IMMUNE SYSTEM RESPONSE TO CHANGES IN TRAINING INTENSITY AND VOLUME IN RUNNERS

IMMUNE SYSTEM RESPONSE TO CHANGES IN TRAINING INTENSITY AND VOLUME IN RUNNERS

By

JASON SAMUEL KAJIURA, B.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Master of Science

McMaster University

April, 1993

(c) Copyright by Jason Samuel Kajiura, April 1993.

MASTER OF SCIENCE (1993)
(Human Biodynamics)

McMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Immune System Response to Changes in Training Intensity and Volume in Runners

AUTHOR: Jason Samuel Kajiura, B.Sc.
(McMaster University)

SUPERVISOR: Dr. J.D. MacDougall (Ph.D.)

NUMBER OF PAGES: (xxii), 245

ABSTRACT

This study examined the acute and chronic effects of changes in training volume and intensity on the blood lymphocyte percentages and immunoglobulin levels in runners. Twelve runners participated in four 10-day phases over a 40-day training period. Phase 1 and phase 3 were "baseline" phases of low volume/low intensity running (LV/LI). Phase 2 and phase 4 consisted of either high volume/low intensity (HV/LI) or high volume/high intensity (HV/HI) training. Subjects were randomly assigned to one of 2 different cross-over training group orders: 1) LV/LI, HV/LI, LV/LI, HV/HI or 2) LV/LI, HV/HI, LV/LI, HV/LI. HV/LI training involved doubling the LV distance run at the same LI of 60-70% $\dot{V}O2$ max. The HV/HI phase consisted of the same volume as the HV/LI phase, but alternate days were replaced with a series of high intensity 1000 m intervals (95-100% $\dot{V}O2$ max). Venous blood samples were drawn at rest on days 1, 4 and 7 and 5 minutes post-exercise on days 1 and 7 of each 10-day phase. Lymphocyte subsets were determined by flow cytometry using monoclonal antibodies for total T (CD3+), T-helper (CD4+), T-suppressor (CD8+) lymphocytes and HLA-DR+ (a B cell and "activated" T-lymphoid cell marker). IgA, IgG and IgM levels were obtained by ELISA analysis. This study revealed

remarkable stability of humoral (antibody) immune components during and after runs of various intensities and volumes. Immunoglobulin levels were not significantly affected by alterations in volume or intensity over the 4 training phases. A transient decrease was observed in the T-lymphocyte percentages of CD3+, CD4+ and the CD4/CD8 ratio 5 minutes post-exercise which was significant ($p < .05$) during the HV/LI and HV/HI phases. Adaptation of lymphocyte subpopulations occurred with repeated exposure to increases in volume and intensity. A training order effect was suggested whereby an initial HI phase, was more immunosuppressive and possibly negated the effects of subsequent HV phases. Results indicate that the exercise-induced lymphocyte subset reduction is transient and suggest that the extent of the reduction is more dependent upon training intensity than volume, and the order of exposure to the high-intensity stimulus may determine the magnitude of subsequent responses.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor Dr. Duncan MacDougall for his readily available assistance, encouragement and advice throughout the course of this thesis. I must also thank the members of my thesis committee, Dr. Digby Sale and Dr. Audrey Hicks, as well as Dr. Michael Houston and Dr. Joe Blimkie for their helpful suggestions.

I am greatly indebted to Dr. Peter Ernst, Barb Bagnarol, Lenore Zettel and Dr. Maria Estrada for their advice and assistance during my learning of the various immunological techniques used in this study. On the other side of the campus, I must thank John Moroz, Joan Martin and Dr. Tim Lee for their always amicable assistance.

Sincere thanks to Karen Woolley for her inspiring advice, humourous naratives and presence during the frequent early mornings and weekends of the first few months of data collection.

I wish to thank my fellow coaches and athletes of North America's oldest active track and field club - the Hamilton Olympic Club (especially the "track masochists" - undoubtedly the top middle-distance group in the area!) who not only provided me with subjects for my other graduate experiments, but gave me tremendous motivation and support during our

seemingly interminable cross-country/road runs, the arduous stair/hill workouts and the awesome track/interval sessions. Special thanks to Jonathan Francis, Bruce Raymond, Paul Dunsford, Jamie Halla, Eric Armstrong, Chris Miller, Rob O'Brien, Steve Moran, Tim Burshtein, Gord Kenny, Manny Bonilla, Marcel Chambers, and Rhona Trott, for enriching my life and demonstrating to me that enthusiasm is often the greatest asset in the world, and unlike the timid souls who know neither victory nor defeat, have frequently exemplified not just greatness, but greatness against the odds!

I also extend my appreciation to my fellow eminent musicians (RL, PC, JP, DS, WP, LL, BC and JS) for providing me over the years, with never enough jam sessions to offer solace from the unexpected complexities of trying to balance academia with high-level athletics (among numerous other things).

Finally, I must thank my family for their tremendous support towards my academic endeavours (though they could have been more supportive and understanding of my musical, artistic, athletic and coaching aspirations and commitments).

PREFACE

The format of this thesis differs from the traditional thesis format in that this thesis is presented in two chapters. Chapter I is a literature review related to the immune system and the effect of exercise upon it. Chapter II embodies the thesis research and is presented in an expanded manuscript format which will be reduced and submitted for journal publication.

TABLE OF CONTENTS

PAGE

CHAPTER 1: THE IMMUNE SYSTEM AND EXERCISE 1

1.1 INTRODUCTION 1

1.2 THE IMMUNE SYSTEM 5

1.2.1 NATURAL IMMUNITY 10

1.2.2 ADAPTIVE IMMUNITY 11

A. ANTIBODY-MEDIATED IMMUNITY 12

B. CELL-MEDIATED IMMUNITY 15

1.3 EXERCISE AND IMMUNE FUNCTION 20

1.3.1 IMMUNOGLOBULIN (ANTIBODY) RESPONSE TO EXERCISE 20

A. RESPONSE TO ACUTE EXERCISE BOUTS 20

B. PROLONGED ACUTE EXERCISE BOUTS 22

C. RESPONSES TO EXERCISE TRAINING 26

1.3.2 EXERCISE AND LYMPHOCYTES 28

A. ACUTE EXERCISE BOUTS AND LYMPHOCYTE COUNTS 30

B. ACUTE EXERCISE AND T AND B CELL COUNTS 31

C. CROSS-SECTIONAL COMPARISON STUDIES 37

D. LONGITUDINAL TRAINING AND LYMPHOCYTE COUNTS 39

1.3.3 EXERCISE AND LYMPHOCYTE FUNCTION 41

1.4 LYMPHOCYTE AND IMMUNOGLOBULIN ANALYSIS 47

1.4.1 FLOW CYTOMETRY 47

1.4.2 ELISA ANALYSIS 49

1.5 POSSIBLE MECHANISMS FOR EXERCISE-INDUCED IMMUNOSUPPRESSION 51

A. CORTISOL 51

B. CATECHOLAMINES 56

C. GLUTAMINE 59

1.6 RATIONALE FOR THE PRESENT STUDY 62

CHAPTER 2: IMMUNE SYSTEM RESPONSE TO CHANGES IN TRAINING INTENSITY AND VOLUME IN RUNNERS 64

2.1 INTRODUCTION 64

2.2 METHODS 70

2.2.1 SUBJECTS 70

2.2.2 DESIGN 70

2.2.3 TREADMILL PERFORMANCE TEST 71

2.2.4 IMMUNOLOGICAL ASSAYS 73

A. LYMPHOCYTE PREPARATION 74

B. FLOW CYTOMETRIC ANALYSIS 75

C. IMMUNOGLOBULIN ANALYSIS OF BLOOD SERUM 75

D. ELISA ANALYSIS 76

2.2.5 RUNNING DIARIES 78

2.2.6 STATISTICAL ANALYSIS 80

2.3 RESULTS 81

2.3.1 SUBJECT CHARACTERISTICS 81

2.3.2 LYMPHOCYTE SUBSET PERCENTAGES 83

A. T-HELPER LYMPHOCYTES (CD4+) 83

B. T-SUPPRESSOR LYMPHOCYTES (CD8+) 89

C. T-HELPER/T-SUPPRESSOR (CD4/CD8) LYMPHOCYTE RATIO 94

D. TOTAL T-LYMPHOCYTES (CD3+) 99

E. HLA-DR+ (B CELLS AND ACTIVATED T-LYMPHOID CELLS) 104

F. CD3+HLA-DR+ 109

2.3.3 IMMUNOGLOBULINS IgG, IgA AND IgM 114

2.3.4 TREADMILL PERFORMANCE TEST 121

2.4 DISCUSSION 130

2.4.1 ACUTE EFFECTS 130

A. CD4+ -HELPER CELLS 130

B. CD8+ -SUPPRESSOR CELLS 131

C. CD4/CD8 -HELPER/SUPPRESSOR T CELL RATIO 132

D. CD3+ -TOTAL T-LYMPHOCYTES 133

E. HLA-DR+ -B CELLS AND "ACTIVATED" T-LYMPHOID CELLS 134

2.4.2 CHRONIC EFFECTS 135

2.4.3 TRAINING ORDER EFFECT 137

2.4.4 STRESS/MOOD STATES 138

2.4.5 CHANGES IN SLEEP PATTERNS 139

2.4.6 CLINICAL SYMPTOMS 140

2.4.7 TREADMILL TEST AND $\dot{V}O2$ MAX 141

2.4.8 IMMUNOGLOBULINS 143

2.4.9 MECHANISMS 145

2.5 CONCLUSIONS 151

2.6 REFERENCES 154

APPENDIX I SAMPLE STATISTICS 193

APPENDIX II SUBJECT DATA TABLES 199

APPENDIX III CALCULATION OF TREADMILL TEST VELOCITY 220

APPENDIX IV LEUKOCYTE PREPARATION PROTOCOL 222

APPENDIX V ELISA PROTOCOL AND 96 WELL-PLATE SET UP 226

APPENDIX VI SAMPLE FAC COMPUTER OUTPUT 232

APPENDIX VII ETHICS APPROVAL, SAMPLE CONSENT FORMS AND TRAINING DIARY. 238

LIST OF FIGURES

		PAGE
CHAPTER 1		
FIGURE 1	FACTORS INVOLVED IN NATURAL AND ADAPTIVE IMMUNITY	19
CHAPTER 2		
FIGURE 2A	GROUP 1 PERCENT CD4+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	85
FIGURE 2B	GROUP 1 MAGNITUDE OF PRE-POST CD4+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	85
FIGURE 3A	GROUP 2 PERCENT CD4+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	87
FIGURE 3B	GROUP 2 MAGNITUDE OF PRE-POST CD4+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	87
FIGURE 4A	GROUP 1 PERCENT CD8+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	90
FIGURE 4B	GROUP 1 MAGNITUDE OF PRE-POST CD8+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	90
FIGURE 5A	GROUP 2 PERCENT CD8+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	92
FIGURE 5B	GROUP 2 MAGNITUDE OF PRE-POST CD8+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	92
FIGURE 6A	GROUP 1 CD4/CD8 VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	95

FIGURE 6B	GROUP 1 MAGNITUDE OF PRE-POST CD4/CD8 DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	95
FIGURE 7A	GROUP 2 CD4/CD8 VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	97
FIGURE 7B	GROUP 2 MAGNITUDE OF PRE-POST CD4/CD8 DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	97
FIGURE 8A	GROUP 1 PERCENT CD3+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	100
FIGURE 8B	GROUP 1 MAGNITUDE OF PRE-POST CD3+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	100
FIGURE 9A	GROUP 2 PERCENT CD3+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	102
FIGURE 9B	GROUP 2 MAGNITUDE OF PRE-POST CD3+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	102
FIGURE 10A	GROUP 1 PERCENT HLA-DR+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	105
FIGURE 10B	GROUP 1 MAGNITUDE OF PRE-POST HLA-DR+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	105
FIGURE 11A	GROUP 2 PERCENT HLA-DR+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	107
FIGURE 11B	GROUP 2 MAGNITUDE OF PRE-POST HLA-DR+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	107
FIGURE 12A	GROUP 1 PERCENT CD3+HLA-DR+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	110

FIGURE 12B	GROUP 1 MAGNITUDE OF PRE-POST CD3+HLA-DR+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	110
FIGURE 13A	GROUP 2 PERCENT CD3+HLA-DR+ VALUES PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	112
FIGURE 13B	GROUP 2 MAGNITUDE OF PRE-POST CD3+HLA-DR+ DIFFERENCES ON DAYS 1 AND 7 OF TRAINING PHASES 1 TO 4	112
FIGURE 14A	GROUP 1 IgG VALUES (g/l) PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	115
FIGURE 14B	GROUP 2 IgG VALUES (g/l) PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	115
FIGURE 15A	GROUP 1 IgA VALUES (g/l) PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	117
FIGURE 15B	GROUP 2 IgA VALUES (g/l) PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	117
FIGURE 16A	GROUP 1 IgM VALUES (g/l) PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	119
FIGURE 16B	GROUP 2 IgM VALUES (g/l) PRE- AND 5 MINUTES POST-EXERCISE DURING TRAINING PHASES 1 TO 4	119
FIGURE 17	GROUP 1 TREADMILL PERFORMANCE TEST TIME (s) AT THE END OF EACH OF THE 4 TRAINING PHASES	122
FIGURE 18	GROUP 2 TREADMILL PERFORMANCE TEST TIME (s) AT THE END OF EACH OF THE 4 TRAINING PHASES	124
FIGURE 19	GROUP 1 NORMALIZED TREADMILL PERFORMANCE (%) AT THE END OF EACH OF THE 4 TRAINING PHASES.	126

FIGURE 20 GROUP 2 NORMALIZED TREADMILL PERFORMANCE (%) AT THE END OF EACH OF THE 4 TRAINING PHASES. 128

LIST OF TABLES

		PAGE
CHAPTER 2		
TABLE 1	CLASSIFICATION OF LEUKOCYTES AND NORMAL RANGE	6
TABLE 2	SUBJECTS' INITIAL PHYSICAL AND TRAINING CHARACTERISTICS	82
APPENDIX II		
TABLE 3	FLOW CYTOMETRY DATA -CD4+, CD8+ AND CD4/CD8 RATIO FOR GROUPS 1 AND 2	200
TABLE 4	DIFFERENCES IN PRE- AND POST-EXERCISE VALUES FOR CD4, CD8 AND CD4/CD8 RATIO FOR GROUPS 1 AND 2	201
TABLE 5	FLOW CYTOMETRY DATA -CD3+, HLA-DR+ AND CD3+HLA-DR+ FOR GROUPS 1 AND 2	202
TABLE 6	DIFFERENCES IN PRE AND POST-EXERCISE VALUES FOR CD3+, HLA-DR+ AND CD3+HLA-DR+ FOR GROUPS 1 AND 2	203
TABLE 7	MEAN ELISA IgG, IgA, AND IgM CONCENTRATIONS (g/l) FOR GROUPS 1 AND 2	204
TABLE 8	SUBJECT'S TREADMILL PERFORMANCE	205
TABLE 9	NORMALIZED TREADMILL PERFORMANCE	206
TABLE 10	TREADMILL $\dot{V}O2$ DATA FOR GROUPS 1 AND 2	207
TABLE 11	HEMATOCRIT VALUES FOR GROUPS 1 AND 2	208
TABLE 12	CLINICAL SYMPTOMS REPORTED BY SUBJECTS DURING EACH PHASE	209
TABLE 13	TRAINING DISTANCE AND TIME COVERED BY EACH SUBJECT IN GROUP 1	210
TABLE 14	TRAINING DISTANCE AND TIME COVERED BY EACH SUBJECT IN GROUP 2	211

TABLE 15	STRESS/MOOD STATES AND SLEEP PATTERNS ON BLOOD SAMPLING AND TREADMILL TEST DAYS	212
TABLE 16	FLOW CYTOMETRY DATA - PERCENT CD4+, CD8+ AND CD4/CD8 RATIO FOR SUBJECTS IN GROUP 1	213
TABLE 17	FLOW CYTOMETRY DATA - PERCENT CD4+, CD8+ AND CD4/CD8 RATIO FOR SUBJECTS IN GROUP 2	214
TABLE 18	FLOW CYTOMETRY DATA - PERCENT CD3+, HLA-DR+ AND CD3+HLA-DR+ FOR SUBJECTS IN GROUP 1	215
TABLE 19	FLOW CYTOMETRY DATA - PERCENT CD3+, HLA-DR+ AND CD3+HLA-DR+ FOR SUBJECTS IN GROUP 2	216
TABLE 20	ELISA IgG CONCENTRATIONS FOR SUBJECTS IN GROUP 1 AND 2	217
TABLE 21	ELISA IgM CONCENTRATIONS FOR SUBJECTS IN GROUP 1 AND 2	218
TABLE 22	ELISA IgA CONCENTRATIONS FOR SUBJECTS IN GROUP 1 AND 2	219

Glossary of Immunologic Terms

For the purpose of this thesis, the following definitions will apply.

Adaptive (acquired) Immunity: Immune resistance that develops following initial exposure to antigen, characterized by extreme antigen specificity and memory mediated by antibody or T cells.

Acute-phase proteins: A set of proteins that are induced rapidly during inflammatory reactions and many infections.

Affinity: The binding strength (equal to the association constant) between a receptor and a monovalent ligand.

Agglutination: An antigen-antibody reaction in which a solid or particulate antigen forms a lattice with a soluble antibody.

Antibody (Immunoglobulin): A set of related proteins capable of binding specifically to an antigen. Produced by B cells in response to the immune challenge.

Antigen: Any substance that reacts specifically with T cells and/or antibodies.

Autologous: Derived from the same individual.

B lymphocyte : A bursa-derived cell that is the precursor of plasma cells that produce antibodies.

CD (cluster of differentiation) markers: Cell-surface molecules used as markers to differentiate cell populations and are recognized by monoclonal antibodies.

CD4: A molecule present on the surface of a population of T cells that recognize antigenic peptides presented by class II MHC molecules. Often associated with helper function and is also the receptor for HIV.

CD8: A molecule present on the surface of a population of T cells that recognize antigenic peptides presented by class I MHC molecules. Often associated with cytotoxic effector function.

Cell-mediated immunity: Immune responses mediated by cells (predominantly lymphocytes and macrophages) as opposed to by humoral factors (antibody).

Class I MHC: Major histocompatibility molecule consisting of a polymorphic, integral membrane polypeptide noncovalently associated with B2-microglobulin and incoded in humans by HLA-A, B, and C loci.

Class II MHC: Major histocompatibility molecules composed of 2 integral membrane polypeptides (α and β). Encoded in humans by HLA-DR, DQ and DP. These molecules present peptide antigen fragments to CD4+ T cells and are expressed on antigen-presenting cells, including B cells, dendritic cells, and some macrophages.

Complement: Serum proteins that, when activated, form a cascade of interactions leading to lytic attack of cell membranes, chemotaxis and phagocyte activation.

Concanavalin A (Con A): A lectin derived from the Jack bean that is a potent mitogen which stimulates predominantly T lymphocytes.

Cytokines: Growth and differentiation factors.

Degranulation: A process whereby cytoplasmic granules of phagocytic cells fuse with phagosomes and discharge their contents into the phagolysosome thus formed.

Disulfide bonds: Chemical S-S bonds between sulfhydryl containing amino acids that bind together H and L chains as well as portions of H-H and L-L chains.

ELISA (enzyme-linked immunosorbant assay): A solid-phase antibody quantitation assay employing enzyme-linked antibody and a coloured substrate to measure the activity of the bound enzyme.

Fab: An antigen-binding fragment of an antibody, consisting of a light chain and part of a heavy chain, produced by enzymatic digestion of an IgG molecule with papain.

Fc fragment: A crystalizable fragment obtained by papain digestion of IgG molecules that consists of the C-terminal half of 2 H chains linked by disulfide bonds. It contains no antigen-binding capability but determines important biologic characteristics of the intact molecule.

FITC (fluorescein isothiocyanate): A fluorescent green dye that can be conjugated to proteins, including antibdies.

Fluorescence: The emission of light of one colour while a substance is irradiated with a light of a different colour.

Heavy chain: A pair of identical polypeptide chains making up an immunoglobulin molecule. It contains twice the number of amino acids and is twice the molecular weight of the light chain.

Helper T cells: A subtype of T lymphocytes that cooperate with B cells in antibody formation and are usually CD4+ and restricted to MHC class II.

Hemagglutinin: A molecule able to cause agglutination of red blood cells (eg: antibodies to red blood cells).

HLA (human leukocyte antigen): The major histo- compatiblity genetic region in humans.

Humoral immunity: Immune responses mediated by antibody and complement in solution in a body fluid.

IgA: The predominant immunoglobulin class present in secretions.

IgD: The predominant immunglobulin class present on human B lymphocytes.

IgE: A reaginic antibody involved in immediate hypersensitivity reactions.

IgG: The predominant immunoglobulin class present in human serum.

IgM: A pentameric immunoglobulin made early in a response, found primarily in the intravascular pool.

Immunogen: A substance that stimulates the immune response when introduced into an animal.

Innate immunity: Various host defences present from birth that do not require immunologic memory.

Interferon: Heterogeneous, low molecular weight proteins elaborated by infected host cells that protect noninfected cells from viral infection.

Interleukin-1: Macrophage derived factor (previously named LAF, or leukocyte activating factor) that promotes short-term proliferation of T cell lines in culture.

Interleukin-2: A lymphocyte-derived factor (previously named TCGF, or T cell growth factor) that promotes long-term proliferation of T cell lines in culture.

Lectins: Glycoproteins that specifically bind sugar residues and are commonly mitogens.

Lysosomes: Granules that contain hydrolytic enzymes and are present in the cytoplasm of many cells.

Macrophage: Large phagocytic myeloid mononuclear cells derived from monocytes.

Major histocompatibility complex (MHC): A complex of polymorphic genes that encode the cell-surface molecules responsible for rapid graft rejection and needed for antigen presentation to T cells.

Mitogens: Substances that cause DNA synthesis, blast transformation, and ultimately division of lymphocytes. (antigens-> in vivo; mitogens-> in vitro).

Monoclonal antibodies: Identical copies of antibody that consist of one H chain class and one L chain type.

NK (natural killer) cells: Cytotoxic cells belonging to the cell class responsible for cellular cytotoxicity without prior sensitization.

Null cell: Cell lacking specific identifying surface markers for either T or B lymphocytes.

Opportunistic infection: During altered or weakened immunity, the ability of organisms of relatively low virulence to cause disease.

