABLE OF CONTENTS

Acknowledgements . . . . . iv
Table of Tables . . . . . v
Table of Figures . . . . . vi

I. Introduction . . . . . 1

II. Review of the Literature . . . . . 17
General Properties of Catecholamines . . . . . 18
Structure-Activity Relationship . . . . . 19
Preclinical Pharmacology of Arbutamine . . . . . 24
Catecholamines and Dynamic Exercise . . . . . 26
Effects of Age and Disease . . . . . 31
Catecholamine Stress Testing . . . . . 32
Historical Development . . . . . 32
Mechanisms of Ischemia . . . . . 35
Infusion Studies . . . . . 40
Arbutamine and Myocardial Ischemia . . . . . 52
Hormonal and Metabolic Effects . . . . . 54
Summary . . . . . 63

III. Results: Journal Manuscript . . . . . 65
Introduction . . . . . 69
Methods . . . . . 71
Results . . . . . 79
Discussion . . . . . 83
References . . . . . 91

IV. Summary and Conclusions . . . . . 110
Recommendations for Future Research . . . . . 115
References Cited . . . . . 119

Appendices
A. List of Abbreviations . . . . . 129
B. The Informed Consent Document . . . . . 132
C. Data Collection Worksheet . . . . . 147
D. Detailed Methodology . . . . . 152
E. Raw Data . . . . . 173
F. Summary ANOVA Tables . . . . . 178

VITA . . . . . 191

# Chapter I

## INTRODUCTION

Coronary artery disease remains the leading cause of mortality in the United States. There are approximately 1.5 million new cases of coronary artery disease diagnosed each year (American Heart Association, 1991). Exercise stress testing is the most widely used, noninvasive screening test for the diagnosis of coronary artery disease (ACC/AHA Guidelines for Exercise Testing, 1986). Exercise stress testing is also used to determine functional capacity in persons with known disease, to evaluate the efficacy of medical and surgical interventions, and to determine prognosis. Unfortunately, many persons are unable to undergo exercise stress testing because of noncardiac concommitant health problems such as arthritis, chronic airway disease, paralysis, or peripheral vascular disease. Over the past decade, pharmacologic stress testing has emerged as a practical alternative to exercise stress testing in persons unable to exercise (Martin, Seaworth, Johns, Pupa, & Condos, 1992).

Exercise induced stress results in integrated autonomic nervous and hormonal system responses which, in turn, accomplishes the necessary circulatory and metabolic adjustments needed to increase blood flow to active muscle and to mobilize fuels to meet the metabolic needs of active

muscle. Endogenous catecholamines, norepinephrine and epinephrine, play a major role in effecting these circulatory and metabolic responses to the physiologic stress imposed by dynamic exercise (Bunt, 1986).

The circulatory effects of catecholamines released in response to the physiologic stress of exercise include increased heart rate, stroke volume, contractility and altered peripheral resistance, thus increasing muscle blood flow or driving pressure (Stone, Dormer, Foreman, Thies, & Blair, 1985). The cardiotonic effects of catecholamines are mediated through stimulation of $B_1$-adrenergic receptors in cardiac muscle. The increased cardiac demand that occurs during dynamic exercise largely accounts for the efficacy of exercise stress testing in the diagnosis of coronary artery disease (Ellestad, 1986).

The metabolic effects of catecholamines released during exercise are due to either the direct effects on effector cells or the indirect effects on hormone intermediaries of metabolism (MacDonald, Bennett, & Fellows, 1985). Direct effects due to adrenergic receptor stimulation include lipolysis, ketogenesis, glycogenolysis, gluconeogenesis, proteolysis, and mineral metabolism. Lipolysis in adipose tissue and glycogenolysis in muscle and liver cells is attributed largely to $B_1$- and $B_2$-adrenergic stimulation.

The indirect effects of catecholamines are mediated

through hormones such as insulin, glucagon, cortisol and growth hormone. Mechanisms of catecholamine control of these hormones are complex and can involve direct sympathetic inhibition or release, catecholamine-induced altered tissue sensitivity to the hormone, or hormone release or inhibition due to changes in metabolite concentration (Woods & Porte, 1974). In addition, cardiovascular responses to catecholamines can alter turnover of metabolites or hormones through perfusion changes which occur during dynamic exercise (MacDonald, Bennet, & Fellow, 1985).

Administration of exogenous catecholamines results in adrenergic receptor stimulation and physiologic effects which are similar to the effects of endogenous catecholamines. Properties of exogenous catecholamines which are known to affect cardiovascular and metabolic response patterns include receptor specificity, relative potency, infusion rate and duration, and half-life of the agent in the body (Tepperman & Tepperman, 1987). Consequently, selective pharmacologic agents have been developed primarily to induce specific circulatory, and to a lesser extent, metabolic effects for therapeutic and diagnostic purposes.

A classic example of applied pharmacology is the development of dobutamine by Tuttle and Mills (1975). In

this case, the isoproterenol molecule was systematically modified for use in persons with heart failure to optimize inotropic properties, but minimize effects on heart rate and peripheral vasodilation. Due to the need for alternatives to exercise stress testing, the use of dobutamine has been extended to mimic exercise stress in the diagnosis of coronary artery disease (Mason, Palac, Freeman, Viruppanavar, Loeb, Kaplan, & Gunnar, 1984).

Exercise simulating agents are pharmacologic agents that mimic the effects of endogenous catecholamines to produce acute and adaptive effects of exercise for diagnostic and therapeutic purposes (Tuttle, 1993). Currently *B*-agonists, namely dobutamine, are being used as pharmacologic stressors in the diagnosis of coronary artery disease in persons who cannot exercise, as well as for echocardiographic and imaging procedures in which bodily movements associated with muscular exercise makes imaging procedures technically difficult (Stratmann & Kennedy, 1989).

Applications of dobutamine as a pharmacologic conditioner compared to exercise training has been examined as well. In dog (Liang, Tuttle, Hood, & Gavras, 1979) and rat studies (Davidson, Banerjee, & Liang, 1986), daily dobutamine infusions in doses that mimic exercise produced conditioning effects on the circulation and in skeletal

muscle similar to exercise. Applications of dobutamine as a pharmacologic conditioner in humans have also been explored. Success has been reported in reversing the deconditioning effects of bedrest in healthy people (Sullivan, Binkley, Unverferth, Ren, Boudoulas, Bashore, Merola, & Leier, 1985) as well as in improving functional capacity in persons with heart failure (Leier & Unverferth, 1982). It has been hypothesized that cardiovascular conditioning effects in patients with heart failure occur due to upregulation of *B*-adrenergic receptors leading to an enhanced inotropic response to endogenous catecholamines (Coats & Adamopoulos, 1991).

Arbutamine, a new synthetic catecholamine, is currently under investigation as a provacative pharmacologic stress agent for detection of coronary artery disease (Gensia, 1994). Arbutamine, a potent $B_1$-adrenergic agonist, is a positive chronotropic and inotropic agent. Arbutamine is of particular interest since it is the first pharmacologic agent specifically designed to mimic the cardiotonic effects of endogenous catecholamines released during high-intensity exercise lasting ten to twenty minutes. Arbutamine has been labeled an exercise simulating agent or ESA.

Statement of the Problem

Catecholamines, either released endogenously due to physiologic stress or by exogenous administration, activate

*a*- and *B*-adrenergic receptors to produce circulatory, hormonal, and metabolic effects. A specific clinical application of catecholamine physiology is provacative testing in the diagnosis of coronary artery disease. The intent of exercise stress testing or exogenous administration of a catecholamine is to activate *B*-adrenergic receptors, produce desired cardiovascular effects, and the desired diagnostic effect, namely, to expose a myocardial supply/demand imbalance. Observed differences in diagnostic and physiologic effects provide evidence of differences in adrenergic receptor specificity.

The adrenergic receptor specificity of arbutamine in humans has yet to be fully characterized. Early proprietary <u>in vitro</u> studies of arbutamine in guinea pig atria and trachea indicated that arbutamine had much greater specificity for $B_1$-adrenergic receptors than $B_2$- or *a*-adrenergic receptors (Gensia, 1992). However, subsequent proprietary <u>in vitro</u> studies suggest little *B*-adrenergic receptor selectivity as previously hypothesized (Gensia, 1994). For purposes of this study, it was assumed that arbutamine was a $B_1$-selective agonist. Characterization of the relative cardiac specificity, potency, and duration of effect of arbutamine is needed.

Arbutamine is similar to exercise in terms of identifying the severity, extent and location of cardiac

ischemia. Arbutamine, however, does so at a lower heart rate, systolic blood pressure, and rate pressure product (Ginzton, Appleton, Mohluddin, Pool, Robertson, Bach, Ismail, & Armstrong, 1993; Ismail, Sada, Shapiro, Cao, French, & Ginzton, 1992). It is likely that observed differences in physiologic effects between arbutamine and exercise stress are due to differences in *B*-adrenergic receptor stimulation. Comparative hormonal and metabolic responses of arbutamine and exercise have not been examined. It is not known whether the magnitude and time course of effects will differ between exercise and arbutamine. However, exercise represents a generalized physiologic stressor which induces wide spread sympathetic nervous system activation while arbutamine represents a more specific *B*-adrenergic stressor.

Significance of the Study

Physiologic research of catecholamines is conducted to understand the nature of adrenergic receptor activation and resultant physiologic effects; circulatory, hormonal, and metabolic. Typically, physiologic studies are conducted with healthy subjects and employ protocols that do not simulate dosing schedules employed clinically. Thus, generalizability of findings to clinical applications is limited. Clinical research is aimed at determination of diagnostic or therapeutic efficacy and is usually limited to

description of selective physiologic effects relative to the therapeutic or diagnostic intent in the clinical target population. There is a need to bridge these two areas of research.

Many catecholamines are employed clinically due to their cardiovascular efficacy. However, metabolic effects of catecholamines have been documented since the early twentieth century (Griffith, 1951). These latter effects occur at cardiotonic doses (Clutter, Bier, Shah, & Cryer, 1980), and become non-selective effects when the catecholamine is employed clinically as a cardiotonic agent. Non-selective effects are of interest as these effects may alert the clinician to possible adverse reactions or side effects. Further, the presence of hormonal and metabolic effects could contribute to future drug development relative to the therapeutic potential of catecholamines as pharmacologic conditioners. Finally, when employed as cardiac stimulants, non-selective effects may potentially counfound clinical interpretation of clinical findings.

Examples of the clinical performance of cardiotonic agents being confounded by non-selective effects have been documented. In the critically ill patient, dobutamine therapy is associated with increased oxygen consumption. This phenomenon is believed to be a result of correction of an oxygen debt as a result of improved perfusion and, thus,

is referred to as oxygen uptake/supply dependency. A rival hypothesis is that the increased oxygen consumption observed with dobutamine therapy is, at least in part, attributable to catecholamine-induced thermogenesis (Vincent, Roman, Backer, & Kahn, 1990). Persistent post-infusion tachycardia is also observed with catecholamines and has been linked to positive thermogenesis (Fellows, Bennet, & MacDonald, 1985).

Clinical trials with arbutamine have been designed to determine the diagnostic efficacy of the agent as a pharmacologic stressor. The hormonal and metabolic effects of arbutamine have not been characterized, although it is likely that metabolic effects do occur and are similar to the effects of other *B*-adrenergic agonists. It is particularly important to characterize the hormonal and metabolic effects of arbutamine in the clinical target population since non-selective effects could have implications for the clinical performance of its use in pharmacologic stress testing. Further, the extent to which these effects do simulate exercise in the clinical target population could potentially lead to the investigation of arbutamine as a pharmacologic conditioning agent and establish the relative *B*-adrenergic specificity of arbutamine.

Research Hypothesis

$H_0$: Arbutamine, a specific $B_1$-adrenergic agonist, will

not cause different metabolic and physiologic effects than the less specific endogenous catecholamines released in response to an exercise stress test in patients with known or suspected coronary artery disease.

Assumptions

The following assumptions were made:

1. Arbutamine infusion and graded exercise stress are acute, high intensity physiologic stressors whose effects are mediated largely by adrenergic receptor stimulation and autonomic nervous system reflex control mechanisms.
2. A peak stress of 85 percent age-predicted maximal heart rate (220 - age x 0.85) is an equipotent diagnostic stressor for arbutamine and exercise.
3. Plasma concentrations of hormones and metabolites reflect pooled tissue levels of these substances.
4. Arbutamine infusion results in physiologically active plasma levels of the drug.
5. No changes in physical training status occurs between the first and second stress tests.

Limitations

1. The sample size of nine subjects limits generalizability to all similar subjects with known or suspected coronary artery disease.
2. Findings are not generalizable to women with known or

suspected coronary disease or healthy people.

3. Due to technical difficulties, microhematocrit results were not to reliable, therefore, adjustments to metabolite concentrations based on changes in plasma volume between treatments or across time could not be made.

4. Different instruments were used in measuring blood pressures and, on occasion, electrocardiographic recordings. Blood pressures were obtained using an electronic blood pressure device in the arbutamine test as this was a part of the ESA System while a cuff sphygmomanometer, a stethoscope, and the manual method of auscultation was used during the exercise trial to determine Korotkoff sounds. In two subjects ECG tracings were obtained from systems which did not report computer averaged ST segment change. However, all computer-averaged ST segment reports were validated manually so it is unlikely that this instrumentation change affected results.

5. The arbutamine trial required two intravenous lines, one for arbutamine infusion and the other for blood sampling. This difference could have induced additional stress on the subjects and different volumes of sodium chloride may have been

infused. The volume of sodium chloride delivered during each trial was not recorded.

6. The design was quasi-experimental; therefore inferences related to causation are tentative.

Delimitations

The following delimitations are present in this study:

1. Metabolic and physiologic effects were limited to a few specific indicators or cardiovascular response, lipid metabolism, glucose metabolism, sympathetic stimulation, and anaerobic metabolism.
2. Subjects were selected from a group of clinically stable patients who had known or suspected coronary artery disease; this was a group representative of the population that would undergo functional or prognostic stress testing.
3. The endpoint for stress intensity was 85 percent age-predicted maximal heart rate.
4. Data were collected at baseline for control purposes and at 0, 5 (cardiovascular only) 10, 30 and 60 minute recovery times to determine treatment effects.
5. Subjects served as their own control within each treatment in an open-label, repeated measures, randomized cross-over design.
6. The subjects were volunteers who participated in two

university-based cardiac rehabilitation programs.

## Definitions of Terms and Symbols

### Indicators or Cardiovascular Response

Heart rate (HR) - the number of heart beats per minute ($bt \cdot min^{-1}$) determined from the R-R interval on the electrocardiogram.

Systolic blood pressure (SBP) - the maximum pressure in millimeters of mercury (mmHg) in the arterial system.

Diastolic blood pressure (DBP) - the minimum pressure in millimeters of mercury (mmHg) in the arterial system.

Rate pressure product (RPP) - the product of heart rate and systolic aortic pressure used as a relative index of myocardial oxygen demand and left ventricular blood flow (Rowell, 1993)

ST segment shift (ST) - deviation of the ST segment in millivolts (mv) from isoelectric recorded 60 milliseconds (msec) from the J-junction and used as a measure of myocardial ischemia (Ribisl, Lui, Mousa, Herbert, Miranda, Froning, & Froelicher, 1993).

### Indicators of Lipid metabolism

Free fatty acids (FFA) - a major source of fat fuel produced by the breakdown of triglyceride

into one glycerol molecule and three fatty acids; measured as serum concentration in milliequivalents per liter ($mEq \cdot l^{-1}$) of non-esterified free fatty acids.

Glycerol (GLY) - a source of fat fuel produced by the breakdown of triglyceride; measured as serum concentration in milligrams per deciliter ($mg \cdot dl^{-1}$).

Indicators of Glucose Metabolism

Glucose (GLU) - the major source of carbohydrate fuel; measured as plasma concentration in milligrams per deciliter ($mg \cdot dl^{-1}$).

Insulin (INS) - a major regulatory hormone produced in the beta islet cells of the pancreas and essential in facilitating uptake and storage of glucose by cells and maintaining normal blood glucose levels; measured as plasma concentration in micro-International Units per milliliter ($uIU \cdot ml^{-1}$).

Glucagon (GCG) - a major regulatory hormone and counter-regulatory hormone to insulin produced in the alpha islet cells of the pancreas and essential for mobilization of stored carbohydrate; prevents hypoglycemia and maintains blood glucose levels; measured as plasma concentration in

picograms per liter ($pg \cdot ml^{-1}$).

Anaerobic metabolite

Lactate - a metabolite of anaerobic glycolysis in muscle and certain other tissues; measured as plasma concentration in millimoles per liter ($mmol \cdot l^{-1}$).

Indicators of sympathetic stimulation

Epinephrine (EPI) - the major sympathetic hormone produced and released by the adrenal medulla; measured as plasma concentration in picograms per milliliter ($pg \cdot ml^{-1}$).

Norepinephrine (NE) - the major sympathetic neurotransmitter produced and released by the post-ganglionic sympathetic neurons; also acts as a sympathetic hormone produced and released by the adrenal medulla or due to spillover from nerve terminals; measured as plasma concentration in picograms per milliliter ($pg \cdot ml^{-1}$).

Dopamine (DOP) - a sympathetic catecholamine released from peripheral sympathetic nerves and from the adrenal medulla; measured as plasma concentration in picogram per milliliter ($pg \cdot ml^{-1}$).

Indicator of Generalized Physiologic Stress

Cortisol (COR) - a regulatory hormone produced and

released from the adrenal cortex in response to adrenocorticotropic hormone (ACTH) stimulation from the pituitary gland; essential to fluid and electrolyte balance and fuel regulation during stress; measured as plasma concentration in micrograms per deciliter ($ug \cdot dl^{-1}$).

# Chapter II

## REVIEW OF THE LITERATURE

Today the exercise stress test is the major noninvasive screening approach for the detection of coronary artery disease. Only recently has pharmacologic stress testing emerged as a significant diagnostic alternative for the evaluation of coronary artery disease. Both exercise and catecholamine stress tests are designed to induce or mimic sympathetic nervous system arousal to increase cardiac demand and thereby expose a myocardial supply/demand imbalance through *B*-adrenergic mechanisms. Isoproterenol, epinephrine, dopamine, and dobutamine have been compared to exercise as pharmacologic stressors in persons with known or suspected coronary artery disease (Stratmann & Kennedy, 1989). Arbutamine is a new agent developed specifically as an exercise simulating agent in the diagnosis of coronary artery disease (Ginzton et al., 1993). In addition to cardiotonic effects, catecholamines, released endogenously during exercise or by exogenous administration, exert substantial hormonal and metabolic effects (Clutter et al., 1980).

The circulatory, hormonal and metabolic effects of catecholamines have important implications for the emerging use of catecholamines as exercise simulating agents. Little

has been documented on the hormonal and metabolic effects of catecholamines in protocols employed in pharmacologic stress testing or on the comparative effects of exercise and catecholamine stress in the clinical target population. The purpose of this review is threefold: a) to examine the comparative physiologic and pharmacologic properties of catecholamines relevant to exercise and pharmacologic stress testing; b) to review the historical development and current use of catecholamine infusions as employed in the diagnosis of coronary artery disease, with emphasis on adrenergic mechanism by which these agents produce myocardial ischemia; and c) to examine catecholamine infusion studies which characterize the physiologic, hormonal, and metabolic effects of these agents, and the adrenergic mechanisms by which these responses occur.

General Properties of Catecholamines

Catecholamines are the initial physiologic defense to maintain homeostasis during stress states. Sympathetic nervous system activation, mediated by the neurotransmitter norepinephrine predominates in cold and exercise stress. Norepinephrine is also activated in hypotension while epinephrine secretion from the adrenal medulla predominates in hypoxia, anxiety states, and hypoglycemia (Tepperman & Tepperman, 1987). The role of dopamine is less clear in stress states; however, dopamine does increase with

sympathetic arousal (Amenta, 1990).

The mechanism of action of catecholamines is due to activation of membrane bound receptors, induction of intracellular second messenger systems, and subsequent molecular and physiologic responses (Gillman, Rael, Nies, & Taylor, 1990). These responses are dependent upon general properties of catecholamines such as the receptor type and receptor distribution in the target tissue. General properties of catecholamines relevant to this review are outlined in Table I. It is recognized that the effects of catecholamines on certain metabolic processes such as glucose metabolism are complex, may involve different mechanisms at rest as opposed to stress states, and involve redundant control mechanisms (Woods & Porte, 1974).

Structure-Activity Relationship

Pharmacologic properties of catecholamines such as relative receptor selectivity influence physiologic responses and can be explained, in part, by the structure-activity relationship. Table II illustrates the chemical structures of catecholamines employed clinically as cardiotonic agents and discussed in this review. Catecholamines are chemical compounds with hydroxy (-OH) substituents on the benzene ring of *B*-phenylethylamine, the parent compound (Gillman et al., 1990). The greatest sympathomimetic activity occurs when two carbons separate

Table I. General Effects of Catecholamines on Tissue Receptors and Responses.

| Receptor Type | Target Tissue | Physiologic Response & Relative Magnitude |
|---|---|---|
| $B_1$ | heart | increase heart rate ++<br>increase automaticity, conduction velocity, and contractility+++ |
| | fat cells | lipolysis+++ |
| $B_2$ | arterioles | dilation<br>coronary++<br>skeletal muscle++<br>abdominal viscera+ |
| | veins | dilation++ |
| | pancreas<br>beta islets | secretion of insulin+ |
| | skeletal muscle | glycogenolysis<br>increase in contractility |
| | liver | glycogenolysis and gluconeogenesis+++ |
| $a_1$ | arterioles | constriction<br>coronary+<br>skin and mucosa+++<br>skeletal muscle++<br>abdominal viscera+++ |
| | veins | constriction++ |
| | fat cells | inhibit lipolysis |
| | liver | gluconeogenesis and glycogenolysis+++ |
| $a_2$ | presynaptic nerve terminals | inhibit release of norepinephrine |
| | pancreas *B* islets | inhibit release of insulin+++ |

(Gillman et al., 1990; Kjaer, 1992)

the catechol ring from the amino group and when the -OH groups are in the three and four positions of the benzene ring. These chemical properties are true of all of the catecholamines which have been examined as pharmacologic stress agents.

Increasing the size of the amino substituent increases *B*-adrenergic activity and decreases *a*-adrenergic activity. To illustrate, norepinephrine is a primary amine and has little $B_2$-adrenergic activity. Adding a methyl group to the amine, as with epinephrine, greatly increases $B_2$-adrenergic activity. Isoproterenol has an isopropyl substituent and high $B_1$- and $B_2$-adrenergic selectivity. This helps explain the well-known receptor selectivity relationships between isoproterenol (ISO), epinephrine (EPI), and norepinephrine (NEPI) as illustrated:

| | |
|---|---|
| $B_1$ | ISO > EPI = NEPI |
| $B_2$ | ISO > EPI >> NEPI |
| $a_1$ | EPI $\geq$ NEPI >> ISO |
| $a_2$ | EPI $\geq$ NEPI >> ISO |

Dobutamine and arbutamine also have large amino substituents and predominant *B*-adrenergic activity.

Epinephrine, even though it is a potent vasoconstrictor, shows greater sensitivity to $B_2$-receptors than $a_1$-adrenergic receptors, thus diastolic blood pressure often falls due to $B_2$ dilation in skeletal muscle beds,

Table II. Chemical Structures of Catecholamines Employed Pharmacolgically as Cardiotonic Agents.

| | Chemical Structure | | | |
|---|---|---|---|---|
| | Aromatic Ring | *B* | *a* | Amine |
| Catecholamine | | CH | CH | NH |
| Dopamine | 3-OH, 4-OH | H | H | H |
| Norepinephrine | 3-OH, 4-OH | OH | H | H |
| Epinephrine | 3-OH, 4-OH | OH | H | $CH_3$ |
| Isoproterenol | 3-OH, 4-OH | OH | H | $CH(CH_3)$ |
| Dobutamine | 3-OH, 4-OH | H | H | $CH(CH_3)-CH(CH_2)_2-$  |
| Arbutamine[1] | 3-OH, 4-OH | OH | H | $CH_2(CH_2)_3-$  |

(Gilman et al., 1990; [1]Young et al., 1994)

Substitution of a methyl group on the alpha carbon (second carbon from the benzene ring) increases duration of action due to the inability of monoamine oxidase to degrade methyl groups (Gillman et al., 1990). None of the catecholamines used in pharmacologic stress testing have an alpha substituent, a beneficial property since rapid degradation and short duration of action allows easy reversibility by simply stopping the infusion.

Substitution of an -OH group on the beta carbon (first carbon from the benzene ring) is associated with increased potency of the catecholamine (Gillman et al., 1990). Arbutamine, epinephrine, norepinephrine, and isoproterenol

Substitution of an -OH group on the beta carbon (first carbon from the benzene ring) is associated with increased potency of the catecholamine (Gillman et al., 1990). Arbutamine, epinephrine, norepinephrine, and isoproterenol all have this property and are potent catecholamines. The absence of the hydroxyl group is also associated with decreased chronotropic activity (Tuttle & Mills, 1975). Dopamine and dobutamine do not have an -OH group at the beta carbon, are less potent than the other catecholamines, and have less chronotropic activity, a desirable property for stress testing in the diagnosis of coronary artery disease.

Isomerism also influences biologic activity (Gillman et al., 1990). For example, the l-isomer of the beta carbon greatly increases *a*- and *B*-adrenergic peripheral effects as opposed to central effects and is the naturally occurring isomer of epinephrine and norepinephrine. Dobutamine is a racemic mixture (i.e., optically inactive due to the presence of two isomers which negate the optical activity of the other in mixture) and this property results in complex pharmacology. To illustrate, each isomer of dobutamine exerts equal *a*-adrenergic agonist and antagonist effects, thus the observed effects are negated. For *B*-adrenergic receptors, both isomers are agonists but one is much more potent than the other. Additionally, dobutamine exerts greater $B_1$- than $B_2$-adrenergic selectivity, thus its

classification as a $B_1$-agonist.

Preclinical Pharmacology of Arbutamine

Young and associates (1994) have reported preclinical pharmacology of arbutamine relatative to receptor binding and relative adrenergic receptor activity. Table III summarizes the relative receptor binding characteristics of arbutamine, dobutamine, and isoproterenol. Receptor binding studies in isolated membranes suggest that arbutamine has similar affinity for $B_1$- and $B_2$-adrenoceptors and less, yet substantial, affinity for $a_1$-adrenoceptors. In vitro studies in atria ($B_1$) and trachea of guinea pigs ($B_2$) and rabbit aorta ($a_1$) support the receptor binding data. Arbutamine increased spontaneous atria rate and relaxed tracheal rings at similar concentrations and contracted aortic rings at a concentration 25 to 30 fold the concentration for *B*-adrenergic activation. The relative *B*-agonist selectivity of arbutamine is consistent with the large amino substituent in its chemical structure.

Preclinical data is consistent with other structure-activity relationships of catecholamines as described above. Dobutamine is approximately 1,000 fold less potent than arbutamine and isoproterenol (Young et al., 1994). The greater potency of arbutamine compared to dobutamine is consistent with the presence of an hydroxyl group on the beta carbon of arbutamine. Arbutamine, isoproterenol, and

dobutamine show rapid recovery of heart rate following cessation of infusion (Young et al., 1994). Short duration of action is consistent with the absence of a methyl group on the alpha carbon of these catecholamines. Arbutamine is an enantomerically pure, d-isomer, and is, therefore, not expected to exhibit the complex pharmacology of dobutamine relative to agonist and antagonist adrenergic activity.

Table III. Relative Adrenergic Receptor Binding Characteristics of Arbutamine, Dobutamine, and Isoproterenol.

| Receptor Type | Relative Receptor Potency |
|---|---|
| $B_1$ | ISO > ARB > DOB |
| $B_2$ | ISO > ARB > DOB |
| $a_1$ | DOB > ARB >> ISO |
| $a_2$ | DOB = ARB > ISO |

Young et al. (1994).

Young et al. (1994) examined the comparative hemodynamic effects of arbutamine, dobutamine, and isoproterenol in dogs. Arbutamine infusion resulted in dose-dependent increases in heart rate and contractility similar to isoproterenol. Arbutamine caused little change in mean arterial pressure, unlike isoproterenol in which mean arterial pressure fell due to peripheral vasodilation. Arbutamine and isoproterenol had less inotropic activity than dobutamine at equal increments in heart rate. Clinical

studies, such as the present one, are needed to further characterize the pharmacologic properties of arbutamine in humans.

Catecholamines and Dynamic Exercise

At rest, plasma norepinephrine levels are three to four times greater than epinephrine levels (Tepperman & Tepperman, 1987). The higher norepinephrine level is attributed to tonic sympathetic activation at rest and the resultant spillover of norepinephrine from nerve terminals. Although epinephrine and norepinephrine are released in a ratio of 4:1 from the adrenal medulla, at rest the output is small so that epinephrine levels remain lower. During acute bouts of exercise, the ratio of norepinephrine to epinephrine remains similar to that seen at rest. However, as exercise duration increases this ratio decreases as the role of the adrenal medulla and epinephrine secretion increases under sustained stress (Mazzeo, 1991). Plasma dopamine levels follow the same trends as do the other catecholamines in response to stress states and comprise approximately 20 percent of total catecholamine levels (Amenta, 1990). It is important to note that plasma catecholamine levels give an indication of whole organism sympathetic stimulation but not the extent and contribution of individual tissues (Mazzeo, 1991). Additionally, plasma levels reflect the sum of turnover rate, synthesis and

removal, and activity of key enzymes related to catecholamine metabolism.

Through adrenergic receptor mechanisms, endogenous catecholamines, norepinephrine and epinephrine, coordinate and regulate key circulatory and metabolic functions during exercise. Catecholamines increase in response to exercise in an exponential manner with exercise intensity and linearly with exercise duration based on relative rather than absolute work rate. In addition, exercise training results in decreased catecholamine response at a given submaximal workload (Mazzeo, 1991).

Sympathetic stimulation of the heart and vasculature occurs to increase blood flow to working muscle. Through $B_1$-adrenergic mechanisms, heart rate and contractility increase with exercise intensity, both factors contributing to the rise in systolic blood pressure necessary to maintain perfusion pressure to active muscle. The vascular effects of catecholamines, mediated predominantly through $a_1$- , $a_2$- and $B_2$- adrenergic activation, serve to redistribute blood flow to metabolically active tissue. Vasoconstriction due to $a_1$-adrenergic stimulation occurs in all vascular beds including the heart, skin, skeletal muscle, brain, lung and abdominal viscera. Conversely, $B_2$ receptor stimulation results in vasodilation only in vascular beds with a significant $B_2$-receptor population such as the heart,

skeletal muscle and liver, i.e., metabolically active tissue. The dominant effect of epinephrine in the latter vascular beds is the $B_2$ vasodilatory effect rather than $a_1$ vasoconstriction, thus resulting in increased flow to active tissue during exercise (Rowell, 1993).