Opsonin: A substance that enhances phagocytosis (eg: antibodies and complement).

Phagocytosis: A process whereby cells (eg: macrophages or PMN's) ingest microorganisms or other particles and enclose it within a vacuole (called a phagosome) in the cytoplasm.

PHA (phytohemagglutinin): A lectin that is derived from the red kidney bean and stimulates predominantly T lymphocytes.

Polyclonal mitogens: Mitogens that activate large subpopulations of lymphocytes.

PPD (purified protein derivative): An antigen prepared by ammonium sulfate precipitation of supernatants of Mycobacterium tuberculosis cultures.

PWM (pokeweed mitogen): A lectin that is derived from pokeweed and that stimulates both B and T lymphocytes.

Serum: Fluid portion of blood that remains after the cells and clotting factors have been removed.

Stem cell: Cell (often regenerating) from which differentiated cells derive.

Suppressor T cells: A subset of T lymphocytes that suppress antibody synthesis by B cells or inhibit other cellular immune reactions by effector T cells.

T (lymphocyte) cell: A thymus-derived lymphocyte cell that participates in a variety of cell-mediated immune reactions.

Thymus: The central lymphoid organ that is present in the thorax and controls the ontogeny of T lymphocytes.

CHAPTER 1

THE IMMUNE SYSTEM AND EXERCISE

1.1 INTRODUCTION

Athletes and coaches have for a long time been aware that increases in training load (intensity and/or duration) are frequently accompanied by an increased propensity towards respiratory infections and other illnesses. Recent studies have reported transient immune depression after a single exhaustive exercise bout (Berk et al., 1985; Brahmi et al., 1985; MacKinnon, 1988), causing speculation that chronic intensive training may lead to a more severe suppression of immune function. In contrast, there is the persistent belief that participation in moderate physical activity will promote "resistance" to infection (Anderson, 1989; Green et al., 1981; Portz, 1984). These two beliefs have resulted in the view that "a little exercise is beneficial but too much may be harmful". Neither relationship has been consistently substantiated (Cannon, 1993; Eichner, 1993; Ferry, 1989; Keast et al., 1988; MacKinnon and Tomasi, 1986, 1989; Simon, 1984, 1987).

Recent longitudinal studies suggest that immune function in sedentary individuals may be enhanced by regular exercise (Watson et al., 1986; Soppi et al., 1982). The conditioned athlete also exhibits healthy immune function provided that a normal training pattern is maintained (Oshida et al., 1988; Green et al., 1981; Hanson and Flaherty, 1981; Busse et al., 1980). Thus moderate exercise may have either no influence or a beneficial effect on one's immune system. The demanding training volumes and intensities of competitive athletes, however, greatly exceed what might be considered as moderate exercise.

Several studies have shown that competitive athletes tend to be more susceptible to infections than their sedentary cohorts. In an outbreak of infectious hepatitis from polluted drinking water at the Holy Cross College in Worcester, Massachusetts, 90 out of 97 football athletes were affected but no employees or students using the same facilities contracted the virus (Fox, 1985; Friedman et al., 1985, Morse et al., 1972). In a high-school asceptic meningitis outbreak, Baron et al. (1982) reported that the incidence of infection was twice as high and the symptoms more severe in the members of the football team than in other students. The researchers concluded that strenuous exercise may contribute to the severity of the infection.

Additionally, reports of persistent sore throats and flu-

like illnesses in athletes have been found to occur more frequently during the competitive season (Fitzgerald, 1991). Douglas and Hansen (1978) at the University of Wisconsin, observed more frequent and severe upper respiratory tract infections in conditioned rowers than in control subjects over a 9-week competitive period. A recent study of 2,300 marathoners linked the occurrence of upper respiratory infections to periods of heavy training and marathon racing (Nieman et al., 1990). A survey by Peters and Bateman (1983) found that of the 150 finishers of a 56 km ultramarathon race, 33.3% of the runners (compared to 15.3% of controls) suffered upper respiratory infections for several days following the race. An even greater incidence of infection was reported in the more highly-trained runners, with almost half of the faster finishing athletes displaying symptoms.

Historically, numerous anecdotal reports have indicated that elite athletes encounter periods of chronic fatigue, staleness and decreased performance (Fry et al., 1991; Ryan et al., 1983). Highly motivated athletes typically endure extreme training loads (volume and intensity) when preparing for competition. Accordingly, as the intensity of this training increases, so do the incidence and severity of their respiratory infections and illnesses (Simon, 1987).

Studies of elite sports women, orienteers, marathon runners and cross-country skiers have revealed that minor

infections and colds accounted for more absences from training than did injuries (Anderson, 1989; Johanssen, 1986; Reid et al., 1989; Tomasi et al., 1982). Moreover, a report by Reilly and Rothwell (1987) found that elite athletes lost more training days due to illness in comparison to club or recreational runners. Sebastian Coe and Diane Edwards are two prominent examples of elite athletes who have contracted toxoplasmosis, an opportunistic infection which affects those with deficient immune systems (Fitzgerald, 1988).

In the present study, the immunological response (in particular lymphocyte and immunoglobulin synthesis) was investigated in runners as a result of changes in training volume and intensity. Lymphocytes are considered to be the critical link in the relationship between exercise and immune function (Simon, 1987) and immunoglobulins (antibodies) are the major components in serum responsible for immunity (Nieman et al., 1991; Welliver and Ogra, 1988).

1.2 THE IMMUNE SYSTEM

For the benefit of readers not familiar with recent advances in immune system physiology, the following summary has been derived from Abbas (1991), Golub (1991), Playfair (1992), Roit (1988), Stites (1991) and Turgeon (1990).

The immune system is responsible for the recognition and disposal of "non-self" or foreign material that enters the body and stimulates antibody production. These non-self materials or "antigens" are often in the form of life-threatening infectious microorganisms (viruses and bacteria), but may also be in the form of life-saving organ grafts.

The majority of cells of the immune system are derived from the "stem cell" precursor (of proven existence but uncertain morphology) found in the bone marrow. The stem cell has the capability of proliferating into red blood cells (erythrocytes) and/or white blood cells (leukocytes). Leukocytes (refer to table 1) are mobilized in the blood to combat an infection as simple as a rhino-virus (common cold) or as complex as a diverse cell neoplasia (such as a cancerous tumor formation). These white blood cells circulate in the blood (normal range of 5000 to 9000 per mm^3) and in the presence of acute infections (appendicitis, pneumonia etc.) may rise as high as 40,000 per mm^3 of blood. There are 5 types of blood leukocytes categorized as granular and non-

Table 1. Classification of Leukocytes and Normal Range.

Leukocytes		Cell type	Average (per mm^3 whole blood)	Relative (%)
/ Granular leukocytes (Granulocytes)	{	Neutrophils	4340	55-60
		Eosinophils	161	1-3
		Basophils	28	0-0.7
\ Non-granular Leukocytes (Mononuclear leukocytes)	{	Lymphocytes (T, B & NK cells)	2100	25-33
		Monocytes	371	3-7
		Total white blood cells	7000	100

Modified from Guyton (1987) and Playfair (1992).

granular (according to the presence or lack of cytoplasmic granules) and mononuclear (single nucleus) or polymorphonuclear (many shaped or lobulated nucleus). The 3 types of granular (polymorphonuclear) leukocytes are the neutrophils, eosinophils and basophils, and the 2 types of non-granular (mononuclear) leukocytes are the lymphocytes and the monocytes.

Neutrophils (or "polys" for polymorphonuclear (or PMN) leukocytes) constitute the largest percentage of leukocytes (55 to 65%) and are part of the first line of defence against bacterial invasion. These short-lived "scavenger" leukocytes adhere to the walls of capillaries and venules at sites of inflammation and then migrate into the connective tissues to phagocytize and destroy bacteria (by the release of powerful bactericidal enzymes from connecting lysosomes). Usually 5 to 20 bacteria may be phagocytized by a neutrophil before it becomes inactivated and dies. The pus that accumulates in abscesses or boils is comprised of millions of dead and dying neutrophil leukocytes.

Eosinophils comprise only 1 to 3% of the leukocyte population, but their numbers increase during various hypersensitivity and allergic situations. It has been speculated that eosinophils selectively phagocytize and destroy antigen-antibody complexes. Since they express Fc (the crystalline fragment of the antibody molecule) receptors

for IgE (a skin sensitizing; hypersensitive antibody), eosinophils are important effector cells in immune reactions to antigens (such as parasites) that induce high levels of IgE. Eosinophils are also activated by secretory IgA. Parasites that are resistant to lysosomal enzymes of neutrophils and macrophages may be destroyed by eosinophils. The eosinophils attach to the parasites that are too large to be phagocytized and release substances to kill them. Eosinophil granules contain highly basic (cationic) proteins which are important in destroying larger parasites including worms. The growth and differentiation of eosinophils is stimulated by a helper T cell-derived cytokine called interleukin-5.

Basophils comprise only 0.5% of the total number of leukocytes. They express high-affinity receptors for IgE, and thus avidly bind IgE antibodies. The large basophilic granules contain heparin and vasoactive amines, important in the inflamatory response.

Monocytes constitute 3 to 8% of the circulating blood leukocytes. These are the largest nucleated cell of the blood and they migrate into various organs and connective tissues where they develop into tissue macrophages (the principal resident phagocyte of tissues which eliminate intracellular microorganisms). Thus monocytes are most useful in tissues (rather than in the blood) where they are a mobile reserve of

scavengers that defensively aid in phagocytosis and intracellular digestion of invading microorganisms. They also are essential for processing many antigens prior to antibody development.

Lymphocytes are the only cells in the body capable of specifically recognizing different antigenic determinants. They constitute 20 to 30% of circulating leukocytes and circulate through the tissues and back via the lymph, "policing" the body for antigens. Specialized surface receptors allow lymphocytes to recognize and acquire a specific "memory" for individual antigens and their long life span makes them ideal for adaptive responses. The major populations of lymphocytes consist of T-cells (thymus-derived), B-cells (bursa or bone marrow-derived) and null cells. T lymphocytes arise in the bone marrow and migrate to and mature in the thymus. They are further subdivided into helper T cells (T_H), cytotoxic T cells (Tc) and suppressor T cells (Ts). The T cells do not produce antibody molecules, although their distinct antigen receptors are structurally related to antibodies. B lymphocytes are the only cells capable of antibody production. Antigens interact with the membrane-bound antibody receptors to initiate B cell activation, which results in the secretion of antibody molecules by developing effector cells. Null cells (or natural killer (NK) cells) are large granular lymphocytes that

do not express the identifying surface markers for T or B cells but are capable of lysing a variety of tumor and virus infected cells without overt antigenic stimulation. Two levels of defence exist against infection by external agents: "natural" (nonspecific, innate and unchanging) or "adaptive" (acquired) immunity; these may be thought of as the first and second lines of defence. The main differences between these two involve immunologic memory and specificity, which are characteristic only of adaptive immunity.

1.2.1 Natural Immunity.

Natural immunity is present from birth and incorporates several processes: 1) physical resistance of the skin and its excretion of bactericidal and bacteriostatic materials, 2) secretion of antibacterial substances (e.g., i) lysozyme (an enzyme which splits bonds in cell walls of bacteria) in tears, saliva and mucous, ii) α-interferons (proteins which bind to cell surface receptors and block virus replication), iii) acidic secretions and enzymes of the digestive system) 3) phagocytosis of bacteria, viruses and other antigens by circulating leukocytes and the tissue macrophage system cells. The system is comprised of natural killer (NK) cells, various phagocytes such as neutrophils (PMN), eosinophils, basophils, monocytes and macrophages (MAC), along with soluble factors

(lysozymes, acute phase proteins, complement (serum enzymes which when activated, produce widespread inflammatory effects and bacterial lysis) and interferons).

1.2.2 Adaptive Immunity.

The adaptive or acquired immune response is activated by any foreign agents which avoid the defense of the innate immune system. Adaptive immunity is directed at specific disease organisms and occurs after the initial exposure to the antigen. The process of vaccination results in acquired immunity and thus protects against specific diseases. Vaccination involves the injection of either dead organisms (as used in typhoid fever, whooping cough and diphtheria), chemically detoxified toxins (used in tetanus and botulism) or specially mutated live organisms (used in poliomyelitis, yellow fever, measles and small pox) that do not cause the disease, but still carry the specific antigens.

Adaptive immunity involves lymphocytes (T and B cells) and soluble factors (immunoglobulins or antibodies) which selectively respond to thousands of different antigens, resulting in a specific memory and an altered pattern of response. These adaptive mechanisms may function single-handedly against certain antigens, but the majority of responses are through the interaction of an antibody with

complement and the phagocytic cells of natural immunity and T cells with macrophages. Adaptive immunity consists of 2 basic types: antibody-mediated (or humoral) immunity and cell-mediated immunity (Bellanti, 1985). Antibody-mediated immunity involves the B lymphocytes and the production and/or directing of the circulating antibodies towards the antigen. Cell-mediated immunity involves specialized T lymphocytes which bind to the antigen and destroy it either by releasing lysozymes or by stimulating the phagocytic activity of macrophages (refer to figure 1 for a summary of natural and adaptive immune processes).

A. Antibody-mediated immunity. The 2 major lymphocyte subpopulations (B and T lymphocytes) develop from the lymphocytic stem cell found in the bone marrow. Antigen exposure and/or collaboration with macrophages, T lymphocytes and their soluable products in the lymph node, cause the B lymphocytes to replicate and mature into plasma cells. These plasma cells synthesize and secrete serum glycoprotein molecules called antibodies. Antibodies are a subgroup of gammaglobulins called immunoglobulins which diffuse into the circulating lymph and blood for transport to the site of infection. The antibodies may bind to and neutralize a wide range of bacterial toxins and by binding to the surface of viruses, bacteria and other parasites, they increase their

adherance to, and phagocytosis by, myleoid (from bone marrow) cells. This process may be further enhanced by the ability of antibodies to activate complement and properdin (a group of proteins involved in resistance to infection) (Guyton, 1986; Goodman, 1987; Turner, 1989).

The basic antibody molecule is made up of 4 polypeptide chains (2 long (heavy) and 2 short (light)), linked in pairs through cysteine residues by disulphide bonds, giving the whole molecule a flexible 'Y' shape. The Fc (crystalline) fragment (the lower part of the "Y") binds to complement molecules and cell-surface receptors such as neutrophils, lymphocytes and macrophages. The "arms" (the upper part of the "Y") consist of 2 Fab (antigen binding) fragments which are variable and interact with the antigens of invading organisms. Each "arm" is specific for a particular antigen, allowing for numerous different antigen binding sites. In each polypeptide chain, this N-terminal domain is the most variable while the remainder is relatively constant. The physical, antigenic and functional variations between the constant regions give rise to 5 main classes of antibodies: IgM, IgG, IgA, IgE and IgD (Benjamini and Leskowitz, 1988; Burton and Gregory, 1986; Goodman, 1987).

IgM is the predominant antibody made early in a response and is believed to have been the first Ig class to appear during evolution. It represents approximately 10% of total

serum immunoglobulin (adult range, 0.3-4.5 g/l) and is found primarily in the intravascular pool. IgM is the major immunoglobulin expressed on the B cell surface. Its pentameric structure (5 linked monomers) yields ten antigen binding sites, making it extremely effective at binding and agglutinating micro-organisms. Examples of IgM include heterophile antibodies (eg: human infectious mononucleosis antibody), cold agglutinins (antibodies which induce clumping only at relatively low temperatures) and isohemagglutinins (antibodies that stimulate clumping of red blood cells) (Lydyard, 1989; Playfair, 1992).

IgG is the major antibody class (75% of total immunoglobulins) in the serum (adult range, 3.2-23.1 g/l). It is the most common immunoglobulin found in the airway and alveolar space secretions, diffusing into the lungs from the blood. In addition, IgG has specific activity against microbial agents or antigens in the lower respiratory tract. IgG is distributed equally between intra- and extravascular pools and is diversified into 4 subclasses (IgG1 to IgG4) which differ in their heavy polypeptide chains.

IgA (consisting of 2 subclasses IgA1 and IgA2) forms approximately 15% of total serum immunoglobulins (adult range, 0.3-7 g/l). It is the primary antibody of body secretions such as tears, sweat, saliva, nasal secretions and the contents of the lungs, gut, urine and breast milk. A

polypeptide secretory piece on IgA enables it to avoid digestion and to be transported for external secretion across the epithelium. The main function of IgA is to prevent the entry of microorganisms from external surfaces to the tissues (e.g., secretory IgA coats the mucosal surfaces of the conducting airways, nasopharynx, oropharynx, eyes, gut and bladder)(Reynolds, 1987; Weliver and Ogra, 1988).

The biological significance of IgD and IgE is uncertain, and both are found in minuscule quantities in human serum (3 mg/dl and .005 mg/dl, respectively). IgD appears to have some regulatory role on the surface of B cells whereas IgE (a skin sensitizing; hypersensitive antibody) binds to mast cells and promotes their degranulation.

Serum immunoglobulin levels have been found to increase during illness (Shimokata et al., 1988) and resistance to infection and reinfection is attributed to sufficient levels of antigen-specific serum and secretory immunoglobulins (Welliver and Ogra, 1988).

B. Cell-mediated immunity. Cell-mediated immunity involves the formation of activated T cells (T lymphocytes) which are leukocytes found in the lymph, modified in the thymus and specialized for cell-mediated hypersensitivity and immunity. After being sensitized by an antigen, the T cells may either destroy it or assist the B-lymphocytes and

macrophages in the production of antibodies. Several subtypes of T lymphocytes exist, the more significant being the helper T cells (T_H, T4 or CD4+), the suppressor T cells (T_S, T8 or CD8+) and cytotoxic T cells (T_C or "killer" T cells). The analysis and identification of these T cell subsets has been simplified by the discovery of specific membrane proteins which serve as phenotypic markers. Cell surface molecules are recognized by specific antibodies and are useful markers of various leukocytes that are involved in immune and inflamatory responses. The CD ("cluster of differentiation") nomenclature is used to name these surface markers or cell surface antigens (eg: helper T cells express a surface protein called CD4+ and suppressor T cells express a different surface protein marker called CD8+) that may be distinguished by monoclonal antibodies.

Helper T cells promote rapid immune system function by recognizing abnormal body cells and minute quantities of antigen. They also activate proliferation of B-cells and plasmocytes to release antibodies. The helper T cells are activated by interleukin-1 (or IL-1) released by macrophages and they produce a second lymphokine, interleukin-2 (or IL-2) which synergises with interferons to further increase T cell activity and stimulate the T-helper cells to secrete a third lymphokine, interleukin-3 (IL-3) (Bonavida and Wright, 1987; Henry et al., 1981). IL-3 initiates a chemical messenger

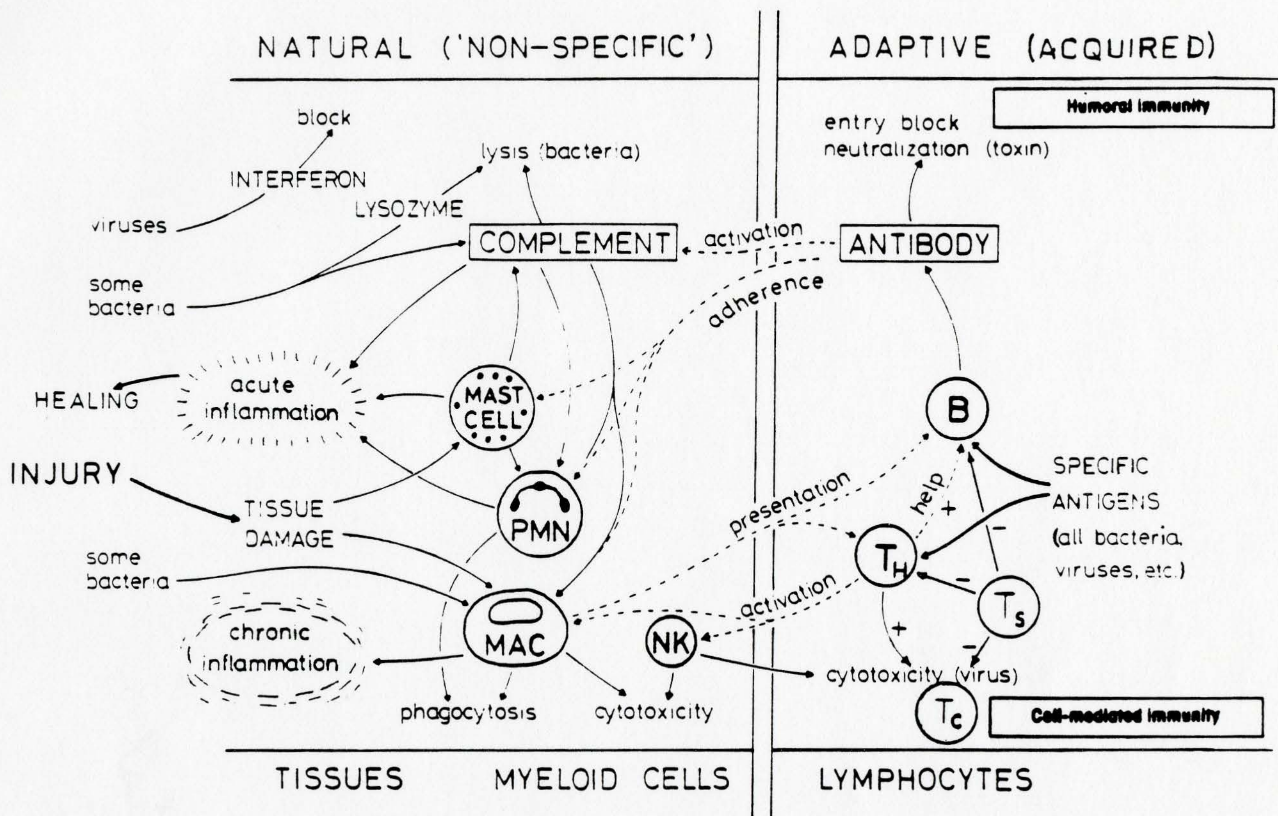
(probably IL-2) to the cytotoxic or killer T cells (Farrar et al., 1981; Hefeneider et al., 1983; Rocha et al., 1989). The rejuvenation of the immune system occurs through the secretion of IL-3 and colony stimulating factors (CSF) by CD4+ T cells which in turn, stimulate differentiation of the lymphocyte precursors in the bone marrow. AIDS (acquired immune deficiency syndrome) is due to a viral infection of the T-helper cells (ie: CD4+ is thought to be the receptor by which the HIV (human immunodeficiency) virus enters the cells). Those who develop AIDS become susceptible to opportunistic infections that those with normal immune systems are able to handle with ease. The dramatic loss of CD4+ cells results in an altered CD4/CD8 ratio. Normal individuals have a CD4/CD8 ratio of 1.7 to 2.2 (Stites et al., 1987), yet ratios of AIDS patients have been reported to be less than 0.9 (Kimball, 1987).

The cytotoxic or "killer" T cells are direct attack cells, able to detect and destroy antigenically abnormal or virus-infected cells (and interrupt virus replication) or to lyse tumor cells exhibiting recently produced antigenic surface markers. The cytotoxic T cell receptor recognises "self" antigens and "non-self" virus antigens (highly specific with different T cells for different viruses). The CD8+ (suppressor/cytotoxic) T cell receptor interacts with class I MHC (Major Histocompatibility Complex - a region of highly

polymorphic genes that encode the cell surface molecules responsible for graft rejection and antigen presentation to T cells) peptide complexes on macrophages in a manner similar to CD4+ T cells. CD8+ T cells use the IL-2 provided by activated CD4+ T cells during cell proliferation and differentiation.

Suppressor (CD8+) T cells are downregulating cells providing a negative feedback mechanism to recognise, control and inhibit the destructive process of both cytotoxic and T-helper cells as well as other self-reactive lymphocytes. Thus the suppressor cells often prevent undue damage to the body by limiting the ability of the immune system to attack the person's own body tisues (ie: to allow immune tolerance and prevent autoimmunity).

A ratio of helper to suppressor T cells (CD4/CD8 ratio) below 1.5 is indicative of an increased susceptibility to infections. Keast et al. (1988) have reported a decreased peripheral lymphocyte stimulation by polyclonal T cell mitogens at a CD4/CD8 ratio below 1.5. Consequently, if the helper/suppressor T cell ratio is low, the host may be more susceptible to infection and less able to combat it.



Where:
B = B lymphocyte
T_H = T-helper lymphocyte
Ts = T-suppressor lymphocyte
Tc = T-cytotoxic lymphocyte
PMN = polymorphonuclear leukocyte (neutrophil)
MAC = macrophage
NK = natural killer cell

Figure 1. Factors involved in natural and adaptive immunity. Modified from Playfair, 1992.

1.3 EXERCISE AND IMMUNE FUNCTION.

1.3.1 Immunoglobulin (Antibody) Response to Exercise.

Immunoglobulin concentrations in serum and other body fluids are affected by alterations in blood volume, catabolism, immune reactions and protein migration between intra- and extravascular compartments (Parving et al., 1974).

A. Response to acute exercise bouts. Increases in serum immunoglobulins have been found during short-term submaximal exercise or graded maximal exercise in both athletes and sedentary individuals. Stephenson et al. (1985) reported a significant (6%) increase in IgG and non-significant increases in IgA and IgM (13 and 11%, respectively) in 5 healthy males during maximal treadmill exercise. These acute increases in serum immunoglobulins were largely due to the 11.2% reduction in plasma volume. However, immediately following maximal cycle exercise in 28 olympic athletes, Poortmans (1970) reported significant increases in IgG (11.8%) and IgA (14.4%) which was more than could be explained by the decrease in plasma volume. In the absence of plasma volume changes during acute moderate submaximal exercise (eg, a 45 min bout of walking), the transient rise in serum immunoglobulins may be explained by increased lymph flow and input from extravascular

protein pools (Nehlsen-Cannarella et al., 1991).

There are numerous conflicting reports as to the effect of acute submaximal exercise on serum immunoglobulin levels. Eberhardt (1971) measured pre- and post-exercise immunoglobulin levels in 7 subjects who strenuously cycled at 1300 kg•m•min^{-1} for 20 minutes. IgG levels were unchanged, but IgA and IgM increased 23% and 43% after exercise, but these changes were not statistically significant. Hanson and Flaherty (1981) compared pre- and 10 min post-exercise immunoglobulin values in 6 subjects who ran 13 km at 72% $\dot{V}O2$ max. IgA and IgG levels were unchanged, but the IgM level increased by 34% and was still elevated by 29%, 18 to 25 h later. Again, these changes were not statistically significant. In neither study was the change in plasma volume reported.

Minor or statistically insignificant changes in total serum immunoglobulins have been found after running less than 40 km, with IgG levels lowest at 1.5 h post-exercise. The most pronounced effect of acute submaximal exercise is upon IgM levels (the first antibody class produced in an immune response). The results, however, have been inconsistent, and plasma volumes were not reported (Hanson and Flaherty, 1981). The IgM increase was possibly due to non-specific noradrenergic sympathetic neural interactions and antigen stimulation from increased ventilation and drying of the

natural mucosal immunity secretions (Nieman et al., 1989).

B. Prolonged acute exercise bouts. Nieman et al. (1991) measured serum immunoglobulin changes in 10 marathoners after a 3 h treadmill run. Blood samples were taken at rest, 1 h of exercise and 5 min, 1.5, 6 and 21 h post-exercise. IgM values increased 7.2% during exercise and decreased to baseline levels during the recovery period. IgG values increased during exercise and decreased during recovery, achieving the lowest value at 1.5 h post-exercise and rising back to baseline values after 21 h. IgA did not change during exercise and then followed the same recovery pattern as IgG. There were no changes in plasma volume and none of these changes were significantly correlated with changes in cortisol or catecholamines.

Decreases in serum immunoglobulin levels (24, 18 and 20% below baseline values for IgM, IgG and IgA, respectively) for as long as 2 days have been induced by intense ultramarathon running (45 or 75 km). Yet only the changes in IgG were statistically significant due to the large between-subject variance (Israel et al., 1982). Poortmans and Haralambie (1979) found a significant 7% increase in IgG, 15 min after a 100 km run, despite no mean change in plasma volume. However, at 18 to 25 h post-exercise, they reported a greater and more

prolonged immunoglobulin decrease of 4 and 12% below baseline values for IgG and IgA, respectively. Following an ultratriathalon competition, venous blood samples were taken pre- and within 15 min post-exercise in 18 male athletes. Bosenberg et al. (1988) reported a significant reduction in IgG from 67.63 to 38.99 ug/ml. It was suggested that this reduction was due to the autoimmune response to lipopolysaccharide that had leaked into the the blood stream during the extensive exercise bout.

Salivary immunoglobulin A (s-IgA), is the first line of defence against invasion of certain pathogenic viruses on the mucosal surface of the upper respiratory tract. It has been proposed that depression of s-IgA may cause an increased susceptibility to upper respiratory infections. McDowell et al. (1991) reported that running at intensities of 50 to 80% of $\dot{V}O2$ max for durations of 15 to 45 min did not affect s-IgA levels taken at rest, immediately post, or 1 and 2 h post-exercise. In contrast, salivary IgA levels in male and female members of the U.S. national cross-country ski team were found to be significantly lower than controls at rest and further decreased by 2-3 h of exhaustive skiing. It was suggested that the salivary IgA antibody secretion was primarily affected by breathing the colder air (Tomasi et al., 1982). Other studies (Tomasi et al., 1982; Tharp and Barnes, 1990) also reported significant decreases in s-IgA following

strenuous exercise bouts. Differences may be due to the considerably higher exercise duration and intensities in these studies and that the subjects were studied under competitive conditions and thus under much more stress due to training and competition. In contrast, no differences in resting salivary IgA levels were found between highly trained cyclists and sedentary controls when exercising on a cycle ergometer for 2 h at 70 to 75% $\dot{V}O_2$ max (MacKinnon et al., 1987). Since Tomasi's skiers were studied during the winter in New York and MacKinnon's cyclists during the summer in New Mexico, perhaps the ambient temperature had an effect on resting salivary immunoglobulin levels. Accordingly, Housh et al. (1991) took IgA saliva samples of 9 males performing 30 min treadmill runs at 80% $\dot{V}O_2$ max in an environmental chamber at temperatures of 6, 19 and 34°C. The results revealed that 30 min of moderate exercise at temperatures ranging from 6 to 34°C had no effect on salivary IgA and thus should not increase the susceptibility to upper respiratory infection. Although there were no significant mean changes in s-IgA, there were large intra-and inter-individual differences in the s-IgA response to exercise at the 3 temperatures. In these studies, IgA and IgG immunoglobulins (found in airway and alveolar space secretions) may have diffused from the serum during exercise recovery non-specifically or in response to antigens and microbial agents entering into the airways during exercise.

Since endurance exercise is associated with local inflammation and muscle cell damage, IgM autoantibodies may assist macrophages in the disposal of muscle cell breakdown products. As a result, IgM may bind to breakdown products in the blood, which is cleaned from the circulation, or the antibodies may travel to the tissues to perform this function.

During the competitive season, many elite athletes experience low concentrations of serum and secretory immunoglobulins (Rocker, 1976). When changes in plasma volume are adjusted for, however, athletes show comparable patterns of serum immunoglobulins as non-athletes at rest during graded, maximal exercise (Green et al., 1981; Hanson and Flaherty, 1981; Haralambie and Keul, 1970; Israel et al., 1982; Nieman et al., 1989; Poortmans, 1970), but in these studies there was a wide range in values as well as in analytical techniques. Immunoglobulin injections have proven to be beneficial in curtailing the duration but not the incidence of these infections (Frolich et al., 1987).

Antibody production was reportedly normal when 4 marathon runners were injected with antitetanus toxoid antigen, 30 min after the race (Eskola et al., 1978). In contrast, pokeweed-stimulated production of IgG and IgM was decreased after only 15 min of submaximal exercise in untrained subjects (Hedfors et al., 1983). In contrast, an increase in pokeweed-stimulated IgG production _in vitro_ was found 5 min after a 30

min bout of submaximal treadmill exercise in trained runners (Verde et al., 1992). These conflicting results may reflect variations in exercise duration, blood culture methodology and fitness of subjects.

C. Responses to exercise training. Although competitive exercise training and the psycho-physiological stress involved may depress serum immunoglobulin levels in elite athletes, chronic moderate exercise training may improve serum immunological levels. Liesen et al. (1976) reported significantly higher serum immunoglobulin levels in 22 elderly (aged 55 to 70) male subjects after 11 weeks of endurance training. This study, however, did not include a randomized control group. Nehlsen-Cannarella et al. (1991) studied the effects of moderate exercise training (45 min of brisk walking, 5 times per week for 15 weeks) on serum immunoglobulins in sedentary, mildly obese, middle aged women. Moderate exercise training was not associated with improved lymphocyte function but was associated with a 20% increase in serum immunoglobulins (IgM, IgG and IgA) and significant decreases in circulating lymphocytes, particularly the T cell subpopulation. Changes were apparent at the 6-week testing period with some attenuation by 15 weeks.

Other chronic training studies involving the effects of exercise on immunoglobulin levels are limited. Swim training

apparently decreases salivary IgA levels while moderate basketball training increases salivary IgA levels (Tharp et al., 1990 a and b). Yet in these two studies, saliva samples were taken only once per month (pre- and post-exercise) for the four months of October to December. Verde et al. (1992) reported a decrease of mitogen-stimulated IgG synthesis after 3 weeks of training. In Verde's study, however, (as in the studies by Tharp, Nehlsen-Cannarella and Liesen) sampling occurred only once at the end of each training or specified time period (as long as 6 weeks in Nehlsen-Cannarella's study). Thus inter- and intra-individual differences on a given day may have partially affected results. Moreover, many of these preliminary studies may be confounded by the sedentary types of subjects used since highly trained athletes would experience a greater likelihood of decreased immunoglobulin levels due to the more intensive nature and competitive psychosocial-physiological stress of their training.

1.3.2 Exercise and Lymphocytes.

Strenuous exercise has a transient effect on immune function, comparable to that incited by various infections (Cannon and Kluger, 1983; Lewis et al., 1986; Schaefer et al., 1987; Simon, 1987). Numerous reviews have reported changes in such immune indicies as granulocytosis, lymphocytosis, increases in antibody-dependent cytotoxic and NK activity and plasmal interleukin-1-like activity (Simon, 1984, 1987; Nash, 1986; MacKinnon and Tomasi, 1986, 1989; Keast et al., 1988, Shephard et al., 1991). Although the biological and clinical significance of these exercise-induced changes is not yet fully understood, decreases in lymphocyte subsets and the incidence of increased systemic infections are thought by some investigators to indicate a state of overtraining and result in a decrease in athletic performance (Simon, 1987; Keast et al., 1988).

Increases in leukocyte counts depend upon exercise duration and intensity, with a greater increase occurring in untrained than in trained subjects (McCarthy and Dale, 1988). Dorner et al. (1987) found that leukocytosis in trained subjects with high work capacities was due mainly to increases in neutrophils. Conversely, in subjects with low work capacities, leukocytosis was due mainly to increases in lymphocytes.

A number of factors can influence the total number of leukocytes in the circulation :i)activation of alpha and beta adrenoceptors, ii) location of the non-circulating leukocyte pool, iii) rates of margination-demargination, sequestration of leukocytes, iv) the hemodynamics of blood flow and cardiac output, v) leukocyte-endothelium interactions, vi) hormonal (cortisol and catecholamines) and neural (sympathetic and parasympathetic) input at the spinal cord and hypothalamus. Although these factors may affect the total leukocyte count, their specific influence upon lymphocytosis during exercise remains obscure (McCarthy and Dale, 1988).

The loci for leukocyte pooling have been investigated by injecting subjects with radio-labelled autologous leukocytes. The liver and spleen are the primary locations for non-circulating lymphocytes with the remainder diffusing among other tissues (McCarthy and Dale, 1988). Exercise-induced movement of these non-circulating lymphocytes is less obvious. Hedfors (1978) has demonstrated minor effects of exercise on the number of leukocytes leaving the spleen and this was supported by the minimal effects of a splenectomy on exercise-induced leucocytosis. Similarly, the lungs are reportedly not the principal source of exercise-induced mobilization of non-circulating lymphocytes. Changes in leukocyte and lymphocyte numbers reflect a mobilization of cells from the liver (Hedfors et al., 1976).

The following section will be limited to the discussion of exercise and lymphocytes. The focus of the present study was to determine the effects of training on the various lymphocyte counts (CD4+, CD8+, CD4/CD8 ratio, CD3+ and HLA-DR+ activation). Since it was not the purpose of the study to examine the effects of exercise on lymphocyte function, this area will only be discussed briefly.

A. Acute exercise bouts and lymphocyte counts. Immediately following exercise, there is an increase in the peripheral venous leukocyte count (leukocytosis)(Andersen, 1955; Gimenez et al., 1987; Gray et al., 1992; Haq et al., 1993; McCarthy and Dale, 1988; Nieman and Nehlsen-Cannarella, 1991; Nieman et al., 1992) and an increase in the number of circulating lymphocytes (lymphocytosis) often corresponding to the duration and intensity of exercise (Berk et al., 1986; Edwards et al., 1984; Gray et al., 1992; Hedfors et al., 1976; McCarthy and Dale, 1988; Nieman et al., 1992; Robertson et al., 1981; Shinkai et al., 1992; Soppi et al., 1982; Vishnu-Moorthy and Zimmerman, 1978; Yu et al., 1977). Prolonged exercise (particularly marathon running), however, may result in little or no lymphocytosis (Davidson, 1987; Eskola et al., 1978; Moorthy and Zimmerman, 1978; Oshida et al., 1988; Wells et al., 1982) and a decrease in leukocytes before the end of exercise due to the migration of cells into the injured muscle

(Galun et al., 1987). A delayed leukocytosis was observed 30 min to 3 h following a marathon (Eskola et al., 1978) possibly due to a cortisol stimulated release of white cells from the bone marrow. In contrast, others have reported decreased leukocyte counts at this point (Davidson et al., 1987; Dickson et al., 1982; Haq et al., 1993; Nieman et al., 1989; Pedersen et al., 1988). Nieman et al., (1989) have reported complete leukocyte count recovery usually within 6 h post-exercise. Wells et al. (1982) observed that leukocytosis and granulocytosis persisted for more than 8 h, returning to normal 24 h later.

B. Acute exercise and T and B cell counts. Absolute numbers of T and B cells have been shown to increase immediately following as little as 30 s (Nieman et al., 1992), 60 s (Gray et al., 1992) and 15 min of strenuous cycle ergometry exercise (Tomasi et al., 1982). Some studies have found the proportion of B cells to increase relative to the T cells using surface immunoglobulins or the Fc receptor as markers (Edwards et al., 1984; Hedfors et al., 1976, 1983; Landman et al., 1984; McCarthy and Dale, 1988; Robertson et al., 1979, 1981; Steel et al., 1964; Tomasi et al., 1982; Yu et al., 1977) whereas others have not (Bongers and Betrams, 1984; Hanson and Flaherty, 1981; Kanonchoff et al., 1984; Soppi et al., 1982). Moorthy and Zimmerman (1978) reported an

85% increase in B cell number and no change in T cell number following a marathon run while Oshida et al. (1988) found no changes in B cell numbers and a significant T cell reduction following 2 h of cycle ergometry. More recently, Verde et al. (1992) found a marginal decrease in the proportion of B cells immediately after 30 min of submaximal treadmill exercise. Verde's approach was more specific than many previous studies in that he used monoclonal antibodies and fluorescent cell sorting. No effort was made to investigate any plasma volume shifts in these studies.

Some studies have reported no significant changes in the CD4+ (helper)/CD8+ (suppressor) ratio (Haq et al., 1993; Kanonchoff et al., 1984; Nieman et al., 1989; Verde et al.,1992) whereas others have found a reduction in this ratio following exercise (Berk et al., 1985; Brahmi et al., 1985; Cameron et al., 1989; Edwards et al., 1984; Hedfors et al., 1983; Landmann et al., 1984; Lewicki et al., 1988; Nguyen et al., 1984; Oshida et al., 1988).

Cameron et al. (1989) selectively tested each of the 3 main exercise energy systems by varying treadmill exercise duration and intensity and reported that the CD4/CD8 lymphocyte ratio was significantly lowered following exercise involving glycolytic and phosphate exercise protocols but not after an aerobic protocol. Recent studies have reported significant decreases in the CD4/CD8 ratio immediately after

30 (Nieman et al., 1992) and 60 s (Gray et al., 1992) of maximal cycle ergometry.

Flow cytometry was used to demonstrate increases in absolute lymphocyte counts from 2,400 to 2,900 / mm^3 after a maximal treadmill exercise bout in 12 males (mean age 45 years) with no overall T cell percentage change (Berk et al., 1986). CD4+ helper cells decreased from 53.8 to 43.4% , CD8+ suppressor cells increased from 32.7 to 36.4% and the CD4/CD8 ratio decreased from 1.94 to 1.36. Landmann et al. (1984) observed a reduction in the T cell percentage after 15 min of submaximal cycle ergometry. A 19% decrease in the CD4/CD8 ratio was explained by the proportionately larger increase in CD8+ suppressor than CD4+ helper cells. In 10 male volunteers, Hedfors et al. (1983) explained the decrease in T lymphocytes (after 15 min of submaximal cycle ergometry) by the reduction in CD4+ helper cells with the proportion of CD8+ suppressor cells remaining unchanged.

Following graded maximal exercise on a cycle ergometer, Brahmi et al. (1985) reported a 29.8% reduction in the total T cell count. CD4+ helper cells decreased by 33.7%, CD8+ suppressor cells did not change and NK cells increased by 53.9%. The decreased CD4/CD8 ratio was accounted for by the post-exercise movement of lymphocyte subsets out of the circulation. The CD4/CD8 ratio rose above baseline levels 2 h post-exercise and returned to baseline 20 h post-exercise.

In contrast, in 11 highly-trained cyclists, an increase in the overall percentage of T cells, the number of CD8+ suppressor cells (per mm^3) and a moderate increase in the number of CD4+ helper and NK cells (per mm^3) was observed by Lewicki et al. (1988) 3 min after a maximal exercise bout. As in Landmann's study, the decrease in the CD4/CD8 ratio was explained by the larger increase in CD8+ suppressor cells than CD4+ helper cells.

A decrease in the CD4/CD8 ratio was demonstrated by Nieman et al. (1989) and Hoffman-Goetz et al. (1990) immediately following 30 min to 2 h of endurance cycling. Oshida et al. (1988) also reported a significant decrease in the helper/suppressor T cell ratio following 2 h on a cycle ergometer at 60% $\dot{V}O2$ max and the ratio returned to normal after 24 h of recovery. Both Oshida and Pederson et al. (1988) reported an increase in T cytotoxic cell numbers and lymphocytes and a significant reduction in helper cells in athletes immediately after exercise. In a group of untrained subjects, Oshida reported a significant suppression of suppressor cells 72 h post-exercise. In addition, the peripheral T cell count was found to significantly decrease in both trained and untrained subjects immediately after (and 72 h after in untrained subjects) 2 h of cycle ergometry at 60% $\dot{V}O2$ max. It should be noted , however, that Oshida used a different T cell marker (known as OKT (Ortho-King)) from the

more commonly used CD classification marker. A decrease in the percentage of CD4+ helper cells was found by Verde et al. (1992) with a non-significant decrease in the CD4/CD8 ratio and no change in the T and B cell percentages, 5 min after 30 min of submaximal exercise. Contrastingly, Nieman et al. (1989) observed no change in the CD4/CD8 ratio immediately after a 3 h exhaustive treadmill run. However, the ratio was elevated at 1.5 and 21 h of recovery due to the significant decrease in the number of CD8+ suppressor cells.

Reductions in total T cell counts 15 min post-exercise have been attributed to the reduction in CD4+ helper cell number (Hedfors et al., 1983), while the CD8+ suppressor cell number has been shown to increase (Lewicki et al., 1988; Masuhara et al., 1987) or remain unchanged (Hoffman-Goetz et al., 1990; Tvede et al., 1989; Verde et al., 1992). Twenty-four hours post-exercise, an elevation in the CD4/CD8 ratio has been found which has been attributed primarily to a cortisol-induced reduction in the suppressor cells (Brahmi et al., 1985; Nieman et al., 1989; Order et al., 1990; Pederson et al., 1988).

Lymphocyte subsets may be affected during and after exercise by variations in beta-adrenoceptor numbers. B cells have 3 times as many receptors as T cells, and T helper cells have 4 times as many receptors as T suppressor cells (Khan et al., 1986; Plaut, 1987). Additionally, the receptor density

is further modulated by catecholamine levels (Fraser et al., 1981), cortisol levels (Davies and Lefkowitz, 1980) and training (Butler et al., 1982).