Acute hormonal responses to exercise are important for regulation of fuel and energy metabolism through changes in target tissue (e.g., muscle, fat, liver) permeability and changes in enzyme activity (Galbo, 1983). These effects are observed as increased lipolysis, glycogenolysis, or gluconeogenesis. Hormone responses which are inhibited during exercise are those which regulate lipogenesis and glucogenesis. Catecholamines, glucagon, and cortisol are included in the former group, while insulin is of the latter. Cortisol enhances lipolysis and gluconeogenesis but has a lag time in response to exercise, thus may be important in potentiating or maintaining free fatty acids and glucose levels during prolonged exercise.

Many of these metabolic effects of catecholamines are mediated through $B_2$ induced glycogenolysis in skeletal muscle and the liver through activation of glycogen phosphorylase and inhibition of gluconeogenesis in the liver through inactivation of glycogen synthase. Stimulation of $B_1$ and/or $B_3$ receptors in adipocytes results in activation of triglyceride lipase which breaks down triglyceride to

glycerol and free fatty acids (Miller, 1992). Lipolysis in fat tissue is enhanced by *B*-adrenergic response to catecholamines. Interesting, in humans *a*-adrenergic inhibition of lipolysis may be the major basal control mechanism during rest while, during exercise, lipolysis is stimulated by *B*-adrenergic receptor activation (Kjaer, 1992).

Lactate is greatly elevated during exercise and increases, as does glucose, on the same curve as catecholamines with exercise intensity and duration (Brooks, 1986). Lactate is a metabolite of glycogenolysis via the enzyme lactate dehydrogenase which increases, when oxygen availability in the mitochondria is reduced, thus reflecting anaerobic metabolism (Miller, 1992). However, an anaerobic condition in the muscle is not the sole determinant of lactate accumulation, since lactate, as with other substrates, reflects the rates of production and removal (Stainsby & Brooks, 1990). Lactate accumulation occurs under conditions such as during aerobic exercise and during catecholamine infusion (Brooks, 1986).

Substrate utilization is also affected by exercise. While free fatty acid uptake and utilization increases during exercise, free fatty acid uptake is a saturable phenomenon so that release and uptake are not proportional (Kjaer, 1992). Hepatic glucose production is increased

during exercise and follows the curve for catecholamine release relative to intensity. However, an increase in glucose does not proportionately increase glucose uptake by the muscle since epinephrine has a blunting effect on glucose uptake. Epinephrine is believed to enhance glycogenolysis in muscle, although muscle glucose output may also be due to gluconeogenesis from other substrates such as lactate (Kjaer, 1992).

Insulin decreases as duration and intensity of exercise increases, a response attributed to *a*-adrenergic inhibition of insulin secretion (Galbo, 1983; Kjaer, 1992). Since insulin increases fat and glycogen synthesis and lowers blood glucose levels, this response preserves euglycemia during exercise and promotes fat mobilization as an energy source. While insulin secretion is inhibited by $a_2$-adrenergic activation and enhanced by $B_2$-adrenergic stimulation, the dominant net effect of epinephrine is inhibition. Insulin, as with many physiologic systems, has redundant control mechanisms to assure tight physiologic control.

The role of catecholamines in glucagon secretion in man is not entirely clear. Glucagon secretion from the alpha pancreatic islet cells is stimulated by *B*-receptor activation, however, its greatest control mechanism appears to be plasma glucose concentration. Glucagon increases the

rate of glycogenolysis in response to decreased serum glucose levels. Glucagon secretion during exercise in humans is believed to be due to changes in plasma glucose or perhaps the ratio of insulin to glucagon (Galbo, 1983; Woods & Porte, 1974).

Catecholamines play a role in regulation of circulatory and metabolic effects not only through peripheral neural and hormonal mechanisms as described above, but also through central control mechanisms. Parasympathetic withdrawal and sympathetic activation from central neural mechanisms (central command) and from motor neural reflexes (exercise pressor reflex) are recognized as feedforward controls important to the regulation of cardiovascular responses to exercise (Mitchell, 1990). Feedforward control mechanisms initiate and provide immediate circulatory and ventilatory adjustments through autonomic efferents based on the type and intensity of muscle work as well as perception of effort (Mitchell, 1990). There is increasing evidence that feed-forward control also activates higher endocrine centers to effect the hormonal and metabolic adjustments to provide fuel to active muscle, particularly glycogenolysis in the liver and lipolysis in fat tissue (Kjaer, 1992).

Effects of Age and Disease on Catecholamines

Age and disease influence catecholamine response to exercise. Aging is associated with an increase in

catecholamine response to absolute and relative workloads. It has been hypothesized that this increase offsets the decreased receptor responsiveness to catecholamines (Galbo, 1983; Korkushko, Frolkis, Shatilo, & Yaroschenkno, 1990). Additionally, catecholamines are believed to play a role in the pathogenesis of diseases such as diabetes, asthma, essential hypertension, hypotensive states, coronary heart disease, hypoglycemia, chronic renal disease and chronic heart failure since catecholamines are elevated in these diseases (Cryer, 1980). In a comparative review, Cryer (1980), examined plasma norepinephrine and epinephrine levels in conditions at rest, stress states such as various levels of exercise, and disease states. Moderate to heavy exercise and hypoglycemia produce catecholamine responses similar to levels induced by many disease states. Dopamine is also increased in disease states such as hypertension, primary aldosteronism, and orthostatic hypotension (Amenta, 1990).

## Catecholamine Stress Testing

### Historical Development

In 1908, Einthoven published the first tracing of ST segment depression following exercise (Chaitman, 1986). However, the significance of these findings was not apparent until reports began to emerge of ST and T wave changes occurring during spontaneous angina episodes (Blousefield,

1918), experimental coronary ligation in dogs (Smith, 1918), and coronary occlusion in man (Pardee, 1920). Feil and Seigel (1928) hypothesized that these changes, if inducible, could be used diagnostically to identify persons with coronary disease. To test this hypothesis, these investigators conducted a series of provocative tests such as vagal massage, cold water drinking, and respiratory maneuvers but were unsuccessful in inducing ST segment changes. However, when employing exercise as the stressor, Wood and Wolferth (1931) were successful in inducing ST changes in persons with angina. This work was the first published account of an exercise stress test as a diagnostic tool in the diagnosis of cardiac disease.

The first use of epinephrine (Levine, Ernstene, & Jacobson, 1930; Katz, Hamburger, & Lev, 1932) as a diagnostic agent for angina pectoris was also being described around this time. Levine and associates (1930), pursued the use of epinephrine as a diagnostic agent based on a previous observation of a patient with a history of angina, that when given epinephrine subcutaneously for a bronchial asthma attack, developed severe angina. Using subcutaneous epinephrine, they were able to induce a typical angina episode in cardiac patients but not in control subjects, thus concluding that epinephrine held promise as a diagnostic tool for angina. Surprisingly, there were no ST

changes observed in the electrocardiogram (ECG) of these subjects.

Subsequently, Katz, Hamburger, and Lev (1932) examined the use of epinephrine to clarify differences reported in the ECG reported by Feil and Seigel (1928) but not observed by Levine et al. (1930). Katz et al. (1932) found that most, but not all, patients with angina exhibited the characteristic ST depression and T wave inversion. However, ECG tracings of normals also showed ST deviation toward isoelectric causing them to conclude that epinephrine was not reliable. An untoward reaction in one subject occurred in this study shedding doubt not only on the efficacy of the provocative epinephrine test, but also on its safety.

Consequently, exercise stress emerged as the leading screening test for coronary artery disease. Intense research was conducted over the next 50 years to develop standard exercise protocols, improve the ECG, and the diagnostic and prognostic value of the exercise stress test (Ellestad, 1986). However, by the 1970s there was a growing need to develop an alternative, noninvasive stress test for persons who could not achieve an adequate exercise stimulus and for use in imaging studies. Other tests such as the hypoxemia test, subcutaneous epinephrine, pitressin, and ergonovine had not gained widespread acceptance due to

safety concerns, side-effects, slow-reversibility or medical-legal issues. The clinical need coupled with the safe therapeutic use of catecholamine infusions in cardiac patients resulted in the reemergence of catecholamines as diagnostic agents. Isoproterenol, a *B*-agonist, was the first to be examined with promising results (Combs and Martin, 1974; Kuromoto, Matsushita, Mifune, Sakai, & Murakami, 1978; Wexler, Kuaity, & Simonson, 1971), thus opening the door for subsequent testing of catecholamines as pharmacologic stress agents in the diagnosis of coronary artery disease.

Mechanisms of Myocardial Ischemia

The diagnostic intent of exercise and catecholamine stress tests is to induce a myocardial supply/demand imbalance which would be reflected by symptoms of angina, ECG abnormalities, left ventricular dysfunction, or perfusion abnormalities (Ellestad, 1986). Key correlates of myocardial oxygen consumption or demand include heart rate, ventricular wall tension, and contractility, all of which increase with $B_1$-adrenergic stimulation. Ventricular wall tension is a function of both preload, the end-diastolic volume or stretch, and afterload, the pressure which must be generated during systole to overcome arterial vascular resistance. Dynamic exercise stress testing increases cardiac output and myocardial demand by placing a

predominantly greater volume stress on the heart than a pressure stress as does isometric exercise (Froelicher & Marcondes, 1989). The double product of heart rate and systolic blood pressure known as the rate pressure product, is often used as a reliable non-invasive determinant of myocardial oxygen consumption (Ellestad, 1986; Froelicher & Marcondes, 1989).

The major determinants of myocardial flow include coronary perfusion pressure and coronary flow distribution (Ellestad, 1986). Perfusion occurs during diastole, thus aortic diastolic pressure and diastolic time will affect coronary flow. As heart rate increases, diastolic time decreases disproportionately to the decrease in systolic time. This disproportionate decrease in diastolic time limits diastolic filling and coronary flow. Likewise, if diastolic blood pressure decreases, coronary flow will also decrease. Decreasing coronary vascular resistance though $B_2$-adrenergic vasodilation or due to local autoregulation offset these changes under normal conditions. During exercise, coronary flow may increase up to five times basal flow levels (Froelicher & Marcondes, 1989). However, in the presence of coronary artery disease, flow is limited through fixed lesions so that the distribution of flow becomes unequal and the ratio of flow from the epicardium to the endocardium decreases creating a supply/demand imbalance

(Ellestad, 1986).

Many of the determinants of myocardial oxygen supply and demand are similar for exercise stress and pharmacologic stress, but differences do exist and reflect mechanistic differences between the general sympathetic arousal which occurs during exercise and the more selective *B*-agonist stress imposed by catecholamine infusion. In a comparative review, Stratmann and Kennedy (1989) summarized the hemodynamic effects of exercise compared to *B*-agonists used as pharmacologic stressors in normals and in persons with coronary artery disease. In both populations, upright exercise has been shown to increase heart rate, stroke volume, systolic blood pressure, cardiac output and myocardial oxygen consumption. Diastolic blood pressure typically does not change in persons with coronary artery disease and may remain stable or may decrease somewhat in healthy people. Beta-agonists exhibit essentially the same hemodynamic profile as upright exercise with the exception of blood pressure responses. In general, systolic blood pressure may increase, although to a lesser extent than exercise, or may show no change in normals and in persons with cardiac disease. Diastolic blood pressure typically decreases.

Several invasive studies have examined the effect of various catecholamines on determinants of myocardial supply

and demand. Vasu and associates (Vasu, O'Keefe, Kapellakis, Vezeridis, Jacobs, Daggett, & Powell, 1978) compared the effect of epinephrine, isoproterenol, dopamine, norepinephrine, and dobutamine in low and high doses using a canine model to determine if there were differences in myocardial oxygen consumption independent of the hemodynamic correlates of myocardial oxygen consumption. The study was conducted in nonfailing hearts under conditions of controlled hemodynamics. When mean aortic pressure, heart rate, and cardiac output were controlled as constants, dobutamine resulted in higher myocardial oxygen consumption than epinephrine and isoproterenol at high doses and epinephrine produced larger increases in myocardial oxygen consumption than isoproterenol. In each case, the greater myocardial oxygen demand could be accounted for by a higher maximum left ventricular time/tension index. This increased velocity of contraction indicated that a greater chronotropic demand was the major determinant of differences in myocardial oxygen consumption among the catecholamines rather than differences in myocardial oxidative metabolism which could contribute to myocardial oxygen consumption must be similar.

Buckberg and Ross (1973) demonstrated in dogs that the increase in myocardial demand due to increases in heart rate and contractility is offset to some extent by $B_2$-adrenergic

coronary vasodilation. However, the shortened diastolic time coupled with a decreased diastolic blood pressure, limits any beneficial effect of sympathetic coronary vasodilation. Using the tension time index as a measure of demand and the diastolic pressure time index to represent supply, Buckberg and Ross demonstrated that the potential beneficial and harmful effects of isoproterenol were dose-dependent. At cardiotonic doses within a normal physiologic range, the increased demand was offset by increased flow in a homogenous manner such that the subendocardial: subepicardial flow ratio remained homogenous (0.97). At doses that produced cardiac stimulation near the upper physiologic limit for a given animal, the subendocardial: subepicardial flow ratio fell to 0.57 and myocardial performance began to decline. These investigators hypothesized that the decreased diastolic pressure had a greater impact on the flow maldistribution than did the shortened diastolic time. This hypothesis was further supported by observations in five of the six dogs, that as myocardial performance declined, aortic constriction occurred. While this increased afterload resulted in an increase in tension time index, there was a proportionately greater increase in diastolic pressure time index due to the increase in aortic diastolic pressure. Overall, this resulted in an absolute and relative improvement in

subendocardial flow and improved ventricular performance.

Dobutamine has been shown to produce regional myocardial perfusion abnormalities in humans. In a study by Meyer and associates (Meyer, Curry, Donsky, Twieg, Parkey, & Willerson, 1976), the effects of dobutamine on myocardial flow were studied in selected patients undergoing cardiac catheterization for chest pain. Perfusion studies on three patients with normal arteries and three patients with significant disease showed that dobutamine (8 $ug \cdot kg^{-1} \cdot min^{-1}$) increased regional flow and homogeneity of flow in persons without coronary artery disease while producing much smaller increases in flow in patients with coronary artery disease and possibly increasing the inhomogeneity of flow with increasing disease severity.

It appears as if one of the major differences between exercise and *B*-agonists in producing myocardial ischemia lies in the contribution of enhanced contractility as a component of myocardial demand with *B*-agonist stress. On the supply side, *B*-agonists may impair flow to a greater extent than exercise due to decreases in diastolic blood pressure (i.e., coronary sinus filling pressure) or due to greater maldistribution of flow caused by *B*-adrenergic coronary vasodilation.

Infusion Studies

Wexler (Wexler et al., 1971) studied the

electrocardiographic effects of low-dose isoprenaline infusion (1-2 $ug \cdot min^{-1}$) in patients with coronary heart disease compared to age-matched healthy subjects. The end-point of the test was a target heart rate of 130 $bt \cdot min^{-1}$. These investigators found significantly more ST depression and characteristic T wave changes such as lowering, inversion, or reversal of baseline inversion in patients with coronary heart disease compared with normals, thus establishing the diagnostic potential of catecholamine stress testing.

Combs and Martin (1974) extended the preliminary report of Wexler (Wexler et al., 1971) in a prospective study comparing the isoproterenol test as described by Wexler to a standard symptom-limited, maximal graded treadmill exercise test in persons with suspected coronary artery disease. The diagnostic efficacy of the isoproterenol test was found to be comparable to the exercise test in predicting coronary artery disease. However, for both exercise and isoproterenol tests, there was a high rate of false negative tests which was attributed to the inability to achieve adequate heart rates and the high number of persons with single vessel disease. Hemodynamic data other that average peak heart rates were not reported.

Kuramoto and associates (Kuramoto et al., 1978) conducted a retrospective study in which isoproterenol test

data, including hemodynamic data, were correlated with extent of coronary stenosis at autopsy. The isoproterenol test in this study consisted of a steady state infusion of 0.02 $ug \cdot kg^{-1} \cdot min^{-1}$ for five minutes with test endpoint being 0.5mm ST segment depression rather than a target heart rate. A positive isoproterenol test was positively correlated with severity of stenosis and number of vessels involved at autopsy. Additionally, the degree of ST depression increased with disease severity. Hemodynamic responses were also examined. In general, heart rate, cardiac index and systolic blood pressure increased with isoproterenol infusion, diastolic blood pressure decreased slightly and stroke index increased slightly. The increase in heart rate and cardiac index was significantly greater and total peripheral resistance index lower in persons with severe stenosis than in persons with mild stenosis or myocardial infarction. The authors attributed this finding to a hyper-responsiveness to beta-adrenergic stimulation in persons with more severe disease. However, this subgroup showed a lower resting heart rate and cardiac index at baseline.

Manca and associates (Manca, Bianchi, Bolognesi, Cucchini, & Visioli, 1979) compared, among other methods, graded exercise stress with isoproterenol and dopamine stress tests. Of note in this study was: a) there were different criteria among the stressors for maximal test

endpoints and b) an incremental dosing schedule was employed for pharmacologic stressing unlike previous test protocols. Dopamine was infusion started at 200 $ug \cdot min^{-1}$ (i.e., 2.5 $ug \cdot kg^{-1} \cdot min^{-1}$ in an 80 kg man) and increased by 200 $ug \cdot min^{-1}$ every three minutes to a systolic blood pressure response of at least 50% above rest values. The isoproterenol test started at 1 $ug \cdot min^{-1}$ with a 1 $ug \cdot min^{-1}$ increase every three minutes to a peak heart rate response of 130 $bt \cdot min^{-1}$, while exercise test end-point was 85% age-predicted heart rate. Dopamine, represented a pressor test, isoproterenol was an absolute chronotropic test, and exercise was an age-adjusted chronotropic test. The sensitivity of the exercise, isoproterenol and exercise tests were similar (88%, 82%, and 85% respectively). Specificity was similar for exercise and isoproterenol (66% and 70%, respectively) but poor for dopamine (41%). As found previously, the sensitivity of each test improved when compared to the number of vessels involved and the rate pressure product was significantly lower with the catecholamine tests than with exercise.

Hemodynamic effects were reported in this study (Manca et al., 1979). The heart rate responses with dopamine was only 76 $bt \cdot min^{-1}$ and, in spite of the highest systolic blood pressure response achieved with dopamine (191 mmHg), rate pressure product was the lowest ($147 \cdot 10^2$). Isoproterenol, on the other hand, achieved a heart rate response of 115

$bt \cdot min^{-1}$, a systolic blood pressure response of 157 mmHg and a rate pressure product of $181 \cdot 10^2$. Treadmill exercise resulted in the highest heart rate (125 $bt \cdot min^{-1}$) and rate pressure product ($225 \cdot 10^2$) with a systolic blood pressure response of 177 mmHg. Diastolic pressures were not reported. It was hypothesized that isoproterenol induced ischemic ST depression at a lower rate pressure product than did exercise due to either a greater inotropic effect or due to greater maldistribution of epicardial to endocardial coronary flow. It was concluded that dopamine was an inferior test for the diagnosis of coronary artery disease.

Fujita and coworkers (Fujita, Ajisaka, Matsumoto, Iida, Iida, Sugishita, Ito, & Takeda, 1986) studied the isoproterenol test with tow-dimensional echocardiography and compared it to other stress tests such as the exercise test and exercise radionuclide angiography. The isoproterenol dose rate was constant at 0.02 $ug \cdot kg^{-1} \cdot min^{-1}$ and the test termination criterion was angina pain or ST deviation of greater than 0.1mv. Sensitivity was similar for all tests at 71% with the exception of exercise ECG which achieved a sensitivity of 86%. Specificity was highest with exercise radionuclide angiography (100%), followed by isoproterenol two dimensional echocardiography (83%), exercise ECG (50%) and isoproterenol ECG (33%). Heart rates increased

significantly during the isoproterenol infusion to a peak average heart rate of 106 $bt \cdot min^{-1}$. Systolic blood pressure did not change from near 130 mmHg at rest, while diastolic blood pressure fell significantly eight mmHg to 69 mmHg. During exercise, all hemodynamics increased significantly with heart rate increased to 114 $bt \cdot min^{-1}$, systolic blood pressure increased to 166 mmHg pressure, and diastolic blood pressure increased 11 mmHg to 92 mmHg. This study, illustrated the effects of unopposed *B*-adrenergic vasodilation seen with isoproterenol but not with exercise.

Schecter and colleagues (Schecter, Wilson, & Yin-Suen, 1983) first re-examined epinephrine using a graded dosing schedule from .03 to .30 $ug \cdot kg^{-1} \cdot min^{-1}$ with five minutes at each dose. In this dose schedule, a predetermined hemodynamic endpoint was not set as had been done in previous studies, rather a symptom-limited graded test similar to the exercise stress test was employed. Epinephrine produced an increase in cardiac work due to a modest increase in heart rate to 86 $bt \cdot min^{-1}$, increase in systolic blood pressure to 158 $bt \cdot min^{-1}$min, and rate pressure product of $133 \cdot 10^2$, notably lower than attained during exercise. Diastolic blood pressure decreased. Contractility, determined by the ratio of rate-corrected pre-ejection period to left ventricular ejection time decreased in a dose-dependent manner. In addition,

myocardial supply, defined as the endocardial viability ratio, decreased as reflected by a shortened diastolic time and decreased diastolic blood pressure.

Subsequent to the investigation by Schechter et al. (1983), Ferrara and associates (Ferrara, Leosco, Longobardi, Abete, Papa, Vigorito, & Rengo, 1986) examined the use of epinephrine in conjunction with two-dimensional echocardiography and ECG monitoring. The dose rates were the same as those employed by Schechter et al. (1983) and, again, a dose-dependent increase in heart rate and systolic blood pressure occurred with an average peak rate pressure product (heart rate x systolic blood pressure) of $140 \cdot 10^2$, while diastolic blood pressure decreased. The mechanism of ischemia was attributed to the increase in myocardial demand due to the increasing heart rate, systolic blood pressure, and contractility coupled with a decreased supply due to a decrease in diastolic time and diastolic pressure. Two dimensional echocardiographic findings demonstrated increased asynergy and new wall motion abnormality as additional evidence of ischemia.

Dopamine was again examined (Weisenberg, Zawadowski, Gebhardt, Prato, Goddard, Nichol & Rechnitzer, 1985). As a pharmacologic stress agent compared to exercise stress, dopamine performed with less diagnostic accuracy than exercise in spite of protocol changes with atropine. Both

exercise and dopamine were symptom-limited maximal stress tests conducted 30 minutes apart with exercise first. These investigators evaluated dopamine RNA with exercise RNA and correlated the findings with coronary angiography. The dopamine protocol started at 2.5 $ug \cdot kg^{-1} \cdot min^{-1}$ and increased every five minutes by 2.5 $ug \cdot kg^{-1} \cdot min^{-1}$ to a maximal dose of 15 $ug \cdot kg^{-1} \cdot min^{-1}$ with images obtained at the end of each five minute stage. Dopamine has dopaminergic effects on the renal and intestinal vasculature at low dose rates of 1-2 $ug \cdot kg^{-1} \cdot min^{-1}$ and *B*-agonist effects at doses of 5-20 $ug \cdot kg^{-1} \cdot min^{-1}$. Dopamine also exerts *a*-adrenergic activity at doses above 10 $ug \cdot kg^{-1} \cdot min^{-1}$ (Kuhn, 1991). At higher dose rates, the marked systolic blood pressure response frequently causes a reflex bradycardia. In this study, in order to prevent reflex bradycardia with increasing systolic blood pressure and peripheral vascular resistance, pretreatment with atropine was used prior to the dopamine protocol.

Weisenberg et al. (1985) found similar peak heart rates, diastolic blood pressure responses, and ST changes for exercise and dopamine while systolic blood pressure and rate pressure product were higher for dopamine in persons found to have disease. In addition, dopamine caused a significant increase in maximal stress ejection fraction in spite of the higher afterload, while ejection fraction fell during exercise. Unlike the other catecholamines which are

able to induce ischemia at a lower rate pressure product than exercise, dopamine does not. The authors suggested that inotropic stress due to catecholamines released during exercise was greater than that induced by dopamine in spite of similar rate pressure products or that dissimilar myocardial metabolic effects occur with more efficient metabolic pathways in response to dopamine infusion.

In the above study protocol (Weisenberg et al., 1985), baseline differences between tests were observed. Systolic blood pressure and ejection fraction were lower before dopamine, thus, differences in ventricular volume could have contributed to differences in myocardial demand. Further, these baseline differences could not be accounted for by differences due to supine versus standing positions and, baseline plasma catecholamine levels were not different. It is possible that a loss of plasma volume due to exercise could have contributed to baseline differences since only thirty minutes were allowed between the exercise and dopamine test. An additional limitation of this study was that many patients remained on *B*-blocking agents.

Dobutamine is the most recent catecholamine to be examined as a diagnostic agent. One of the earliest accounts of dobutamine was a comparative study of dobutamine thallium scintigraphy to the exercise ECG (Mason et al., 1984). Dobutamine infusions started at 5 $ug \cdot kg^{-1} \cdot min^{-1}$ and

increased every five minutes by 5 $ug \cdot kg^{-1} \cdot min^{-1}$ up to 20 $ug \cdot kg^{-1} \cdot min^{-1}$. At doses of 2-10 $ug \cdot kg^{-1} \cdot min^{-1}$, dobutamine has primarily $B_1$- effects, mild $B_2$- and equal *a*-adrenergic effects (Kuhn, 1991). High dose dobutamine of 20-40 $ug \cdot kg^{-1} \cdot min^{-1}$ was used for pharmacologic stressing in order to achieve an adequate chronotropic response and pre-treatment with atropine is occasionally employed (Mazeika, Aleksander, & Oakley, 1992). A symptom-limited graded exercise treadmill test or supine bicycle ergometer was used as the exercise stress. Dobutamine thallium scintigraphy for detection of coronary flow abnormality was significantly more accurate in detecting myocardial ischemia than the exercise ECG although the dobutamine ECG alone was less accurate than exercise. Hemodynamic comparisons indicated a slightly lower rate pressure product for exercise with the average peak heart rate and systolic blood pressures 114 $bt \cdot min^{-1}$ and 170 mmHg for exercise and 123 $bt \cdot min^{-1}$ and 175 mmHg for dobutamine. However, the lower accuracy with the exercise ECG could have been due to an inadequate exercise stimulus since the 85% age-predicted heart rate in this sample (mean age of 59 years) should have been near 137 $bt \cdot min^{-1}$. Diastolic blood pressure was not reported, therefore differences in perfusion pressure cannot be inferred.

Dobutamine has been compared to exercise in post-MI

patients (Mannering, Cripps, Leech, Mehta, Valantine, Gilmour, & Bennett, 1988). A symptom limited Bruce protocol was compared to graded dobutamine infusion similar to the protocol previously described. Unlike the previous study (Manca et al., 1979), peak heart rate and thus rate pressure product were significantly higher for exercise versus dobutamine with no difference in peak systolic blood pressure. However, the maximum acceleration measured by doppler ultrasound in the ascending aorta was significantly higher with dobutamine, supporting the hypothesis that inotropic demand with dobutamine contributes more to myocardial demand than it does in exercise stress. Stroke volume was higher with dobutamine. However, this was attributed to positional changes (upright versus supine) since stroke volume within each test was not significantly different between rest and peak stress. ST depression between the two tests showed an 88% concordance and in all cases appeared in the same lead(s). Heart rate at the onset of ST depression was lower with dobutamine than exercise, but in both cases was greater as disease severity increased. In addition, the increase in systolic blood pressure was significantly less in patients with three vessel disease than in those with one or two diseased vessels unlike the findings with isoproterenol (Kuramoto et al., 1978). The dobutamine test gives clinically similar information as

exercise, perhaps with the advantage of technically easier echocardiographic readings than exercise.

Dobutamine has now gained widespread use as a useful diagnostic tool in persons who cannot exercise or in conjunction with imaging procedures that are technically more difficult with exercise. Recently, the experience with dobutamine stress echocardiography was reviewed (Mazeika et al., 1992). Dobutmamine protocols may use peak dose rates up to 40 $ug \cdot kg^{-1} \cdot min^{-1}$ using three to five minute stages or lower peak infusion rates (20 $ug \cdot kg^{-1} \cdot min^{-1}$ of longer durations of six to eight minutes per stage, and atropine is occasionally used if there is a failure to induce an adequate chronotropic response, particularly if *B*-blockers are not discontinued prior to testing. Withdrawal from *B*-blockers varies from not at all to up to 48 hours or, alternatively, up to four half-lives to account for differences in clearance times among individual drugs. In general, the dobutamine ECG has been found to have low sensitivity compared to the exercise ECG. However, the improved sensitivity and specificity attained when adding imaging techniques makes dobutamine echocardiography a useful diagnostic test and provides data regarding the location of ischemia as opposed to the stress ECG alone.

Hemodynamic data reported with dobutamine use were reviewed in this paper (Mazeika et al., 1992).

Comparatively, exercise stress produces a higher heart rate response but similar systolic blood pressure response as dobutamine stress, thus a higher rate pressure product is seen with exercise. No significant change in diastolic blood pressure has been observed with dobutamine unlike isoproterenol. This is not unexpected, however, since dobutamine has weak $B_2$-agonist properties (Mazeika et al., 1992). Data during recovery indicate that heart rate and systolic blood pressure responses return toward baseline more quickly following exercise.

Arbutamine and Detection of Coronary Artery Disease

Preclinical studies of arbutamine indicate that arbutamine is effective in detecting myocardial ischemia by producing wall motion abnormalities and coronary flow heterogeneity. In studies by Hammond and McKirnan (1992; 1994), pigs were made chronically ischemic by constriction of the left circumflex coronary artery and wall motion abnormalities were examined during exercise and arbutamine infusion. In this study, ventricular wall thickening was measured directly by sonomicrometers and myocardial blood flow was determined by labeled microspheres. Arbutamine was superior to dobutamine in differentiating ischemic (left circumflex artery bed) from control coronary artery beds (left anterior descending artery bed). Arbutamine infusion resulted in increased wall thickening in the control bed but

decreased wall thickening in the ischemic bed while dobutamine increased wall thickening similarly in both beds. Differences occured despite similar effects on endocardial to epicardial blood flow ratios and hemodynamic effects. In this study, arbutamine and dobutamine increased heart rate and contractility similarly. In a $^{201}$thallium study on dogs with coronary stenosis, arbutamine increased regional coronary flow through non-ischemic coronary beds but decreased flow through stenotic beds demonstrating its diagnostic potential in revealing perfusion defects due to coronary artery disease (Sinusas, Daher, & Shi, 1993).

In persons with coronary artery disease, arbutamine produced ischemia similar to the exercise stress electrocardiogram (Ginzton et al., 1993) and exercise stress echocardiography (Jaarsma & Sutherland, 1992; Ismail et al., 1992). Arbutamine does so at a lower rate pressure product than does exercise due to achievement of lower systolic blood pressures and heart rates than attained during exercise stress testing. It is likely that this finding is due to enhanced contractility similar to dobutamine and its ability to produce flow heterogeneity.