Numerous reports suggest that a strong leukocytosis but mild lymphocytosis occurs after long-endurance exercise (Davidson et al., 1987; Eskola et al., 1978; Moorthy and Zimmerman, 1978; Wells et al., 1982). Following exhaustive endurance exercise, cortisol is greatly elevated, thus inducing a strong leukocytosis and possibly reducing circulating lymphocytes, temporarily sequestering the T helper cells in the bone marrow (Robertson et al., 1981; Yu et al., 1977). Cortisol also impairs the release of interleukin-1, which is required to induce the release of interleukin-2 necessary for lymphocyte proliferation (Fauci, 1975). In contrast, a transient, mild leukocytosis and strong lymphocytosis is observed after acute moderate or maximal exercise (Deuster et al., 1986; Hedfors et al., 1976, 1983; Landmann et al., 1984; Masuhara et al., 1987; Robertson et al., 1981; Soppi et al., 1982; Steel et al., 1974; Stevenson et al., 1985). During maximal or submaximal exercise, catecholamine levels increase early in the exercise bout and induce a mild leukocytosis along with a rapid and strong lymphocytosis from lung storage sites (MacKinnon and Tomasi, 1986; Muir et al.,1984).

C. Cross-sectional comparison studies. Cross-sectional comparison studies involving athletes and sedentary subjects provide conflicting data. While some show little or no differences in resting immune status (Busse et al., 1980; Green et al., 1981; Hanson and Flaherty, 1981) others have reported lower lymphocyte and leukocyte levels in athletes than sedentary control subjects (Davidson et al., 1987; Deuster et al., 1988; Dorner et al., 1987; McCarthy and Dale, 1988). Still others have found athletes to display lymphocytosis, increased antibody-dependent cytotoxic activity and granulocytosis (Fernandes et al., 1985; Nieman et al., 1990; Pedersen et al., 1989) and increased plasma interleukin-1-like activity (Evans et al., 1986).

McCarthy et al. (1987) have reported that athletes display a lower leukocytosis than sedentary controls at any given absolute work-rate. The leukocytosis in athletes has been attributed to a neutrophilia rather than lymphocytosis (Dorner et al., 1987). Masuhara et al. (1987), however, found a greater lymphocytosis in subjects with high aerobic powers.

Athletes have demonstrated lower phagocytic activity, which is an important defence against certain microorganisms in cell- mediated immunity (Kono et al., 1988; Lewicki et al., 1987). Moreover, athletes involved in controlled intensity training displayed lower total lymphocyte, T cell, CD4+ helper and CD4/CD8 ratio levels than healthy untrained men (Liesen et

al., 1990).

Brahmi et al. (1985) reported no differences between 5 male athletes and 10 untrained subjects with regards to the percentage of T cells, CD4+ helper cells, CD8+ suppressor cells and NK cells following a maximal cycle ergometry test. In contrast, Oshida et al. (1988) found both trained distance runners and non-athletes to display a significant decrease in the percentage of lymphocytes that were T cells and helper cells after maximal exercise. However, only the trained athletes displayed a marked increase in the percentage of suppressor cells and NK cells. Neither group demonstrated a change in the percentage of B cells.

Lower complement levels (but not immunoglobulin levels) during exercise and post-exercise, were reported in marathoners compared to age-matched controls after a graded maximal treadmill test using the Balke protocol (Nieman et al., 1989). It was theorized that long distance running overworked the liver's capability to synthesize complement. Alterations in blood volume, catabolism and immune reactions may also have contributed to the lower complement levels.

These conflicting results may be caused by the failure of some studies to differentiate between relative and absolute exercise intensity. When both athletes and sedentary controls are stressed maximally, the leukocytosis would appear to be comparable (Gimenez et al., 1987).

D. Longitudinal training and lymphocyte counts. Longitudinal studies investigating the immune response to training are very limited in number. The lymphocyte response to training is influenced by the initial fitness level of the subject, the duration, the frequency and intensity of the training stimulus. Presumably these variables would be greater in well-trained athletes before the immune system becomes strained (Ferry et al., 1989).

After 6 weeks of conditioning, T cells account for a larger percentage of total lymphocytes (Soppi et al., 1982; Watson et al., 1986). Watson and co-workers reported a significant increase in the percentage of mature T cells but no changes in the lymphocyte percentage in 15 young males (ages 17 to 34 years) who walked/jogged for 15 weeks. The increase in the percentage of T cells may have been due to the maturation of NK cells into mature T cells (Watson et al., 1986). In this study, however, total leukocyte number was not reported, and thus changes in lymphocyte numbers is not known, nor was there a randomized control group of sedentary subjects for comparison. Ndon et al. (1992) reported that 28 days of exercise training (cycling, running, swimming and weight training) did not alter the baseline resting values or magnitude of the circulating leukocytic response to acute exercise on a cycle ergometer.

The resting CD4/CD8 ratio has been found to either

decrease after training (Verde et al., 1992) or remain unchanged (Nehlsen-Cannarella et al., 1991). In response to 15 weeks of moderate-walking exercise training in 36 sedentary, mildly obese women, Nehlsen-Cannarella et al. (1991) reported a significant but mild decrease in the number of lymphocytes, especially T cells. These responses were strongest after 6 weeks of training with some attenuation between 6 and 15 weeks.

LaPerriere et al. (1991) found a significant increase in T helper cells (the primary immunologic target of the AIDS virus) after 10 weeks of cycle ergometry (45 min, 3 times per week) in HIV-positive subjects. However, Rigsby et al. (1992) reported statistically unchanged total leukocyte, lymphocyte, CD4+, CD8+ and CD4/CD8 ratio counts in 45 men seropositive for HIV-1 after 12 weeks of 1 hour sessions (3 days per week) of cycle exercise and strength training. As with most of the previous studies, differences may be due to the mode, duration and intensity of exercise.

1.3.3 Exercise and Lymphocyte Function.

Very few studies have dealt with exercise and lymphocyte function. Furthermore, the large variations in exercise duration, relative intensity, and (most importantly) the methodological assessment of lymphocyte function, have resulted in numerous discrepancies in the literature.

Functional assays are used to evaluate the lymphocyte response to nonspecific mitogen stimulation. Mitogens are substances which nonspecifically provoke DNA synthesis, blast transformation, mitosis or production of antibodies in order to determine which cells are functioning abnormally (Abbas, 1991). For T lymphocytes, mitogens such as concanavalin A (Con-A), phytohaemagglutinin (PHA) (lectins derived from the jack bean and red kidney bean, respectively) are commonly used. Pokeweed mitogen (PWM) is generally considered to be a B cell mitogen, although it also stimulates T cell proliferation (Shinkai et al., 1992). Some studies have reported an increased proliferation of absolute cells (per mm^3 of blood) in response to PWM, PHA and Con-A mitogen stimulation after a maximal cycle ergometer test (Robertson et al., 1981; Soppi et al., 1982). Shinkai et al. (1992) have reported a decreased lymphocyte responsiveness to PHA and PWM during 60 min of cycle ergometry (60% $\dot{V}O2$ max) which returned to normal by 2 h of recovery. In contrast, other studies

involving a 10 min treadmill run (Edwards et al., 1984), 30 min of submaximal treadmill exercise (Verde et al., 1992), a 32.1 km race (Vishnu-Moorthy and Zimmerman, 1978) or a marathon (Eskola et al., 1978) have reported no change in mitogen response. Another study by Nieman et al. (1992) has found that brief, heavy exertion (a modified 30 s Wingate cycle ergometer test) resulted in no significant changes in lymphocyte proliferative response (PHA and Con-A) or serum immunoglobulin (IgG, IgA, and IgM) levels when adjustments were made for plasma volume and the rapid changes in circulating NK and T lymphocytes.

Apparent discrepancies in lymphocyte function may also depend upon whether cell counts are expressed in relative or absolute terms. Robertson et al. (1981) expressed lymphocyte function (proliferation) as a percentage of the total lymphocytes (in relative terms) and found a decreased mitogen response, mainly because exercise induces a concurrent lymphocytosis. If the results were expressed as the absolute number of cells responding to mitogen stimulation, an enhanced response (a 60% increase) was found. Soppi et al. (1982) also noted a decreased response when the results were expressed in relative terms and an enhanced response when expressed in absolute units. Results are commonly expressed in relative terms and a decreased mitogen response has been inferred in several studies (Brahmi et al., 1985; Eskola et al., 1978;

Hedfors et al., 1983; Oshida et al., 1988; Robertson et al., 1981; Soppi et al., 1982). However, changes in the total lymphocyte count (per mm^3 of blood) do not appear to be solely responsible for the reported changes in lymphocyte function (Eskola et al., 1978).

Keller and Calvanico (1982) have suggested that studies using whole blood (rather than isolated lymphocytes or mononuclear cells) to assess _in vitro_ lymphocyte transformation may introduce complications due to other macro-molecules in the plasma which may affect the immune response. Shinkai et al. (1992), however, have argued that the whole blood method is preferable for detecting changes in both absolute counts and relative proportions of lymphocyte subsets during exercise. Shinkai reported greater (more than 95%) recovery of leukocytes using the whole blood method whereas the PBMC (peripheral blood mononuclear cell) method yielded a lower and unstable recovery rate (large between-sample variance) of mononuclear cells. Very few researchers, however, have used the whole blood method (Eskola et al., 1978; Espersen et al., 1990; Nieman et al., 1992; Shinkai et al., 1992; Soppi et al., 1982) in comparison to the PBMC method (Berk et al., 1990; Hoffman-Goetz et al., 1990; Kendall et al., 1990; Nieman et al., 1989; Oshida et al., 1988; Pedersen et al., 1988, 1990; Ricken et al., 1990). An additional problem involves the apparent decrease of T cell

proliferation, due to the incorrect inclusion of non-proliferating NK cells in the total lymphocyte count (Shephard et al., 1991). Accordingly, Hedfors et al. (1976) isolated highly purified blood lymphocytes (rather than using unsorted peripheral blood mononuclear cells) but still found a decreased proliferative response to mitogen stimulation after 15 min of submaximal cycle ergometry. Fry et al. (1992) reported that upper body interval exercise (kayaking) induced similar reduced lymphocyte proliferative response (*in vitro* to the T cell mitogen Con-A) as those described for lower body (treadmill) exercise. This post-exercise suppression could not be accounted for by depressed IL-2 production nor the inhibitory action of prostaglandin secretion.

The literature suggests that an acute bout of submaximal exercise (30 min or less) leads to an immediate suppression of lymphocyte responsiveness to mitogen stimulation in untrained subjects and a marginal suppression in the trained subject. An acute bout of maximal exercise will immediately suppress the lymphocyte responsiveness to mitogen stimulation in both the trained and untrained subject. Recovery of lymphocyte responsiveness apparently occurs within 30 min post-exercise to levels equal to or greater than pre-exercise values in the untrained subject or non-fatigued athlete. Fatigued athletes are speculated to respond to an acute bout of exercise with a more severe suppression of lymphocyte function (Verde et al.,

1992).

It is not clear whether exercise training enhances or suppresses immune function in humans. Houston (1987) found a significant 43% and non-significant 17% decrease in resting lymphocyte proliferation in response to Con-A and PHA stimulation, respectively, after an 8 week endurance cycling program (40 one-hour sessions at 70% $\dot{V}O2$ max). The investigator, however, was unable to reproduce these results using a second cohort of subjects. Longitudinal studies by Soppi et al. (1982) and Watson et al. (1986) suggest that regular moderate physical exercise is beneficial to immune function. Enhanced resting lymphocyte proliferation in response to mitogen stimulation (using PHA, PWM and Con-A) was observed after 6 weeks of basic naval training (Soppi et al., 1982), after a 15 week walk/jog/run aerobic study of 52 university students (Watson et al., 1986) and after 3 weeks of increased (38%) training volume (Verde et al., 1992). Furthermore, Verde reported that following the heavy training period, 30 min of additional submaximal treadmill running resulted in a 17.9% post-exercise suppression of PHA-induced lymphocyte proliferation and a non-significant 12% suppression of Con-A induced lymphocyte proliferation. Unfortunately, in Verde's study the time of testing varied from early morning to evening for different subjects, and the effects of food intake were not controlled for. This may have influenced the _in_

vitro proliferation capacity of isolated lymphocytes (Hedfors et al., 1976; Houston, 1987). In addition, Verde's study did not carefully control the training stress imposed on the athletes since the interpretation of the subject's training diary was the determinant of the extent of the training stimulus. Verde reported variance in training time (mornings, midday and evenings), seasons (July versus September), as well as sporatic uncontrolled hill and interval workouts (and variation in recovery of the intervals) during the heavy training regimen.

Keast et al. (1988) observed day-to-day variations in individual in vitro response to polyclonal mitogens. It was noted that some individuals may demonstrate a suppressed mitogen response on one day, and on another day may produce lymphocytes with an enhanced response. Overall, there was a greater incidence of post-exercise suppression of mitogen responsiveness than enhanced activity, but the significance of these transient changes is unknown. Accordingly, a potential problem with previous studies was that blood was sampled on only one day at the end of each training period. Thus inter- and intra-individual differences on a given day may have affected the results.

1.4 LYMPHOCYTE AND IMMUNOGLOBULIN ANALYSIS.

1.4.1 Flow Cytometry.

For years, microscopes have been used to visually analyse biochemical and biophysical characteristics of single cells. Even with the most sophisticated microscopes and monoclonal fluorescent antibodies, the estimation of individual cells in a complicated mixture was tedious and often imprecise. With the development of flow cytometry, multiparameter analysis was available in immunologic research with cellular analysis being more accurate, and very rapid (Mayall, 1976).

Flow cytometry can differentiate between the global cellular effects and the heterogeneous effects that affect subpopulations of cells to express varying amounts of antigen. Flow cytometers are sophisticated instruments which can analyse properties of individual cells as they move singly past one or more measuring stations at high velocity. Cytochemical or immunocytochemical probes may be used to label up to 8 or more properties of each cell. Flow cytometry also allows flow cell sorting, in which cells satisfying a predetermined criterion may be separated, counted, examined and used for other biochemical tests (Shapiro, 1983, 1986).

In flow cytometry, a single cell suspension (from blood or other tissues) is labelled with a fluorescent antibody or

a fluorochrome DNA staining dye. The cells are forced under pressure through a nozzle in a liquid jet surrounded by a sheath of water or saline. Vibration of the nozzle tip assembly breaks up the liquid jet stream into a series of droplets, and the droplet size may be adjusted so each droplet will contain exactly one cell. The droplets are illuminated by a monochromatic laser beam and electrically monitored by fluorescence detectors. Since the cells are treated with fluorescently labelled monoclonal antibodies, when they pass through a laser beam of a specific wavelength, fluorescent light is emitted. With the addition of volume and light scatter measurements, numerous subpopulations of T cells may be easily identified. Other applications include the separation of live from dead cells, detection of rare cells, analysis of cell cycle kinetics by various DNA stains, cell cloning by substituting microtitre plates for collection tubes and separation of lymphoid cells by size or antibody marker sorting. Various measurements that may be obtained include physical characteristics such as size (volume and mass), refractive index, cytokinetics, viscosity and biochemical features such as membrane potential, intracellular pH, membrane fluidity, surface antigens, proteins, enzymatic activity, DNA and RNA content (Andreef, 1986).

Advanced flow cytometers may simultaneously excite 2 different fluorescent dyes (eg: bound to antibodies) that

absorb light at similar wavelengths but emit light in red and green. The resulting 2-colour immunofluorescence analysis is a 2-dimensional "scatter plot" graph (see Appendix VI) where the relative intensity of one dye is plotted against that of the other for each cell (represented by a single dot). The coordinate of each individual dot indicates whether it is labelled with one or both fluorescently stained antibodies and allows visualization of the shifts and heterogeneities within the leukocyte classes. Computer software allows powerful analysis of multiple parameters such as 2-colour fluorescence, foward-angle light scatter and 90-degree-angle light scatter. For example, cells smaller and larger than lymphocytes may be "gated out" electronically allowing the analysis to focus on lymphocytes. Granulocytes may also be identified with 90-degree angle light scatter and thus electronically "gated out" allowing measurement of only lymphoid cell analysis in whole blood. Accurate and highly reproducible measurements of single cell suspensions with large cell counts may be analysed at rapid rates of thousands of cells per second (Mayall, 1986; Shapiro, 1986).

1.4.2 ELISA Analysis.

ELISA (enzyme-linked immunosorbent assay) analysis of immunoglobulins (Ig) is an extremely sensitive assay (readings

in ng/ml) available for measuring a range of antigen/antibody reactions. It is similar to immunofluorescence in that a purified enzyme is linked to a specific antibody. The assay is unique in that one of the reactants (commonly the antigen) is adsorbed or immobilized onto the surface of a microtitre well (Roit, 1988).

In order to determine the amount of antibody in a sample, the antiserum is reacted with the antigen which was adsorbed onto the plate's surface. Any unbound or unreacted Ig are washed away and an enzyme-linked anti-Ig is then added. A colourless substrate is added and the amount of enzyme-linked second antibody reacted is proportional to the amount of colour that develops. The amount of antibody (Ab) is measured by using optical density scanning of the plate to assess the amount of coloured product. A standard curve is then derived from a known amount of antibody added to the antigen (Ag) and enzyme-linked anti-Ig (Abbas et al., 1991).

Another variation called the sandwich ELISA involves adsorbing unlabelled antibody to the solid well plate surface followed by the antigen. Quantification of the Ag-Ab complex is attained by the addition of an enzyme-linked antibody directed against the antigen (ie: rather than an anti-Ig, a labelled Ab of the same specificity as the unlabelled Ab adsorbed to the surface). Since 2 Ab molecules are bound to the same Ag, the Ag must have 2 accessible binding sites.

ELISA methods may be automated, are stable and are not as hazardous as radioisotope (^{125}I,^{131}I) labelling (Turgeon, 1990).

1.5 Possible mechanisms for exercise-induced immunosuppression.

Several theories exist as to how exercise affects the immune system. These will only be briefly discussed since it was not the purpose of the present study to investigate underlying mechanisms.

A. Cortisol.

Physiological or psychological stress results in the adrenal cortical secretion of cortisol which is considered to be immunosuppressive and a clinical indicator of stress (Bunt, 1986; Cupps and Fauci, 1982; Keast et al., 1988; Selye, 1956). This stress is repeatedly experienced by athletes and is less common to the untrained subject. Accordingly, Eskola et al. (1987) has reported that the cortisol levels and composite psycho-physical stress asociated with a recreational runner running 7 km (0.37 umol/l before, 0.70 umol/l after) might be equivalent to a national level marathoner running a marathon (0.41 umol/l before, 0.79 umol/l after).

The addition of cortisol *in vitro* has been shown to

reduce PHA-, Con-A- and pokeweed mitogen-induced transformation (Gillis et al., 1979; Goodwin et al., 1980; Gordon and Nouri, 1981; Neifield and Tormey, 1979; Nowell, 1961; Smith et al., 1977; Webel and Ritts, 1977). Cortisone has induced suppression of the mixed lymphocyte reaction _in vitro_ (Ilfeld et al., 1977; Katz and Fauci, 1979) and enhanced Ig synthesis when added to pokeweed mitogen-stimulated lymphocyte cultures (Cooper et al., 1987). Mitogen-stimulated lymphocyte response and Ig production _in vitro_ has been significantly altered by the _in vivo_ administration of hydrocortisone, dexamethasone and prednisolone (Fauci, 1976; Gillis et al., 1979; Saxon et al., 1978). _In vivo_ administration of corticosteroids at low dosages has slightly suppressed immune function, whereas lethal modification of the immune system in mice has resulted from physiologically excessive dosages (Keast, 1968, 1969).

Though the stress response probably differs between animals and humans, early studies with rats have suggested immunologic suppression of mitogen-induced lymphocyte proliferation proportional to the intensity of physical stress (an electric shock) (Keller et al., 1981). In addition, a negative relationship has been established between the plasma level of glucocorticosteroids and the proliferative response of isolated splenic lymphocytes in mice (Gisler, 1974; Monjan and Collector, 1977). Furthermore, experimentation with

guinea-pigs has revealed differences in cell-mediated lymphocyte function in acute versus chronic administration of glucocorticosteroids (Balow et al., 1975). Numerous studies have also shown both in vitro (Heilman et al., 1973) and in vivo (Fauci and Dale, 1974; Fauci et al., 1974; Yu et al., 1974) that administration of corticosteroid affects the human lymphocyte response to mitogens and antigens. Following an intravenous dose of either 100 mg or 400 mg of hydrocortisone, the human lymphocyte response to Con-A was significantly decreased whereas the response to PHA was unaffected. As well, the 400 mg dosage of hydrocortisone greatly reduced the lymphocyte response to pokeweed mitogen but the 100 mg dosage had no effect (Fauci and Dale, 1974).

Resting plasma cortisol levels are subject to diurnal variation in the secretory rate with elevated levels in the morning (about 20 ug/ml) and low levels in the evening (about 5 ug/ml) (Guyton, 1986). The secretion rate of cortisol is not entirely reflected by the total cortisol concentration in the plasma. In the basal state, only 4% of the total plasma cortisol is unbound (free) while the remaining 96% is bound to cortisol-binding globulin (CBG). If total plasma cortisol exceeds 552 nmol/l, saturation of CBG binding sites occurs and any excess plasma levels will result in an elevated free cortisol level (Galbo, 1983). Furthermore, changes in plasma volume may alter the total cortisol concentration, but may not

reflect specific concentration changes in free cortisol or the secretion rate. The ratio of bound to free cortisol may vary between and within individuals during longitudinal studies with the effect of training unknown (Galbo, 1983).

The intensity and duration of exercise as well as the fitness level of the subject all affect the serum cortisol concentration. Low intensity exercise leads to a lower secretion rate than at rest but the rate of removal of cortisol from the plasma is increased. During high intensity exercise, the removal rate is increased, but is exceeded by an increased rate of secretion (Few, 1974). Short-term (less than 20 min) submaximal exercise does not appear to affect plasma cortisol levels, although acute exhaustive exercise will (Hartley et al., 1972; Nieman et al., 1992; Shephard and Sidney, 1975; Van Helder et al., 1985). If submaximal exercise is, however, greater than 20 min it has been found to cause an eventual increase in plasma cortisol concentrations (Hartley et al., 1972; Eskola et al., 1978; Kinderman et al., 1982). It has been suggested that plasma cortisol concentrations decrease at workloads below 50% $\dot{V}O2$ max and increase at work loads greater than 60% $\dot{V}O2$ max (Few, 1974). Following acute maximal workloads, plasma cortisol concentration does not peak until 15 min or longer into recovery (Robertson et al., 1981; Kinderman et al., 1982). A 1 to 4 h time delay has been reported between hydrocortisone

injections and the influence upon lymphocyte counts and function (Fauci and Dale, 1974).

During exercise training, increases in blood cortisol levels may contribute to an enhanced free fatty acid mobilization and gluconeogenesis (Keast et al., 1988). Trained distance runners have demonstrated significantly higher resting serum cortisol levels during normal training in comparison to non-athlete matched controls. The cortisol response to a given bout of high intensity acute exercise, however, decreases with training (Mathur et al., 1986). Similarly, following prolonged exercise such as a marathon run, lower plasma cortisol levels have been reported in distance runners who were more highly trained (Eskola et al., 1978). Vishnu-Moorthy and Zimmerman (1978) also found that following a 32.3 km race, higher plasma cortisol levels were inversely related to the volume of training (km/week) reported by each runner. Furthermore, Yakovlev and Viru (1985) reported plasma cortisol concentrations that during extreme exhaustion, decreased below pre-exercise levels and remained so for several days.

In contrast, other investigators have reported an increase in resting plasma cortisol following chronic exercise stress (Barron et al., 1985; Costill et al., 1987; Kuipers, 1987). In addition, Dressendorfer and Wade (1985) reported no change in resting plasma cortisol in 12 experienced marathon

runners during a 20-day 520 km road race. Shephard and Sidney (1975) also found no change in the cortisol response to training despite increases in aerobic power.

Verde et al. (1992) reported no noticeable changes in resting serum cortisol concentrations in response to heavy training though PHA-induced lymphocyte proliferation was significantly suppressed. This relationship between cortisol and immune function is further confounded by the likelihood that the time course for change in the plasma cortisol level differs from the time course for recovery in the immune system. Balow et al. (1978) reported that 2 weeks after injections of cortisone acetate in rats, a 50% lymphopenia was still present even though the plasma cortisol had returned to baseline values. Eskola et al. (1978) also reported an impairment of lymphocyte function (PHA, Con-A and PPD (purified protein derivative antigen)) in marathoners which was still evident after elevated plasma cortisol levels returned to baseline.

B. <u>Catecholamines.</u>

Exercise is accompanied by sympathoadrenal activity, resulting in an increase in the catecholamines adrenaline (epinephrine) and nor-adrenaline (norepinephrine) in the blood (Galbo, 1983). Catecholamine levels increase exponentially with exercise duration and intensity (Bloom et al., 1976;

Davies et al., 1974; Galbo et al., 1973, 1977) with transient effects due to short-term heavy exercise (Hagberg et al., 1979; Nieman et al., 1992) and moderate exercise, and up to an 8-fold increase during prolonged exercise (Galbo et al., 1977). Increases in catecholamine levels result in an increased heart rate, vasoconstriction in the splanchnic region, increased metabolic rates of working muscle fibres, increased substrate mobilization and sweating (Bunt, 1976; Galbo, 1983).

The number of circulating leukocytes and lymphocytes has been shown to be affected by the administration of catecholamines. Adrenaline infusion has lead to leukocytosis with a lymphocytosis followed by neutrophilia (Crary et al., 1983; Eriksson and Hedfors, 1977; Gader and Cash, 1975; Mishler and Sharp, 1976; Steel et al., 1971). Lymphocytes have B-adrenergic surface receptors for sympathetic neurotransmitters, with T cytotoxic/suppressor and NK cells having more than T helper cells. As a result, epinephrine has been reported to increase the number of circulating T cytotoxic/suppressor and NK cells, yet decrease T helper cells, contributing to a decrease in the CD4/CD8 ratio (Crary et al., 1983; Field et al., 1991; Muir et al., 1984; Pedersen et al., 1988). Noradrenaline has been found to cause leukocytosis after infusion (Gader et al., 1975). Erickson and Hedfors (1977) reported a decrease in the proportion of T

lymphocytes after administration of adrenaline whereas Crary et al. (1983) did not, although a reduction in the CD4/CD8 ratio was found. This is similar to the previously mentioned changes in leukocyte populations that have been found after exercise. Kendall et al. (1990) reported that there was no significant relationship between epinephrine and norepinephrine and changes in CD3+ or CD8+ cells and only a weak association with the CD4+ lymphocyte subset percentage in response to exercise. Furthermore, plasma epinephrine returns to basal levels very quickly after short-term maximal exercise ($T1/2 \simeq$ 2-3 minutes), weakening the relationship between catecholamines and lymphocyte subset percent changes (Kjaer et al., 1986).

The site for leukocyte mobilization after adrenaline infuson or exercise is not clear. The lymphocytosis may be due to the increased emptying of lymph into the blood via the thoracic duct, while the neutrophils may arise from the bone marrow due to increased blood flow (Gader and Cash, 1975). An additional possibility is that the cells may have been mobilized from the peripheral marginated pool, the lungs and spleen (Crary et al., 1983; Mishler and Sharp, 1976; Muir et al., 1984). Changes in the mobilization of cell subsets can be accounted for by the different circulation times between T and B lymphocytes and affinity to adrenaline (Crary et al., 1983; Hedfors et al., 1976).

Immunological function may be affected by the catecholamine-induced increase in adenyl cyclase activity, resulting in an increase in intracellular cyclic adenosine monophosphate (cAMP) which inhibits the T and B cell mitogen response (Estes et al., 1971; Goodwin and Webb, 1980; Watson, 1976). Leukocytes from subjects inoculated with epinephrine display significantly suppressed PHA, Con-A and PW mitogen responses *in vitro* (Crary et al., 1983). On the other hand, if norepinephrine is added in physiological concentrations to cultures, human lymphocyte PHA-stimulated mitogen response increases (Hadden et al., 1970, 1971) (although decreases have also been reported (Goodwin et al., 1979)). The author is not aware of any studies to date on chronic catecholamine levels during overtraining.

C. Glutamine.

Glutamine is a critical fuel for macrophages and most, if not all, rapidly dividing cells such as tumor cells and lymphocytes (Szondy and Newsholme, 1989). Glutamine is essential for lymphocyte cell replication in culture (particularly the rate of proliferation, protein, RNA and antibody synthesis, interleukin-2 production, as well as affecting the rate of phagocytosis by macrophages (Parry-Billings et al., 1990, 1992)) and is utilized at a considerably higher rate (four-fold) than that of glucose

(Ardawi and Newsholme, 1985). Most of the glutamine present in the blood is stored in, synthesized and released by skeletal muscle (Ardawi and Newsholme, 1985). Much of the nitrogen necesssary for glutamine synthesis is provided by the transamination of branched-chain amino acids in the muscle. Subsequent release of glutamine from the muscle across the plasma membrane into the blood via a glutamine transporter is facilitated by elevated levels of growth hormone, thyroxine or endotoxin and reduced by increased catecholamine levels (Newsholme, 1990).

Since the immune response to the challenge of microorganisms must be rapid, optimal levels and rates of glutamine utilization must be maintained. It is likely that the glutamine required by lymphocytes is readily satisfied from the muscle. Yet other tissues such as the small intestine and the kidney (especially during acidosis) require glutamine (Newsholme, 1990). It has also been suggested that the glutamine requirement is dramatically increased after burns, injury, surgery and infection due to the large number of rapidly dividing cells involved in the repair process and the increased net rate of protein breakdown (Ardawi and Newsholme, 1985; Parry-Billings et al., 1990).

Newsholme (1990) has proposed a hypothetical link between the brain, muscle and immune system and thus failure of the muscle to release sufficient glutamine could subsequently

impair immune funtion. Plasma glutamine levels were significantly lower in overtrained athletes compared to that of optimally trained athletes (Parry-Billings et al., 1992) although there was no effect on in vitro T-lymphocyte proliferation and plasma IL-1 and IL-6 levels. Thus in the overtrained state, environmental/ psychological stress as well as exercise stress causing muscle fatigue may depress the immune system due to the low availability and outflux of glutamine.

Although there is some scientific verification for the link between glutamine, hormones and neuropeptides, the precise effect of exercise on this relationship is still hypothetical. Further research is also needed to examine exercise-induced immunomodulation of immunological cytokines such as interleukins, interferons, prostaglandins and other differentiation, growth or communicational factors.

1.6 Rationale for the Present Study.

The influence of strenuous exercise on the immune system may support the observation that fatigued or "overtrained" athletes are more susceptible to infections and illnesses (Jokly, 1974; Weinstein, 1973). Since lymphocyte and immunoglobulin levels are believed to be the critical link in the relationship between exercise and immune status, this study focused on these measurements (refer to "adaptive immunity" in Figure 1). While many studies have consistently demonstrated immunosuppression following acute bouts of intense prolonged exercise, the contribution of chronic variations in exercise intensity and volume is not well understood and was considered to warrant further investigation.

A potential problem with previous studies was that blood was sampled on only one day at the end of each training phase. Inter- and intra-individual differences on a given day may thus have partially affected the results. In the present study, frequent measurements were taken throughout the course of each training phase. Another major problem in any longitudinal study is deciding on what constitutes "normal baseline" values so that any deviations from these can be detected. A strength of the design of the present study was that pre- and post-exercise samples were taken over each of

the 4 training phases thus permitting between-phase comparisons as well as within-phase comparisons (for the time course of possible changes). The fact that each group returned to baseline training following the increased training phases permitted an assessment of the possible residual effects of these interventions. Differences in pre-exercise measurements can be interpreted as possible indicators of changes in susceptibility to infection and illness, while differences in post-exercise measurements can be interpreted as indicators of the severity of the exercise stress. The "cross-over" design, whereby the training order of either increased intensity or increased volume was alternated in the 2 groups was used in order to help determine which type of overload had the greatest effect. There have not been any longitudinal studies to date, which have investigated the effects of altering both training intensity and volume separately on the lymphocyte and immunoglobulin levels in athletes. Training programmes for high performance athletes typically involve alterations in both.

CHAPTER II

IMMUNE SYSTEM RESPONSE TO CHANGES IN TRAINING INTENSITY AND VOLUME IN RUNNERS.

2.1 INTRODUCTION

Enhanced resistance to infection is often associated with regular, moderate participation in physical activity (Green et al., 1981; Portz, 1984; McDowell et al., 1991). The demanding training volumes and intensities of competitive athletes, however, greatly exceed what might be considered as moderate exercise. Indeed, anecdotal reports from physicians, coaches and athletes have indicated that increases in training load (intensity and/or volume) are frequently accompanied by periods of staleness, chronic fatigue and decreased performance (Fry et al., 1991; Ryan et al., 1983; Simon, 1987). Furthermore, chronic intensive training may modify the immune system and increase the incidence and severity of respiratory infections and illnesses (Douglas and Hanson, 1978; Eichner, 1993; Peters and Bateman, 1983; Nieman et al., 1988; Simon, 1987).

Most available data regarding exercise and immune function are limited to acute responses following a single exercise bout. There are very few studies pertaining to the effects of physical training on lymphocyte levels and even fewer which have examined immunoglobulin (antibody) and HLA-

DR+ levels. There have not been any longitudinal studies to date which have investigated the effects of altering both training volume and intensity separately, on the lymphocyte and immunoglobulin levels in athletes. Training programs for high performance athletes typically involve alterations in both.

Lymphocytes are considered to be the primary cells in the immune response and thus play a critical role in the relationship between exercise and immune function (Simon, 1987). Numerous studies have reported an increase in the number of circulating lymphocytes (lymphocytosis) often corresponding to the duration and intensity of exercise (Berk et al., 1986; Davidson et al., 1987; Edwards et al., 1984; Gray et al., 1992; Hedfors et al., 1976; Nieman et al., 1992; McCarthy and Dale, 1988; Robertson et al., 1981; Shinkai et al., 1992; Soppi et al., 1982; Vishnu-Moorthy and Zimmerman, 1978; Wells, 1982; Yu et al., 1977), while a few have found lymphocyte counts unchanged (Eskola et al., 1978; Green et al., 1981; Hanson et al., 1981).

A reduction in the T cell subset ratio (or CD4 (helper)/CD8 (suppressor) ratio) has been reported following exercise (Berk et al., 1985; Brahmi et al., 1985; Cameron et al., 1989; Edwards et al., 1984; Gray et al., 1992; Hedfors et al., 1983; Landmann et al., 1984; Lewicki et al., 1988; Nguyen et al., 1984; Oshida et al., 1988) whereas other studies have

found either no significant changes (Haq et al., 1993; Kanonchoff et al., 1984; Nieman et al., 1989; Verde et al., 1992) or variable results over the testing sessions (Hoffman-Goetz et al., 1990). Reductions in total post-exercise T cell counts have been attributed to the reduction in CD4+ (helper) cell number (Hedfors et al., 1983), while the CD8+ (suppressor) cell number has been shown to increase (Lewicki et al., 1988; Masuhara et al., 1987) or remain unchanged (Hoffman-Goetz et al., 1990; Tvede et al., 1989; Verde et al., 1992).

Conflicting data are also found in cross-sectional comparisons of athletes and sedentary subjects. Some report little or no differences in resting immune status (Busse et al., 1980; Green et al., 1981; Hanson and Flaherty, 1981) while others have reported lower lymphocyte and leukocyte levels in athletes than in sedentary controls (Davidson et al., 1987; Deuster et al., 1988; Dorner et al., 1987; McCarthy and Dale, 1988).

Longitudinal studies investigating the immune response to training in humans are very limited in number. A significant but mild decrease in the lymphocyte number was found after 6 weeks of moderate walking exercise (Nehlsen-Cannarella et al., 1991) whereas no change in lymphocyte percentage was reported in a 15 week walk/jog training study (Watson et al., 1986), or during 28 days of cycling, running, swimming and weight

lifting (Ndon et al., 1992). The resting CD4/CD8 ratio has been found to either decrease after training (Verde et al., 1992) or remain unchanged (Nehlsen-Cannarella et al., 1991). As with the acute studies, differences may be due to the mode, duration and intensity of exercise training.

Few studies have examined exercise-induced changes on serum or salivary immunoglobulin levels. There are conflicting reports as to the effect of acute submaximal exercise on serum immunoglobulin levels. Some studies have reported increases in serum immunoglobulins during short-term submaximal exercise or graded maximal exercise in both athletes and sedentary individuals (Nehlsen-Cannarella et al., 1991; Poortmans, 1970; Stephenson et al., 1985), but minor or statistically non-significant changes in serum immunoglobulins have also been reported in response to acute intense submaximal exercise (Eberhard et al., 1971; Hanson and Flaherty, 1981; Israel et al., 1982). Long duration endurance exercise (eg: 45 to 100 km of running) was associated with a greater prolonged depression of serum immunoglobulins despite no mean change in plasma volume and a non-significant correlation with cortisol and catecholamine changes (Bosenberg et al., 1988; Nieman et al., 1991; Poortmans and Haralambie, 1979; Israel et al., 1982). Salivary IgA levels have been found not to be affected by moderate exercise (McDowell et al., 1991; MacKinnon et al., 1987), but to be significantly

decreased following strenuous exercise bouts (Tomasi et al., 1982; Tharp and Barnes, 1990).

Moderate exercise training has been found to improve serum immunoglobulin levels in elderly males (Liesen et al., 1976) and mildly obese middle-aged females (Nelsen-Cannarella et al., 1991). Two training studies by Tharp et al. (1990 a and b) examining salivary IgA levels have diversely reported a decrease with swim training and yet an increase with basketball training. Ambient temperatures were found not to affect salivary IgA levels (Housh et al., 1991).

From the limited literature, it is not clear whether exercise enhances or suppresses immune function in humans. The cell proliferative response to mitogen stimulation has been reported to increase (Robertson et al., 1981; Soppi et al., 1982) after maximal cycle ergometry. In contrast, other studies involving 30 seconds of maximal cycle ergometry (Nieman et al., 1992), a 10 minute treadmill run (Edwards et al., 1984), 30 minutes of submaximal treadmill exercise (Verde et al., 1992), a 32.1 km race (Vishnu-Moorthy and Zimmerman, 1978) or a marathon (Eskola et al., 1978) have reported no change in post-exercise mitogen response. Longitudinal studies suggest that regular moderate exercise is either beneficial to immune function (Soppi et al., 1982, Watson et al., 1986) or has no significant effect (Houston et al., 1987). Nonetheless, day-to-day variations in individual <u>in</u>

vitro response to polyclonal mitogens have also been reported (Keast et al., 1988).

The numerous conflicting studies may reflect variations in exercise duration, mode and intensity, subject fitness level, immunological techniques, assessment of relative versus absolute cell counts, circadian rhythms, seasonal variations, sampling frequency, uncontrolled or unknown exposure to pathogens and other extraneous confounders.

The present study was designed to examine the acute and chronic changes in lymphocyte and immunoglobulin levels in runners in response to alterations in both training volume and intensity. Subsequent immunomodulation may result from the influence of a variety of neuroendocrine hormones (including corticosteroids, endorphins, interferon, interleukins and catecholamines) as well as plasma glutamine levels.

2.2 METHODOLOGY

2.2.1 Subjects.

Twelve male runners (ages 18-23 years) served as subjects for the study. They were fully informed of the procedures and associated risks and gave written consent. Each subject completed a questionnaire, documenting his previous weekly training mileage and personal best racing times in order to determine treadmill test speeds and to control for the initial fitness of each subject for determining increases in training volume. Subjects were randomly assigned to one of two differently ordered training groups. All measurements were performed in accordance with the policies of McMaster University's Committee on the Ethics of Research on Human Subjects (Appendix VII).

2.2.2 Design.

Immune function was assessed in the 12 male runners over a 40-day training period. The 40-day training period consisted of 4 phases (10 days per phase) differing in intensity and volume. Phase 1 and phase 3 were "baseline" phases of low volume/low intensity running (LV/LI). Phase 2 and phase 4 were either high volume/low intensity (HV/LI) or

high volume/high intensity (HV/HI) training. Subjects were randomly assigned to one of 2 different cross-over training group orders (6 subjects in each training group): 1) LV/LI, HV/LI, LV/LI, HV/HI, or 2) LV/LI, HV/HI, LV/LI, HV/LI. The LV/LI "baseline" phase consisted of continuous running at a pace estimated to be equivalent to 60-70% $\dot{V}O2$ max. HV/LI training involved increasing the distance run by 100 percent. The HV/HI phase consisted of the same volume as the HV/LI phase, but on alternate days, was replaced with a series of high intensity 1000 m intervals (with 3 minute walking recovery periods) equivalent to 95 to 100% $\dot{V}O2$ max.

Blood was sampled on 5 occasions during each phase through venipuncture of the anticubital vein at rest (pre-exercise) on days 1, 4 and 7, and 5 minutes post-exercise on days 1 and 7 of each 10-day phase. In general, environmental temperatures remained consistent during the study. Subjects were instructed to maintain the same diets and energy intakes over all 4 phases and not to exercise or consume food, drugs (eg., alcohol, caffeine etc..) 12 hours before blood sampling. Ten subjects fasted for 12 hours before blood sampling and the remaining 2 subjects fasted for 7 hours before blood sampling.

2.2.3 Treadmill Performance Test.

All subjects were familiarized with treadmill running by

means of practice sessions on 3 separate days. The criterion running performance test was a timed run to exhaustion on a level treadmill at a constant velocity equivalent to each athlete's best 1500 m race of that year (See Appendix III for sample calculation procedure). It was given at the end (day 9) of each training phase with rest days placed before and after each treadmill test day. The lab visits were at the same time of day (mornings) for each subject in order to control for possible diurnal variations. All subjects were allowed a preliminary 5 minute warm-up (7.5 mph at 0% grade). During the last 30 seconds of this warm-up the treadmill velocity was increased to the appropriate test velocity in order for the subject to become familiar with it. After a 5 minute rest period, the subjects stepped onto the treadmill which was rotating at the pre-set velocity; timing began when they released their grasp of the handrail and terminated when they re-grasped the handrail or began to stumble. A safety harness suspended from the ceiling was worn by each subject to allow them to push themselves maximally without any apprehension of falling while on the treadmill. Heart rate was continuously recorded throughout the warm-up and performance test, as was oxygen uptake by means of a computerized open circuit system which calculated $\dot{V}O2$ on-line every 20 seconds. Subjects received verbal encouragement throughout the run but were not allowed to view a clock or to

receive any feedback regarding performance times until completion of the study.

2.2.4 Immunological Assays.

During each of the 20 blood sampling occasions (days 1, 4 and 7 pre and days 1 and 7 post-exercise, of each of the 4 training phases), a 10 ml non-heparinized vacutainer and a 5 ml vacutainer (ethylenediaminetetraacetic acid, EDTA) tube was filled with blood for subsequent immunological testing for CD4+, CD8+, CD3+, HLA-DR+, and immunoglobulins (IgM, IgG and IgA). Lymphocyte subpopulations were measured by direct immunofluorescence staining of cell surfaces with mouse antihuman fluorochrome-labelled monoclonal antibodies, and counting in an automated flow cytometer (Epics V flow cytometry system). Serum immunoglobulin levels were determined by ELISA (enzyme-linked immunosorbent assay). An additional 10 ml sodium heparinized vacutainer was filled on day 1 and day 7 of the baseline phase and day 7 of the HV/LI and HV/HI phases for future analysis of plasma cortisol.

The blood samples were transported immediately in a thermally-insulated styrofoam container (a 5 minute walk) to the diagnostic immunology lab (McMaster University Medical Centre) and analysed for CD4+ (T helper), CD8+ (T suppressor), CD3+ (mature T cells) and HLA-DR+ (an activation marker for B

cells and mature T-lymphoid cells) (see Appendix IV for sources of these). Preparation of blood samples for the immunological assays took place under a sterile laminar flow hood and latex medical gloves and protective clothing were worn at all times as a further precaution against contamination.

A. Lymphocyte preparation. Lymphocytes from whole blood were prepared for immunofluorescence by optical flow cytometry using the COULTER Q-PREP Epics immunology work station (Epics division of Coulter Corporation, Hialeah, Florida manufactured by Coulter Diagnostics Hialeah, Fl., 33014). The ImmunoPrep leukocyte/lymphocyte preparation system is a gentle, rapid erythrocyte lysing system which maintains cell surface markers and leukocyte morphology. Moreover, the absence of washing and centrifugation steps allow for greater cell recoveries to be attained.