Animal and human studies demonstrate that arbutamine may be useful in detection of myocarial ischemia determined from characteristic changes in the electrocardiogram, decreased coronary flow detected by scintigraphic imaging,

and decreased wall motion observed by echocardiography in ischemic myocardium. In the present study, the comparative ability of arbutamine and exercise to provoke a latent myocardial supply/demand imbalance in a group of stable cardiac patients was examined. Characterization of circulatory differences between arbutamine and exercise which exist advance our present understanding of the possible mechanisms which contribute to the diagnostic efficacy of arbutamine in detection of coronary artery disease.

Hormonal and Metabolic Effects

Catecholamines induce hormonal and metabolic responses in a dose-dependent fashion. Several studies have examined the physiologic thresholds and metabolic clearance rates of epinephrine infusions relative to circulatory and metabolic actions in healthy adults (Clutter et al., 1983; Staten, Matthews, Cryer, & Bier, 1987; Sjöstrom, Schutz, Gudinchet, & Hegnell, 1983). Clutter et al. (1980) used sixty minute infusions at rates of 0.1, 0.5, 2.5, and 5.0 $ug \cdot min^{-1}$ in men and women to produce steady-state plasma epinephrine concentrations within the normal physiologic range of 24-1020 $pg \cdot ml^{-1}$. Plasma thresholds were lowest for heart rate, systolic blood pressure, and blood glycerol in order of sensitivity and occurring at plasma epinephrine levels of 50-125 $pg \cdot ml^{-1}$. At concentrations between 150 and

200 $pg \cdot ml^{-1}$, increases in glucose, lactate, *B*-hydroxybutyrate, and decreases in diastolic blood pressure were noted. Insulin response had the highest threshold showing a decrease at epinephrine levels greater than 400 $pg \cdot ml^{-1}$. Glucagon, growth hormone, cortisol, and alanine did not change. Finally, epinephrine accelerated its own metabolic clearance in healthy human subjects.

Similar findings were reported by Sjostrom and associates (Sjostrom et al., 1983). In this study, epinephrine was infused in women at 0.01, 0.03, and 0.1 $ug \cdot kg^{-1}$ fat free $mass \cdot min^{-1}$ at separate times. Estimated plasma epinephrine concentrations were calculated based on dose rate and half-life of epinephrine. Estimated physiologic thresholds for physiologic responses occurring between 90 to and 120 $pg \cdot ml^{-1}$ in order of sensitivity were systolic blood pressure, diastolic blood pressure, glycerol, free fatty acids, and metabolic rate. Heart rate and respiratory frequency increased around 140 $pg \cdot ml^{-1}$, while glucose and lactate changed above 160 $pg \cdot ml^{-1}$. Insulin responses were biphasic with an initial early decrease and a later and larger increase which continued to rise following cessation of infusion and which showed a lower threshold sensitivity than did the early rise. The greatest magnitude of change occurred with glycerol and free fatty acids, while the least change was observed in blood pressure responses.

In spite of a fixed infusion rate, free fatty acids, glycerol, and glucose levels decreased toward the end of each infusion. Plasma norepinephrine levels were increased 40 percent in the higher two infusion rates but these increases were not significant.

Staten and associates (1987) extended the findings of Sjostrom et al. (1983) by increasing the infusion duration to 240 minutes and at lower dose rates (0.1, 0.5, and 1.0 $ug \cdot min^{-1}$. Again, a dose-dependent increase in metabolic rate occurred with an increase in metabolic rate occurring around 90 $pg \cdot ml^{-1}$ and lipolysis occurring around 100 $pg \cdot ml^{-1}$. It was noted that these threshold rates correspond with physiologic concentrations of epinephrine in daily activity. A biphasic insulin (and C-peptide) response was noted with an insulin rise noted with low infusion rates. This early rise was attributed to *B*-stimulated release while the later decrease at the highest dose rate was attributed to *a*-adrenergic inhibition. This finding helps to clarify the insulin responses reported in the previous studies. In the study by Clutter et al. (1980), insulin likely decreased at the higher dose rates due to *a*-adrenergic stimulation since this effect was associated with a high physiologic threshold. The later rise in insulin in the study by Sjostrom et al. (1983) occurred in recovery and at a lower physiologic threshold than did the early rise and could have been due to

the unmasking of *B*-adrenergic effects as plasma epinephrine levels decreased.

Fellows, Bennet & MacDonald (1985) examined the effects of exogenous administration of epinephrine in physiologic doses (10 and 50 $ng \cdot kg^{-1} \cdot min^{-1}$) for 30 minutes on separate occasions. Heart rate and metabolic rate rose in a dose dependent fashion, systolic blood pressure rose only in response to the higher dose rate, and diastolic blood pressure decreased similarly for both 10 and 50 $ng \cdot kg^{-1} \cdot min^{-1}$ rates. Increased calf blood flow due to vasodilation was similar for both infusion rates. While blood glucose, glycerol, and lactate rose during the high dose rate, only glycerol increased at the lower infusion rate, a finding consistent with the physiologic threshold studies of epinephrine. Venous plasma norepinephrine increased. It was hypothesized that norepinephrine release was triggered by baroreceptor stimulation in response to the fall in mean blood pressure or mediated via pre-junctional $B_2$-adrenergic stimulation. Metabolic rate remained elevated for up to 30 minutes following administration of the high dose of epinephrine. The persistent recovery tachycardia that was observed was attributed to the increased metabolic rate since plasma catecholamine levels returned to baseline levels shortly after infusion cessation. The investigators concluded that infusion of epinephrine at physiologic doses

induces substantial metabolic and circulatory effects and that *B*-agonist activity is a likely mechanism of many of these effects.

A follow-up study was conducted to determine whether isoproterenol sustained metabolic rate as did epinephrine (Mansell, Fellows, Birmingham & MacDonald, 1988). In this study 5 and 15 $ng \cdot kg^{-1} \cdot min^{-1}$ of isoproterenol was infused for thirty minutes. Dose-dependent cardiovascular responses occurred with isoproterenol similar to those observed with epinephrine. There were no changes in plasma epinephrine or norepinephrine levels. Plasma insulin increased but there were no changes in blood glucose, thus supporting a direct *B*-agonist effect on insulin secretion rather than an increase due to increased blood glucose. Glycerol levels increased and were attributed to *B*-mediated lipolysis. There were no changes in glucose or lactate levels. In contrast to epinephrine infusion, metabolic rate and heart rate returned rapidly to resting levels. It was suggested that this difference in recovery may be due to differences in metabolism between the two catecholamines. Further, epinephrine may prolong its effect through presynaptic uptake and subsequent release which would stimulated norepinephrine secretion from pre-junctional $B_2$-adrenergic receptors, whereas, isoproterenol would not have a prolonged effect since it is not metabolized in this way.

Cobbald, Ginsburg, and Paton (1960) previously investigated the circulatory, respiratory and metabolic effects of isoproterenol at a dose rate of 0.1 $ug \cdot kg^{-1} \cdot min^{-1}$ for 30 minutes. The cardiovascular effects were as reviewed above; an increase in heart rate and systolic blood pressure, a decrease in diastolic blood pressure, a substantial increase in forearm and calf blood flow, and a small and transient increase in hand and foot blood flow. The metabolic effects included a sustained increase in the respiratory rate and depth and an increase in oxygen consumption. There was no change in lactate. Blood glucose increased, although not to the same extent as with epinephrine infusions.

As noted in the studies above, glucose response to catecholamine infusion varies. Sacca and colleagues (1980) compared the effects of epinephrine, norepinephrine and isoproterenol and found differential effects which could be attributed to differential adrenergic mechanisms; mixed *a*- and *B*-adrenergic stimulation with epinephrine, prevalent *a*-adrenergic effects with norepinephrine and prevalent *B*-adrenergic effects with isoproterenol. Equal doses of each drug were used (0.05 $ug \cdot kg^{-1} \cdot min^{-1}$). Changes in plasma glucose, insulin, glucagon, and glucose kinetics were examined. Epinephrine induced a hyperglycemia believed to be mediated by a marked, but transient stimulation of

hepatic glucose output followed by a sustained inhibition of glucose uptake and clearance. There was no change in glucagon, while a transient rise in insulin occurred. On the other hand, isoproterenol induced hyperglycemia due to a smaller but sustained glucose output in spite of increased insulin levels was associated with sustained glucose uptake unlike epinephrine. No change in glucagon occurred. Norepinephrine followed a similar, though less marked response, as did epinephrine for glucose output while no changes occurred in glucose uptake and clearance or on plasma insulin or glucagon concentrations. Based on these differences, it was concluded that glucose output with *B*-adrenergic stimulation is not mediated by glucagon and is not inhibited by insulin, while glucose uptake is mediated through *B*-adrenergic mechanisms.

Based on their previous work with epinephrine and norepinephrine and the observation that circulating dopamine rises similarly to the other catecholamines during stress, Pernett, Hammond, Blesa-Malpica, Burrin, Orskov, Alberti, and Johnston (1984) examined the metabolic effects of dopamine with and without somastatin. Somastatin inhibits intermediary metabolism through inhibition of growth hormone and insulin and the target tissues of these hormones. In this study, six normal males underwent a series of four infusions on separate occasions: a) dopamine at 1.5 and 3.0

$ug \cdot kg^{-1} \cdot min^{-1}$, b) dopamine with somastatin, c) somastatin alone, and d) sodium chloride as a control. Each infusion lasted two hours. Dopamine caused an early and sustained rise in plasma glucagon. This effect was believed to be independent of *a*-or *B*-adrenergic stimulation and due possibly to dopaminergic receptor activation of pancreatic alpha islet cells. Dopamine at the higher dose was associated with an early, small and transient rise in insulin and glucose. It was hypothesized that the insulin rose in response to elevated glucose and was independent of dopamine, although the investigators cited that dopamine had been previously shown to have a direct stimulatory effect on the beta islet cells of the pancreas. The hyperglycemic response was inhibited by somastatin indicating that the glucose is mediated through glucagon, particularly since insulin and growth hormone were also suppressed. Dopamine alone did not increase plasma nonesterified fatty acids, however, with somastatin, dopamine enhanced the lipolysis (non-esterified fatty acids) and ketogenesis (3-hydroxybutyrate) induced by the somastatin-induced insulin deficiency. Finally, dopamine infusions were associated with a small rise in norepinephrine levels.

Metabolic effects of dopamine infusion were also examined by Regan, Duckworth, Fairhurst, Maycock, Frayn, & Campbell (1990). In this study, subjects received

increasing doses of 2, 5, and 10 $ug \cdot kg^{-1} \cdot min^{-1}$ infusions of 45 minutes each over 135 minutes. Infusions at 2 $ug \cdot kg^{-1} \cdot min^{-1}$ had little metabolic effect. Dopamine at 5 $ug \cdot kg^{-1} \cdot min^{-1}$ resulted in increased norepinephrine and blood glucose concentrations. Plasma norepinephrine, oxygen consumption, blood glucose, free fatty acids and glycerol increased during the 10 $ug \cdot kg^{-1} \cdot min^{-1}$ infusion rate. Dopamine increased norepinephrine concentration by 230% during the 10 $ug \cdot kg^{-1} \cdot min^{-1}$ infusion rate and was attributed to dopamine-induced norepinephrine release from nerve terminals and subsequent spillover. No changes in blood lactate were observed at any dose rate. The rise in blood glucose in the dopamine study was attributed to possible hepatic glycogenolysis or norepinephrine inhibition of insulin release. Increased free fatty acids and glycerol demonstrated the lipolytic effects of catecholamines mediated via $B_1$-adrenoceptors. The lack of change in lactate levels with dopamine infusion indicated that no effect on muscle glycogen occurred. Since epinephrine infusion (mixed *B*-effect) resulted in an increase in blood lactate while dopamine infusion (predominant $B_1$-agonist effect) did not, it is possible that blood lactate accumulation can be influenced through $B_2$-adrenergic effects on skeletal muscle metabolism.

In a recent study by Green, Frazer, Underhill, Maycock,

Fairhurst, & Campbell (1992), the metabolic effects of dobutamine in normal man were examined. In this study, eight healthy male subjects received dobutamine infusions at 2, 5, and 10 $ug \cdot kg^{-1} \cdot min^{-1}$ dose rates in a design similar to the dopamine study reviewed above. In this study, norepinephrine levels were significantly lower during dobutamine infusion, epinephrine tended to decrease and there was no change in dopamine concentrations. The decrease in norepinephrine levels was attributed to baroreceptor stimulation in response to the marked $B_1$-induced increase in cardiac output and blood pressure. Additional findings attributed to the $B_1$-adrenoceptor stimulation included a rise in free fatty acids and glycerol concentrations, increased plasma insulin concentrations and oxygen consumption. The rise in insulin was associated with a fall in blood glucose. Glucagon was not measured. Overall, the lipolytic and thermogenic effects of dobutamine were greater than those seen with dopamine infusion.

Summary

Catecholamines released endogenously during exercise stress or by exogenous administration exert circulatory, metabolic, and hormonal responses through adrenergic receptor activation. In the diagnosis of coronary artery disease, exercise and catecholamine stress tests are designed to induce a myocardial supply/demand imbalance.

Differential effects in *B*-adrenergic stimulation of the heart, coronary vessels, and peripheral vasculature contribute to observed differences in the mechanisms by which exercise and catecholamine stress produce a myocardial supply/demand imbalance. In addition to the cardiotonic effects, catecholamine infusions produce hormonal and metabolic effects in cardiotonic doses. Many of the hormonal and metabolic effects are due to *B*-agonist receptor stimulation in skeletal muscle, the liver, adipose tissue, and the beta islets cells of the pancreas. While the cardiotonic effects of arbutamine have been described in clinical trials to determine its diagnostic efficacy, other physiologic effects have not been adequately described.

The present study was designed to characterize the circulatory, hormonal, and metabolic effects of arbutamine as employed clinically, thus bridging physiologic and clinical research. Additionally, the effects of arbutamine, a potent *B*-agonist stress agent, are compared to exercise stress, a generalized sympathetic stressor, to clarify differences in adrenergic receptor activation between the two stressors. This information will assist in evaluation of the clinical performance of arbutamine as an exercise simulating agent in the diagnosis of coronary artery disease.

# Chapter III

The results chapter is presented as a journal manuscript. A detailed description of the methodology is provided in Appendix D and summary ANOVA tables are presented in Appendix F.

---

Journal Manuscript

to

Medicine and Science in Sports and Exercise

# Circulatory, Hormonal, and Metabolic Effects of Arbutamine Compared to Exercise in Persons with Known or Suspected Coronary Artery Disease

Running Head: Effects of Arbutamine

Karen Toft Dorn

Laboratory for Exercise, Sport and Work Physiology

Division of Health and Physical Education

Virginia Polytechnic Institute and State University, Blacksburg, VA

Author responsible for correspondence and to whom correspondence should be addressed:

Karen Toft Dorn, Ph.D.
Assistant Professor
Augustana College
29th and Summitt
Sioux Falls, SD 57197
(605) 336-4721

ABSTRACT

The purpose of this study was to compare the circulatory, hormonal, and metabolic effects of arbutamine (ESA), a new synthetic beta-agonist stress agent, to exercise (ETT). Nine male patients, mean age 66 years, completed symptom-limited ESA and ETT (Naughton protocol) stress tests in a randomized cross-over study. The ESA delivery device controlled infusion rate to induce a graded heart rate increase of 8 $bt \cdot min^{-1}$. Heart rate, systolic blood pressure, diastolic blood pressure, rate pressure product, ST segment shift, and specimens for epinephrine, norepinephrine, dopamine, cortisol, insulin, glucagon, glucose, free fatty acids, glycerol, and lactate were collected at baseline, immediate post-stress, and 5 (cardiovascular only), 10, 30, and 60 minutes post-stress. Repeated measures analysis of variance demonstrated significant ($p \leq .05$) time·treatment interactions in heart rate, systolic blood pressure, rate pressure product, insulin, glucagon, glycerol, free fatty acids, and lactate responses, and a significant ($p \leq 0.05$) time effect for cortisol. Circulatory differences included higher systolic blood pressure and rate pressure product responses for ETT than ESA and a more rapid recovery of hemodynamic variables following ETT. Metabolic differences were due to higher free fatty acid and glycerol responses and a later rise in

lactate for ESA than ETT, and a slower recovery of these metabolites following ESA. Hormonal differences included an earlier and greater magnitude rise in insulin response for ESA than ETT. There were no significant differences by treatment, time, or time by treatment interactions for diastolic blood pressure, ST segment shift, catecholamines, or glucose responses. In conclusion, arbutamine caused different circulatory, hormonal, and metabolic effects than exercise stress consistent with differences in adrenergic receptor activation. Arbutamine caused substantial $B_{1+2}$ agonist effects on hormonal and metabolic responses in cardiotonic doses employed clinically for diagnostic stress testing and may impact clinical interpretation of stress test results.

Keywords: Arbutamine, Exercise Stress Testing, Hormones, Hemodynamics, Glucose Metabolism, Lactate Metabolism, Lipid Metabolism, Catecholamines, Cortisol, Coronary Artery Disease

INTRODUCTION

The efficacy of exercise stress testing in the diagnosis of coronary artery disease is due to the cardiotonic effects of endogenous catecholamines released during high intensity exercise. The increased demand for oxygen and fuel is satisfied through increased perfusion to active skeletal muscle; an effect closely coupled with increases in epinephrine and norepinephrine as exercise intensity increases [22]. In the diagnosis of coronary artery disease, the rising levels of catecholamines result in increased heart rate, stroke volume, systolic blood pressure, cardiac output, and myocardial oxygen consumption and, in the presence of significant coronary disease, will expose a myocardial supply/demand imbalance [5].

Exogenous catecholamines such as epinephrine [7,28], isoproterenol [9,16], dopamine [16,37] and dobutamine [19 21] have been used as exercise simulating agents in the diagnosis of coronary artery disease in persons who cannot exercise or in conjunction with imaging procedures. Dobutamine has emerged as the leading catecholamine in these stress applications [21]. In pharmacologic stress testing, *B*-agonists such as dobutamine are used to mimic the sympathetic nervous system arousal which occurs during an acute bout of exercise. However, it is known that the effects of catecholamines, either released endogenously

during exercise or by exogenous administration, are not limited to circulatory effects. Infusion studies in humans have reported that catecholamines such as epinephrine [6,30,31] isoproterenol [4,18], dobutamine [11] and dopamine [25] elicit significant hormonal and metabolic effects in doses employed clinically.

Arbutamine, a new synthetic catecholamine, is currently under investigation as a diagnostic agent for detection of coronary artery disease [10,13]. Arbutamine, a potent $B_1$-adrenergic agonist, is a positive chronotropic and inotropic agent. It was specifically designed as an exercise simulating agent (ESA) to mimic the cardiotonic effects of endogenous catecholamines released during high-intensity exercise lasting ten to twenty minutes. Due to its potency, arbutamine is being investigated with its delivery device. This delivery system is a computer controlled, "closed-loop" feedback system in which arbutamine is delivered by an infusion pump in a ramping dosage that is based upon achievement of a predetermined patient heart rate slope in beats per minute [14].

Clinical trials have shown that the effects of arbutamine infusion are similar to progressive dynamic exercise in identifying the severity, extent and location of myocardial ischemia, although at peak heart rate and systolic blood pressure levels that are lower compared to

exercise [10]. Differences in the *B*-selective myocardial stress between arbutamine and exercise may impact the comparative clinical safety and clinical interpretation of test results. Further, the hormonal and metabolic effects of catecholamines may influence clinical interpretation of hemodynamic responses [34]. The hormonal and metabolic effects of arbutamine have not been characterized. The comparative responses of arbutamine and exercise are of particular interest since arbutamine was specifically developed as an exercise simulating agent. The objective of this study was to examine cardiovascular, hormonal and metabolic differences between arbutamine and exercise under conditions of graded stress as each is employed in the clinical diagnosis of coronary artery disease.

METHODS

Subjects

Eleven subjects with known or suspected coronary artery disease and who had achieved at least 6 METs on a symptom limited exercise tolerance test (ETT) within 6 months of the study were recruited from two cardiac rehabilitation programs. One subject did not complete the trial, and one subsequently had diabetes mellitus and was excluded from data analyses. The nine remaining subjects completed medical histories and physical exams; individuals were excluded if there were electrocardiographic (ECG)

abnormalities that precluded ST segment analysis. Subjects were excluded from participation if there was a history of certain cardiovascular and or metabolic contraindications to exercise stress or catecholamine administration (e.g., heart failure, valve disease, hypokalemia, cerebral vascular accident, narrow angle glaucoma) or conditions that would interfere with hormonal and metabolic responses (e.g., diabetes mellitus and inability to be withdrawn from *B*-blocker therapy). The research protocol was approved by the institutional review board for research involving human subjects at each study site. All subjects gave written informed consent. A follow-up assessment was conducted to detect potential serious adverse effects of the protocol.

Study Design

After screening, subjects underwent a continuous progressive exercise and arbutamine stress test; the order of testing was randomized for each subject. These trials were administered a minimum of 20 hours and a maximum of 14 days apart. All tests were performed in the early morning and subjects were asked to report in a fasted state. Patients receiving *B*-blockers had these medication discontinued under physician supervision for 48 hours prior to each test, and placed on an alternate medication when indicated. No patients in the present study required removal from *B*-blocker medication. Standard techniques were

employed for ECG assessment in both tests using the Mason-Liker 12 lead recording systems. For the ESA System, additional electrodes were placed adjacent to the limb leads for the purpose of providing R wave frequency responses for use in regulating drug infusion via the ESA delivery device. An indwelling intravenous catheter was inserted in an antecubital vein for blood specimen collection. An additional catheter with minimal dead space was inserted in the opposite arm for arbutamine infusion during the ESA test. The patency of each catheter was maintained by an infusion of 0.9% sodium chloride. The blood pressure cuff was placed on the same arm as the specimen catheter. Blood pressure responses were measured by auscultation and arm cuff for the ETT. Blood pressures were obtained by trained technicians experienced in exercise stress testing and systolic and diastolic blood pressures were determined by the initial appearance to phase 4 Korotkoff sounds. An automatic blood pressure device as part of the ESA delivery device was used to obtain blood pressures during the ESA test. A minimum of 15 minutes of quiet supine rest was required following catheter insertion(s) before baseline blood specimens were collected and ECG and blood pressure values were recorded. Baseline measures were obtained in the supine posture and additionally, for the exercise trials, hemodynamic data were obtained in the standing

posture. During each stress test, heart rate, blood pressure, ECG, symptoms, and any significant signs, were monitored for both tests according to American College of Sports Medicine (ACSM) guidelines for exercise testing [1]. Continuous 12-lead ECG monitoring at 25 $mm \cdot sec^{-1}$ paper speed was conducted using the Mortara X-SCRIBE (Mortara Instrument, Inc, Milwaukee, WI) or Quinton 5000 (Quinton Instrument Co., Seattle WA) electrocardiographic monitoring systems. The endpoint of each test was achievement of 85% of age-predicted maximal HR, flat or downsloping ST segment displacement $\geq$ 0.2 mv, intolerable symptoms, or adverse events according to ACSM guidelines for exercise test termination [1]. Arbutmamine infusion was terminated for additional reasons such as attainment of the maximal allowable dose or failure of the heart rate to rise with increasing dose rates. The supervising physician determined test endpoint.

ESA The ESA test was performed with subjects in the supine position. The pre-determined heart rate per minute slope used for this study was 8 $bt \cdot min^{-1}$. The infusion started at 0.1 $ug \cdot kg^{-1} \cdot min^{-1}$ and was increased to achieve the desired heart rate per minute slope with a maximal allowable dose rate of 0.8 $ug \cdot kg^{-1} \cdot min^{-1}$ and a maximum allowable total dose of 10 $ug \cdot kg^{-1}$. During arbutamine infusion, the ESA device monitored heart rate frequency and blood pressure responses

and calculated delivery rate to assure safe delivery of the drug. In addition, at two minute intervals during the test, 12-lead ECG tracings and blood pressure responses were recorded. Following infusion, the intravenous catheter used for arbutamine infusion was clamped to assure no additional drug was delivered.

ETT The ETT was performed using a modified Naughton protocol [24]. The modified protocol allowed subjects with higher functional capacities to be advanced in the early stages so as to achieve a uniform stress duration in the two stress modes of 8 to 16 minutes. During the test, 12-lead ECG and BP were obtained every two minutes. Immediately following achievement of the test endpoint, the subject was rapidly assisted to a supine position for post-stress data collection.

Data Collection Procedures

Blood pressure, 12-lead ECG recordings, and blood samples were collected at baseline and during recovery times as follows: zero (0R), five (5R)(cardiovascular measures only), ten (10R), thirty (30R) and sixty (60R) minutes. Specimens for free fatty acids and glycerol were collected in serum tubes without additive. Samples for glucose and lactate were collected in plasma tubes containing sodium fluoride and potassium oxalate. Samples for catecholamines were drawn in EGTA plasma tubes with reduced glutathione.

Samples for cortisol, insulin and glucagon were collected in EDTA plasma tubes. Specimens were collected in chilled tubes and placed into a slurry of ice and water for the duration of the test with the exception of free fatty acids and glycerol which were obtained and allowed to clot at room temperature. The iced samples were centrifuged at 4°C for seven minutes at 2500 revolutions per minute (rpm). Free fatty acids and glycerol specimens were centrifuged at 25°C for seven minutes at 2500 rpm. Serum or plasma was transferred to freezer vials. Aprotinin was added to the vial for storage of glucagon. Catecholamines were stored in a -70°C freezer and all other samples were stored at -20°C for later batch analysis.

Data Analysis

Blood specimens. Cortisol, insulin, and glucagon were analyzed using radioimmunoassay kits ($^{125}$I Cortisol, $^{125}$I Insulin, and $^{125}$I Glucagon; ICN Biochemicals, Costa Mesa, CA). Inter-assay coefficients of variation for cortisol, insulin, and glucagon were 6.6%, 2.7%, and 1.6%. Catecholamines were determined by high performance liquid chromatography (HPLC) with post-column electrochemical detection [20]. Enzymatic colorimetric assays were used to analyze free fatty acids (WAKO NEFA C test kit, Wako Chemicals, Dallas, TX), glycerol (Triglyceride GPO-Trinder Procedure No. 337, Sigma Diagnostics, St. Louis, MO), and

glucose (Enzymatic Glucose Procedure No. 1070, Stanbio Laboratory, Inc., San Antonio, TX). Lactate was determined by enzymatic electrochemical detection using a 2300 YSA Lactate Analyzer (Yellow Springs Instruments, Yellow Springs, OH). Standards were used to verify metabolic assays and samples were run in duplicate and achieved a coefficient of variation of $\leq$ 5%.

Cardiovascular responses. Heart rate was determined from the 12-lead ECG using the R-R interval method. ST segment displacement was determined from the computer-averaged ST segment shift from baseline measured 60 msec after the J-point in lead $V_5$. The unfiltered tracings were also inspected visually to exclude the possibility of artifact that might introduce errors in computer signal processing of the ST segment [26].

Statistical Analysis

Possible carry-over effects due to the cross-over design were examined by testing for treatment differences at baseline and by testing the signficance of sequencing as a factor in the analysis of variance model statement. There were no significant differences ($p \leq 0.05$) at baseline between treatments nor did the sequence, sequence by treatment, or sequence by time effects contribute to the statistical model indicating there were no significant carry-over effects. A power analysis was done to determine

the probability of commiting a Type II error in concluding there were no baseline differences. Systolic blood pressure was used as the variable of power analysis. The probability that baseline differences existed in systolic blood pressure is approximately 0.97. Baseline measures and the sequence factor were excluded in the final analysis to increase power of the statistical test. The univariate procedure for repeated measures analysis of variance was performed using the SAS statistical package (SAS System for Linear Models, SAS Institute, Inc., Cary, NC). When the adjusted probability of the analysis of variance was significant (Greenhouse-Giezer adjusted alpha $\leq$ .05), the profiles of the time and treatment contrasts for each successive time interval were examined to determine where significant differences occurred ($p \leq .05$). Treatment differences at OR were also examined. The distribution of the residuals from the analysis of variance were tested for normality. Normal probability plots were constructed to examine the distributions for homogeneity of variance. Log transformations were performed to better approximate normality and homogeneity of the error distributions for epinephrine and glucose. The nonparametric Wilcoxin matched-pairs signed-ranks test was used to test for treatment differences in ST segment displacement.

RESULTS

The nine male subjects had an average age of 66.2 ± 10.8 (S.D.) years ranging from 49 to 80 years and mean weight of 83.1 ± 13.1 kg. Average peak stress intensity achieved in the ETT was 8.7 ± 2.7. METs with subjects' rating of perceived effort ranging from somewhat hard to very hard. The average peak dose rate of arbutamine was 0.43 ± 0.19 $ug \cdot kg^{-1} min^{-1}$. Peak stress HR did not differ between exercise (131.7 ± 15.0 $bt \cdot min^{-1}$) and arbutamine (123.8 ± 15.2 $bt \cdot min^{-1}$) nor did test duration (ETT, 15.2 ± 6.5 minutes; ESA, 14.0 ± 5.5 minutes). Subjects' cardiac histories included one or more of the following; coronary artery bypass surgery (n = 7), myocardial infarction (n = 5), and percutaneous transluminal coronary arteriography (n = 1). By history, subjects typically experienced no angina (n = 6) or a frequency of angina less than once per day (n = 3). No adverse reactions occurred as a result of the study protocol. Symptoms which occurred during the ESA test included sensations of pounding in the chest, racing heart beat, lightheadedness or dizziness, slight headache, arm heaviness, and dry mouth. One subject experienced chest and neck pain which was relieved by sublingual nitroglycerin. During the ETT subjects reported symptoms of dry mouth only. One subject experienced a dull ache in the chest which resolved spontaneously following test termination.

Cardiovascular Responses

There were significant ($p \leq .01$) time by treatment interactions for heart rate (HR), systolic blood pressure (SBP), and rate pressure product (RPP). Heart rate (Figure 1) did not differ between treatments at zero minutes recovery (0R), however, for each successive time interval through 30R, there were significant ($p \leq 0.05$) time and/or treatment effects in HR due to a greater magnitude decline for ETT than ESA. Between 30R and 60R, both treatments decreased significantly ($p \leq 0.05$) due to time but showed no treatment differences in the magnitude of decline. Systolic blood pressure (Figure 2) was higher ($p \leq .01$) at 0R for ETT (181 $\pm$ 14 mmHg) than ESA (142 $\pm$ 22 mmHg). For both treatments, SBP recovery was rapid with differences observed only between 0R and 5R during which ETT showed a more rapid return to baseline. Rate pressure product (Figure 3) was higher ($p \leq .01$) at 0R for ETT ($240 \cdot 10^2 \pm 53$) than ESA ($176 \cdot 10^2 \pm 29$) and the magnitude of decline was greater ($p \leq 0.05$) for ETT than ESA for each interval through 30R. There were no significant effects found for diastolic blood pressure (Figure 2) due to either treatment or time. There were no treatment differences in ST segment shift (not shown). However, ST segment shift of $\geq 0.1$ mv occurred in three patients during or following the ESA while there were no ST changes $\geq 0.1$ mv observed for the ETT.

Hormonal and Metabolic Responses

Glucose metabolism. Glucose responses (Figure 4) were not significantly different over time, by treatment, or by time by treatment interaction. However, insulin (INS) (Figure 5) and glucagon (GCG) (Figure 6) showed significant time by treatment interactions ($p \leq .01$ and .05, respectively). Insulin was significantly higher for ESA than ETT at 0R ($p \leq 0.05$). The magnitude of the INS response to ESA was greater and occurred earlier than for ETT resulting in a significant ($p \leq 0.05$) treatment effect between 0R and 10R. Insulin increased 70% from baseline levels for ESA with a peak response observed at 0R. For ETT, INS increased 46% above baseline levels which was not seen until 10R. Subsequently, the INS response was significant ($p \leq 0.05$) due to time only. There were no peak differences in GCG levels. However, during the interval between 0R and 10R there was a significant ($p \leq 0.05$) treatment effect as GCG decreased for ESA, but rose for ETT. Between 10R and 30R, GCG decreased significantly ($p \leq 0.05$) due to time for both treatments with no further significant effects in GCG response.

Lipid metabolism.

A time by treatment interaction ($p \leq .01$) was seen for free fatty acid (FFA) responses (Figure 7) due to a significantly higher magnitude response ($p \leq 0.01$) for ESA

than ETT at peak stress. Free fatty acids increased to 246% from baseline levels by 10R for ESA. For ETT, FFA levels did not increase until 10R and then rose 77% from baseline levels. The magnitude of decline of FFA following the peak response at 10R was significantly ($p \leq 0.05$) greater for ESA than ETT resulting in time and treatment effects through 60R. Also, there was a significant ($p \leq 0.05$) time by treatment interaction observed for GLY (Figure 8). Glycerol increased for both treatments and was significantly higher at 0R for ESA than ETT ($p \leq .05$). Glycerol continued to rise post-stress for both treatments to 140% above baseline levels for ESA and 78% above baseline for ETT. Subsequently, the change over time for each recovery interval was significant ($p \leq 0.05$) due to time but not treatment.

Physiologic Stress. There was a significant ($p \leq .01$) time effect seen in cortisol (COR) responses (Figure 9). The time effect was due to declining post-stress levels to below baseline levels.

Catecholamine Response. There were no differences catecholamine response (Figure 10-12) by time, treatment, or time by treatment interaction.

Lactic Acid Response. Although blood lactate response (Figure 13) did not reflect substantial accumulation of plasma lactate (HLa) for either treatment, there was a

significant time by treatment effect ($p \leq .01$). Although no significant peak differences occurred between ETT and ESA, the time course of HLa response did differ. Between 0R and 10R, significant ($p \leq 0.05$) treatment differences occurred as HLa continued to rise for ESA but began to decline for ETT. Subsequently, the recovery pattern was similar between ESA and ETT decreasing significantly over time for both treatments.

DISCUSSION

The results of this study demonstrate that pharmacologic stress testing with arbutamine, a potent $B_1$-receptor agonist, causes significantly different circulatory and metabolic responses compared to exercise stress testing in the same clinical subject group. In this group of stable cardiac patients, arbutamine increased heart rate similarly to exercise but did not increase systolic blood pressure or rate pressure product to the same extent as did exercise. Diastolic blood pressure responses and ECG evidence of ischemia did not differ between the two treatments. In addition, substantial hormonal and metabolic treatment differences were observed in insulin, free fatty acids, glycerol, and lactate responses.

Arbutamine has been shown to have similar diagnostic efficacy similar to exercise stress testing, although at a lower rate pressure product than seen with exercise [10].

The lower rate pressure product in our study was attributable to a lower systolic blood pressure. Epinephrine [7,28] and isoproterenol [9,16], when employed as pharmacologic stress agents, increased heart rate and systolic blood pressure to a lesser extent than did exercise. Dobutamine, on the other hand, exerted a higher systolic blood pressure response more similar to the response seen during exercise [17,21,23]. However, dobutamine does not increase heart rate to the same extent as exercise, and thus rate pressure product is lower. The similar heart rate response seen with arbutamine in the present study is likely due to the characteristics of the study sample; stable cardiac patients who are physically active.

Arbutamine, based on our findings, appears not to affect diastolic blood pressure. Dobutamine does not decrease diastolic blood pressure predominantly due to its weak $B_2$-adrenergic activity [33]. This is in contrast to the $B_2$-mediated vasodilation and resulting decrease in diastolic blood pressure observed with epinephrine [28] and isoproterenol [9]. The finding in our study that diastolic pressure response does not decrease, may suggest that arbutamine has greater $B_1$- versus $B_2$-adrenergic selectivity similar to dobutamine, or alternatively, that arbutamine may exhibit some $a_1$-receptor activity at peak dose rates.

The ability of *B*-agonists to induce myocardial ischemia at a lower rate pressure product than seen with exercise has been attributed to a greater myocardial demand imposed by increased $B_1$-agonist inotropism [17,29,34]. In addition, $B_2$-receptor vasodilation may contribute to reduced myocardial supply due to decreases in diastolic pressure which lowers coronary perfusion pressure, or coronary vasodilation which results in modified myocardial perfusion [3,23,28]. Arbutamine has efects similar to dobutamine on contractility and heterogeneity of coronary flow in animal models [12,29]. This finding is likely to contribute to the diagnostic efficacy of arbutamine and explain its ability to produce evidence of myocardial ischemia at a lower rate pressure product than exercise. Since diastolic blood pressure response did not decrease with arbutamine infusion, it is unlikely that changes in coronary sinus perfusion pressure contribute to its diagnostic efficacy.

Recovery of heart rate, systolic blood pressure and rate pressure product are slower for arbutamine stress than exercise stress. This finding is similar to reports of dobutamine compared to exercise [21]. It has been hypothesized that post-infusion tachycardia following cateholamine infusion may be due to the thermogenic effect of catecholamines [6] or due to metabolic clearance mechansisms involving presynaptic reuptake and subsequent

release [18].

Glucose levels remained stable under exercise and arbutamine stress despite substantial observed changes in hormones that regulate glucose, i.e., insulin and glucagon. This finding likely reflects effective regulatory systems for maintenance of blood glucose. However, catecholamines did not affect blood glucose response in a similar fashion. Epinephrine infusion normally results in an early rise in glucose levels followed by a sustained hyperglycemia, the former effect attributed to $B_2$-mediated glycogenolysis in the liver and the latter attributed to inhibition of glucose uptake [27,31]. Endogenous epinephrine released during dynamic exercise serves to maintain euglycemia through increased glucose production, inhibition of insulin secretion, and possibly, through decreased uptake at higher exercise intensities [15]. Conversely, isoproterenol has either no effect [18] or may cause a transient rise in blood glucose due to hepatic glucose output accompanied by uninhibited glucose uptake in peripheral tissues [27]. Dobutamine infusion has been found to decrease blood glucose [11]. The lack of change in blood glucose observed with arbutamine would suggest that arbutamine dose not inhibit glucose uptake as does epinephrine and that an increase in glucagon was necessary to prevent a fall in blood glucose.

The substantial early rise in insulin levels seen with

arbutamine is consistent with infusion studies of other catecholamines [11,18,31]. During graded epinephrine infusion in physiologic doses, Staten and colleagues [31] found that the insulin response was biphasic and dose-dependent. At low infusion rates, insulin levels rise, an effect attributed to direct $B_2$-mediated insulin secretion from the beta islet cells of the pancreas. With increasing dose rates, insulin levels decreased, an effect believed to be due to *a*-adrenergic inhibition. The marked increase in insulin seen with arbutamine would suggests that $B_2$-adrenergic stimulation of insulin secretion occurs with arbutamine. Insulin secretion is believed to be inhibited during exercise via $a_2$-adrenergic inhibition [15] which would explain the failure of insulin to rise during exercise.

The lipolytic effects seen with arbutamine are consistent with the known thermogenic effects of endogenous catecholamines due to $B_1$-adrenergic stimulation of fat cells [15]. Sjostrom and associates [30] found that lipolysis due to epinephrine infusion is large in magnitude and occurs at a low physiologic threshold. Isoproterenol [18] and dobutamine [11] also have lipolytic effects in doses employed clinically for cardiac stimulation. Since free fatty acid production and uptake increase with the duration of exercise [15], the lower magnitude responses in glycerol

and free fatty acids observed during exercise compared to arbutamine may be related to the short duration of exercise stress. Alternately, the lower levels observed may have been due to increased uptake of free fatty acid and glycerol into active muscle.

In the present study, lactate increased due to arbutamine infusion and exercise, although neither treatment resulted in substantial increases in plasma lactate levels. Glycogenolysis in contracting skeletal muscle does occur under aerobic conditions; a finding attributed to $B_2$-adrenergic activation of glycolytic enzymes by endogenous catecholamines [2]. Exogenous administration of epinephrine [31] and dobutamine [11] have been found to increase lactate levels, although isoproterenol has not shown an effect on lactate metabolism [4,18].

Exercise stress testing generally would result in substantial anaerobiosis but was not seen in the present study. The lack of substantial change in serum catecholamines and the lack of significant rise in serum cortisol might suggest an inadequate exercise stimulus. The use of 85% age-predicted heart rate as a test end-point may have underestimated maximal heart rate [8]. Additionally, these subjects regularly participated in a training program which may have increased the ability of these subjects to maintain aerobic metabolism [36]. The

differences in recovery lactates between exercise and arbutamine suggest differences in lactate production or clearance between arbutamine and exercise, the latter a more probable explanation during recovery due to increased uptake into muscle active during exercise.

Catecholamine infusions have been shown to have varying effects on endogenous catecholamines. Norepinephrine levels decrease due to dobutamine infusion presumably due to reflex autonomic inhibition with increases in systolic blood pressure [11]. No such inhibition has been observed with isoproterenol which does not cause the same magnitude response in systolic blood pressure [18]. Findings of this study would suggest that arbutamine does not substantially affect catecholamine metabolism although it is recognized that the small sample size and the large between subject variability possibly due to psychologic arousal, age, or disease severity may have made treatment effects in our investigation difficult to detect.

In summary, pharmacologic stress testing with arbutamine results in circulatory and metabolic responses which differ in magnitude and time course from responses due to exercise testing as employed in diagnostic testing for coronary artery disease. At an equipotent $B_1$-adrenergic chronotropic stress, arbutamine causes a smaller rise in systolic blood pressure and rate pressure product than does

exercise, and the recovery rate for heart rate, systolic blood pressure, and rate pressure product are slower for arbutamine. Arbutamine results in greater increases in free fatty acids and glycerol levels than exercise and suggests a direct $B_1$-adrenergic effect on lipolysis in fat cells. Arbutamine also appears to increase plasma insulin and lactate. Both of these responses indicate that arbutamine has a direct stimulatory effect on $B_2$-adrenergic receptors in cardiotonic doses. These effects generally support the interpretation that arbutamine is a mixed *B*-agonist. The circulatory and metabolic response due to this exercise simulating agent and potent *B*-agonist differ from the generalized autonomic responses which occur in response to exercise stress. The responses need to be considered by the clinician in comparative interpretation of clinical findings between exercise and arbutamine stress tests. Further, the hormonal and metabolic effects of arbutamine suggest that the agent may have therapeutic potential as a pharmacologic conditioning agent.

Acknowledgements

The authors thank Pete Brubaker, Jeff Ocel, Laura Craft, and Scott VanGeluwe for assistance with study coordination and data collection.

References

1. American College of Sports Medicine, (1991). Guidelines for exercise testing and prescription 4th ed. Philadelphia: Lea & Febiger.

2. Brooks, G.A. (1986). Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. Federation Proceedings, 45: 2924-2929.

3. Buckberg, G.D., & Ross, G. (1973). Effects of isoprenaline on coronary blood flow: its distribution and myocardial performance. Cardiovascular Research, 7: 429-437.

4. Cobbald, A.F., Ginsburg, J., & Paton, A. (1960). Circulatory, respiratory and metabolic responses to isopropylnoradrenaline in man. Journal of Physiology, 151: 539-550.

5. Ellestad, M.H. (1986). Stress testing: Principles and practice, 3rd ed. Philadelphia: F.A. Davis Company.

6. Fellows, I.W., Bennett, T., & MacDonald, I.A. (1985). The effect of adrenaline upon cardiovascular and metabolic functions in man. Clinical Science, 69: 215-222.

7. Ferrara, N., Leosco, D., Longobardi, G., Abete, P., Papa, M., Vigorito, C., & Rengo, F. (1986). Use of epinephrine test in diagnosis of coronary artery disease. American Journal of Cardiology, 58: 256-260.

8. Froelicher, V. F., & Marcondes, G. D. (1989). Manual of exercise testing. Chicago: Year Book Medical Publisher, Inc.

9. Fujita, T., Ajisaka, R., Matsumoto, R., Iida, K., Iida, K., Sugishita, Y., Ito, I., & Takeda, T. (1986). Isoproterenol infusion stress two-dimensional echocardiography in diagnosis of coronary artery disease in elderly patients. Japanese Heart Journal, 27: 287-297.

10. Ginzton, L.E., Appleton, C., Mohluddin, S., Pool, P.E., Robertson, W., Bach, D., Ismail, G., & Armstrong, W. (1993). Arbutamine, a new agent for diagnosing coronary artery disease: Comparison with exercise testing. JACC, 21: 229A-761-4.

11. Green, C.J., Frazer, R.S., Underhill, S., Maycock, P., Fairhurst, J.A., & Campbell, I.T. (1992). Metabolic effects of dobutamine in normal man. Clinical Science, 82: 77-83.

12. Hammond, H.K., & McKirnan, M.D. (1994). Effects of dobutamine and arbutamine on regional myocardial function in a porcine model of myocardial ischemia. Journal of the American College of Cardiology, 23: 475-482.

13. Ismail, G., Sada, M., Shapiro, S., Cao, T., French, W., & Ginzton, L. (1992). Quantitative stress echocardiography: Comparison of exercise and pharmacologic stress with arbutamine. Circulation, 86: I-127-0505.

14. Jaarsma, W., & Sutherland, G. (1992). Computerized closed loop delivery of arbutamine, a new agent for the diagnosis of coronary artery disease. Circulation, 86: I-126-0503.

15. Kjaer, M. (1992). Regulation of hormonal and metabolic responses during exercise in humans. Exercise and sport sciences reviews, 20: 161-184.

16. Manca, C., Bianchi, G., Bolognesi R., Cucchini, F., & Visioli, O. (1979). Comparison of five different stress testing methods in the ECG diagnosis of coronary artery disease. Cardiology, 64: 325-332.

17. Mannering, D., Cripps, T., Leech, G., Mehta, N., Valantine, H., Gilmour, S., & Bennett, E.D. (1988). The dobutamine stress test as an alternative to exercise testing after acute myocardial infarction. British Heart Journal, 59: 521-526.

18. Mansell, P.I., Fellows, I.W., Birmingham, A.T., & MacDonald, I.A. (1988). Metabolic and cardiovascular effects of infusions of low doses of isoprenaline in man. Clinical Science, 75: 285-291.

19. Mason, J.R., Palac, R.T., Freeman, M.L., Virupannavar, S., Loeb, H.S., Kaplan, E., & Gunnar, R.M. (1984). Thallium scintigraphy during dobutamine infusion: nonexercise-dependent screening test for coronary artery disease. American Heart Journal, 107: 481-485.

20. Maycock, P.F., & Frayn, K.N. (1987). Use of alumina columns to prepare plasma samples for liquid-chromatographic determination of catecholamines. Clinical Chemistry, 33: 286-287.

21. Mazeika, P.K., Aleksander, N., & Oakley, C.M. (1992). Dobutamine stress echocardiography for detection and assessment of coronary artery disease. Journal of the American College of Cardiology, 19: 1203-1211.

22. Mazzeo, R.S. (1991). Catecholamine responses to acute and chronic exercise. Medicine and Science in Sports and Exercise, 23: 839-845.

23. Meyer, S.L., Curry, G.C., Donsky, M.S., Twieg, D.B., Parkey, R.W., & Willerson, J.T. (1976). Influence of dobutamine on hemodynamics and coronary blood flow in patients with and without coronary artery disease. The American Journal of Cardiology, 38: 103-108.

24. Naugton, J.B., Balke, B., & Nagle, F. (1964). Refinements in methods of evaluation and physical conditioning before and after myocardial infarction. American Journal of Cardiology, 14: 837.

25. Pernett, A., Hammond, V.A., Blesa-Malpica, G., Burrin, J., Orskov, H., Alberti, K.G.M.M., & Johnston, D.G. (1984). The metabolic effects of dopamine in man. European Journal of Clinical Pharmacology, 26: 23-28.

26. Ribisl, P.M., Liu, J., Mousa, I., Herbert, W.G.,

Miranda, C.P., Froning, J.N., & Froelicher, V.F. (1993). Comparison of computer ST criteria for diagnosis of severe coronary artery disease. American Journal of Cardiology, 71: 546-551.

27. Sacca, L., Morrone, G., Cicala, M., Corso, G., & Ungaro, B. (1980). Influence of epinephrine, norepinephrine, and isoproterenol on glucose homeostasis in normal man. Journal of Clinical Endocrinology and Metabolism, 50: 680-684.

28. Schechter, E., Wilson, M.F., & Yin-Suen, K. (1983). Physiologic responses to epinephrine infusion: the basis for a new stress test for coronary artery disease. American Heart Journal, 105: 554-560.

29. Sinusas, A., Daher, E., & Shi, Q. (1993). Arbutamine: a new pharmacologic stress agent for potential use with thallium-201 scintigraphy. Journal of the American College of Cardiology, 21: 377A.

30. Sjostrom, L., Schutz, Y., Gudinchet, F., & Hegnell, L. (1983). Epinephrine sensitivity with respect to metabolic rate and other variables in women. American Journal of Physiology, 245: E431-E442.

31. Staten, M.A., Matthews, D.E., Cryer, P.E., & Bier, D.M. (1987). Physiologic increments in epinephrine stimulate metabolic rate in humans. American Journal of Physiology, 253: E322-E330.

32. Stratmann, H.G., & Kennedy, H.L. (1989). Evaluation of coronary artery disease in the patient unable to exercise: alternatives to exercise stress testing. American Heart Journal, 117: 1344-1365.

33. Tuttle, R.R., & Mills, J. (1975). Development of a new catecholamine to selectively increase cardiac contractility. Circulation Research, 36: 185-196.

34. Vasu, M.A., O'Keefe, D.D., Kapellakis, G.Z., Vezeridis, M.P., Jacobs, M.L., Daggett, W.M., & Powell, W.J. (1978). Myocardial oxygen consumption: Effects of epinephrine, isoproterenol, dopamine, norepinephrine, and dobutamine. American Journal of Physiology, 235: H237-H241.

35. Vincent, J., Roman, A., De Backer, D., & Kahn, R. (1990). Oxygen supply/dependency. Amercian Review of Respiratory Disease, 142: 2-7.

36. Wasserman, K., Hansen, J.E., Sue, D.Y., Whipp, B.J., & Casaburi, R. (1994). Principles of exercise testing and interpretation, 2nd ed. Philadelphia: Lea & Febiger.

37. Wisenberg, G., Zawadowski, A.G., Gebhardt, V.A., Prato, F.S., Goddard, M.D., Nichol, P.M., & Rechnitzer, P.A. (1985). Dopamine: its potential for inducing ischemic left ventricular dysfunction. Journal of the American College of Cardiology, 6: 84-92.

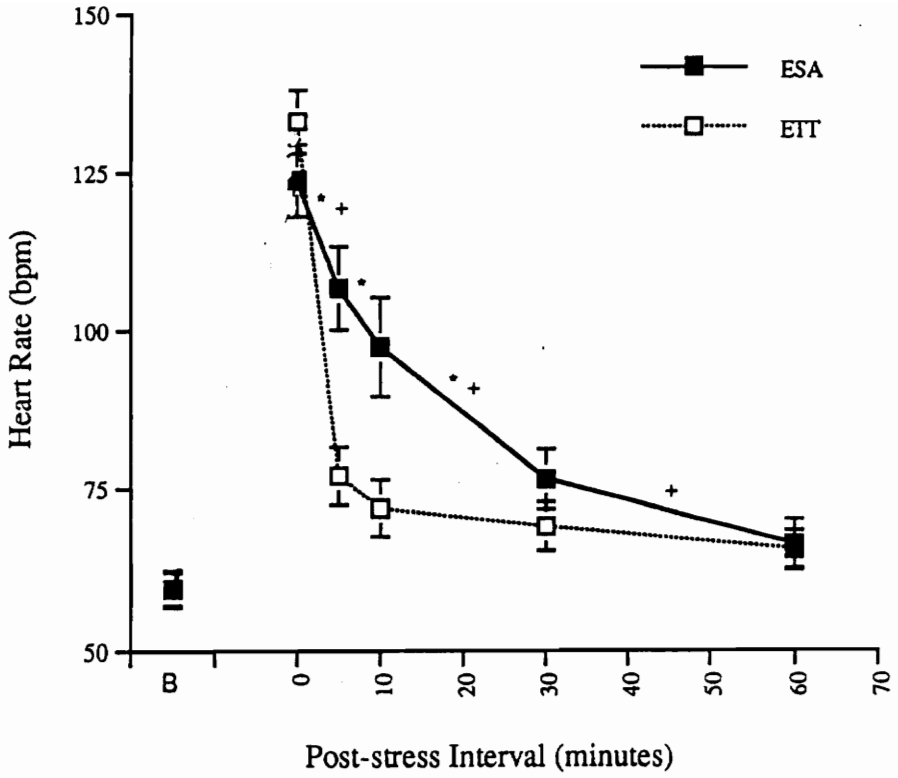


Figure 1. Heart rate responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction was significant; $F(4,56) = 19.80$, $p < 0.01$. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM.

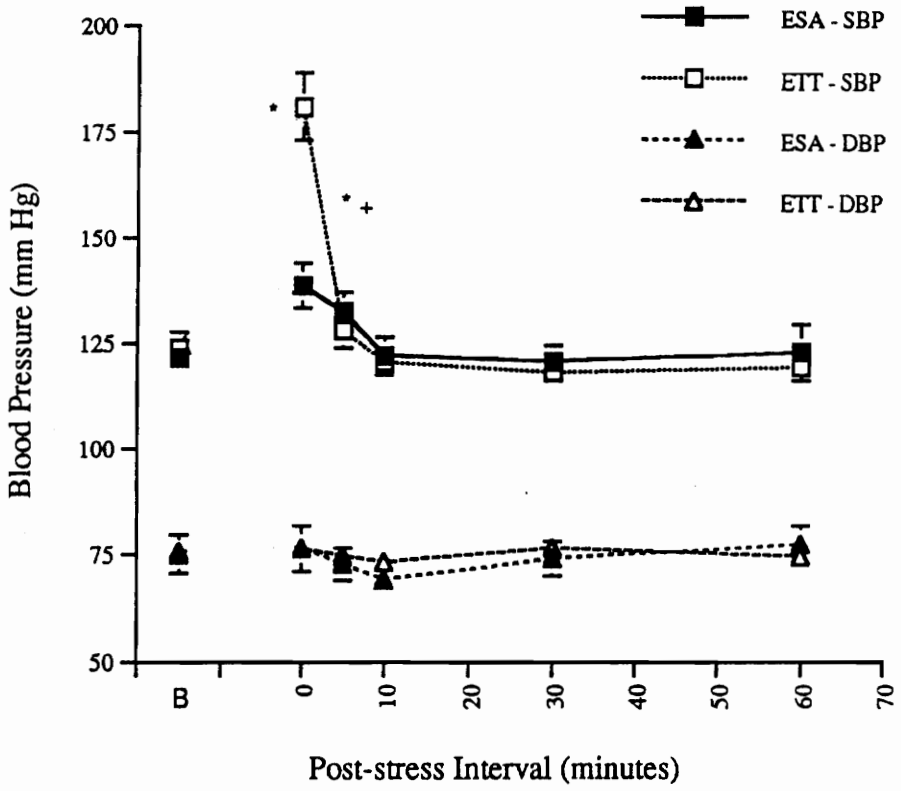


Figure 2. Systolic (SBP) and diastolic blood pressure (DBP) responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction for SBP was significant; $F(4,48) = 12.19$, $p < 0.01$. DBP was not significant for any effect. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM. Vertical bars representing the SEM do not appear where the SEM is very small.

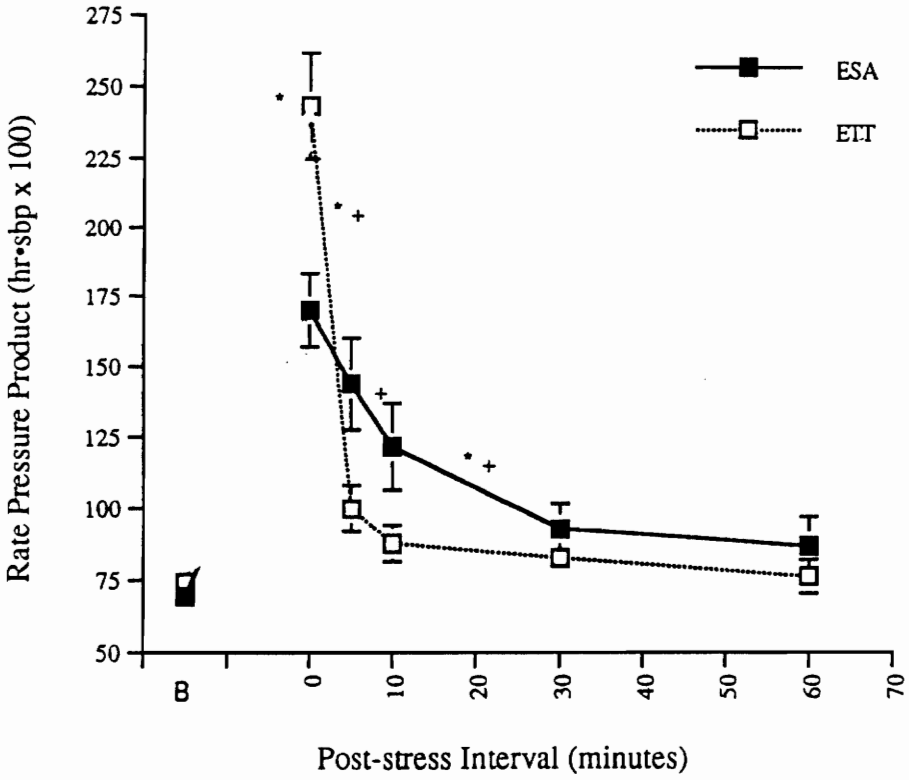


Figure 3. Rate pressure product responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction for RPP was significant; $F(4,48) = 18.92$, $p < 0.01$. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM. Vertical bars representing the SEM do not appear where the SEM is very small.

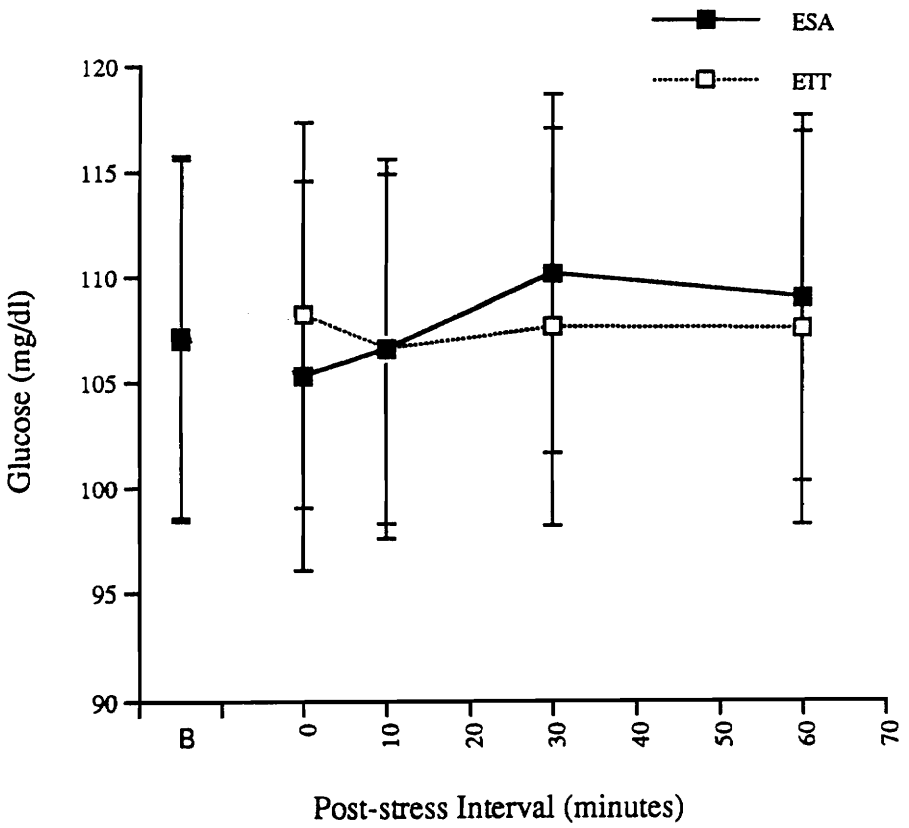


Figure 4. Glucose responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA was not significant for any effect. Values are means ± SEM.

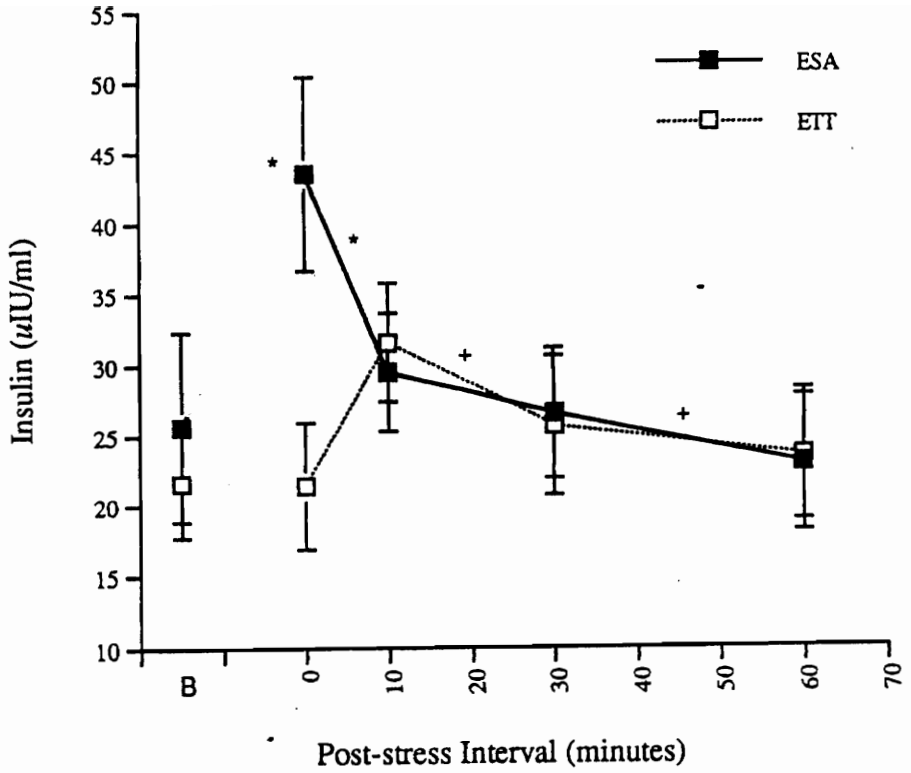


Figure 5. Insulin responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction was significant; $F(3,48) = 14.37$, $p < 0.01$. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM.