Anticoagulated whole blood was pipetted into labelled test tubes, and the following diluted liquid fluorochrome-labelled monoclonal antibodies were added to the individual tubes: 1) MsIgG-RDI and MsIgG-FITC, 2) T4 RDI and T8 FITC and 3) T3 RDI and HLA-DR FITC (refer to Appendix IV for sources). This was followed by a 10 minute incubation period at room temperature in the absence of light. An erythrocyte lytic agent, leukocyte stabilizer and cell membrane fixative were added to each sample at the COULTER Q-PREP Epics immunology

work station (set at the 35 second cycle) (See Appendix IV for a more detailed description of procedures and reagents used). The prepared samples were then analysed on an Epics V flow cytometry system.

B. Flow cytometric analysis. Analysis was performed in the diagnostic immunology lab using an EPICS 541 flow cytometry system incorporating an argon laser (488 nm wavelength). Analytic reproducibility was found to be within 1 to 1.5 %. An example and explanation of the computer output from the fluorescence activated cell sorting (FACS) analysis is presented in Appendix VI.

C. Immunoglobulin analysis of blood serum. After collection of blood by venipuncture, 10 ml non-heparinized tubes were incubated in a water bath for 30 minutes at 37°C to facilitate clotting. The tubes were then balanced and centrifuged (Beckman TJ-6) at 3000 rpm for 7 minutes, utilizing a swinging bucket rotor. The serum was suctioned off with sterile Pasteur pipettes (Maple Leaf Brand Cat. No. 14672-029) and placed in labelled Eppendorf tubes. The remaining contents were emptied into biohazard autoclave bags and appropriately disposed. The labelled blood serum aliquots were then frozen until all 20 samples for each of the 12 subjects were collected for determination of IgM, IgG and IgA

concentrations by ELISA analysis.

D. <u>ELISA analysis.</u> Human serum antibody measurement by Enzyme-Linked Immunosorbent Assay (ELISA) analysis was performed to determine IgM, IgG and IgA concentrations in the serum samples. The ELISA is based on the alkaline phosphatase enzyme covalently bound to ExtrAvidin and anti-human phosphatase enzyme covalently bound to ExtrAvidin and anti-human Ig antibody substituted with biotin (see Appendix V). The antigen (Ag) of interest was bound to the plastic wells of a 96-Well (200 ul) flat bottom polystyrene sterile/gamma irradiated tissue culture plate (FALCON 3072, Becton Dickinson). The procedure presumes that the antigen is a soluble protein.

The antigen was diluted (as determined) in Borate buffer solution (BBS), with no other protein as carrier and plated out at 100 ul per well. The plates were then allowed to incubate for 3 hours (allowable range 2.5 to 4 hours) at room temperature (R/T). Ag-negative wells were coated with an appropriate "sham" Ag (Bovine Serum Albumin or BSA). These Ag-negative wells control for variability of non-specific sticking of human Ig to the wells, a problem when the serum is not very dilute. After antigen coating of wells, excess antigen was aspirated. BSA (10 mg/ml) in TBS was then added at 200 ul per well, and incubated at R/T for 1 hour to "block"

any remaining binding sites of plastic.

Blocking BSA was removed by flicking out (again no washing necessary) wells and blotting the plates on paper towels. Sera were diluted in TBS-BSA (1 mg/ml), and plated out at 100 ul per well. Overnight incubation at 4°C was employed to ensure that low affinity interactions could equilibrate.

All wells were then washed 3 times with 200 ul of wash solution per well, flicking out before washing. This is the most important wash step, especially if the serum samples are not very dilute. Plates were then patted dry on a paper towel. One hundred ul of Biotin anti-human Ig reagent at a predetermined dilution in TBS-BSA (1 mg/ml) was then added to each well. Plates were allowed to incubate at room temperature for 3 hours.

The plates were washed 3 times with wash solution and patted dry on paper towels. One hundred ul of ExtrAvidin-Alk/Phos (at a predetermined dilution in TBS-BSA) were added and plates were allowed to incubate for 1 hour at room temperature.

Plates were again washed 3 times with wash solution and patted dry on paper towels. One hundred ul of phosphatase substrate dilute in substrate buffer (1M diethenolamine) (1 small tablet for every 5 ml) were added to each well. The plates were incubated at room temperature for 15 to 20 minutes

and positive wells were checked for colour. The reaction may be stopped with 20 ul of 10 N HCl per well, but if the substrate is added at the appropriate time intervals, the plates may be read at intervals with no stopping of the reaction. The automated ELISA MR600 Dynatech (Allied Fischer Scientific) microplate reader read the optical density of each well at a wavelength of 410 nm for the detection of the yellow colour emitted by the substrate-conjugate reaction. Care was taken when devising the serum dilutions so as not to develop positive signals beyond optical density values of 1.5, since the ELISA microplate reader gave non-linear signals beyond optical density readings of 1.5. Optical density values were automatically stored on computer diskettes for future analysis.

Appropriate control (coated/non-coated, serum/no serum) wells and standard curves (in duplicate) were established for each plate. The immunoglobulin concentrations were determined by taking the average of duplicate optical density readings and interpolating the IgM, IgG, and IgA concentrations from the standard curve. The arrangement of the 96-well plate for ELISA analysis of IgG, IgM and IgA is depicted in appendix V.

2.2.5 Running diaries.

All runners were given a training/clinical diary and

asked to keep daily records of the following information:

a) distance run each day.

b) time spent covering that distance.

c) their perceived exertion of the exercise (ie: was their run "easy", "steady" or "hard").

d) any physical problems or pain acquired during the run.

e) their psychological stress/mood state (a score from 1 (tired) to 3 (OK) to 5 (good)).

f) their perceived virus exposure (ie: whether family, roommates, friends or the subjects themselves were sick).

g) body weight before exercise.

h) the number of hours of sleep they obtained the previous night.

The same information (as well as pre-and post-exercise hematocrit values (in triplicate) and the ambient temperature at the time of training) was gathered by the investigator when the subjects visited the lab for blood sampling (pre- and post- exercise) in order to get an immediate (and possibly more accurate in the case of post- exercise states) informative account. See Appendix VII for a sample running diary and the corresponding data collection sheets used by the investigator.

2.2.6 Statistical Analysis.

Acute effects of training load upon the lymphocyte subset percentages in groups 1 and 2 across the 4 training phases were analyzed with a 4-way analysis of variance (ANOVA) (1 between and 3 within factors) as well as selective comparisons using two-tailed t-tests. Chronic effects were analyzed using a 3-way ANOVA (1 between and 2 within factors). Treadmill performance data was analyzed using a 2-way (between-within) ANOVA. A significant main effect was determined at $p < 0.05$ and further analyzed using a Tukey post hoc analysis.

2.3 RESULTS

2.3.1 Subjects' Characteristics.

The physical characteristics and training histories of the 12 runners who participated in this study are presented in Table 2. No significant differences were found between the physical characteristics and weekly training volumes between group 1 or group 2. Each subject's personal training history was used to determine the absolute increase in training volume during the 2 HV phases. The objective was to increase the training volume by a maximum of 100% (much more than any previous training study involving runners). Tables 10 and 11 (Appendix II) document the appropriate training distance and time incurred by each athlete in groups 1 and 2 (as recorded by the investigator and by the subjects in their training diaries). It was assumed that each subject's running efficiency would remain constant throughout training. The subjects also utilized the same running routes during the HV and baseline (LV) training phases in order to prevent changes in terrain and keep the intensity constant.

Table 2. Subjects' Physical Characteristics and Training Histories.

	Subj.	Age	Height (m)	Weight (kg)	Average Training Distance/week (km)
Group #1.	JR	21	1.83	80.5	30
	MTM	21	1.73	67.0	50
	CM	23	1.73	71.1	50
	KL	20	1.78	64.0	25
	JB	23	1.69	60.0	40
	MM	18	1.68	71.0	25
	MEAN	21.0	1.74	68.9	36.7
	±SD	±1.90	±0.06	±7.1	±11.7
Group #2.	DK	19	1.88	75.5	50
	GB	19	1.75	74.0	30
	IB	19	1.85	72.0	30
	SC	19	1.80	69.9	30
	SR	22	1.83	88.0	30
	BA	23	1.88	84.5	35
	MEAN	20.2	1.83	77.3	34.2
	±SD	±1.83	±0.05	±7.3	±8.0

2.3.2 Lymphocyte Subset Percentages.

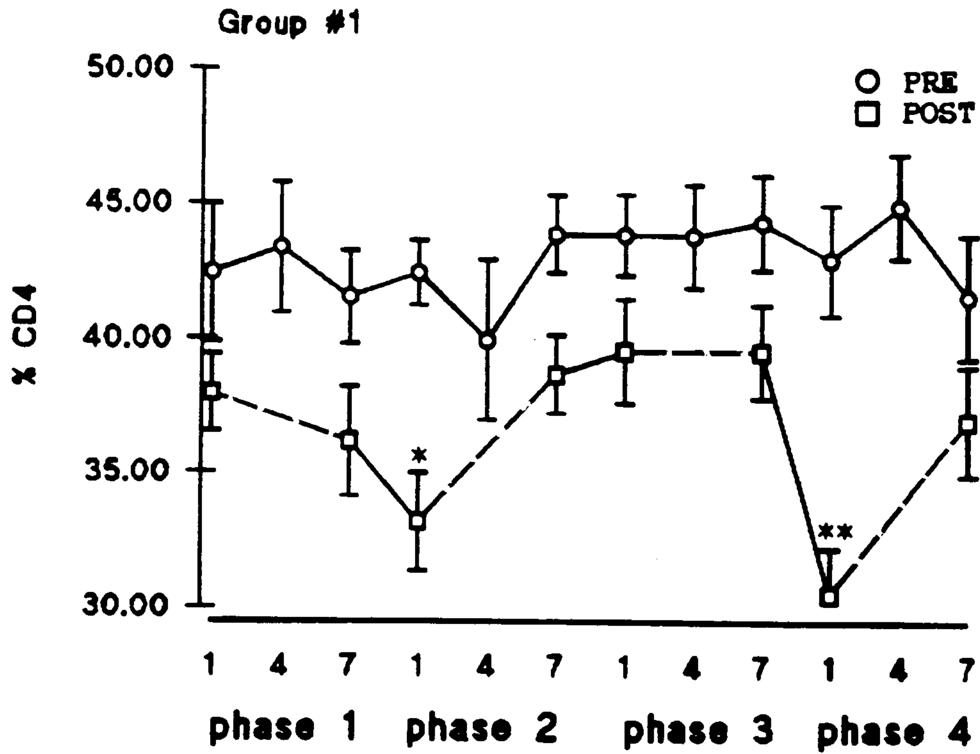
A. T-helper lymphocytes (CD4+). Figure 2A illustrates the T-helper lymphocyte percentages pre and 5 minutes post-exercise during the 4 training phases for group 1. No significant differences were found for resting CD4+ lymphocyte percentages over time. Minor but non-significant differences between pre- and post-exercise CD4+ percentage values were observed during the baseline LV/LI phases (1 and 3). The T-helper lymphocyte percentage was significantly depressed post-exercise during day 1 of phase 2 (HV/LI) ($p < 0.05$) and day 1 of phase 4 (HV/HI)($p < 0.001$). The magnitude of the pre-post differences for T-helper lymphocytes in group 1 is illustrated in Figure 2B. The mean T-helper lymphocyte percentage fell by approximately 4 to 5% during the baseline LV/LI phases. During the first day of the HV/LI phase the T-helper lymphocyte percentage decreased by 9.3% and showed a larger 12.5% decrease during the HV/HI phase. These percentages had returned to baseline levels by the 7th day of the HV/LI and HV/HI phases.

The T-helper lymphocyte percentage response for group 2 is presented in Figure 3A. No significant differences were found for resting CD4+ lymphocyte percentages across the 4 training phases. Minor but statistically non-significant differences were found between the pre- and post-exercise values during the baseline (LV/LI) phases (1 and 3). The T-

helper lymphocyte percentage was significantly depressed post-exercise on both day 1 ($p < 0.005$) and day 7 ($p < 0.05$) of phase 2 (HV/HI) but the change was not as great during phase 4 (HV/LI). The magnitude of the pre-post exercise differences in T-helper lymphocytes for group 2 is illustrated in Figure 3B. The mean T-helper lymphocyte percentage fell by approximately 5 to 7% during the baseline LV/LI phases. A mean T-helper lymphocyte percent reduction of 10.9% and 9.6% was observed on day 1 and day 7, respectively, of phase 2 (HV/HI). The percent reduction in T-helper lymphocytes during phase 4 (HV/LI) was similar to that of the baseline (LV/LI) phases.

FIGURE 2: A. Group #1 percent CD4+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means $\pm$ SE.
* $p \leq 0.05$
** $p \leq 0.001$

B. Group #1 magnitude of pre-post CD4+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means $\pm$ SE.


Group #1
PRE
POST
50.00
45.00
40.00
35.00
30.00
% CD4
*
**
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

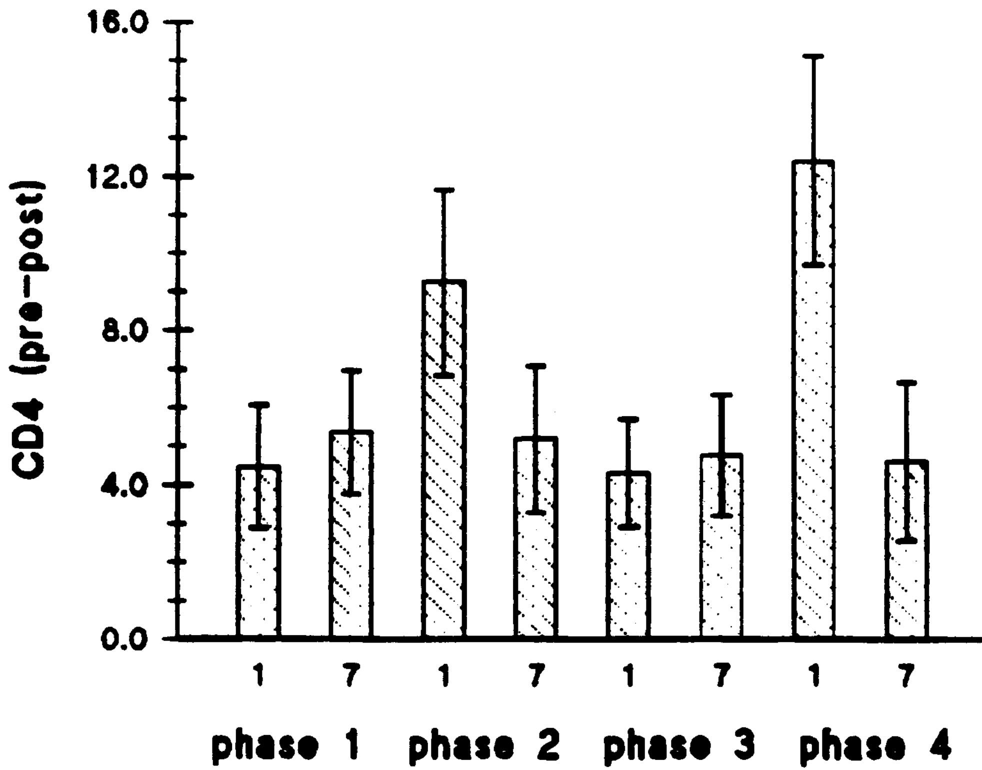
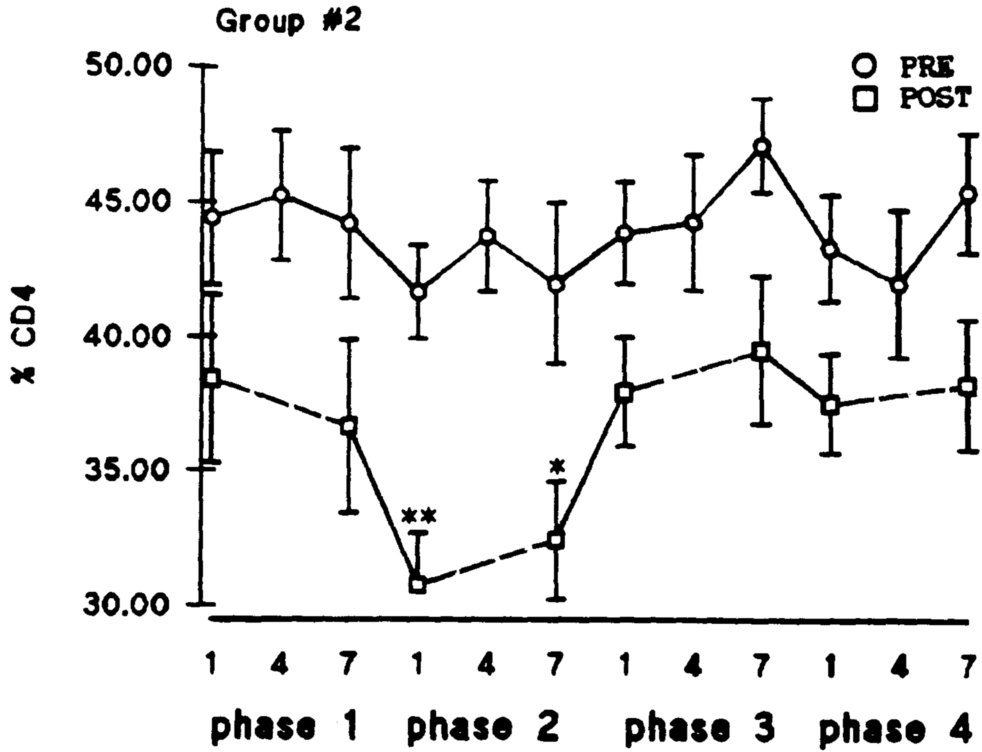
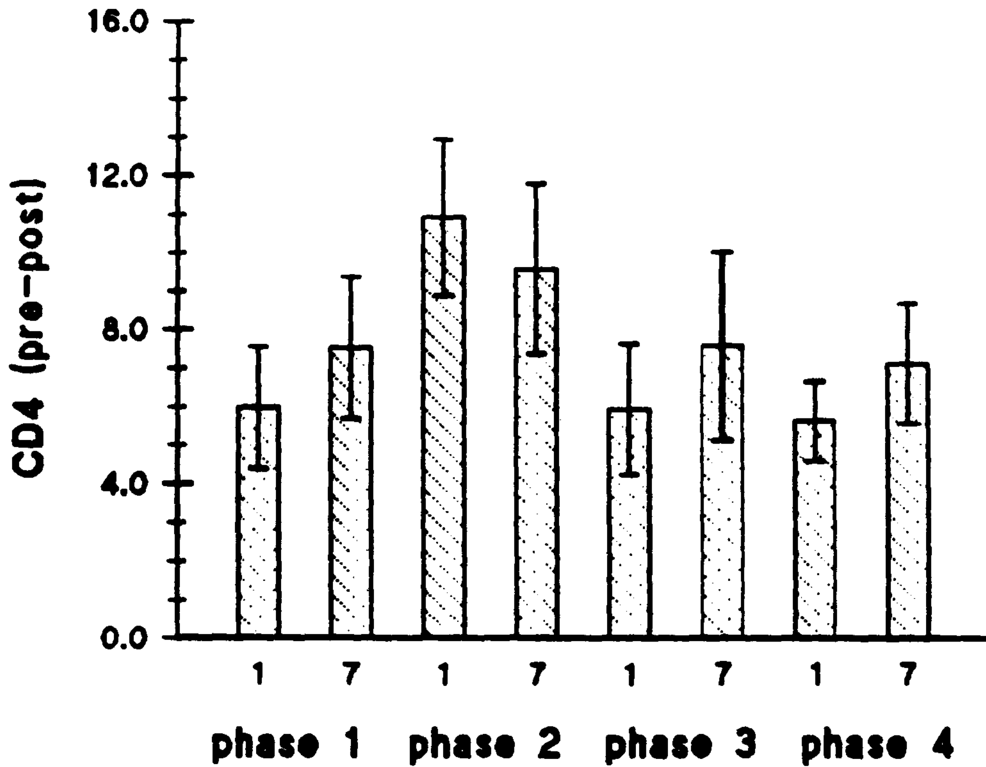

16.0
12.0
8.0
4.0
0.0
CD4 (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

FIGURE 3: A. Group #2 percent CD4+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.
* $p \leq 0.05$
** $p \leq 0.005$

B. Group #2 magnitude of pre-post CD4+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.

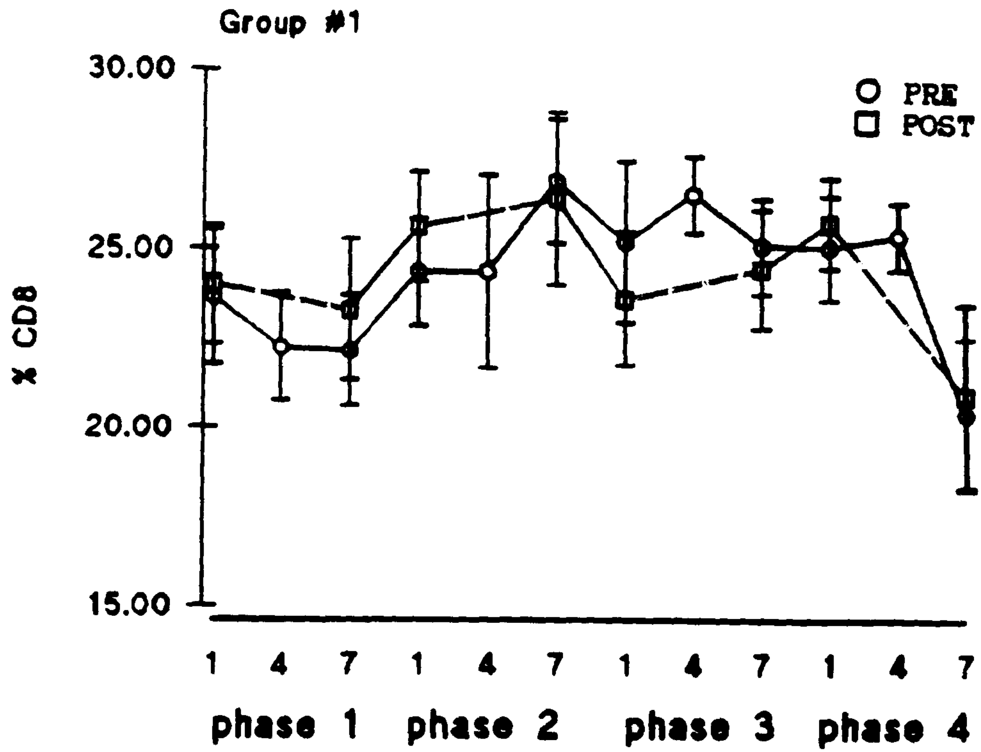

Group #2
50.00
45.00
40.00
35.00
30.00
% CD4
O PRE
□ POST
**
*
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4


16.0
12.0
8.0
4.0
0.0
CD4 (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

B. <u>T-suppressor lymphocytes (CD8+).</u> Figure 4A and 5A illustrate the T-suppressor lymphocyte percentages pre- and post-exercise for groups 1 and 2. The T-suppressor lymphocyte population was highly resistant to change during all 4 training phases. There was no overall main effect over time, nor was there a significant difference between pre-and post-exercise values on days 1 and 7 of each of the 4 training phases. The magnitude of pre-and post-exercise differences for the T-suppressor lymphocytes in groups 1 and 2 are presented in Figures 4B and 5B, respectively. There was a slight tendency for the post-exercise CD8+ percentages to increase above the pre-exercise values (evident by the negative pre-post values) particularly during the HV/LI and HV/HI phases, though this change was not significant.