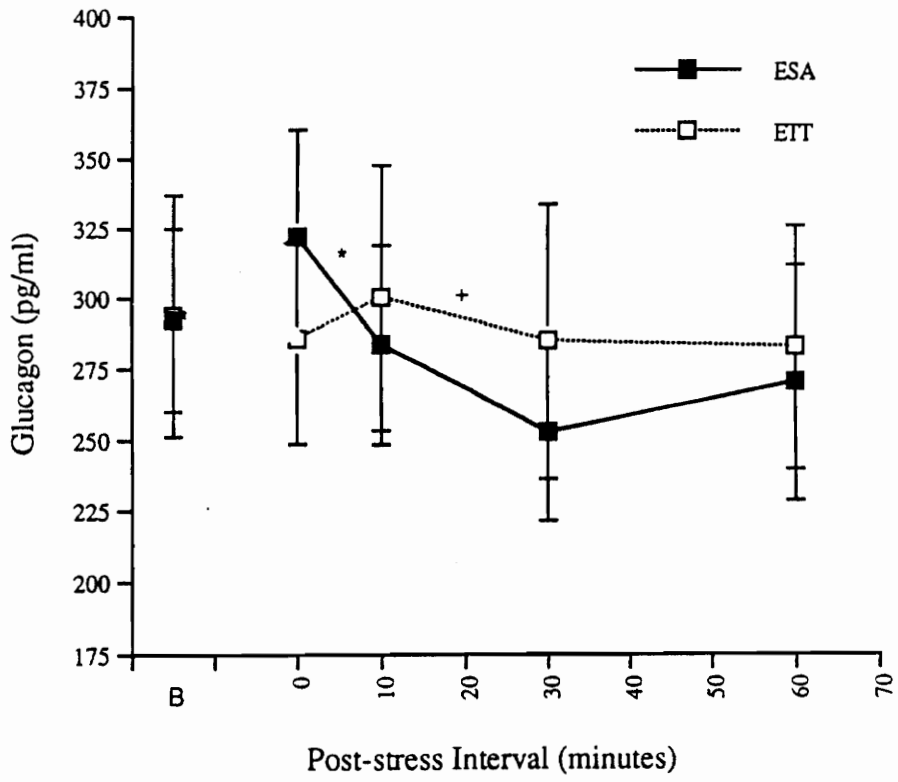


Figure 6. Glucagon responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction was significant; $F(3,48) = 3.60$, $p < 0.05$. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM.

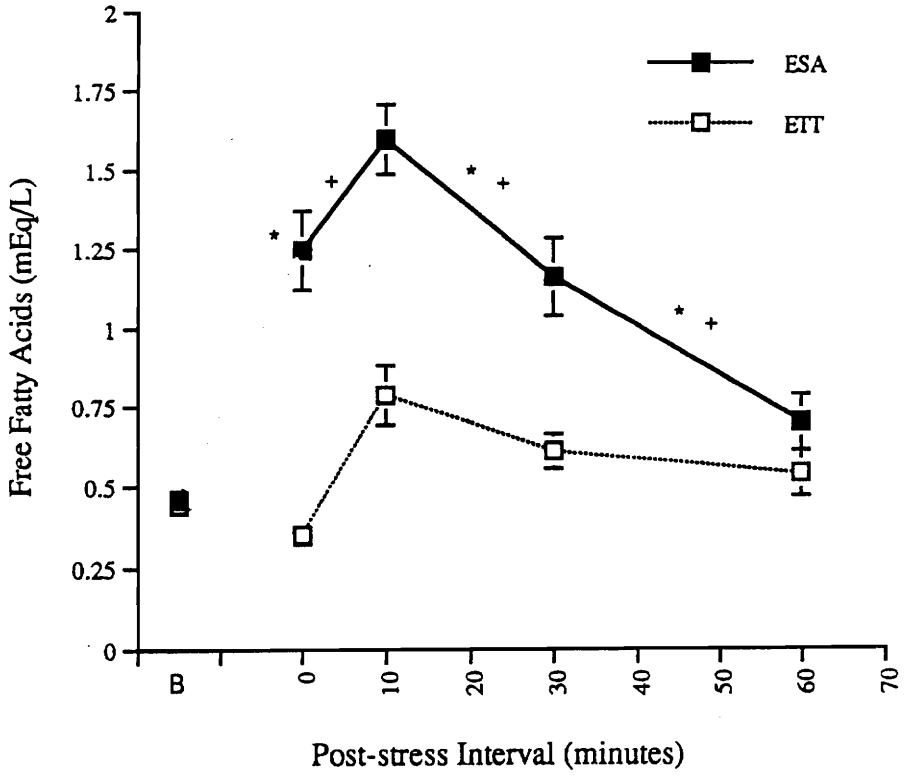


Figure 7. Free Fatty Acid responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction was significant; $F(3,48) = 14.23$, $p < 0.05$. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.01$. Values are means $\pm$ SEM. Vertical bars representing the SEM do not appear where the SEM is very small.

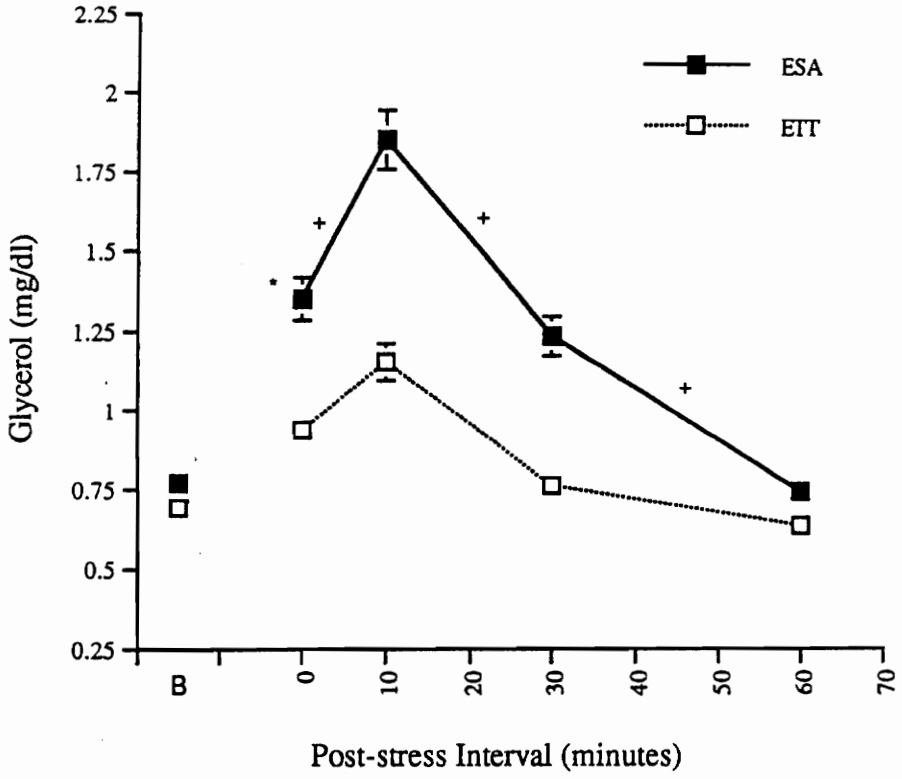


Figure 8. Glycerol responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction was significant; $F(3,48) = 3.76$, $p < 0.05$. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM. Vertical bars representing the SEM do not appear where the SEM is very small.

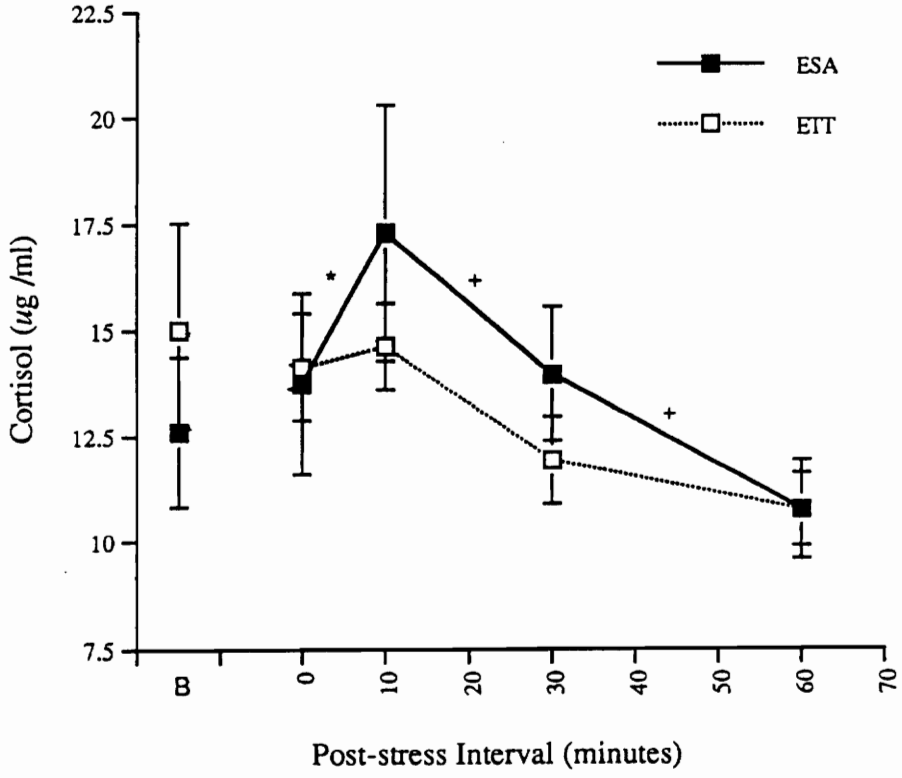


Figure 9. Cortisol responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time effect was significant; $F(3,48) = 6.13$, $p < 0.01$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM.

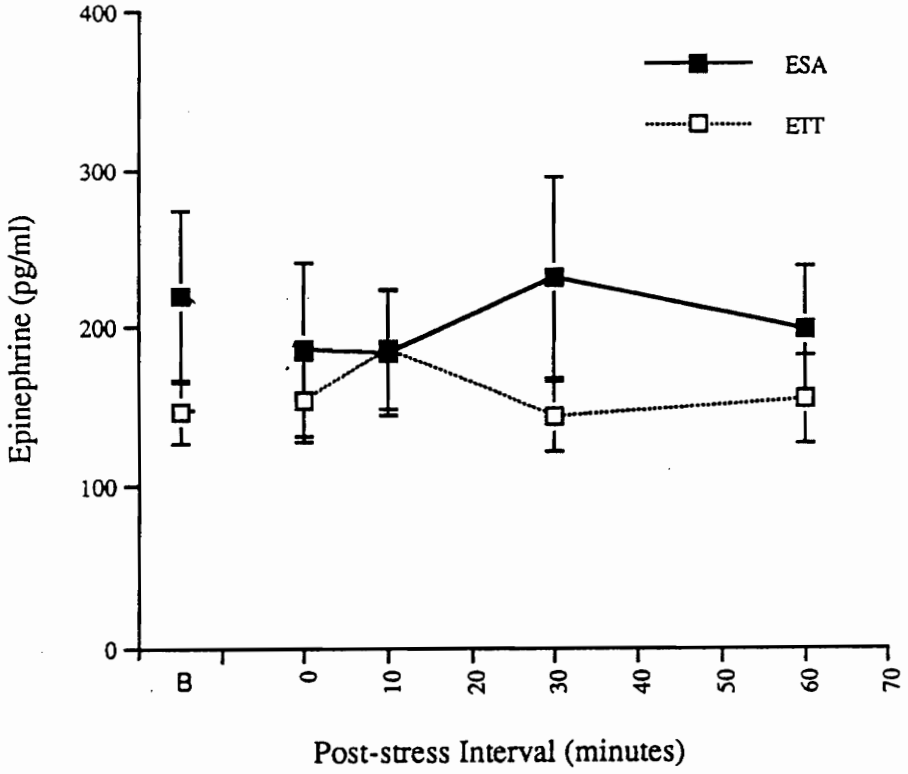


Figure 10. Epinephrine responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA was not significant for any effect. Values are means ± SEM.

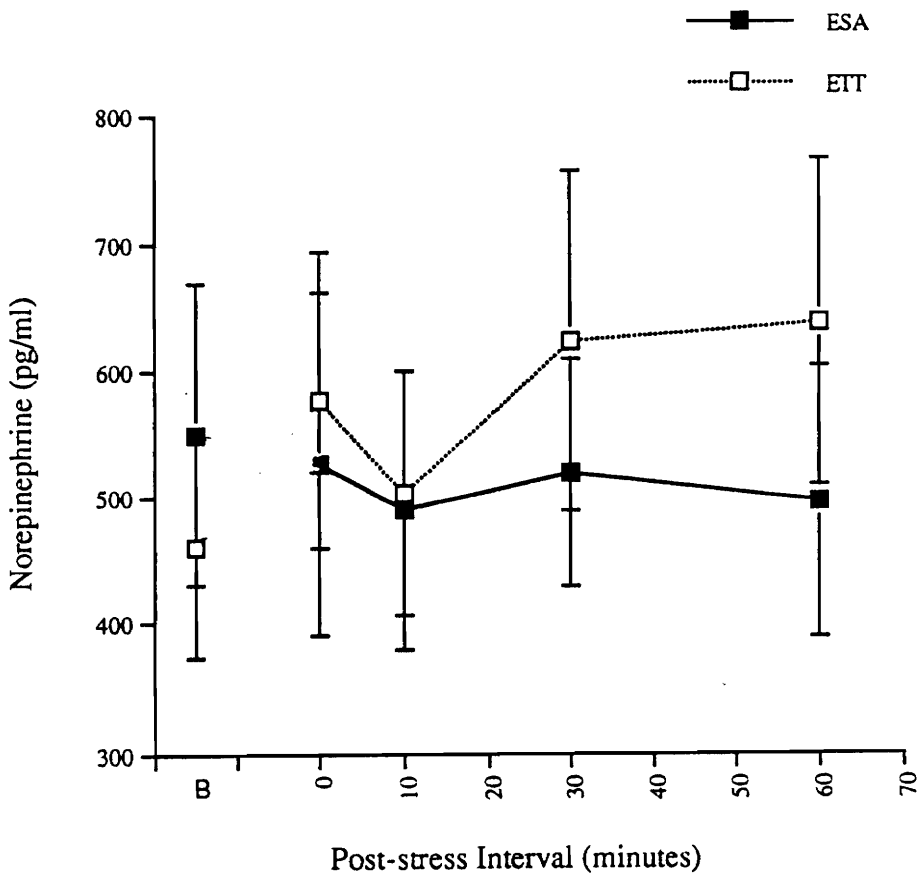


Figure 11. Norepinephrine responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA was not significant for any effect. Values are means ± SEM.

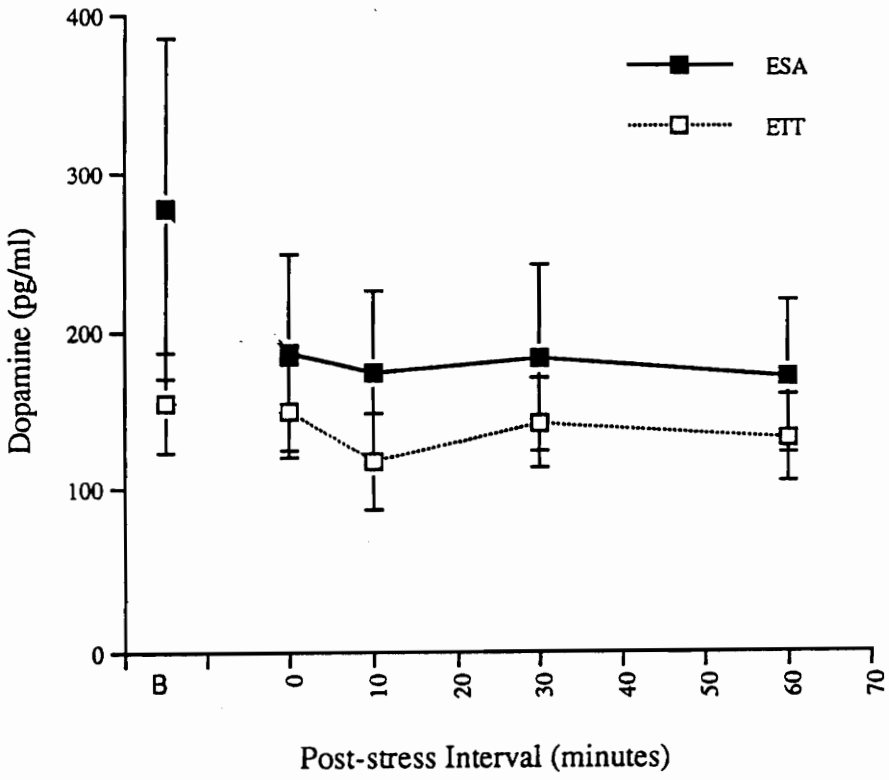


Figure 12. Dopamine responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA was not significant for any effect. Values are means ± SEM.

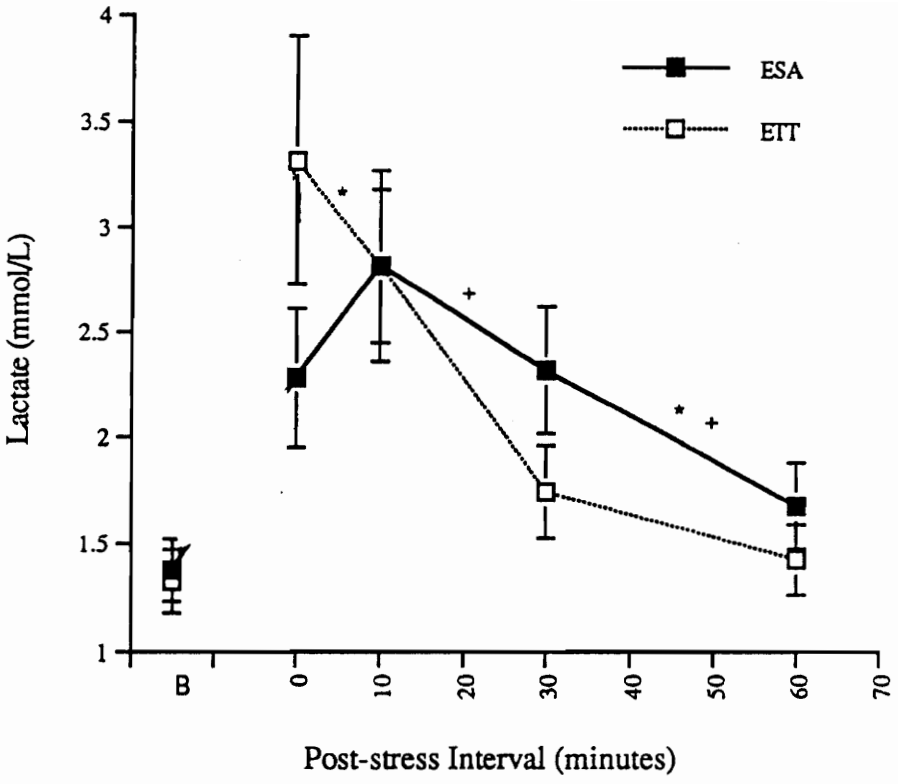


Figure 13. Lactate responses of arbutamine (ESA) compared to exercise (ETT) stress testing in persons (n=9) with known or suspected coronary artery disease. B = baseline measures not included in ANOVA. Repeated measures ANOVA time·treatment interaction was signficant, $F(3,48) = 2.178$, $p < 0.01$. * = Treatment differences, $p \leq 0.05$. + = Time effect, $p \leq 0.05$. Values are means $\pm$ SEM.

# Chapter IV

## SUMMARY AND CONCLUSIONS

The term exercise simulating agent has been used to describe agents which mimic the effects of endogenous catecholamines to produce the acute and adaptive effects of exercise (Tuttle, 1993). Arbutamine was developed as an exercise simulating agent to simulate the cardiotonic effects of endogenous catecholamines released during exercise stress testing as employed in the diagnosis of coronary artery disease. Other catecholamines which have substantial $B_1$-agonist properties have previously been employed as pharmacologic stressors (Stratmann & Kennedy, 1989). Clinical trials involving these agents have been aimed at determining the diagnostic efficacy of these agents compared to exercise and, thus, findings are limited to diagnostic properties and hemodynamic effects. However, metabolic effects can confound interpretation of clinical findings (Vincent et al., 1989) and the potential therapeutic value of catecholamines as pharmacologic conditioners is related to both circulatory and metabolic effects (Liang et al., 1979; Davidson et al., 1986; Sullivan et al., 1985; Leier & Unverferth, 1982).

Studies aimed at describing hormonal and metabolic responses to catecholamine infusion are not designed to be comparative studies in relation to exercise stress. In

addition, healthy people are used as subjects and dosing schedules are typically of longer duration than those employed in pharmacologic stress testing. This study was designed to bridge these two areas of research by examining the circulatory, hormonal, and metabolic effects of arbutamine compared to exercise in the clinical target population and to employ a protocol used for pharmacologic stress testing. Additionally, comparative differences observed in diagnostic efficacy or physiologic effects between arbutamine and exercise stress could be used to infer differences in adrenergic receptor stimulation. This study demonstrated that arbutamine infusion, as employed in the diagnosis of coronary artery disease, caused different circulatory, hormonal and metabolic effects than exercise stress testing in men with known or suspected coronary artery disease, thus supporting the research hypothesis.

The circulatory differences between arbutamine and exercise were similar to those reported in previous studies (Ginzton et al., 1993; Ismail et al., 1992) although, in the present study of stable cardiac patients all subjects achieved the target heart rate for both exercise and arbutamine. A major difference between catecholamine stress and exercise stress is the ability of catecholamines to produce ischemia at a lower rate pressure product than exercise. This property of catecholamine stress has been

attributed to either a greater inotropic demand or to decreased coronary perfusion occurring during catecholamine stress (Stratmann & Kennedy, 1989). In this study of stable patients, arbutamine, at an equipotent chronotropic stress, did not achieve the same systolic blood pressure response and thus rate pressure product at peak stress as did exercise. In view of previous studies which demonstrate that arbutamine produces evidence of myocardial ischemia at a lower rate pressure product than does exercise (Ismail et al., 1992; Ginzton et al., 1993), a given heart rate response with arbutamine versus exercise likely represents a greater myocardial demand due to additional inotropic stress as compared to exercise. Additional study is needed to better characterize the mechanisms by which arbutamine produces myocardial ischemia and the comparative diagnostic and prognostic value of arbutamine compared to other stress tests. In addition, it would be beneficial to characterize the relative cardiac stress of arbutamine compared to exercise so that arbutamine stress test results could be used to determine an exercise prescription. Characterization of comparative prognostic indicators between exercise and arbutamine stress tests is warranted.

Arbutamine infusion, in addition to the circulatory effects, resulted in substantial hormonal and metabolic changes in cardiac patients similar to findings with other

catecholamines observed in normal subjects. Comparing the effects of arbutamine to exercise, the magnitude and time course of circulatory, hormonal and metabolic effects differed from those observed following exercise and can be explained, largely, due to differences in sympathetic activation between the two stressors. This study demonstrated that the $B_1$ agonist effects of arbutmamine are not limited to cardiac effects as evidenced by the substantial lipolytic effects observed. These lipolytic effects confirm that arbutamine, like other catecholamines, has thermogenic effects similar to other catecholamines, an effect which may contribute to the post-infusion tachycardia observed.

Although not expected at the outset of the study, the effects of arbutamine are not limited $B_1$-adrenergic agonism as hypothesized. The increase in insulin and lactate observed, strongly suggest that arbutamine is an equi-potent $B_1$- and $B_2$-agonist. Further, the increase in plasma insulin concentrations would indicate that arbutamine directly stimulates insulin secretion from the beta islet cells of the pancreas. The increase in lactate levels provides evidence of $B_2$-agonism in skeletal muscle. Knowledge that these non-cardiac effects do occur may be useful to the clinician in assessing the clinical response to arbutamine stress testing. For instance, the increase in lactate

levels would likely result in an increased respiratory rate during infusion. Further, even though plasma glucose levels remained stable in this study, it is possible that insulin secretion could potentially result in hypoglycemia in some patients. In addition, the effect of arbutamine on glucose response must be interpreted with caution since some subjects had elevated fasting glucose levels.

Neither exercise or arbutamine in the present protocol appeared to produce significant changes in catecholamine responses. This may be attributable to the small sample size, large variability in catecholamine levels between subjects, or sampling interval. Psychologic arousal related to the nature of the study protocol may have contributed to the large variability seen. The lack of significant effects in catecholamines due to exercise stress is problematic from a theoretical perspective. Inferences regarding differences in adrenergic receptor activation due to differences between endogenous catecholamines release in response to exercise compared to exogenous administration of arbutamine must be tentative since endogenous catecholamines did not differ between the two treatments.

While cortisol levels did not appear to increase due to the treatments, it is possible that the sampling interval for blood collection did not capture intervals of greatest change. Alternatively, there may not have been a treatment

effect and the decline in cortisol which occurred post-stress may have been due to normal circadian changes (Galbo, 1983). Finally, it is possible that the lack of adjustment of metabolites for changes in plasma fluid shifts between treatments and over time within treatments cannot be overlooked as a rival hypothesis for some of the observed effects in this study.

Although the magnitude and time course of responses between arbutamine and exercise differ, the presence of substantial *B*-adrenergic stimulation of circulatory and metabolic processes would indicate that arbutamine may have potential value as a pharmacologic conditioner. For example, it has been suggested that pulsed *B*-agonist therapy may be of value in improving the functional status of persons with heart failure due to mechanisms related to up-regulation of *B*-adrenergic receptors (Coats & Adamopoulos, 1991). Clearly more research is needed in examining the use of catecholamines as pharmacologic conditioners.

Recommendations for future research

Based on the findings of the present study and relevant literature, the following studies seem warranted:

1. A more through understanding is needed on the mechanisms by which arbutamine contributes to myocardial demand. Comparative effects of arbutamine on properties of contractility using noninvasive indices of contractility

such as the rate of rise of ventricular pressure (dP/dt) could be examined during exercise and arbutamine echocardiography. It is possible that data may become available on this topic as results of Phase III trials begin to appear in the literature.

2. Similarly, there is a need to examine effects arbutamine may have on myocardial perfusion. More thorough examination of existing data involving arbutamine may reveal a dose-response effect on diastolic pressure. Use of adrenergic blocking agents in humans are suggested to further characterize effects on diastolic blood pressure. In addition, in vivo animal studies or in vitro studies could be used to better characterize mechanisms of myocardial demand/supply imbalances. For instance, $B^2$-blockade compared to $B^1$-blockade would be expected to prevent any drop in diastolic blood pressure. On the other hand, a-blockade would be expected to result in a persistent decrease in diastolic pressure as infusion rates increased.

3. Persons undergoing pharmacologic stress may not have also undergone an exercise stress test, yet could benefit from an exercise program. A possible advantage of pharmacologic stress testing with arbutamine as opposed to a coronary vasodilator is that the information obtained during a graded chronotropic test could be used in determination of an exercise prescription without having to undergo an

exercise test. Studies designed to determine the best hemodynamic predictors of equipotent cardiac stress are needed.

4. The generalizability of the present study is limited. Further studies are needed to examine the effects due to age versus disease by using an age-matched control group and studying young, middle-aged, and older adults.

5. Autonomic responses, lipid and glucose metabolism may differ due to gender. Studies in women, pre- and post-menopausal, and comparative studies of men and women would enhance our present understanding of physiologic differences due to gender.

6. Examination of the potential use of arbutamine as a pharmacologic conditioner is warranted for persons who may benefit from the adaptive effects of exercise. A study comparing a daily exercise bout with daily arbutamine infusion could be conducted for potential use to improve functional capacity in persons with cardiac disease or prevent muscle wasting in persons on bed rest or under conditions of weightlessness as in flight conditions. In a conditioning study, a steady state infusion for a longer duration than used in pharmacologic stress testing would be needed.

7. Future studies comparing exercise and arbutamine could be strengthened by considering the following modifications

to the present study:

a. Base exercise intensity on a preliminary maximal stress test rather than 85% age-predicted maximal heart rate.

b. Screen subjects for resting hyperglycemia.

c. Adjust for fluid volume shifts.

8. Evaluate the use of various statistical models in repeated measures designs such as the present one.

## REFERENCES CITED


Amenta, F. (1990). Peripheral dopamine pathophysiology. Boca Raton: CRC Press.

American College of Cardiology/American Heart Association, (1986). Guidelines for Exercise Testing. JACC, 8: 725-738.

American Heart Association, (1991). 1992 heart and stroke facts. Dallas: American Heart Association.

American College of Sports Medicine, (1991). Guidelines for exercise testing and prescription. Philadelphia: Lea & Febiger.

Blousefield, G. (1918). Angina pectoris: variations in electrocardiogram during paroxysm. Lancet, 2: 457.

Brooks, G.A. (1986). Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. Federation Proceedings, 45: 2924-2929.

Buckberg, G.D., & Ross, G. (1973). Effects of isoprenaline on coronary blood flow: its distribution and myocardial performance. Cardiovascular Research, 7: 429-437.

Bunt, J.C. (1986). Hormonal alterations due to exercise. Sports Medicine, 3: 331-345.

Chaitman, B.R. (1986). The changing role of the exercise electrocardiogram as a diagnostic and prognostic test for chronic ischemic heart disease. Journal of the American College of Cardiology, 8: 1195-1210.

Clutter, W.E., Bier, D.M., Shah, S.D., & Cryer, P.E. (1980). Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic actions in man. Journal of Clinical Investigation, 66: 94-101.

Coats, A.J., & Adamopoulos, S. (1991). Physical and pharmacological conditioning in chronic heart failure: a proposal for pulsed inotrope therapy. Postgraduate Medical Journal, 67 Supplement 1: S69-73.

Cobbald, A.F., Ginsburg, J., & Paton, A. (1960). Circulatory, respiratory and metabolic responses to isopropylnoradrenaline in man. Journal of Physiology, 151: 539-550.