FIGURE 4: A. Group #1 percent CD8+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.

B. Group #1 magnitude of pre-post CD8+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #1
30.00
25.00
20.00
15.00
% CD8
PRE
POST
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

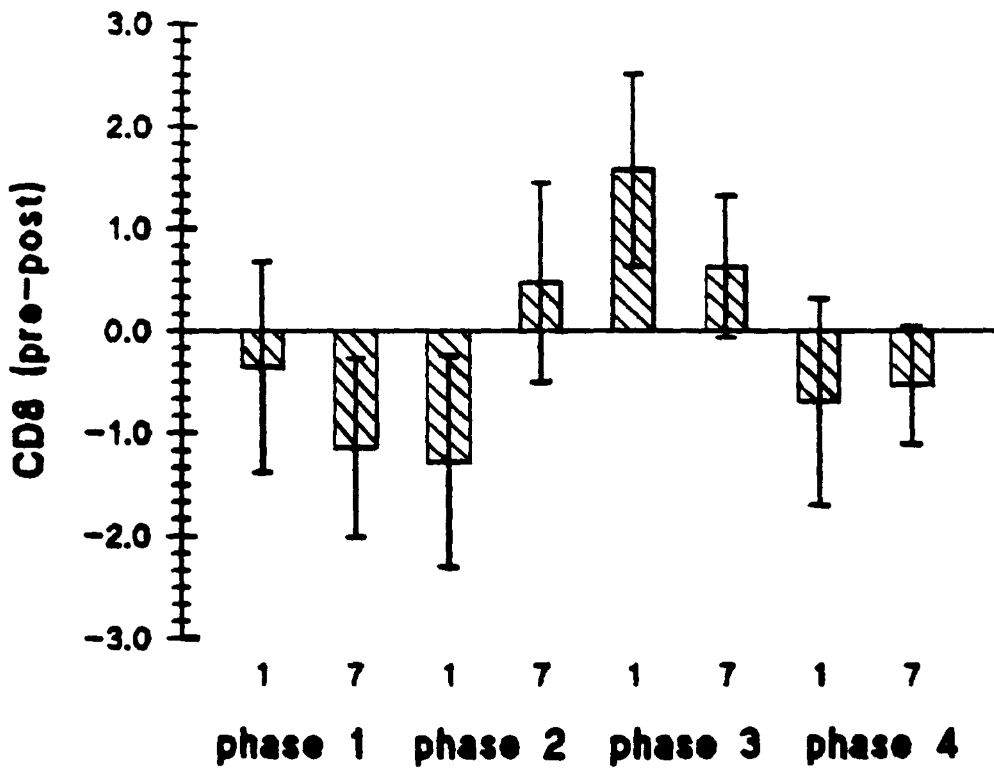
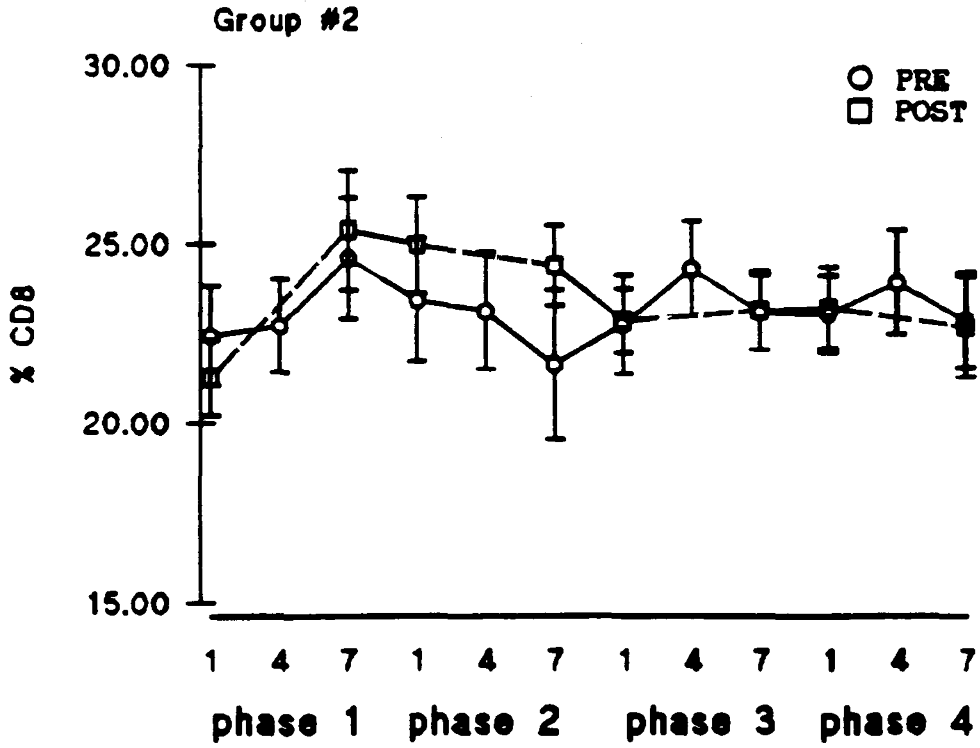
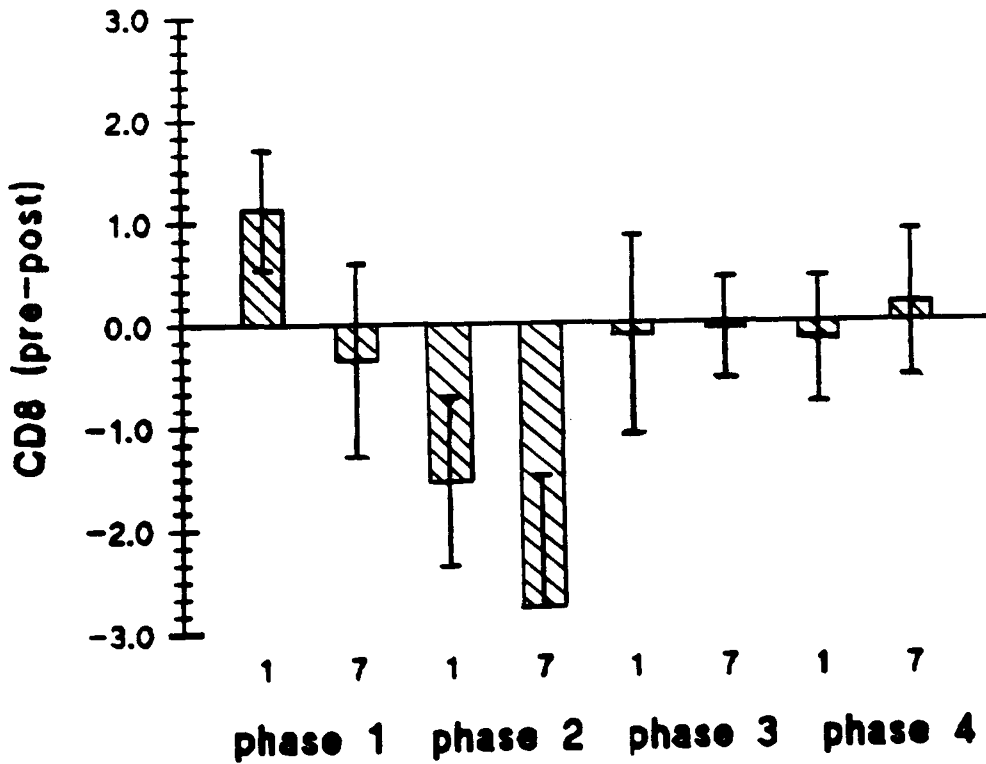

3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
CD8 (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

FIGURE 5: A. Group #2 percent CD8+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.

B. Group #2 magnitude of pre-post CD8+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #2
30.00
25.00
20.00
15.00
% CD8
PRE
POST
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

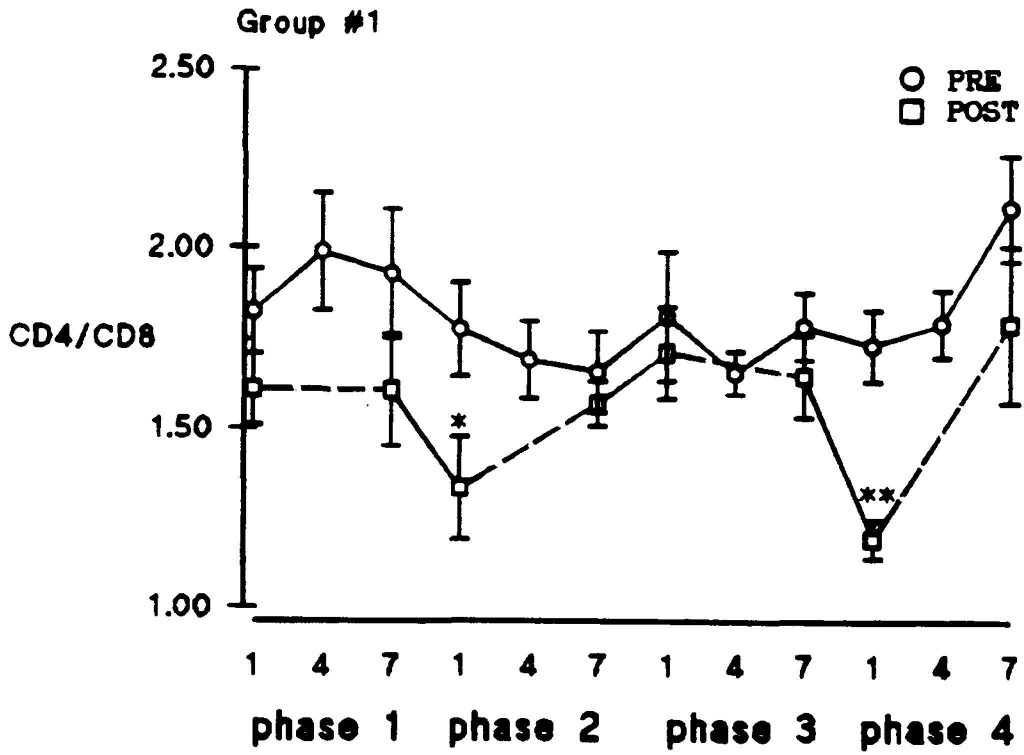

3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0
CD8 (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

C. <u>T-helper/T-suppressor (CD4/CD8) lymphocyte ratio.</u> The mean CD4/CD8 ratio for group 1 is presented in Figure 6A. The pre-exercise CD4/CD8 values did not vary significantly over the 4 training phases, although there was a slight rise at the end of phase 4 (HV/HI). Post-exercise values were lower than pre-exercise values, but only significantly so on day 1 of phase 2 (HV/LI) ($p < 0.05$) and day 1 of phase 4 (HV/HI) ($p < 0.001$). The CD4/CD8 ratios were below the critical 1.50 value (Keast et al., 1988) only during these 2 occasions. The magnitude of the pre-post exercise differences in the CD4/CD8 ratio for group 1 is presented in Figure 6B. Pre-post exercise differences were the lowest near the end of phase 2 and throughout phase 3 (baseline). Significant differences in this ratio were found at the beginning of phase 2 (0.45 ($p < 0.05$)) and phase 4 (0.54 ($p < 0.001$)).

Figure 7A illustrates the pre and post-exercise CD4/CD8 ratios for group 2. The pre-exercise values were resistant to change over the 4 training phases. Post-exercise CD4/CD8 values were marginally lower than pre-exercise values and below the critical 1.50 (Keast et al., 1988) value only during days 1 and 7 of phase 2 (HV/HI). The magnitude of the pre-post exercise difference in the CD4/CD8 ratio for group 2 is illustrated in Figure 7B. The smallest pre-post CD4/CD8 differences appeared during phases 1, 3 and 4. The 2 largest (and significant; $p < 0.05$) differences occurred during days 1 and 7 of phase 2 (HV/HI) with pre-post CD4/CD8 differences

FIGURE 6: A. Group #1 CD4/CD8 ratio values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means $\pm$ SE.
* $p \leq 0.05$
** $p \leq 0.001$

B. Group #1 magnitude of pre-post CD4/CD8 ratio differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means $\pm$ SE.


Group #1
2.50
2.00
1.50
1.00
CD4/CD8
O PRE
□ POST
*
**
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

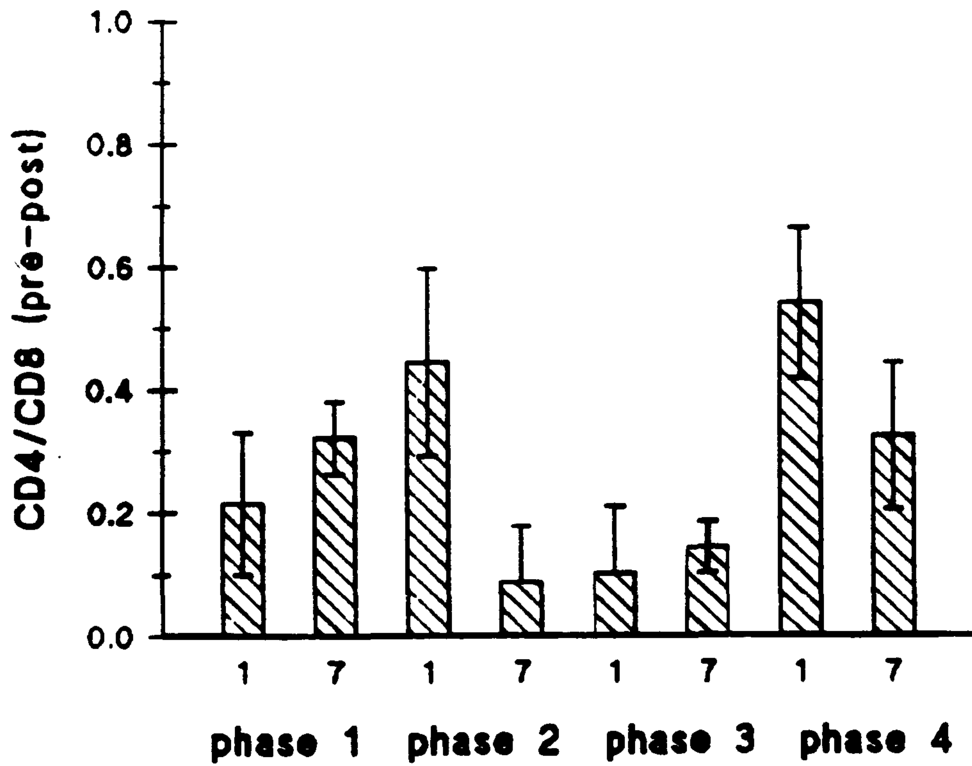
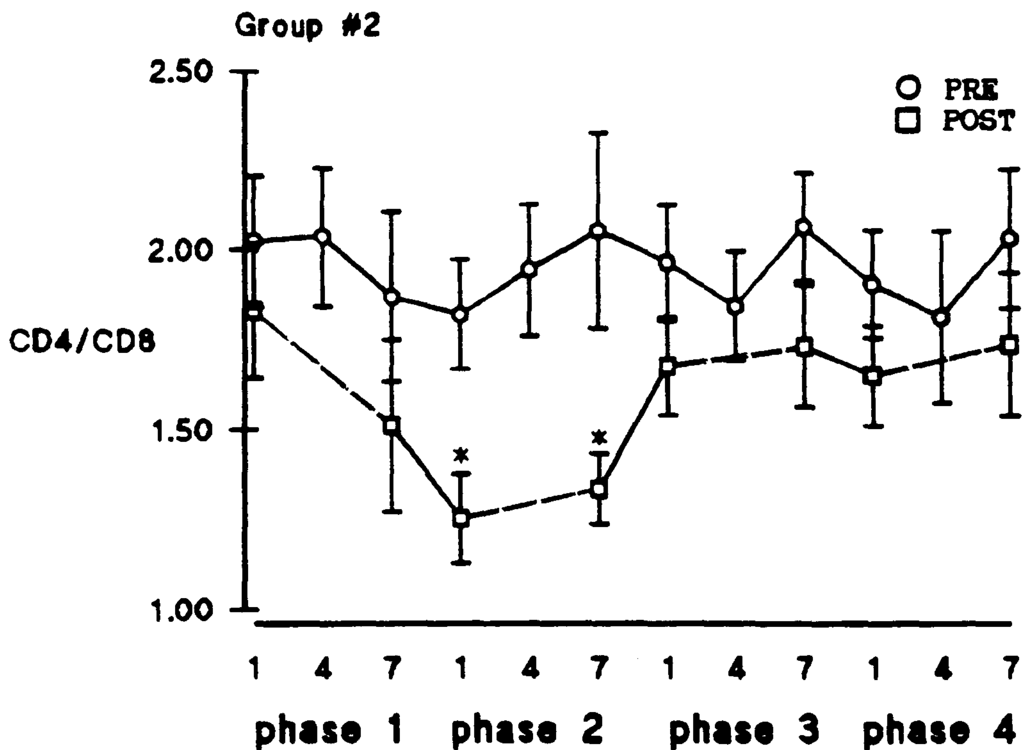
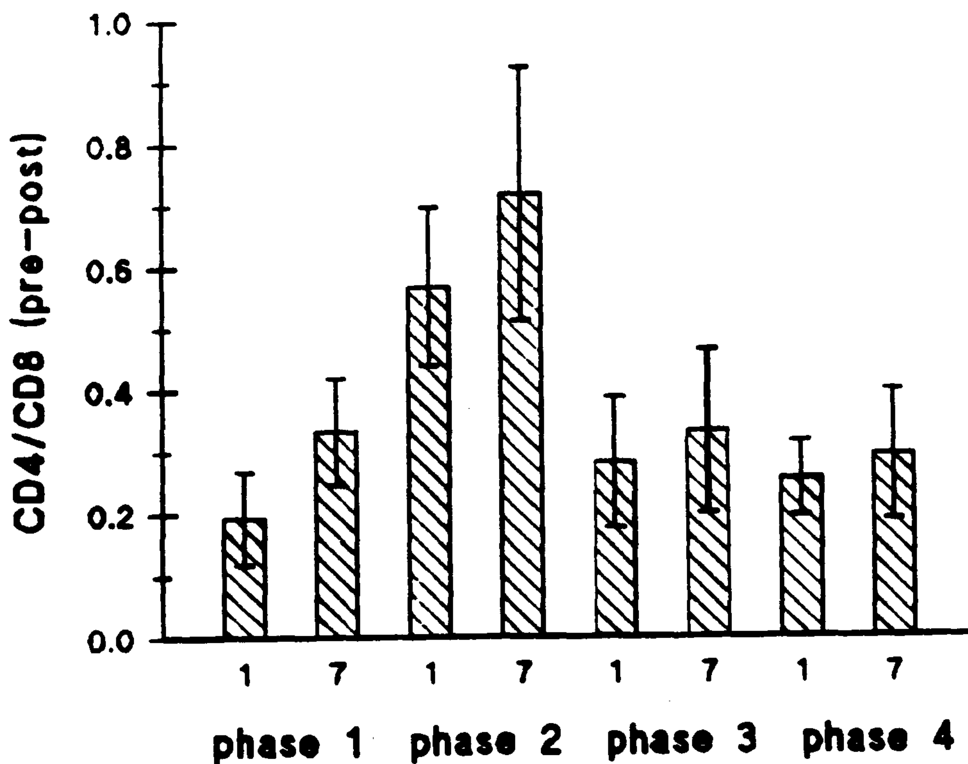

1.0
0.8
0.6
0.4
0.2
0.0
CD4/CD8 (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

FIGURE 7: A. Group #2 CD4/CD8 ratio values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.
* $p \leq 0.05$

B. Group #2 magnitude of pre-post CD4/CD8 ratio differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #2
2.50
2.00
1.50
1.00
CD4/CD8
O PRE
□ POST
*
*
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4


1.0
0.8
0.6
0.4
0.2
0.0
CD4/CD8 (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

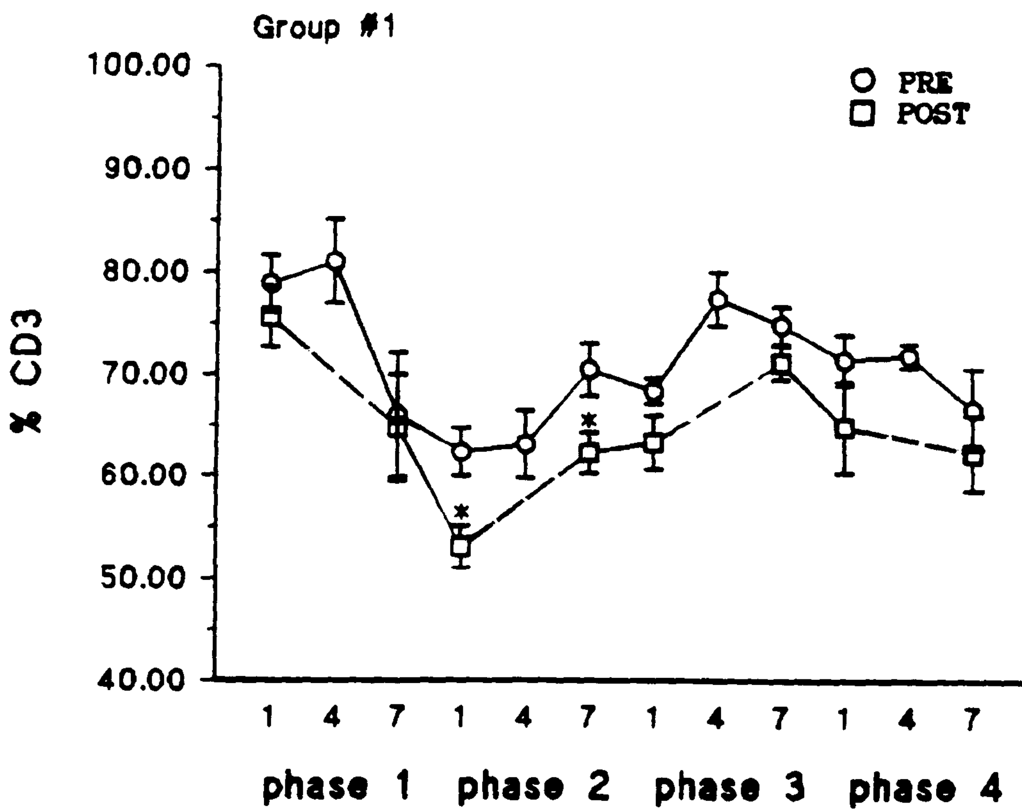
of 0.57 and 0.75, respectively.