Combs, D.T., & Martin, C.M. (1974). Evaluation of isoproterenol as a method of stress testing. American Heart Journal, 87: 711-715.

Cryer, P.E. (1980). Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. The New England Journal of Medicine, 303: 436-444.

Davidson, W.R., Banerjee, S.P., & Liang, C.S. (1986). Dobutamine-induced cardiac adaptations: comparison with exercise-trained and sedentary rats. American Journal of Physiology, 250: H725-H730.

Ellestad, M.H. (1986). Stress testing: Principles and practice, 3rd ed. Philadelphia: F.A. Davis Company.

Feil, H., & Siegel, M.L. (1928). Electrocardiographic changes during attacks of angina pectoris. The American Journal of Cardiology, 175: 255-269.

Fellows, I.W., Bennett, T., & MacDonald, I.A. (1985). The effect of adrenaline upon cardiovascular and metabolic functions in man. Clinical Science, 69: 215-222.

Ferrara, N., Leosco, D., Longobardi, G., Abete, P., Papa, M., Vigorito, C., & Rengo, F. (1986). Use of epinephrine test in diagnosis of coronary artery disease. American Journal of Cardiology, 58: 256-260.

Finney, D.J., (1990). Repeated measurements: what is measured and what repeats? Statistics in Medicine, 9: 639-644.

Froelicher, V. F., & Marcondes, G. D. (1989). Manual of exercise testing. Boca Raton: Year Book Publishers, Inc.

Fujita, T., Ajisaka, R., Matsumoto, R., Iida, K., Iida, K., Sugishita, Y., Ito, I., & Takeda, T. (1986). Isoproterenol infusion stress two-dimensional echocardiography in diagnosis of coronary artery disease in elderly patients. Japanese Heart Journal, 27: 287-297.

Galbo, H. (1983). Hormonal and metabolic adaptations to exercise. New York: Thieme-Stratton, Inc.

Gensia Pharmaceuticals, Inc. (1992). Arbutamine: Investigator drug brochure, 4th Ed. San Diego: Gensia Pharmaceuticals, Inc.

Gensia Pharmaceuticals, Inc. (1994). Arbutamine: Investigator drug brochure, 7th Ed. San Diego: Gensia Pharmaceuticals, Inc.

Gillman, A.G., Rael, T.W., Nies, A.S., & Taylor, P. (1990). Goodman and Gilman's the pharmacologic basis of therapeutics, 8th Ed. New York: Pergman Press.

Ginzton, L.E., Appleton, C., Mohluddin, S., Pool, P.E., Robertson, W., Bach, D., Ismail, G., & Armstrong, W. (1993). Arbutamine, a new agent for diagnosing coronary artery disease: Comparison with exercise testing. JACC, 21: 229A-761-4.

Green, C.J., Frazer, R.S., Underhill, S., Maycock, P., Fairhurst, J.A., & Campbell, I.T. (1992). Metabolic effects of dobutamine in normal man. Clinical Science, 82: 77-83.

Griffith, F.R. (1951). Fact and theory regarding the calorigenic action of adrenaline. Physiologic reviews, 31: 151-187.

Hammond, H.K., & McKirnan, M.D. (1992). Arbutamine is more effective than dobutamine as a diagnostic stress agent in a porcine model for myocardial ischemia. Circulation, 86: I-126-0504.

Hammond, H.K., & McKirnan, M.D. (1994). Effects of dobutamine and arbutamine on regional myocardial function in a porcine model of myocardial ischemia. Journal of the American College of Cardiology, 23: 475-482.

Ismail, G., Sada, M., Shapiro, S., Cao, T., French, W., & Ginzton, L. (1992). Quantitative stress echocardiography: Comparison of exercise and pharmacologic stress with arbutamine. Circulation, 86: I-127-0505.

Jaarsma, W., & Sutherland, G. (1992). Computerized closed loop delivery of arbutamine, a new agent for the diagnosis of coronary artery disease. Circulation, 86: I-126-0503.

Katz, L.N., Hamburger, W.W., & Lev, M. (1932). The diagnostic value of epinephrine in angina pectoris. The American Heart Journal, 7: 370-379.

Kjaer, M. (1992). Regulation of hormonal and metabolic responses during exercise in humans. Exercise and sport sciences reviews, 20: 161-184.

Korkushko, O.V., Frolkis, M.V., Shatilo, V.B., & Y Aroschenko, Y.T. (1990). Hormonal and autonomic reaction to exercise in elderly healthy subjects and patients with ischemic heart disease. Acta Clinica Belgica, 45: 164-175.

Kuramoto, K., Matsushita, S., Mifune, J., Sakai, M., & Murakami, M. (1978). Electrocardiographic and hemodynamic evaluation of isoproterenol test in elderly ischemic heart disease. Japanese Circulation Journal, 42: 955-960.

Leier, C.V., Huss, P., Lewis, R.P., & Unverferth, D.V. (1982). Drug-induced conditioning in congestive heart failure. Circulation, 65: 1332-1337.

Levine, S.A., Ernstene, A.C., & Jacobson, B.M. (1930). The use of epinephrine as a diagnostic test for angina pectoris. Archives of Internal Medicine, 45: 191-200.

Katz, L.N., Hamburger, W.W., & Lev, M. (1932). The diagnostic value of epinephrine in angina pectoris. The American Heart Journal, 7: 371-379.

Kuhn, M. M. (1991). Pharmacotherapeutics, 2nd Ed. Philadelphia: F.A. Davis Company.

Liang, C.S., Tuttle, R.R., Hood, W.B., & Gavras, H. (1979). Conditioning effects of chronic infusions of dobutamine. Journal of Clinical Investigation, 64: 613.

MacDonald, I.A., Bennett, T., & Fellows, I.W. (1985). Catecholamines and the control of metabolism in man. Clinical Science, 68: 613-619.

Manca, C., Bianchi, G., Bolognesi R., Cucchini, F., & Visioli, O. (1979). Comparison of five different stress testing methods in the ECG diagnosis of coronary artery disease. Cardiology, 64: 325-332.

Mannering, D., Cripps, T., Leech, G., Mehta, N., Valantine, H., Gilmour, S., & Bennett, E.D. (1988). The dobutamine stress test as an alternative to exercise testing after acute myocardial infarction. British Heart Journal, 59: 521-526.

Mansell, P.I., Fellows, I.W., Birmingham, A.T., & MacDonald, I.A. (1988). Metabolic and cardiovascular effects of infusions of low doses of isoprenaline in man. Clinical Science, 75: 285-291.

Martin, T.W., Seaworth, J.F., Johns, J.P., Pupa, L.E., & Condos, W.R. (1992). Comparison of adenosine, dipyridamole, and dobutamine in stress echocardiography. Annals of Internal Medicine, 116: 190-196.

Mason, J.R., Palac, R.T., Freeman, M.L., Virupannavar, S., Loeb, H.S., Kaplan, E., & Gunnar, R.M. (1984). Thallium scintigraphy during dobutamine infusion: nonexercise-dependent screening test for coronary artery disease. American Heart Journal, 107: 481-485.

Matthews, J.N.S., Altman, D.G., Campbell, M.J., & Royston, R. (1990). Analysis of serial measurements in medical research. British Medical Journal, 300: 230-236.

Maycock, P.F., & Frayn, K.N. (1987). Use of alumina columns to prepare plasma samples for liquid-chromatographic determination of catecholamines. Clinical Chemistry, 33: 286-287.

Mazeika, P.K., Aleksander, N., & Oakley, C.M. (1992). Dobutamine stress echocardiography for detection and assessment of coronary artery disease. Journal of the American College of Cardiology, 19: 1203-1211.

Mazzeo, R.S. (1991). Catecholamine responses to acute and chronic exercise. Medicine and Science in Sports and Exercise, 23: 839-845.

Meyer, S.L., Curry, G.C., Donsky, M.S., Twieg, D.B., Parkey, R.W., & Willerson, J.T. (1976). Influence of dobutamine on hemodynamics and coronary blood flow in patients with and without coronary artery disease. The American Journal of Cardiology, 38: 103-108.

Miller, W.C. (1992). The biochemistry of exercise and metabolic adaptations. Dubuque: Brown & Benchmark.

Mitchell, J.H. (1990). Neural control of the circulation during exercise. Medicine and Science in Sports and Exercise, 22: 141-154.

Naughton, J.B., Balke, Nagle, F. (1964). Refinements in methods of evaluation and physical conditioning before and after myocardial infarction. American Journal of Cardiology, 14: 837.

Pardee, H.E.B. (1920). An electrocardiographic sign of coronary artery obstruction. Archives of Internal Medicine, 26: 244.

Pernett, A., Hammond, V.A., Blesa-Malpica, G., Burrin, J., Orskov, H., Alberti, K.G.M.M., & Johnston, D.G. (1984). The metabolic effects of dopamine in man. European Journal of Clinical Pharmacology, 26: 23-28.

Regan, C. J., Duckworth, R., Fairhurst, J. A., Maycock, P. F., Frayn, K. N., & Campbell, I. T. (1990). Metabolic effects of low dose dopamine infusion in normal volunteers. Clinical Science, 79: 605-611.

Ribisl, P.M., Liu, J., Mousa, I., Herbert, W.G., Miranda, C.P., Froning, J.N., & Froelicher, V.F. (1993). Comparison of computer ST criteria for diagnosis of severe coronary artery disease. American Journal of Cardiology, 71: 546-551.

Rowell, (1993). Human cardiovascular control. New York: Oxford University Press.

Sacca, L., Morrone, G., Cicala, M., Corso, G., & Ungaro, B. (1980). Influence of epinephrine, norepinephrine, and isoproterenol on glucose homeostasis in normal man. Journal of Clinical Endocrinology and Metabolism, 50: 680-684.

SAS Institute, Inc. (1990). SAS system for linear models. Cary: SAS Institute, Inc.

Schechter, E., Wilson, M.F., & Yin-Suen, K. (1983). Physiologic responses to epinephrine infusion: the basis for a new stress test for coronary artery disease. American Heart Journal, 105: 554-560.

Sinusas, A., Daher, E., & Shi, Q. (1993). Arbutamine: a new pharmacologic stress agent for potential use with thallium-201 scintigraphy. Journal of the American College of Cardiology, 21: 377A.

Sjostrom, L., Schutz, Y., Gudinchet, F., & Hegnell, L. (1983). Epinephrine sensitivity with respect to metabolic rate and other variables in women. American Journal of Physiology, 245: E431-E442.

Smith, F.M. (1920). Further observations on the T wave of the electrocardiogram of the dog following the ligation of the coronary arteries. Archives of Internal Medicine, 25: 673.

Stainsby, W.N., & Brooks, G.A. (1990). Control of lactic acid metabolism in contracting muscles and during exercise. Exercise and sport sciences reviews, 18: 29-63.

Staten, M.A., Matthews, D.E., Cryer, P.E., & Bier, D.M. (1987). Physiologic increments in epinephrine stimulate metabolic rate in humans. American Journal of Physiology, 253: E322-E330.

Stone, H.L., Dormer, K.J., Foreman, R.D., Thies, R., &

Blair, R.W. (1985). Neural regulation of the cardiovascular system during exercise. Federation Proceedings, 44: 2271-2278.

Stratmann, H.G., & Kennedy, H.L. (1989). Evaluation of coronary artery disease in the patient unable to exercise: alternatives to exercise stress testing. American Heart Journal, 117: 1344-1365.

Struthers, A.D., Reid, J.L., Whitesmith, R., & Rodger, J.C. (1983). The effects of cardioselective and non-selective B-adrenergic blockade on the hypokalemic and cardiovascular responses to adrenomedullary hormones in man. Clinical Science, 65: 143-147.

Sullivan, M.J., Binkley, P.F., Unverferth, D.V., Ren J.H., Boudoulas, H. Bashore, T.M., Merola, A.J., & Leier, C.V. (1985). Prevention of bedrest-induced physical conditioning by daily dobutamine infusions. Journal of Clinical Investigation, 76: 1632-1642.

Tepperman, J., & Tepperman, H.M. (1987). Metabolic and endocrine physiology: an introductory text, 5th Ed. Chicago: Year Book Medical Publishers.

Tuttle, R.R. (1993). Tutorial lecture. Amercian College of Sports Medicine 39th Annual Meeting, Dallas, TX.

Tuttle, R.R., & Mills, J. (1975). Development of a new catecholamine to selectively increase cardiac contractility. Circulation Research, 36: 185-196.

Vasu, M.A., O'Keefe, D.D., Kapellakis, G.Z., Vezeridis, M.P., Jacobs, M.L., Daggett, W.M., & Powell, W.J. (1978). Myocardial oxygen consumption: Effects of epinephrine, isoproterenol, dopamine, norepinephrine, and dobutamine. American Journal of Physiology, 235: H237-H241.

Vincent, J., Roman, A., De Backer, D., & Kahn, R. (1990). Oxygen uptake/supply dependency. American Review of Respiratory Disease, 142: 2-7.

Wasserman, K., Hansen, J.E., Sue, D.Y., Whipp, B.J., & Casaburi, R. (1994). Principles of exercise testing and interpretation, 2nd ed. Philadelphia: Lea & Fegiger.

Wexler, H., Kuaity, J., & Simonson, E. (1971).

Electrocardiographic effects of isoprenaline in normal subjects and patients with coronary atherosclerosis. British Heart Journal, 33: 759-764.

Wisenberg, G., Zawadowski, A.G., Gebhardt, V.A., Prato, F.S., Goddard, M.D., Nichol, P.M., & Rechnitzer, P.A. (1985). Dopamine: its potential for inducing ischemic left ventricular dysfunction. Journal of the American College of Cardiology, 6: 84-92.

Wood, F.C., Wolwerth, C.C., & Livsey, M.M. (1931). Angina pectoris: the clinical electrocardiographic phenomena of the attack and their comparison with the effects of experimental temporary artery occlusion. Archives of Internal Medicine, 47: 339.

Woods, S.C., & Porte, D. (1974). Neural control of endocrine pancreas. Physiologic Reviews, 54: 596-612.

Young, M., Pan, W., Wiesner, J., Bullough, D., Browne, G., Balow, G., Potter, S., Metzner, K., & Mullane, K. (1994). Characaterization of arbutamine: A novel catecholamine stress agent for diagnosis of coronary artery disease. Drug Development Research, 32: 19-28.

# APPENDIX A

## List of Abbreviations of Terms and Units of Measure

## LIST OF ABBREVIATIONS

Abrreviations of Terms

| | |
|---|---|
| ACSM | American College of Sports Medicine |
| AHA | American Heart Association |
| CAD | coronary artery disease |
| COR | cortisol |
| DBP | diastolic blood pressure |
| DOP | dopamine |
| ECG | electrocardiogram |
| EPI | epinephrine |
| ESA | exercise simulating agent (arbutamine) |
| ETT | exercise stress test |
| FFA | free fatty acids |
| GCG | glucagon |
| GLU | glucose |
| GLY | glycerol |
| HLa | lactate |
| HPLC | high performance liquid chromatography |
| HR | heart Rate |
| INS | insulin |
| ISO | isoproterenol |
| MET | metabolic equivalent of oxygen consumption |
| NEPI | norepinephrine |
| -OH | hydroxyl |
| RPP | rate pressure product |
| SBP | systolic blood pressure |
| 2-DE | two dimensional echocardiography |
| 0R | zero minutes recovery |
| 5R | five minutes recovery |
| 10R | ten minutes recovery |
| 30R | thirty minutes recovery |
| 60R | sixty minutes recovery |

Abbreviations of Units of Measure

| | |
|---|---|
| bt | beats |
| $^0$C | degree centrigrade |
| dl | deciliter |
| kg | kilogram |
| l | liter |
| mEq | milliequivalent |
| min | minutes |
| ml | milliliter |
| mmHg | millimeters of mercury |
| mmol | millimole |
| msec | millisecond |
| mv | millivolt |
| mg | milligram |
| ng | nanogram |
| pg | picogram |
| rpm | revolutions per minute |
| ug | microgram |
| uIU | micro-International Unit |

# APPENDIX B

## Informed Consent

Informed Consent for the ESA System

Protocol #0135 - Virginia Tech Extension

by

Michael E. Slayton, M.D.,

William G. Herbert, Ph.D.,

J. Edwin Wilder, M.D.,

and

J. Michael Payne, M.D.

Laboratory for Health & Exercise Sciences

Virginia Polytechnic Institute and State University

Blacksburg, Virginia, 24061-0326

Short Title of Research Project:

A Multicenter Study of the ESA (Arbutamine) System and Exercise Stress Testing on Selected Hormones and Substrates, Oxygen Uptake and Ventilatory Parameters.

Investigators: Michael E. Slayton, Telephone#: 702/231-6565

J. Edwin Wilder,

William G. Herbert

and

J. Michael Payne

I have been asked to participate in a research study sponsored by Gensia Pharmaceuticals, Inc., 11025 Roselle Street, San Diego, California, 92121-1204, U.S.A. A

description of the study and other important study information have been provided for me in this consent form. Before I decide whether or not I want to participate, I will discuss the study with the medical staff conducting the research who will answer my questions. I have had at least 48 hours to study this informed consent document and decide on my participation.

Voluntary Participation:

I understand that if I refuse to participate in this research study or choose to discontinue my participation at anytime, no penalties or loss of benefits to which I am otherwise entitled will occur. If I discontinue study participation, the medical staff will advise me regarding other courses of treatment and the best way for me to terminate my participation. Regardless of whether I withdraw from the study voluntarily or my participation is ended by the investigator, I agree to complete certain termination procedures including laboratory tests electrocardiogram and physical examination to protect my safety.

Purpose:

Exercise stress tests are frequently used to help determine if patients have coronary artery disease; this is a disease affecting the blood vessels that provide nutrition and oxygen to the heart. Arbutamine is a new drug which has not yet been approved for use by the Food and Drug Administration of the U.S. Arbutamine increases the strength and rate of the heart beat in a manner similar to the effect of exercise. Arbutamine is administered by a device that controls the amount of drug a person is given according to how much his/her heart rate increases. The device has also not yet been approved by the Food and Drug Administration. Arbutamine and its delivery device is referred to as the ESA System. The purpose of this study is to determine the effects of arbutamine an exercise on hormones (body chemicals) and respiration (breathing and use of oxygen) parameters.

Description of Procedures:

Approximately 230 patients with suspected coronary artery disease throughout the U.S. have already participated in a similar study; 10 patients will participate at the Virginia Tech site. The time to complete each individual's participation will total 9 to 10 hours divided over 4 visits to the test site at Virginia Tech in Blacksburg. The Cardiac Therapy and Intervention Center in War Memorial

Hall will be the laboratory site. These visits will take place within an interval of 4 to 35 days, depending on when procedures are scheduled.

To participate in this study, my personal physician must have determined that I have known or suspected coronary artery disease, prior history of myocardial infarction, coronary artery bypass surgery, or received other treatments for coronary artery disease. If my personal physician agrees that I may participate, and if I choose to participate, I will sign the informed consent. During the first visit to the Cardiac Therapy Center I will undergo screening procedures including a review of my medical history, a physical examination including vital signs, and a resting electrocardiogram. An electrocardiogram is a simple study of electrical activity of the heart, also called "ECG". I agree to disclose any medications, including alcohol and tobacco, that I have recently taken, as well as disclose any changes in my medications during the study. In addition, blood and urine specimens will be obtained from me for clinical laboratory evaluations, If I have not had an exercise test performed within the last 6 months, I agree to undergo a screening exercise test.

If the results of my screening procedures show that I am an appropriate subject for this study, I will undergo both an ESA System test and an exercise stress test on

separate visits. If I am currently receiving therapy with "beta blocking" agents such as propranolol or atenolol, it will be necessary for me to gradually stop taking my beta blocking medication for at least 48 hours prior to each test. My personal physician must agree for my to be withdrawn from my beta blocking medication. My physician will specify how I will decrease my dosage, and whether an alternative medication will be necessary. I understand that if I stop my beta blocker medication suddenly and without supervision of a physician, I may endanger my health as a result of increased heart rate and possible elevation of blood pressure. My personal physician will work with the medical staff of the study to keep track of any problems that I may have with stopping this medicine. The ESA System and exercise tests will be performed on separate days. The order or the tests will be assigned to me by chance. On each test day, the functions of my heart will be monitored by a physician and nurse by periodic electrocardiograms as well as measurements of blood pressure and heart rate. Blood specimens will be collected several times: prior to each test, at the end of exercise or arbutamine therapy, and at 10, 30, and 60 minutes following exercise and arbutamine therapy. A special tube called an intravenous catheter will be inserted into a vein in my arm to collect blood samples. It will remain in place for the entire test: approximately

2 hours. Through the course of the study, a total of approximately 250 mls (amount is a little more than one cup) of blood will be taken for laboratory analyses. In addition, for each stress test, I will be fitted with a breathing apparatus which will measure my respiratory function.

During the ESA System test, I will receive a quantity of arbutamine solution in my vein while my response to the drug is monitored. The drug delivery device will control how much and how quickly arbutamine is administered. The delivery device has been designed to signal if device problems are detected and drug delivery should stop if a potentially hazardous situation is discovered.

During the exercise test, a treadmill that increases the required level of exercise every 2 minutes will be used.

If problems occur that prevent the completion of either test, I agree to return to the laboratory in War Memorial Hall and repeat the test on another day. I understand that the Cardiac Therapy Center is not a hospital; however, a board certified cardiologist will supervise the ESA system test and a board certified internal medicine physician will supervise the exercise test. An emergency medical technician will be present in the lab at all times. The ambulance and emergency rescue squad is located on the Virginia Tech campus. Its average time to reach our

facility is 3 to 4 minutes and it is 4 miles from the nearest hospital.

Arbutamine infusion and exercise will end: (1) when electrocardiogram signs of coronary artery disease have been detected, (2) symptoms which prevent me from completing the tests occur, (3) my targeted heart rate has been achieved, (4) the supervising physician believes it to be in my best interest to stop, and/or (5) I no longer wish to participate.

After the tests have been completed or if I stop participation before I complete both tests, I agree to return for a final follow-up assessment. At this visit, monitoring tests including a physical examination, vital signs, electrocardiogram and collection of laboratory specimens will be repeated for my safety.

Risks and Discomforts:

Many people who have been given this drug note that their heart beats faster and/or stronger. These are expected effects of arbutamine. Other side effects noted during arbutamine therapy include headache, brief decreases in blood pressure, chest pain, irregular heart beat, dizziness, decreased blood potassium levels, tremor, flushing of the skin and decreased heart rate. Most of these adverse events have been mild and all have gone away once administration of the drug was stopped or appropriate

treatment was administered. In approximately 900 subjects who received arbutamine, 4 patients were observed overnight following significant arrhythmias (irregular heart beat). Some effects of arbutamine may also be reversed by readily available treatments that the medical staff will have available when you are tested, such as the drugs esmolol or metoprolol. Because the safe use of arbutamine in pregnancy and by lactating mothers has not been established, women who are pregnant or breast feeding are not allowed to participate in this study.

Although the experimental device used to administer arbutamine has many safety alert and alarm systems, the possibility exists that the device could malfunction. To our knowledge, it has failed only on one previous occasion as a result of computer software problems; this problem has already been corrected. During ESA System tests, the performance of the device will be monitored by checks built into the system itself as well as by the medical staff conducting the study. The administration of arbutamine can be stopped at anytime by the medical staff. Remote risks which may occur from use of the device include an inadvertent delivery of air or larger than expected dose of drug into the veins and the unlikely possibility of electrical shock due to inadequate grounding.

Exercise testing may cause severe shortness of breath,

chest pain often called angina, fatigue, dry mouth or irregular heart beat. During the study, heart rate, electrocardiograms and symptoms will be carefully monitored to minimize the chances of any problems occurring.

Although unlikely, it is remotely possible that either the ESA System test or the exercise test could cause a problem that would result in hospitalization, such as heart attack, heart rhythm changes or even death. To date, no deaths have been reported. Other study risks include the possibility of pain, bruising or oozing of blood at the site of the insertion of needles for blood specimens or drug administration. Another possibility, although rare, is the development of thrombophlebitis; this is inflammation of the vein. In addition, as yet unknown side effects or conditions associated with study tests may occur. I will be made aware of any important new findings about the drug or the device that delivers it that may affect my decision to participate or continue participation in the study.

Benefits:

Benefits from the study procedures are not guaranteed. I will be provided with information from the physical examinations, laboratory tests and the exercise tolerance test that I will have completed in this study. If my personal physician agrees and I am interested and able to do so, I will also receive exercise recommendations on how I

might improve my physical fitness and reduce my current heart disease risk factors. I also am eligible to receive the equivalent of $200 in clinical services as a participant in the health-fitness program of Virginia Tech. If I reside closer to another cardiac rehabilitation center outside of Blacksburg, Virginia, then these benefits may be transferred to that center. If I so request, my doctor will receive copies of results from my physical exams, blood chemistries, and my exercise test. In addition, reports of any rehabilitation services provided to me by the Virginia Tech staff can also be sent to my doctor if I agree. It is possible that information obtained from these tests may be of benefit to my doctor in managing my health care. I may also have the satisfaction of participating in a research trial that could result in improved treatment for other patients.

I understand that no payment is available to compensate me specifically for my time and effort as a subject in this research study. I understand that should my participation be discontinued for any reason, I will receive whatever pro-rated Virginia Tech health-fitness program benefits are due, in accordance with any initial agreement with the staff.

Alternate Procedures:

The exercise stress testing and the electrocardiograms performed in this study are just some of the methods used to

evaluate patients with suspected coronary artery disease. Other methods available, but not employed in this study, include the use of radio-labelled isotopes injected into the blood stream for heart imaging studies and use of echocardiograms. These are noninvasive studies of heart muscle movement.

Costs to the Patient:

Any medical procedures such as additional blood tests that the physician investigators decide are needed for my participation will be provided without cost to me.

Compensation for Injury:

If physical injury directly resulting from research procedures occurs, I understand that both emergency and non-emergency care will be provided. I understand that I will not have to pay for any reasonable and customary costs associated with the diagnosis and treatment provided to me for research related injuries.

Confidentiality:

The medical information gathered during this study by the research staff at Virginia Tech will be reviewed by Gensia Europe, Ltd, and Gensia Pharmaceuticals, Inc., and may be disclosed to appropriate regulatory authorities, including the U.S. Food and Drug Administration. Appropriate staff from the sponsor and/or regulatory authorities will review my medical records to verify the

accuracy of the information collected. This information will be treated confidentially. If the results of the study are published, my identity will not be disclosed. By signing this consent form, I grant my permission for review of such confidential information.

Questions or Research Related Problems:

This research plan has been reviewed and approved by a committee at Virginia Tech created for the protection of human research subjects known as the Institutional Review Board. If I have questions about this research, I can contact the Clinical Studies Coordinator, Karen Dorn, R.N., (703/951-3311 during office hours). In the event of a research related injury, I should contact the laboratory director, William G. Herbert, Ph.D., at 703/231-6565. If I need assistance outside of normal office hours, the following 24-hour telephone number will put me in contact with a member of the medical staff: Michael E. Slayton, M.D. or J. Edwin Wilder, M.D., 703/951-1111. The hospital switchboard operator will contact them. Questions about research subjects' rights should be addressed to Janet M. Johnson, Ph.D., 335A Wallace Hall, Virginia Tech, Blacksburg, VA 24061, who can be reached at 703/231-6168.

Summary

I understand the conditions and procedures as written above. I agree to undergo screening procedures including a

review of my medical history, a physical examination, electrocardiogram, blood test and, if needed, a preliminary exercise test. I agree to disclose any medications, including alcohol and tobacco, that I have recently taken, as well as disclose any changes in my medications during the study. If I am on "beta-blocking" medication, I agree to be withdrawn form this medication only under the direction of my personal physician.

I understand that it is my right to withdraw from the study at anytime and that the study could be discontinued without my consent by the investigator or by the sponsors. I agree to complete certain termination procedures including laboratory tests, check of vital signs, an electrocardiogram and physical examination to protect my safety.

I understand the risks of my participation and the nature of any potential benefits. I understand that the study site is not a hospital. If I am on "beta-blocking" medication, I understand that stopping this medication suddenly and without supervision of a physician may be dangerous to my health. I understand that I will receive free services (as listed on page ten and written at the time of signing of this consent form).

I have had the opportunity to ask questions. Any questions which I have asked have been answered to my complete satisfaction.

Of my own free will I consent to participate in this study. I will be given a copy of this consent form for my future reference.

Notes of Questions and Answers:

_____

_____

Acceptance of $200 equivalent in ree Services to be provided by Virginia Tech Cardiac Therapy and Intervention Center (list): (see attached literature about services offered).

_____

_____ _____
Signature of Patient/Legal Guardian Date

_____ _____
Signature of Investigator Date

_____ _____
Signature of Witness Date

# APPENDIX C

## Data Collection Work Sheets

Data Collection Worksheet
ESA

Subject _____-_____ (site - randomization #)

Initials _____ Date _____

Time of last oral intake _____

Caffeine day of test? YES NO

IV start: Time _____ (Use Mortara time)

15 MINUTES QUIET REST

BP #1 _____ HR _____ Time _____

BP #2 _____ HR _____ Time _____

$VO_2$ baseline for 3-5 minutes

| Measures | ECG | BP | Blood |
|---|---|---|---|
| Baseline | _____ | _____ | _____ (all) |
| BEGIN TEST | _____ | _____ | Comment/Symptoms |
| 2 min | _____ | _____ | |
| 4 min | _____ | _____ | |
| 6 min | _____ | _____ | |
| 8 min | _____ | _____ | |
| 10 min | _____ | _____ | |
| 12 min | _____ | _____ | |
| 14 min | _____ | _____ | |
| 16 min | _____ | _____ | |
| END INFUSION | _____ | _____ | _____ ($K^+$ , HOR) |

END INFUSION TIME _____ (0 min post-test)

MAX INFUSION RATE _____ TOTAL DOSE RATE _____

| Recovery | ECG | BP | Blood | |
|---|---|---|---|---|
| + 1 min | _____ | _____ | | |
| + 2 min | _____ | _____ | | |
| + 4 min | _____ | _____ | | |
| + 5 min | _____ | _____ | | |
| + 8 min | _____ | _____ | | |
| + 10 min | _____ | _____ | _____ | (HOR) |
| + 12 min | _____ | _____ | | |
| + 16 min | _____ | _____ | | |
| + 20 min | _____ | _____ | | |
| + 24 min | _____ | _____ | | |
| + 28 min | _____ | _____ | | |
| + 30 min | _____ | _____ | _____ | ($K^+$, HOR) |
| + 60 min | _____ | _____ | _____ | (all) |

NOTES:

| Event | Onset | Offset | Comments |
|---|---|---|---|

Data Collection Worksheet
ETT

Subject _____-_____ (site - randomization #)

Initials _____ Date _____

Time of last oral intake _____

Caffeine day of test? YES NO

IV start: Time _____ (Use Mortara time)

15 MINUTES QUIET REST

Supine Position

BP #1 _____ HR _____ Time _____

$VO_2$ Supine Baseline 3-5 min _____

BP #2 _____ HR _____ Time _____ Blood _____

Exercise Position

BP #1 _____ HR _____ Time _____

$VO_2$ baseline for 3-5 minutes

BP #2 _____ HR _____ Time _____

| Measures | ECG | BP | RPE/Symptoms |
|---|---|---|---|
| BEGIN TEST | _____ | _____ | Comment/Symptoms |
| Stage I | _____ | _____ | |
| Stage II | _____ | _____ | |
| Stage III | _____ | _____ | |
| Stage IV | _____ | _____ | |
| Stage V | _____ | _____ | |
| Stage VI | _____ | _____ | |
| Stage VII | _____ | _____ | |

| | | | |
|---|---|---|---|
| END TEST | _____ | _____ | _____ ($K^+$ , HOR) |
| NOTE TIME _____ (0 min post-test) | | | PEAK METS _____ |
| Recovery | ECG | BP | Blood |
| + 1 min | _____ | _____ | |
| + 2 min | _____ | _____ | |
| + 4 min | _____ | _____ | |
| + 5 min | _____ | _____ | |
| + 8 min | _____ | _____ | |
| + 10 min | _____ | _____ | _____ (HOR) |
| + 12 min | _____ | _____ | |
| + 16 min | _____ | _____ | |
| + 20 min | _____ | _____ | |
| + 24 min | _____ | _____ | |
| + 28 min | _____ | _____ | |
| + 30 min | _____ | _____ | _____ ($K^+$, HOR) |
| + 60 min | _____ | _____ | _____ (all) |

NOTES:

| Event | Onset | Offset | Comments |
|---|---|---|---|

# APPENDIX D

## Detailed Methodology

## DETAILED METHODOLOGY

This study was part of a larger study, Gensia Protocol 0135: A Multicenter Study to Describe the Effects of the ESA (Arbutamine) System an Exercise Stress Testing on Selected Hormones and Substrates, Oxygen Uptake and Ventilatory Parameters. The study employed an open-label, randomized cross-over, repeated measures design. Preliminary work was done during an addendum study to Gensia Protocol 0122B. Protocols 0122B and 0135 were part of the Phase Three Clinical Trials of the ESA System. Karen Dorn served as the Study Coordinator at the Virginia Tech Site for Protocols 0125 and 0135. The study coordinatory was responsible for subject recruitment, coordination of the clinical trials, data collection, handling of specimens, completion of case report forms, and coordination of data analysis.

Gensia protocol 0135 included objectives beyond the scope of the present study to include continued evaluation of the safety and tolerance of the ESA System and assessment of oxygen uptake and ventilatory parameters of arbutamine. In accordance with the first objective, a detailed study protocol (Gensia Protocol 0135 and Amendment A, May 1993) and study procedures (ESA System Directions for Use, ECG Data Collection and Analysis Plan for The ESA System Phase Three Protocol

0135, Case Report Forms for Protocol 0135) were followed as required for any site employing the ESA System. Additionally, documentation of clinical data and any adverse events were recorded and filed in case report forms at each study site. The plan described below includes the study plan relevant to the present study to determine the comparative effects of exercise and arbutamine on selected cardiovascular, hormonal, and metabolic responses.

Subject Selection

Potential subjects were identified from two university-based cardiac rehabilitation centers and referring physicians. The study coordinator at each site met with the potential subject to discuss participation. If the potential subject expressed an interest in participating, the study was then explained in detail, a copy of the informed consent document was provided, and the subject was interviewed for principal inclusion criteria, and any questions the potential subject may have had were answered. If the subject agreed to participate, each potential subject's participation was approved according to the inclusion and exclusion criteria by the patient's attending cardiologist or internist with permission of the primary physician.

Subjects were included who met all of the following

inclusion criteria:

a) provided written informed consent,

b) legal age of consent or older,

c) males; and females not of childbearing potential (e.g., tubal ligation, hysterectomy, post-menopausal) or using hormonal or barrier contraceptive methods,

d) known or suspected coronary artery disease (i.e., patients with a history of ischemic responses to exercise, prior history of myocardial infarction, coronary artery bypass graft operation, percutaneous coronary angiography, or angiographic evidence of coronary artery disease defined as $\geq$ 50% stenosis of one or more major coronary arteries)

e) achieved at least six METs on a symptom limited ETT performed within six months of the first study stress test. MET capacity was estimated from the highest treadmill stage maintained for a least two minutes.

Subjects were excluded who met any of the following exclusion criteria:

a) ETT is contraindicated,

b) low exercise tolerance (< 6 METs on ETT),

c) screening ECG abnormalities which preclude ST

segment analysis such as resting ST segment elevation or depression > 0.05 mv in more than three of twelve leads, left bundle branch block, and left ventricular hypertrophy with ST-T changes,

d) cardiovascular diseases to include unstable angina within six weeks preceding screening, myocardial infarction within 30 days preceding screening, history of sustained ventricular tachycardia (at least ten beats) or cardiac arrest except when associated with acute myocardial infarction, cardiac pacemaker or automated cardiac defibrillator in situ, congestive heart failure - New York Heart Association Class III or IV, dilated cardiomyopathy, idiopathic hypertrophic subaortic stenosis or hemodynamically significant aortic stenosis or other hemodynamically significant valvular disease, uncontrolled systolic hypertension at screening or during baseline period before either study stress test (supine SBP > 180 mmHg), and known aortic aneurysm or dissection,

e) laboratory exclusions to include hypokalemia, inadequate renal function indicated by a blood

urea nitrogen or creatinine > 2 times the upper limit of normal, and inadequate hepatic function indicated by total bilirubin, SGOT and/or SGPT > 3 times upper limit of normal,
f) a history of other diseases to include diabetes mellitus, cerebrovascular accident, narrow angle glaucoma, uncontrolled hyperthyroidism, and hypersensitivity to sulfites,
g) medication contraindications to include patients in whom catecholamines are contraindicated, *B*-adrenoceptor antagonists within 48 hours prior to either test, class 1 antiarrhythmic drugs or tricyclic antidepressants within one week prior to the screen visit, digitalis therapy within two weeks of the screen visit, amiodarone within 30 days of the screen visit, any other unlicensed drug within 30 days prior to the start of the study,
h) females who are pregnant or lactating as well as females of childbearing potential who are not utilizing barrier or hormonal contraceptive methods,
i) inability or unwillingness to abide by the protocol.

Protection of Human Subjects

The research protocol and informed consent document (Appendix A) was approved by the Internal Review Board (IRB) for research involving human subjects at each study site. The IRB was informed in a timely manner of any adverse effects which occurred during the course of the study at any study site involved in this or other trials involving the ESA System. Subjects underwent a comprehensive medical screen and follow-up examination to assure patient safety and identification of any adverse events. Additionally, approval to participate according to the selection criteria was obtained from the subject's personal physician. Testing was conducted in cardiac rehabilitation laboratories with extensive experience in exercise testing, in the presence of a physician investigator, a registered nurse, exercise physiologist, and support personnel. The physician investigators and study coordinators received training in the use of the ESA System. Cardiac resuscitation equipment and medication cart with parenteral *B*-blockers were available.

Study Plan

Screening. Following written informed consent the subject underwent screening procedures which included a

medical history and physical exam. Vital signs, a resting 12-lead ECG, and blood for hematology and clinical chemistry (and HCG if female of child-bearing potential) were obtained as part of the exam. For patients on *B*-blockade agents, subjects had to be able to safely withdraw from *B*-blocker therapy for at least 48 hours prior to each study stress test. Following review of screening results by a study physician, determination that selection criteria had been met, and the subject was medically eligible to participate, the subject was enrolled in the study.

Testing. The order of testing was determined according to a randomization schedule based on enrollment of ten subjects at each site. The order of testing for each subject was not revealed to the investigators until the subject was enrolled in the study. The first test was scheduled 1 to 14 days from the first screening procedure. The second test was scheduled 20 hours to 14 days after the first test. The follow-up assessment was conducted 24 hours to 7 days following the second test or upon premature study discontinuation to assure subject safety and determine if there had been any adverse effects of the protocol.

The ESA and ETT were performed in the morning as close to the same time of day as possible. Prior to

testing, patients fasted for an 8 to 12 hour period. Patients receiving *B*-blockers had the medication discontinued under physician supervision for 48 hours prior to each test. Following skin preparation, ECG electrodes for the 12-lead ECG were placed with limb leads in the usual chest positions (ACSM, 1992). Continuous 12-lead ECG monitoring was conducted and ECG recordings at 25 $mm \cdot sec^{-1}$ were obtained using the Mortara monitoring system. On occasion, when technical difficulties with the Mortara system occurred, a Quinton 2000 monitoring systems was used. For the ESA System, additional electrodes were placed in the LL (-), RA (+), and LA (reference) positions for R-wave detection of heart rate by the delivery device.

An indwelling intravenous catheter was inserted in an antecubital vein for blood specimen collection. A three-way adapter was attached to the catheter for ease in obtaining blood specimens. An additional catheter with minimal dead space was inserted in the opposite arm for arbutamine infusion during the ESA test. This catheter was discontinued after termination of the infusion. The patency of each catheter was maintained by an infusion of 0.9% sodium chloride.

The blood pressure cuff was placed on the same arm as the specimen catheter. Electronic blood pressures

using the ESA device was used to determine appearance and cessation of Korotkoff sounds during the ESA test. A cuff and stethoscope was used to obtain blood pressures during the exercise test.

A minimum of 15 minutes of quiet supine rest following catheter insertion(s) preceded baseline blood specimen collection and baseline ECG, HR and BP measures. During each stress test, HR, BP, ECGs, symptoms, and any significant signs, were monitored in accordance with accepted guidelines for exercise testing (ACSM, 1992) and the Gensia Protocol 0135. Oxygen uptake measures were obtained at baseline and during each test but not during recovery. Data collection worksheets (Appendix B) for each study test was used to assure standardization and completeness of data collection required for each test. The detailed protocol for blood specimen collection is provided below.

The ESA test was performed in the supine position. Arbutamine infusion was controlled by the ESA System; a computer controlled, "closed-loop" feedback device for the delivery of arbutamine based upon patient heart rate slope in $bt \cdot min^{-1}$. The heart rate slope for this study was 8 $bt \cdot min^{-1}$. The rate of infusion was programmed to begin at 0.1 $ug \cdot^{-1} \cdot min^{-1}$ and not to exceed a dose rate of 0.8 $ug \cdot^{-1} \cdot min^{-1}$ or to deliver a total dose greater than 10

$ug \cdot kg^{-1}$. The peak arbutamine dose rate was recorded for data analysis. During the infusion, the device automatically recorded HR at five second intervals and BP at two minute intervals. ECG tracings were recorded every two minutes. Following infusion, the intravenous catheter used for arbutamine infusion was clamped immediately to assure no additional drug was delivered and was discontinued as soon as was feasible for subject comfort.

The ETT was performed in the upright position using the Naughton protocol. The protocol was modified for subjects with higher functional capacities to start beyond stage I to assure that total test times did not differ significantly between arbutamine and exercise. During the test, ECG and BP were obtained at the end of each two minute stage. The peak treadmill workload was recorded for data analysis. Immediately following achievement of the test endpoint, the subject was rapidly assisted to a supine position for post-test data collection.

The endpoint of each test was a) achievement of target HR defined as 85% of age-predicted maximal HR, b) horizontal or downsloping ST segment depression $\geq$ 2.0 mm (0.2 mv) measured 60 msec after the J point or $\geq$ 0.2 mm (0.2 mv) ST segment elevation in leads without Q waves,

c) intolerable anticipated events (e.g., angina or exercise limiting fatigue), d) intolerable adverse events, e) mechanical or device malfunction or failure, f) subject request to terminated the test, or g) other reasons (e.g., total dose of arbutamine received, failure of HR to rise with increasing dose rates of arbutamine). The supervising physician, a cardiologist for the ESA and an internist or cardiologist for the ETT, determined test endpoint.

During the recovery period, HR, BP, and ECG recordings, and blood for chemistry and hematology were obtained to assure patient safety and according to the research protocol.

Blood collection and handling

The total blood collected for each stress test was approximately 117 mls (34 ml for clinical analyses and 83 ml for the hormonal and metabolic analyses). Specimens were collected at baseline and in recovery at 0, 10, 30 and 60 minutes post-stress according to the table above. Specimen collection tubes were selected based on the recommendations from each analysis kit or procedure to be performed and to assure compatibility with additives and methods when more than one analyte was drawn in the same tube. Free fatty acid and glycerol specimens were drawn in room temperature red top serum tubes without additives

and allowed to clot at room temperature for at least one hour. All other blood specimens were collected in chilled tubes and placed into a slurry of ice and water for the duration of the test. Glucose and lactate were drawn in a grey top tube with sodium fluoride as an anticoagulant and potassium oxalate as an anti-oxidant to prevent glycolysis by glucose oxidase. Catecholamines were drawn in a 4.5 ml red top tube with EGTA as the anticoagulant and reduced glutathione as the antioxdant to prevent degradation by monoamine oxidase. Cortisol, insulin and glucagon were drawn in lavender top tubes with sodium EDTA as the anticoagulant. The following table summarizes the blood collection protocol.

Summary of Blood Specimen Collection

| Analyte | Additive | Pre-test | Post-test (minutes) | | | |
|---|---|---|---|---|---|---|
| | | Baseline (mls) | 0 | 10 (mls) | 30 | 60 |
| COR, INS, GLU | $Na_2EDTA$ (plasma) | 3 | 3 | 3 | 3 | 3 |
| FFA, GLY | none (serum) | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| EPI NEPI, DOP | EGTA + reduced glutathione (plasma) | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| HLa GLU | $NaF + K_2C_2O_4$ (plasma) | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |

To control for plasma volume shifts which may effect hormone concentration, hematocrit was determined at each time point by drawing approximately 0.1 ml of blood from the $Na_2EDTA$ tube into two capillary tubes. Hematocrit was determined by microhematocrit centrifuge technique. The numerical average $\pm$ 0.5% of the two microhematocrit results for each collection time was used to determine hematocrit.

The iced blood samples taken from the slurry were centrifuged at 4°C for seven minutes at 2500 revolutions per minute (rpm). Free fatty acids and glycerol specimens were centrifuged at 25°C for sevem minutes at 2500 rpm. After centrifugation, serum or plasma was transferred to labeled 2 ml screw cap freezer vials. Due to the instability of glucagon, aprotinin was added to the freezer vials for glucagon. Care was taken to keep specimens for catecholamines, cortisol, insulin and glucagon assays cold at all times. These specimens were handled first and immediately placed in a minus 20°C freezer. After all specimens had been aliquoted, the catecholamine specimens were transferred to a minus 70°C freezer.

Specimens from both study sites were analyzed by batch analysis. Frozen specimens were packed in dry ice

for transport to the study site performing the analyses. To assure consistency of data collection and handling procedures at both study sites, all collection tubes and freezer vials were prepared by Karen Dorn, study coordinator at the Virginia Tech study site. Research assistants at both study sites were trained by the study coordinator and detailed written instructions and diagrams were provided for blood collection and handling procedures.

Data Analysis

Microhematocrit results were found to underestimated hematocrit results determined by the central laboratory as part of the clinical monitoring protocol. In addition, the underestimation did not appear equal across time. The greatest underestimation occurred at baseline. This was believed to be due to the effect of red cell sedimentation with time. Since all tubes used to obtain hematocrits needed to be kept in an ice bath until transferred to the cold centrifuge, the tubes were not rotated to assure equal distribution of red cells in plasma. Thus, adjustments for shifts in plasma volume between treatments and across time could not be made. However, all samples were obtained in the supine position to minimize the effect of position on fluid volume shifts.

Cortisol, insulin, and glucagon were analyzed using the following radioimmunoassays (ICN Biochemicals, Inc., Costa Meca, CA): $^{121}$I Cortisol (Cat. No. 07-221), $^{125}$I Insulin (Cat. No. 07-160102), and $^{125}$I glucagon (Cat. No. 07-1522101). Catecholamines were determined by high performance liquid chromatography (HPLC) on a Beckman 244 modular system (Beckman Instruments Inc, San Ramon, CA) connected in series to a BAS Model LC-4B (Bioanalytical Systems Inc., West Lafayette, IA) post column electrochemical detector using an aluminum chloride reference electrode. Dihydrobenzoic acid (DHBA) was used as the internal standard. Radioimmunoassays for hormones and HPLC determination of catecholamines were done by Delbert Jones, Senior Laboratory Specialist at the Veterinary Medicine Biochemistry Laboratory, Virginia Tech, Blacksburg, VA. Enzymatic colorimetric assays were used to analyze free fatty acids (WAKO NEFC C test kit; Wako Chemicals, Dallas, Texas), glycerol (Triglyceride GPO-Trinder Procedure No. 337, Sigma Diagnostics, St. Louis, MO), and glucose (Enzymatic Glucose Procedure No. 1070, Stanbio Laboratory, Inc., San Antonio, Texas). Kathy Reynolds, Lab Specialist, performed the assays for glucose, glycerol, and free fatty acids at the Metabolic Reasearch Laboratory, Virginia Tech, Blacksburg, VA. Lactate was determined by enzymatic electrochemical

detection using a 2300 YSA Lactate Analyzer (Yellow Springs Instruments, Yellow Springs, OH) and run by Jeff Ocel and Laura Craft, Lab Specialists, Laboratory for Exercise, Sport & Work Physiology, Virginia Tech, Blacksburg, VA. Circulatory data including ST segment analysis was completed by Karen Dorn.

Statistical Analysis

It is recognized that controversy exists in the appropriateness of a given statistical analysis for repeated measures designs in which the same set of subjects receive more than one treatment. (Finney, 1990; Matthews, Altman, Campbell, & Royton, 1990). The confounding of the treatment effects with time trends can be controlled to some extent by use of the cross-over design. The most appropriate statistical model is determined not only by the design, but to a greater extent on the objectives of the study, the structure of the data, and the number of times each subject is measured (Finney, 1990).

The primary objective of this study was to determine differences in physiologic responses between arbutamine and exercise. In this study, determination of the effects of treatment sequencing was not a research objective as is the case in some cross-over designs. The research protocol was designed to minimize carry-over

effects. In order to minimize any possible residual effects due to the previous treatment, a washout time of at least twenty hours between treatments was included in the design. With the washout period, carry-over effects due to arbutamine were not anticipated due to the short half-life of the drug. Likewise, carry-over effects were not anticipated due to exercise as subjects regularly participated in a physical activity program and the exercise stimulus was not a maximal effort test. Randomization of subjects to a treatment sequence also served to distribute any residual effects due to treatment equally between arbutamine and exercise treatment groups. The study design can be illustrated as follows:

| | | Sequence | |
|---|---|---|---|
| | | I | II |
| Treatment | R | Arbutamine (n = 3) | Exercise |
| | | Exercise (n = 5) | Arbutamine |

Statistical methods can be used to determine if carry-over effects are present to a significant extent. In this study, two statistical procedures were used to test for carry-over effects. First, differences in baselines among the four treatment-sequence blocks were

determined. No baseline differences were found. Baseline measures were not used in the subsequent analysis of variance test.

Second, the effect of sequencing on the recovery time trend was determined. This can be accomplished by a split-plot analysis (Finney, 1990). However, when a carry-over effect does occur, data obtained in the second sequence are eliminated from the hypothesis test. The spit-plot approach was not considered appropriate with this data set due to the small sample size and unequal cell sizes. The statistical approach employed with this data was to determine whether, sequence, when included as a factor in the analysis of variance model, contributed significantly to the statistical model. If a sequencing effect did occur, evidence of some treatment differences could still be determined after adjustment of the treatment sums of squares for the sequence effect. When the sequence effect was not significant, the sequence factor could be dropped from the analysis of variance model to increase the statistical power of the hypothesis test. Using this approach, the effect of sequencing was found to be not significant ($p \leq 0.05$) due to main effect or interaction effects with time or treatment for any variable and was dropped from the analysis of variance model.

The SAS statistical package was used to perform the analysis of variance (SAS System for Linear Models, 3rd ed., SAS Institute, Inc., Cary, NC). The SAS program uses a general linear models procedure (GLM) for repeated measures analysis of variance to test the effect of time, treatment, and time by treatment interaction. Either a univariate of multivariate procedure can be used and there are advantages and disadvantages to each. In general, the univariate procedure is statistically more powerful but assumes equal correlation and variance between the repeated measures within each time interval. This is the assumption of sphericity. The multivariate procedure does not assume equal correlation and adjusts for unequal correlations between the repeated-measurements based on a correlation matrix of the repeated-measurement observations. The SAS statistical program used the Machley's Criterion to test for sphericity. When the test for sphericity applied to orthogonal components is significant, an adjusted probability for the univariate procedure should be used. Alternatively, the multivariate test for the time and time by treatment interaction effects could be used.

In this data set, the univariate procedure was used and, to reduce the Type I error rate, the conservative Greenhouse-Geizer adjusted probability was used to

determine statistical significance at alpha $\leq 0.05$. When the overall analysis of variance was significant, the profiles of the time and treatment contrasts for each successive time interval was examined for where significant differences occurred ($p \leq 0.05$). In addition, differences at peak stress were examined.

A limitation of the SAS GLM procedure for repeated measures is that all cases in a time series are eliminated where any of the repeated measures are missing. In this study, missing data on heart rate and blood pressure resulted in loss of data in the statistical analysis due missing data.

Analysis of variance model assumptions relative to error variance were also examined. The distribution of the residuals from the analysis of variance was tested for normality ($p \leq 0.05$). Normal probability plots were constructed to further examine the distributions for homogeneity of variance. Log transformations were performed where residuals were not normally distributed or where variance heterogeneity existed and was necessary for glucose and epinephrine. The nonparametric Wilcoxin matched-pairs signed-ranks test was used to analyze ST segment shift since transformation of this variable did not result in normal distribution of the error term.

# APPENDIX E

Raw Data

Cardiovascular Variables

```
subject treatment sequence time HR SBP DBP RPP ST;*
01 1 1 1 060 122 058 073.2 0
01 1 1 2 124 161 072 199.6 -50
01 1 1 3 111 127 068 141.0 -30
01 1 1 4 101 109 071 110.1 -40
01 1 1 5 081 128 075 103.7 0
01 1 1 6 068 139 075 094.5 0
01 2 2 1 057 123 059 070.1 0
01 2 2 2 121 184 064 222.6 -20
01 2 2 3 064 114 058 073.0 -30
01 2 2 4 057 110 057 062.7 -10
01 2 2 5 068 107 065 072.8 0
01 2 2 6 057 109 060 062.1 0
02 1 2 1 057 130 083 074.1 0
02 1 2 2 127 124 067 157.5 -20
02 1 2 3 119 129 077 153.5 -50
02 1 2 4 109 123 074 134.1 -70
02 1 2 5 078 126 082 098.3 -10
02 1 2 6 063 129 092 118.7 0
02 2 1 1 064 135 087 086.4 0
02 2 1 2 136 216 098 293.8 -30
02 2 1 3 091 150 082 136.5 -20
02 2 1 4 084 122 078 102.5 -10
02 2 1 5 076 120 078 091.2 0
02 2 1 6 053 114 076 060.4 0
03 1 2 1 061 132 086 080.5 0
03 1 2 2 124 143 084 177.3 0
03 1 2 3 127 144 081 182.9 -20
03 1 2 4 129 127 069 163.8 -20
03 1 2 5 092 126 087 115.9 -20
03 1 2 6 073 133 083 097.1 -10
03 2 1 1 064 142 086 090.9 0
03 2 1 2 126 174 074 219.2 0
03 2 1 3 083 136 080 112.9 -20
03 2 1 4 075 140 080 105.0 0
03 2 1 5 072 134 084 096.5 0
03 2 1 6 072 136 088 097.9 0
04 1 2 1 060 117 080 070.2 0
04 1 2 2 148 136 072 201.3 -220
04 1 2 3 119 146 077 173.7 -100
04 1 2 4 109 135 078 147.2 -80
04 1 2 5 088 118 072 103.8 -40
04 1 2 6 081 111 079 089.9 -10
04 2 1 1 058 112 084 065.0 0
04 2 1 2 147 192 088 282.2 50
04 2 1 3 080 128 084 102.4 0
04 2 1 4 077 120 086 092.4 0
04 2 1 5 076 124 090 094.2 0
04 2 1 6 076 122 084 092.7 0
05 1 2 1 043 106 065 045.6 0
05 1 2 2 090 128 087 115.2 110
05 1 2 3 062 115 056 071.3 30
05 1 2 4 052 109 052 056.7 -20
05 1 2 5 052 103 058 053.6 -10
05 1 2 6 045 096 060 043.2 0
```

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 05 | 2 | 1 | 1 | 044 | 110 | 068 | 048.4 | 0 |
| 05 | 2 | 1 | 2 | 115 | 144 | 054 | 165.6 | 0 |
| 05 | 2 | 1 | 3 | 051 | 112 | 058 | 057.1 | 0 |
| 05 | 2 | 1 | 4 | 051 | 108 | 058 | 055.1 | 0 |
| 05 | 2 | 1 | 5 | 049 | 108 | 064 | 052.9 | 0 |
| 05 | 2 | 1 | 6 | 046 | 106 | 068 | 048.8 | 0 |
| 26 | 1 | 1 | 1 | 066 | 151 | 084 | 100.0 | 0 |
| 26 | 1 | 1 | 2 | 122 | 166 | 066 | 202.5 | -50 |
| 26 | 1 | 1 | 3 | 104 | 186 | 078 | 193.4 | -30 |
| 26 | 1 | 1 | 4 | 096 | 160 | 072 | 153.6 | -40 |
| 26 | 1 | 1 | 5 | 086 | 160 | 088 | 137.6 | 0 |
| 26 | 1 | 1 | 6 | 075 | . | . | . | 0 |
| 26 | 2 | 2 | 1 | 062 | 120 | 066 | 074.4 | 0 |
| 26 | 2 | 2 | 2 | 134 | 166 | 060 | 222.4 | -20 |
| 26 | 2 | 2 | 3 | 087 | 130 | 070 | 113.1 | -20 |
| 26 | 2 | 2 | 4 | 084 | 120 | 068 | 100.8 | 0 |
| 26 | 2 | 2 | 5 | 078 | 108 | 068 | 084.2 | 0 |
| 26 | 2 | 2 | 6 | 068 | 120 | 064 | 081.6 | 0 |
| 27 | 1 | 1 | 1 | 071 | 112 | 076 | 079.5 | 0 |
| 27 | 1 | 1 | 2 | 134 | 148 | 074 | 198.3 | 0 |
| 27 | 1 | 1 | 3 | 106 | 142 | 088 | 150.5 | -10 |
| 27 | 1 | 1 | 4 | 092 | 126 | 078 | 115.9 | -10 |
| 27 | 1 | 1 | 5 | 071 | 120 | 082 | 085.2 | 0 |
| 27 | 1 | 1 | 6 | 068 | . | . | . | . |
| 27 | 2 | 2 | 1 | 070 | 116 | 078 | 081.2 | 0 |
| 27 | 2 | 2 | 2 | 162 | 208 | 082 | 337.0 | 10 |
| 27 | 2 | 2 | 3 | 088 | 120 | 082 | 105.6 | 0 |
| 27 | 2 | 2 | 4 | 084 | 118 | 080 | 099.1 | 0 |
| 27 | 2 | 2 | 5 | 079 | 120 | 086 | 094.8 | 10 |
| 27 | 2 | 2 | 6 | 075 | 122 | 082 | 091.5 | 0 |
| 29 | 1 | 2 | 1 | 077 | 135 | 071 | 104.0 | 0 |
| 29 | 1 | 2 | 2 | 124 | 133 | 061 | 164.9 | -60 |
| 29 | 1 | 2 | 3 | 126 | 144 | 086 | 181.4 | -50 |
| 29 | 1 | 2 | 4 | 111 | 118 | 066 | 131.0 | -50 |
| 29 | 1 | 2 | 5 | 095 | 107 | 075 | 101.7 | -10 |
| 29 | 1 | 2 | 6 | . | . | . | . | . |
| 29 | 2 | 1 | 1 | 071 | 118 | 070 | 083.8 | 0 |
| 29 | 2 | 1 | 2 | 120 | 178 | 088 | 213.6 | -90 |
| 29 | 2 | 1 | 3 | 096 | 122 | 084 | 117.1 | -30 |
| 29 | 2 | 1 | 4 | 087 | 122 | 080 | 106.1 | -20 |
| 29 | 2 | 1 | 5 | . | 112 | 080 | . | . |
| 29 | 2 | 1 | 6 | . | . | . | . | . |
| 06 | 1 | 1 | 1 | 059 | 122 | 079 | 072.0 | 0 |
| 06 | 1 | 1 | 2 | 121 | 139 | 078 | 168.2 | -50 |
| 06 | 1 | 1 | 3 | 105 | 133 | 078 | 139.7 | -100 |
| 06 | 1 | 1 | 4 | 091 | 129 | 072 | 117.4 | -50 |
| 06 | 1 | 1 | 5 | 065 | 123 | 071 | 080.0 | 0 |
| 06 | 1 | 1 | 6 | 059 | 129 | 076 | 076.1 | 0 |
| 06 | 2 | 2 | 1 | 058 | 132 | 078 | 076.6 | 0 |
| 06 | 2 | 2 | 1 | 124 | 164 | 092 | 203.4 | 0 |
| 06 | 2 | 2 | 1 | 073 | 134 | 084 | 097.8 | -50 |
| 06 | 2 | 2 | 1 | 066 | 126 | 080 | 083.2 | 0 |
| 06 | 2 | 2 | 1 | 059 | 124 | 078 | 073.2 | 0 |
| 06 | 2 | 2 | 1 | 059 | 126 | 076 | 074.3 | 0 |

*Treatment 1 = ESA, Treatment 2 = ETT; Time 1 = Baseline, Time 2 = 0R, Time 3 = 5R, Time 4 = 10R, Time 5 = 30R, Time 6= 60R; Sequence 1 = first treatment received, Sequence 2 = second treatment received.

Hormonal and Metabolic Variables

| subj | treatment | sequence | time | COR | INS | GCG | EPI | NEPI | DOP | FFA | GLY | GLU | HLa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01 | 1 | 1 | 16.5 | 08.9 | 465.5 | 0438 | 0360 | 0850 | 0.59 | 0.36 | 080.4 | 0.699 | |
| 01 | 1 | 2 | 18.0 | 17.6 | 481.7 | 0260 | 0309 | 0580 | 1.08 | 0.92 | 077.3 | 0.958 | |
| 01 | 1 | 3 | 22.4 | 11.6 | 463.9 | 0244 | 0226 | 0242 | 1.44 | 1.08 | 082.2 | 1.385 | |
| 01 | 1 | 4 | 19.2 | 11.1 | 379.3 | 0638 | 0371 | 0524 | 1.08 | 0.61 | 083.2 | 1.120 | |
| 01 | 1 | 5 | 12.6 | 09.2 | 472.2 | 0308 | 0168 | 0381 | 0.72 | 0.40 | 084.6 | 0.885 | |
| 01 | 2 | 1 | 16.7 | 05.9 | 450.0 | 0136 | 0134 | 0109 | 0.28 | 0.29 | 087.6 | 0.635 | |
| 01 | 2 | 2 | 18.2 | 06.3 | 421.5 | 0100 | 0059 | 0261 | 0.20 | 0.48 | 086.4 | 1.545 | |
| 01 | 2 | 3 | 13.8 | 13.0 | 429.6 | 0075 | 0082 | 0057 | 0.42 | 0.48 | 087.6 | 1.055 | |
| 01 | 2 | 4 | 13.7 | 09.4 | 396.1 | 0081 | 0088 | 0126 | 0.40 | 0.45 | 088.8 | 0.760 | |
| 01 | 2 | 5 | 15.2 | 09.8 | 413.4 | 0060 | 0081 | 0110 | 0.32 | 0.35 | 089.5 | 0.733 | |
| 02 | 1 | 2 | 1 | 10.1 | 22.6 | 267.3 | 0531 | 1033 | 0809 | 0.46 | 0.71 | 105.6 | 1.215 |
| 02 | 1 | 2 | 2 | 12.2 | 22.5 | 436.4 | 0550 | 1162 | 0349 | 1.68 | 1.64 | 103.4 | 3.105 |
| 02 | 1 | 2 | 3 | 13.1 | 26.7 | 279.8 | 0460 | 1164 | 0520 | 1.86 | 1.60 | 109.3 | 3.305 |
| 02 | 1 | 2 | 4 | 16.4 | 25.8 | 257.2 | 0441 | 0991 | 0384 | 1.01 | 0.83 | 114.6 | 2.660 |
| 02 | 1 | 2 | 5 | 12.8 | 22.0 | 242.9 | 0421 | 0903 | 0398 | 0.54 | 0.62 | 105.4 | 1.810 |
| 02 | 2 | 1 | 1 | 29.7 | 22.3 | 378.0 | 0136 | 0888 | 0313 | 0.42 | 0.68 | 101.7 | 1.135 |
| 02 | 2 | 1 | 2 | 11.6 | 39.6 | 299.5 | 0100 | 1060 | 0220 | 0.32 | 1.29 | 106.0 | 5.975 |
| 02 | 2 | 1 | 3 | 14.8 | 28.4 | 382.2 | 0363 | 0688 | 0241 | 0.64 | 1.34 | 101.0 | 4.050 |
| 02 | 2 | 1 | 4 | 07.7 | 20.3 | 340.8 | 0135 | 1195 | 0241 | 0.65 | 0.72 | 101.3 | 2.095 |
| 02 | 2 | 1 | 5 | 06.6 | 16.1 | 312.0 | 0101 | 1234 | 0242 | 0.86 | 0.80 | 097.8 | 1.375 |
| 03 | 1 | 2 | 1 | 09.9 | 39.8 | 255.4 | 0139 | 0798 | 0241 | 0.42 | 0.56 | 128.8 | 1.585 |
| 03 | 1 | 2 | 2 | 09.2 | 55.2 | 303.5 | 0050 | 0769 | 0240 | 1.42 | 1.64 | 129.6 | 3.155 |
| 03 | 1 | 2 | 3 | 16.2 | 43.5 | 229.6 | 0134 | 0624 | 0240 | 2.00 | 2.04 | 126.8 | 3.615 |
| 03 | 1 | 2 | 4 | 13.0 | 42.9 | 241.6 | 0123 | 0680 | 0240 | 1.62 | 1.45 | 135.6 | 2.920 |
| 03 | 1 | 2 | 5 | 10.7 | 44.7 | 256.5 | 0060 | 0682 | 0239 | 1.05 | 0.85 | 130.7 | 2.065 |
| 03 | 2 | 1 | 1 | 11.3 | 34.8 | 363.4 | 0239 | 0600 | 0240 | 0.32 | 0.94 | 138.0 | 1.755 |
| 03 | 2 | 1 | 2 | 10.7 | 35.6 | 298.8 | 0101 | 0582 | 0240 | 0.25 | 1.16 | 138.0 | 3.075 |
| 03 | 2 | 1 | 3 | 08.8 | 52.2 | 287.6 | 0127 | 0766 | 0242 | 0.56 | 1.34 | 127.2 | 4.915 |
| 03 | 2 | 1 | 4 | 07.5 | 51.3 | 279.5 | 0133 | 1198 | 0279 | 0.54 | 0.94 | 132.8 | 2.545 |
| 03 | 2 | 1 | 5 | 05.6 | 47.2 | 254.5 | 0130 | 0979 | 0240 | 0.45 | 0.80 | 126.6 | 1.925 |
| 04 | 1 | 2 | 1 | 10.6 | 34.2 | 410.5 | 0101 | 0824 | 0241 | 0.41 | 0.53 | 161.8 | 1.815 |
| 04 | 1 | 2 | 2 | 08.1 | 63.9 | 430.6 | 0138 | 0754 | 0242 | 1.34 | 1.10 | 164.7 | 3.335 |
| 04 | 1 | 2 | 3 | 12.8 | 34.3 | 375.6 | 0125 | 0642 | 0239 | 1.74 | 1.72 | 166.0 | 4.400 |
| 04 | 1 | 2 | 4 | 13.4 | 38.7 | 380.5 | 0123 | 0699 | 0239 | 1.38 | 1.31 | 161.4 | 4.120 |
| 04 | 1 | 2 | 5 | 12.9 | 40.4 | 348.9 | 0125 | 0639 | 0240 | 0.92 | 0.90 | 165.4 | 3.000 |
| 04 | 2 | 1 | 1 | 13.1 | 34.3 | 472.2 | 0101 | 0580 | 0238 | 0.32 | 0.58 | 154.8 | 1.845 |
| 04 | 2 | 1 | 2 | 11.8 | 27.3 | 447.4 | 0144 | 0587 | 0240 | 0.20 | 0.98 | 164.0 | 6.135 |
| 04 | 2 | 1 | 3 | 15.8 | 41.0 | 555.8 | 0136 | 0637 | 0232 | 0.60 | 1.30 | 161.5 | 4.205 |
| 04 | 2 | 1 | 4 | 11.6 | 42.3 | 550.2 | 0050 | 0624 | 0239 | 0.56 | 0.77 | 168.5 | 2.615 |
| 04 | 2 | 1 | 5 | 08.5 | 44.4 | 519.9 | 0126 | 0665 | 0239 | 0.40 | 0.65 | 173.4 | 2.200 |
| 05 | 1 | 2 | 1 | 12.0 | 09.1 | 312.0 | 0170 | 0255 | 0053 | 0.17 | 0.23 | 083.9 | 1.130 |
| 05 | 1 | 2 | 2 | 25.9 | 39.2 | 288.7 | 0070 | 0110 | 0020 | 1.70 | 0.89 | 085.6 | 3.515 |
| 05 | 1 | 2 | 3 | 36.1 | 17.0 | 236.0 | 0038 | 0076 | 0040 | 1.62 | 1.19 | 084.8 | 4.030 |
| 05 | 1 | 2 | 4 | 21.8 | 10.5 | 158.8 | 0133 | 0403 | 0045 | 0.87 | 0.95 | 085.4 | 2.665 |
| 05 | 1 | 2 | 5 | 10.3 | 07.9 | 228.2 | 0145 | 0338 | 0061 | 0.30 | 0.54 | 088.1 | 1.610 |
| 05 | 2 | 1 | 1 | 22.2 | 10.2 | 146.5 | 0110 | 0346 | 0210 | 0.48 | 0.19 | 077.9 | 0.885 |
| 05 | 2 | 1 | 2 | 16.8 | 06.1 | 184.8 | 0253 | 0953 | 0119 | 0.42 | 0.35 | 078.8 | 1.670 |
| 05 | 2 | 1 | 3 | 17.6 | 28.4 | 155.4 | 0339 | 0390 | 0056 | 1.36 | 0.85 | 083.3 | 1.510 |
| 05 | 2 | 1 | 4 | 14.1 | 13.7 | 152.0 | 0203 | 0582 | 0103 | 0.57 | 0.25 | 076.2 | 1.070 |
| 05 | 2 | 1 | 5 | 14.1 | 19.3 | 164.7 | 0176 | 0674 | 0070 | 0.42 | 0.14 | 083.9 | 0.891 |
| 06 | 1 | 1 | 1 | 14.0 | 16.3 | 278.1 | 0289 | 0956 | 0068 | 0.46 | 0.54 | 093.0 | 1.000 |
| 06 | 1 | 1 | 2 | 17.1 | 40.0 | 265.6 | 0301 | 1000 | 0068 | 0.87 | 1.22 | 087.9 | 1.175 |
| 06 | 1 | 1 | 3 | 14.6 | 27.8 | 231.5 | 0213 | 0639 | 0074 | 1.40 | 1.91 | 089.2 | 1.430 |
| 06 | 1 | 1 | 4 | 12.4 | 19.3 | 180.7 | 0218 | 0682 | 0080 | 0.90 | 0.86 | 091.6 | 1.315 |
| 06 | 1 | 1 | 5 | 09.2 | 14.1 | 154.1 | 0190 | 0907 | 0060 | 0.63 | 0.68 | 093.0 | 1.135 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 06 | 2 | 2 | 1 | 18.5 | 14.7 | 182.6 | 0087 | 0340 | 0093 | 0.47 | 0.58 | 099.5 | 0.989 |
| 06 | 2 | 2 | 2 | 18.3 | 13.6 | 182.8 | 0196 | 0627 | 0102 | 0.30 | 0.91 | 097.0 | 1.955 |
| 06 | 2 | 2 | 3 | 15.4 | 23.8 | 177.1 | 0240 | 0688 | 0020 | 0.90 | 1.21 | 099.0 | 1.600 |
| 06 | 2 | 2 | 4 | 12.4 | 19.9 | 154.4 | 0250 | 0866 | 0093 | 0.69 | 0.71 | 095.7 | 1.115 |
| 06 | 2 | 2 | 5 | 10.7 | 12.5 | 162.5 | 0270 | 0824 | 0089 | 0.51 | 0.57 | 097.1 | 1.035 |
| 26 | 1 | 1 | 1 | 24.6 | 11.0 | 291.7 | 0138 | 0137 | 0133 | 0.68 | 0.77 | 092.9 | 1.300 |
| 26 | 1 | 1 | 2 | 18.0 | 30.4 | 363.1 | 0080 | 0171 | 0082 | 1.55 | 2.33 | 090.0 | 1.760 |
| 26 | 1 | 1 | 3 | 24.4 | 20.8 | 400.3 | 0133 | 0224 | 0080 | 1.91 | 2.61 | 095.8 | 2.080 |
| 26 | 1 | 1 | 4 | 13.0 | 20.4 | 343.7 | 0076 | 0260 | 0046 | 1.80 | 3.16 | 107.4 | 2.020 |
| 26 | 1 | 1 | 5 | 07.8 | 13.1 | 441.2 | 0286 | 0102 | 0050 | 1.04 | 1.00 | 098.9 | 1.310 |
| 26 | 2 | 2 | 1 | 09.6 | 13.0 | 313.0 | 0179 | 0698 | 0052 | 0.65 | 0.76 | 087.6 | 1.375 |
| 26 | 2 | 2 | 2 | 18.7 | 07.1 | 382.4 | 0241 | 0808 | 0054 | 0.48 | 1.16 | 088.6 | 3.265 |
| 26 | 2 | 2 | 3 | 10.9 | 18.4 | 367.0 | 0206 | 0818 | 0068 | 0.94 | 1.47 | 092.8 | 3.075 |
| 26 | 2 | 2 | 4 | 09.5 | 16.6 | 393.5 | 0090 | 0415 | 0078 | 0.81 | 1.06 | 094.0 | 1.860 |
| 26 | 2 | 2 | 5 | 14.4 | 12.3 | 369.3 | 0254 | 0677 | 0060 | 0.82 | 1.00 | 093.4 | 1.305 |
| 27 | 1 | 1 | 1 | 06.4 | 17.7 | 143.8 | 0087 | 0480 | 0060 | 0.43 | 0.31 | 099.3 | 1.465 |
| 27 | 1 | 1 | 2 | 08.5 | 40.6 | 157.4 | 0177 | 0409 | 0060 | 0.82 | 1.06 | 092.0 | 1.735 |
| 27 | 1 | 1 | 3 | 07.7 | 32.6 | 178.3 | 0143 | 0539 | 0075 | 1.43 | 1.62 | 088.8 | 2.885 |
| 27 | 1 | 1 | 4 | 05.5 | 20.9 | 146.6 | 0268 | 0487 | 0064 | 1.12 | 0.77 | 096.6 | 2.110 |
| 27 | 1 | 1 | 5 | 06.4 | 15.3 | 139.9 | 0190 | 0609 | 0054 | 0.65 | 0.54 | 094.4 | 1.690 |
| 27 | 2 | 2 | 1 | 06.6 | 21.2 | 160.3 | 0239 | 0447 | 0054 | 0.33 | 1.14 | 094.0 | 1.785 |
| 27 | 2 | 2 | 2 | 12.1 | 21.1 | 153.2 | 0221 | 0466 | 0056 | 0.43 | 0.76 | 098.0 | 4.060 |
| 27 | 2 | 2 | 3 | 17.5 | 36.1 | 169.4 | 0164 | 0448 | 0089 | 0.70 | 0.79 | 090.9 | 2.955 |
| 27 | 2 | 2 | 4 | 14.9 | 16.6 | 116.7 | 0202 | 0499 | 0073 | 0.40 | 0.87 | 092.6 | 2.000 |
| 27 | 2 | 2 | 5 | 08.9 | 19.0 | 175.8 | 0231 | 0568 | 0099 | 0.33 | 0.30 | 092.6 | 1.800 |
| 29 | 1 | 2 | 1 | 09.3 | 71.2 | 207.9 | 0081 | 0103 | 0047 | 0.53 | 2.93 | 118.6 | 2.150 |
| 29 | 1 | 2 | 2 | 06.6 | 82.4 | 171.3 | 0045 | 0054 | 0038 | 0.76 | 1.36 | 117.1 | 1.790 |
| 29 | 1 | 2 | 3 | 08.3 | 51.3 | 158.0 | 0160 | 0278 | 0054 | 0.95 | 2.88 | 116.1 | 2.220 |
| 29 | 1 | 2 | 4 | 11.0 | 49.5 | 185.7 | 0060 | 0097 | 0025 | 0.68 | 1.17 | 115.4 | 1.920 |
| 29 | 1 | 2 | 5 | 14.1 | 39.9 | 146.8 | 0057 | 0120 | 0060 | 0.46 | 1.15 | 120.4 | 1.565 |
| 29 | 2 | 1 | 1 | 07.3 | 38.4 | 181.6 | 0090 | 0109 | 0084 | 0.70 | 1.07 | 121.7 | 1.500 |
| 29 | 2 | 1 | 2 | 09.0 | 35.7 | 201.7 | 0023 | 0048 | 0052 | 0.55 | 1.35 | 116.9 | 2.145 |
| 29 | 2 | 1 | 3 | 17.0 | 43.3 | 180.6 | 0021 | 0010 | 0049 | 0.97 | 1.60 | 115.9 | 1.960 |
| 29 | 2 | 1 | 4 | 16.0 | 41.1 | 181.3 | 0143 | 0142 | 0045 | 0.87 | 1.09 | 118.6 | 1.610 |
| 29 | 2 | 1 | 5 | 12.8 | 31.5 | 172.4 | 0037 | 0038 | 0040 | 0.75 | 1.10 | 113.6 | 1.530 |

*Treatment 1 = ESA, Treatment 2 = ETT; Time 1 = Baseline, Time 2 = 0R, Time 3 = 5R, Time 4 = 10R, Time 5 = 30R, Time 6= 60R; Sequence 1 = first treatment received, Sequence 2 = second treatment received.

# APPENDIX F

## Summary ANOVA Tables

Repeated Measures ANOVA for Epinephrine

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 0.465 | 1 | 0.465 | 0.3 | 0.5912 |
| Error | 24.766 | 16 | 1.548 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 0.227 | 3 | 0.076 | 0.36 | 0.7348 |
| Time·Treat | 0.225 | 3 | 0.749 | 0.36 | 0.7374 |
| Error(Time) | 9.969 | 48 | 0.208 | | |

Repeated Measures ANOVA for Norepinephrine

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 107184.500 | 1 | 107184.500 | 0.25 | 0.6232 |
| Error | 6831558.500 | 16 | 426972.406 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 63760.056 | 3 | 21253.352 | 1.07 | 0.3597 |
| Time·Treat | 43772.944 | 3 | 14590.981 | 0.74 | 0.5031 |
| Error(Time) | 950355.500 | 48 | 19799.073 | | |

Repeated Measures ANOVA for Dopamine

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 26030.014 | 1 | 26030.014 | 0.39 | 0.5429 |
| Error | 1077460.389 | 16 | 67341.274 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 9466.708 | 3 | 3155.569 | 1.09 | 0.3403 |
| Time·Treat | 3222.264 | 3 | 1074.088 | 0.37 | 0.6540 |
| Error(Time) | 139134.278 | 48 | 2898.631 | | |

Repeated Measures ANOVA for Glucose

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 0.000 | 1 | 0.000 | 0.00 | 0.9785 |
| Error | 3.277 | 16 | 0.205 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 0.006 | 3 | 0.002 | 2.14 | 0.1187 |
| Time·Treat | 0.008 | 3 | 0.003 | 2.77 | 0.0621 |
| Error(Time) | 0.048 | 48 | 0.001 | | |

Repeated Measures ANOVA for Insulin

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 464.109 | 1 | 464.109 | 0.61 | 0.4454 |
| Error | 12128.041 | 16 | 758.003 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 942.476 | 3 | 314.759 | 7.65 | 0.0037 |
| Time·Treat | 1770.003 | 3 | 590.001 | 14.37 | 0.0001 |
| Error(Time) | 1970.271 | 48 | 41.047 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 64.222 | 1 | 64.222 | 0.58 | 0.4563 |
| Treatment | 2649.920 | 1 | 2649.920 | 24.05 | 0.0002 |
| Error | 1762.958 | 16 | 110.185 | | |
| Time.2 | | | | | |
| Time | 354.667 | 1 | 354.667 | 9.76 | 0.0065 |
| Treatment | 40.201 | 1 | 40.201 | 1.11 | 0.3085 |
| Error | 581.402 | 16 | 36.338 | | |
| Time.3 | | | | | |
| Time | 147.920 | 1 | 147.920 | 7.43 | 0.0149 |
| Treatment | 9.976 | 1 | 9.976 | 0.50 | 0.4892 |
| Error | 318.444 | 16 | 19.903 | | |

Repeated Measures ANOVA for Lactate

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 0.0458 | 1 | 0.0458 | 0.01 | 0.9100 |

| | | | | | |
|---|---|---|---|---|---|
| Error | 55.550 | 16 | 3.472 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 20.734 | 3 | 6.911 | 19.93 | 0.0001 |
| Time*Treat | 6.534 | 3 | 2.178 | 6.28 | 0.0084 |
| Error(Time) | 16.648 | 48 | 0.347 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 0.006 | 1 | 0.006 | 0.01 | 0.9288 |
| Treatment | 4.828 | 1 | 4.8280 | 6.91 | 0.0182 |
| Error | 11.174 | 16 | 0.698 | | |
| Time.2 | | | | | |
| Time | 11.131 | 1 | 11.131 | 29.66 | 0.0001 |
| Treatment | 1.476 | 1 | 1.476 | 3.93 | 0.0648 |
| Error | 6.004 | 16 | 0.375 | | |
| Time.3 | | | | | |
| Time | 4.163 | 1 | 4.163 | 42.90 | 0.0001 |
| Treatment | 0.469 | 1 | 0.469 | 4.83 | 0.0431 |
| Error | 1.553 | 16 | 0.097 | | |

Repeated Measures ANOVA for Cortisol

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 20.801 | 1 | 20.801 | 0.36 | 0.5560 |
| Error | 920.181 | 16 | 57.511 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 252.196 | 3 | 84.065 | 6.13 | 0.0076 |
| Time·Treat | 30.523 | 3 | 10.175 | 0.74 | 0.4710 |
| Error(Time) | 658.428 | 48 | 13.717 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 73.609 | 1 | 73.609 | 3.44 | 0.0822 |
| Treatment | 4.828 | 1 | 4.8280 | 6.91 | 0.0182 |
| Error | 342.371 | 16 | 21.398 | | |
| Time.2 | | | | | |
| Time | 162.601 | 1 | 162.601 | 8.14 | 0.0115 |
| Treatment | 1.805 | 1 | 1.805 | 0.09 | 0.7675 |
| Error | 319.464 | 16 | 19.967 | | |
| Time.3 | | | | | |
| Time | 86.681 | 1 | 86.681 | 6.12 | 0.0249 |
| Treatment | 18.605 | 1 | 18.605 | 1.31 | 0.2685 |
| Error | 226.544 | 16 | 14.159 | | |

Repeated Measures ANOVA for Glucagon

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 732.807 | 1 | 732.807 | 0.01 | 0.9108 |

| | | | | | |
|---|---|---|---|---|---|
| Error | 904756.214 | 16 | 56547.263 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 13385.364 | 3 | 4461.788 | 4.06 | 0.0231 |
| Time*Treat | 11861.745 | 3 | 3953.915 | 3.60 | 0.0345 |
| Error(Time) | 52787.579 | 48 | 1099.741 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 2513.405 | 1 | 2513.405 | 0.90 | 0.3569 |
| Treatment | 12688.245 | 1 | 12688.245 | 4.54 | 0.0489 |
| Error | 44685.160 | 16 | 2792.823 | | |
| Time.2 | | | | | |
| Time | 9758.045 | 1 | 9758.045 | 8.94 | 0.0087 |
| Treatment | 1068.761 | 1 | 1068.761 | 0.98 | 0.3372 |
| Error | 17465.404 | 16 | 1091.588 | | |
| Time.3 | | | | | |
| Time | 1036.642 | 1 | 1036.642 | 0.54 | 0.4746 |
| Treatment | 1732.642 | 1 | 1732.642 | 0.90 | 0.3579 |
| Error | 30930.836 | 16 | 1933.177 | | |

Repeated Measures ANOVA for Free Fatty Acids

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 6.570 | 1 | 6.570 | 31.92 | 0.0001 |
| Error | 3.293 | 16 | 0.206 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 3.072 | 3 | 1.024 | 29.83 | 0.0001 |
| Time·Treat | 1.465 | 3 | 0.488 | 14.23 | 0.0001 |
| Error(Time) | 1.648 | 48 | 0.034 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 2.777 | 1 | 2.777 | 56.85 | 0.0001 |
| Treatment | 0.036 | 1 | 0.036 | 0.75 | 0.4004 |
| Error | 0.782 | 16 | 0.049 | | |
| Time.2 | | | | | |
| Time | 1.674 | 1 | 1.674 | 28.46 | 0.0001 |
| Treatment | 0.291 | 1 | 0.291 | 4.95 | 0.0408 |
| Error | 0.942 | 16 | 0.059 | | |
| Time.3 | | | | | |
| Time | 1.269 | 1 | 1.269 | 61.08 | 0.0001 |
| Treatment | 0.688 | 1 | 0.688 | 33.12 | 0.0001 |
| Error | 0.332 | 16 | 0.021 | | |

Repeated Measures ANOVA for Glycerol

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 3.213 | 1 | 3.213 | 5.20 | 0.0367 |
| Error | 9.890 | 16 | 0.618 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 6.156 | 3 | 2.052 | 29.11 | 0.0001 |
| Time*Treat | 0.795 | 3 | 0.265 | 3.76 | 0.0321 |
| Error(Time) | 3.383 | 48 | 0.070 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 2.297 | 1 | 2.297 | 20.21 | 0.0004 |
| Treatment | 0.361 | 1 | 0.361 | 3.18 | 0.0936 |
| Error | 1.819 | 16 | 0.114 | | |
| Time.2 | | | | | |
| Time | 4.560 | 1 | 4.560 | 20.71 | 0.0004 |
| Treatment | 0.227 | 1 | 0.227 | 1.03 | 0.3254 |
| Error | 3.523 | 16 | 0.220 | | |
| Time.3 | | | | | |
| Time | 1.720 | 1 | 1.720 | 7.65 | 0.0138 |
| Treatment | 0.598 | 1 | 0.598 | 2.64 | 0.1236 |
| Error | 3.619 | 16 | 0.226 | | |

Repeated Measures ANOVA for Heart Rate

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 2464.200 | 1 | 2464.200 | 2.66 | 0.1253 |
| Error | 12976.950 | 14 | 926.925 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 4899.170 | 4 | 1224.793 | 23.48 | 0.0001 |
| Time*Treat | 4131.675 | 4 | 1032.919 | 19.80 | 0.0001 |
| Error(Time) | 2920.800 | 56 | 55.108 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 756.900 | 1 | 756.900 | 5.92 | 0.2090 |
| Treatment | 6045.063 | 1 | 6045.063 | 47.26 | 0.0001 |
| Error | 1790.875 | 14 | 127.920 | | |
| Time.2 | | | | | |
| Time | 297.025 | 1 | 297.025 | 18.05 | 0.0008 |
| Treatment | 76.563 | 1 | 76.563 | 4.65 | 0.0489 |
| Error | 230.375 | 14 | 16.455 | | |
| Time.3 | | | | | |
| Time | 2418.025 | 1 | 2418.025 | 28.27 | 0.0001 |
| Treatment | 1314.063 | 1 | 1314.063 | 15.36 | 0.0015 |
| Error | 1197.375 | 14 | 85.527 | | |
| Time.4 | | | | | |
| Time | 308.025 | 1 | 308.025 | 6.65 | 0.0219 |
| Treatment | 56.250 | 1 | 56.250 | 1.21 | 0.2891 |
| Error | 648.750 | 14 | 46.339 | | |

Repeated Measures ANOVA for Systolic Blood Pressure

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 644.876 | 1 | 644.876 | 1.45 | 0.2525 |
| Error | 5355.067 | 12 | 446.256 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 1148.822 | 4 | 287.205 | 2.46 | 0.1096 |
| Time*Treat | 5683.231 | 4 | 1420.808 | 12.19 | 0.0003 |
| Error(Time) | 5592.683 | 48 | 116.514 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 2088.982 | 1 | 2088.982 | 5.51 | 0.0368 |
| Treatment | 7520.095 | 1 | 7520.095 | 19.85 | 0.0008 |
| Error | 4546.833 | 12 | 278.903 | | |
| Time.2 | | | | | |
| Time | 218.982 | 1 | 218.982 | 3.30 | 0.0941 |
| Treatment | 27.524 | 1 | 27.524 | 0.42 | 0.5314 |
| Error | 795.333 | 12 | 66.278 | | |
| Time.3 | | | | | |
| Time | 0.107 | 1 | 0.107 | 0.00 | 0.9703 |
| Treatment | 3.720 | 1 | 3.720 | 0.05 | 0.8269 |
| Error | 893.208 | 12 | 74.434 | | |
| Time.4 | | | | | |
| Time | 12.009 | 1 | 12.009 | 0.30 | 0.5924 |
| Treatment | 2.881 | 1 | 2.881 | 0.07 | 0.7922 |
| Error | 476.333 | 12 | 39.694 | | |

Repeated Measures ANOVA for Diastolic Blood Pressure

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 20.743 | 1 | 20.743 | 0.05 | 0.8293 |
| Error | 5123.500 | 12 | 426.958 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 167.6009 | 4 | 41.900 | 1.00 | 0.3679 |
| Time·Treat | 94.602 | 4 | 23.651 | 0.69 | 0.5379 |
| Error(Time) | 2007.083 | 48 | 41.814 | | |

Wilcoxin Matched-Pairs Signed-Ranks Test for ST Change
(SPSS for MS Windows)

| Ranks | Cases |
|---|---|
| + | 3 |
| - | 6 |
| | 9 Total |

z = -1.6030 2-tailed p = .1088

Repeated Measures ANOVA for Rate Pressure Product

Between Subjects Effects

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Treatment | 425.292 | 1 | 425.292 | 0.13 | 0.7255 |
| Error | 39504.051 | 12 | 3292.004 | | |

Within Subjects Effects

| Source | SS | df | MS | F | G-G adj p |
|---|---|---|---|---|---|
| Time | 8723.237 | 4 | 2180.809 | 5.62 | 0.0156 |
| Time*Treat | 29333.610 | 4 | 7333.402 | 18.92 | 0.0001 |
| Error(Time) | 18609.579 | 48 | 387.700 | | |

Post-hoc Profile of Each Successive Difference in Time

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Time.1 | | | | | |
| Time | 10492.804 | 1 | 10492.804 | 7.25 | 0.0196 |
| Treatment | 47181.412 | 1 | 47181.412 | 32.61 | 0.0001 |
| Error | 17361.008 | 12 | 1446.751 | | |
| Time.2 | | | | | |
| Time | 1298.869 | 1 | 1298.869 | 19.15 | 0.0009 |
| Treatment | 338.301 | 1 | 338.301 | 4.99 | 0.0453 |
| Error | 813.853 | 12 | 67.821 | | |
| Time.3 | | | | | |
| Time | 3531.493 | 1 | 3531.493 | 17.98 | 0.0011 |
| Treatment | 1954.339 | 1 | 1054.339 | 9.95 | 0.0083 |
| Error | 2356.895 | 12 | 196.408 | | |
| Time.4 | | | | | |
| Time | 39.908 | 1 | 39.908 | 0.27 | 0.6095 |
| Treatment | 0.4101 | 1 | 0.4101 | 0.00 | 0.9585 |
| Error | 1741.442 | 12 | 145.122 | | |

VITA

Karen Toft Dorn was raised on a farm in rural Iowa where she gained an appreciation for the mystery of living things. Karen pursued her interest in life sciences at Luther College, Decorah, Iowa majoring in nursing and biology, graduating summa cum laude with a B.A. degree in 1978. Following graduation from Luther, she served as a registered nurse in the U.S. Army Nurse Corps stationed at Brooke Army Medical Center, San Antonio, TX. As a registered nurse, Karen specialized in adult coronary and medical intensive care nursing. Following a move to Georgia, Karen pursued graduate study in adult nursing and received an M.S.N degree from the Medical College of Georgia, Augusta, GA in 1987. With a desire to advance her knowledge in human physiology, she decided to pursue doctoral studies in exercise physiology. She received a Presidential Fellowship at Virginia Tech, Blacksburg, VA graduating in 1994. Her studies were interrupted in 1991 during Operation Desert Storm where she served in Riyadh, Saudi Arabia. Karen has taught nursing at the Medical College of Georgia, Augusta, GA and Radford University, Radford, VA. Currently, she is Assistant Professor at Augustana College where she teaches in the Department of Nursing.

Karen Dorn