D. Total T-lymphocytes (CD3+). Total T-lymphocyte percentages pre- and post-exercise are shown for group 1 in Figure 8A. Pre-exercise percent CD3+ values did not vary significantly across the 4 training phases. Post-exercise percent CD3+ values closely tracked the pre-exercise values and were significantly ($p < 0.05$) lower during phase 2 (HV/LI). Figure 8B illustrates the magnitude of pre-post exercise differences in percent CD3+ values. The largest pre-post exercise differences appeared during phase 2 and phase 4. Statistically significant ($p < 0.05$) decreases in percent CD3+ occurred during day 1 and 7 of phase 2 with CD3+ percentage reductions of 9.3 and 8.25, respectively.

Figure 9A illustrates the total T-lymphocyte percentages pre- and post-exercise for group 2. No main effect was found for pre-exercise values over the 4 training phases. The post-exercise CD3+ percentages were only slightly lower than the pre-exercise values and were only significantly ($p < 0.05$) lower on day 1 of phase 2 (HV/HI). This reduction of 10.49% in the CD3+ pre-post exercise difference is presented in Figure 9B. The largest CD3+ percentage pre-post exercise differences appeared during phase 2 (HV/HI) and phase 4 (HV/LI) and the smallest differences were found during baseline phases 1 and 3.

FIGURE 8: A. Group #1 percent CD3+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.
* $p \leq 0.05$

B. Group #1 magnitude of pre-post CD3+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #1
% CD3
100.00
90.00
80.00
70.00
60.00
50.00
40.00
O PRE
□ POST
*
*
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

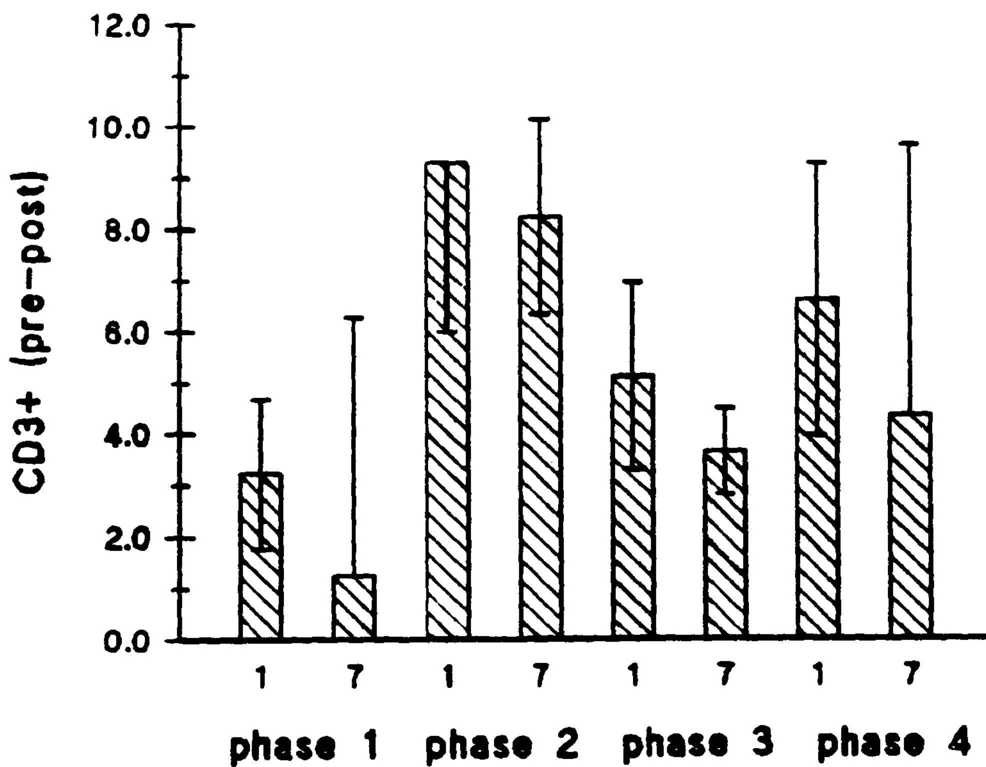
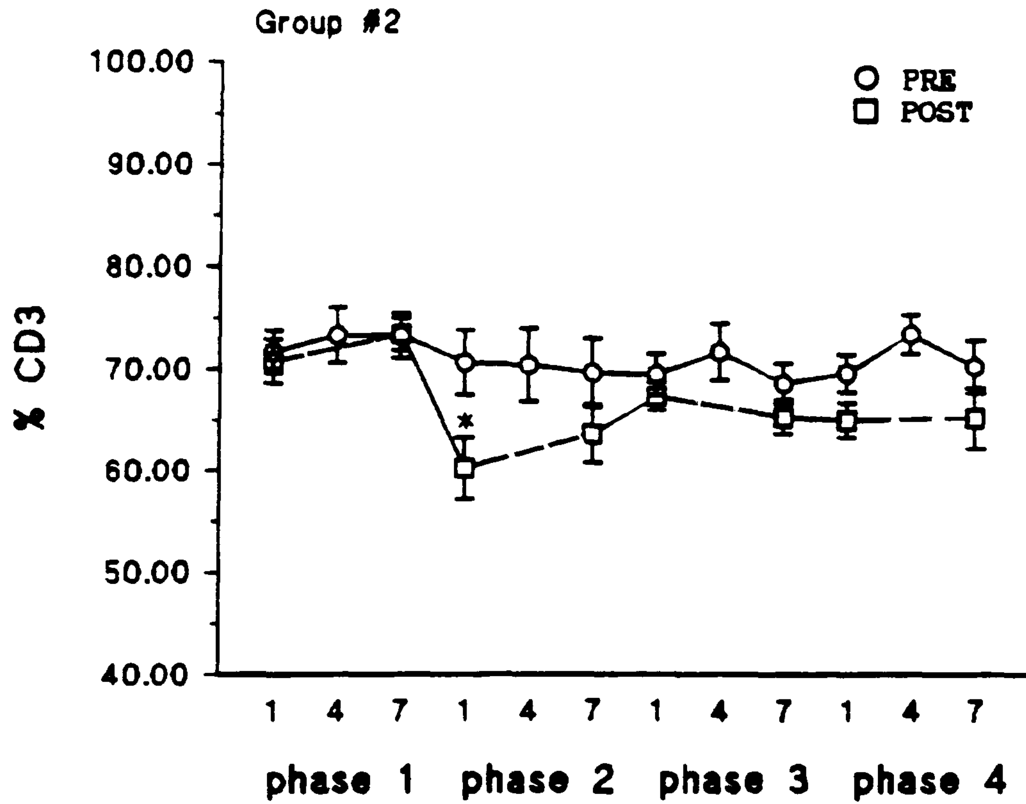
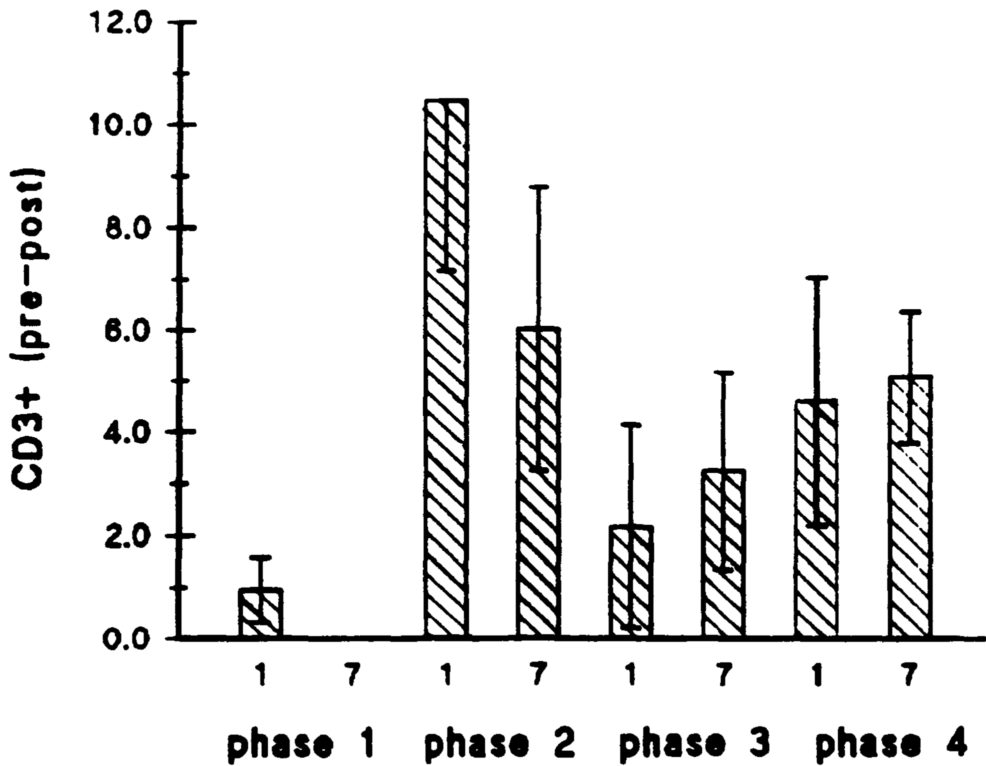

CD3+ (pre-post)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

FIGURE 9: A. Group #2 percent CD3+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.
* $p \leq 0.05$

B. Group #2 magnitude of pre-post CD3+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #2
100.00
90.00
80.00
70.00
60.00
50.00
40.00
% CD3
PRE
POST
*
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

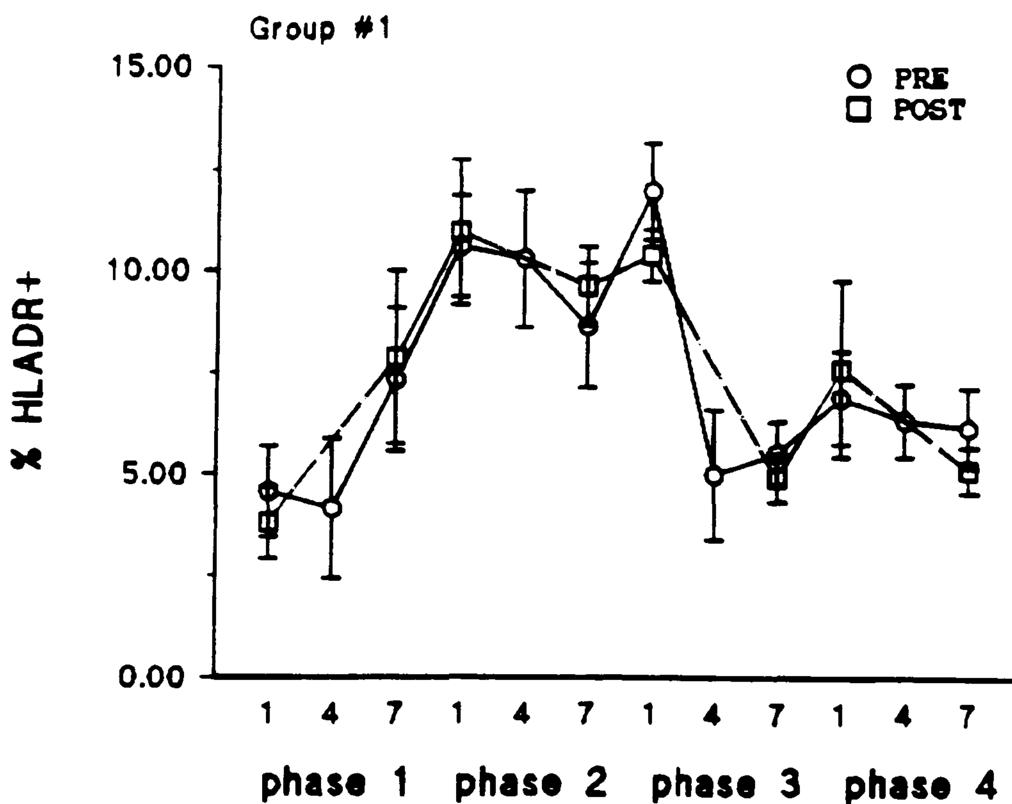

12.0
10.0
8.0
6.0
4.0
2.0
0.0
CD3+ (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

E. HLA-DR+ (B cells and activated T-lymphoid cells) The percent HLA-DR+ values for group 1 are illustrated in Figure 10A. Pre- and post-exercise values did not significantly differ from each other. Values obtained during phase 2 were marginally (but not significantly) higher than the other 3 phases. The magnitude of pre-post exercise differences in percent HLA-DR+ values for group 1 are illustrated in Figure 10B. Although post-exercise levels tended to be slightly higher than pre-exercise levels in phase 2 and the first part of phase 4, pre-post exercise differences were not significantly different throughout all 4 training phases.

The percent pre-post exercise HLA-DR+ values for group 2 are presented in Figure 11A. In general, pre- and post-exercise values were not significantly different from each other and did not appear to vary significantly over the 4 training phases. The magnitude of pre-post exercise differences in percent HLA-DR+ values for group 2 are presented in Figure 11B. Although post-exercise values appear to be higher at the beginning of phase 2 (HV/HI), the pre-post exercise differences throughout all 4 training phases were not significantly different.

FIGURE 10: A. Group #1 percent HLA-DR+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.

B. Group #1 magnitude of pre-post HLA-DR+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #1
15.00
10.00
5.00
0.00
% HLADR+
PRE
POST
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

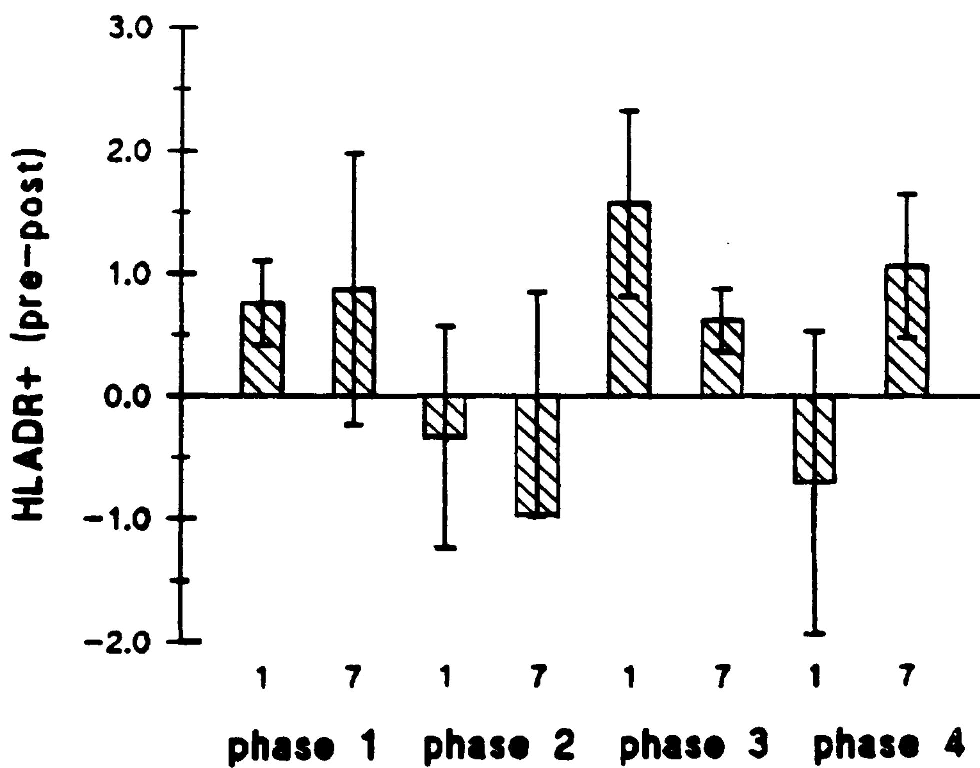
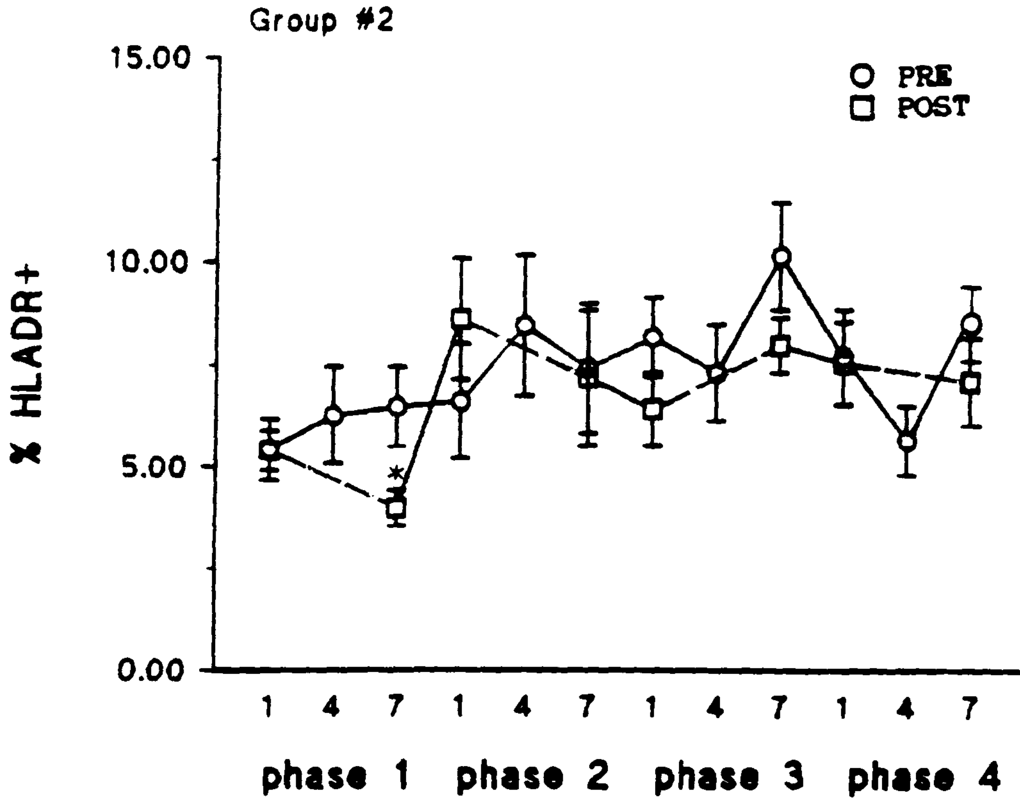
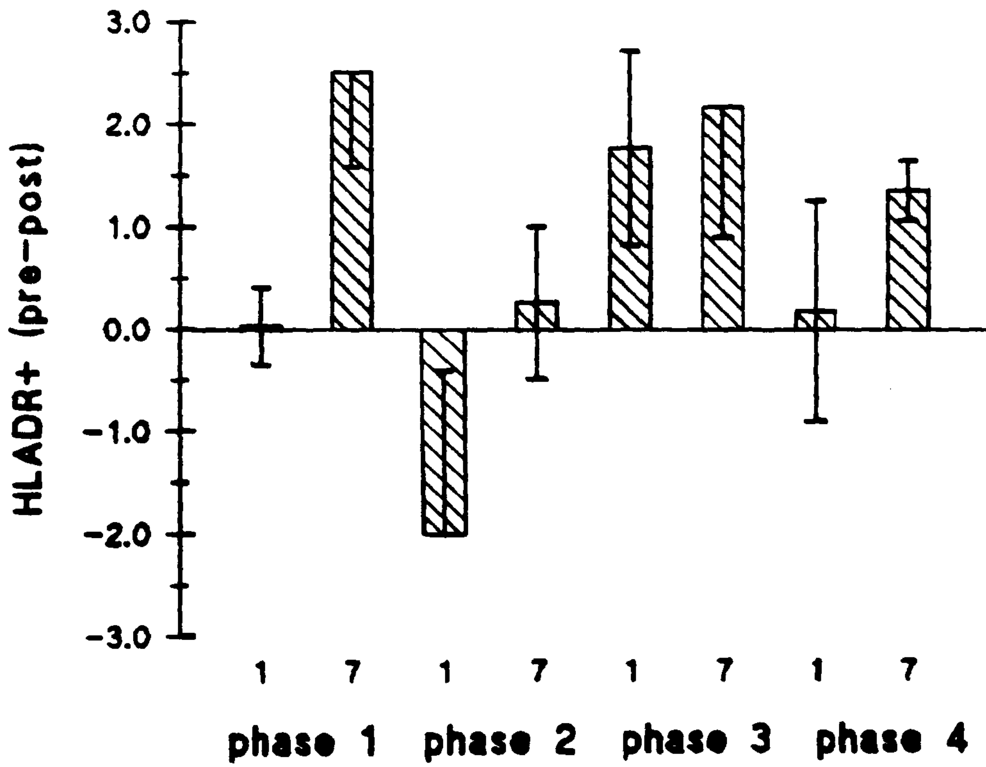

3.0
2.0
1.0
0.0
-1.0
-2.0
HLADR+ (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

FIGURE 11: A. Group #2 percent HLA-DR+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.
* $p \leq 0.05$

B. Group #2 magnitude of pre-post HLA-DR+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #2
15.00
10.00
5.00
0.00
% HLADR+
O PRE
□ POST
*
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

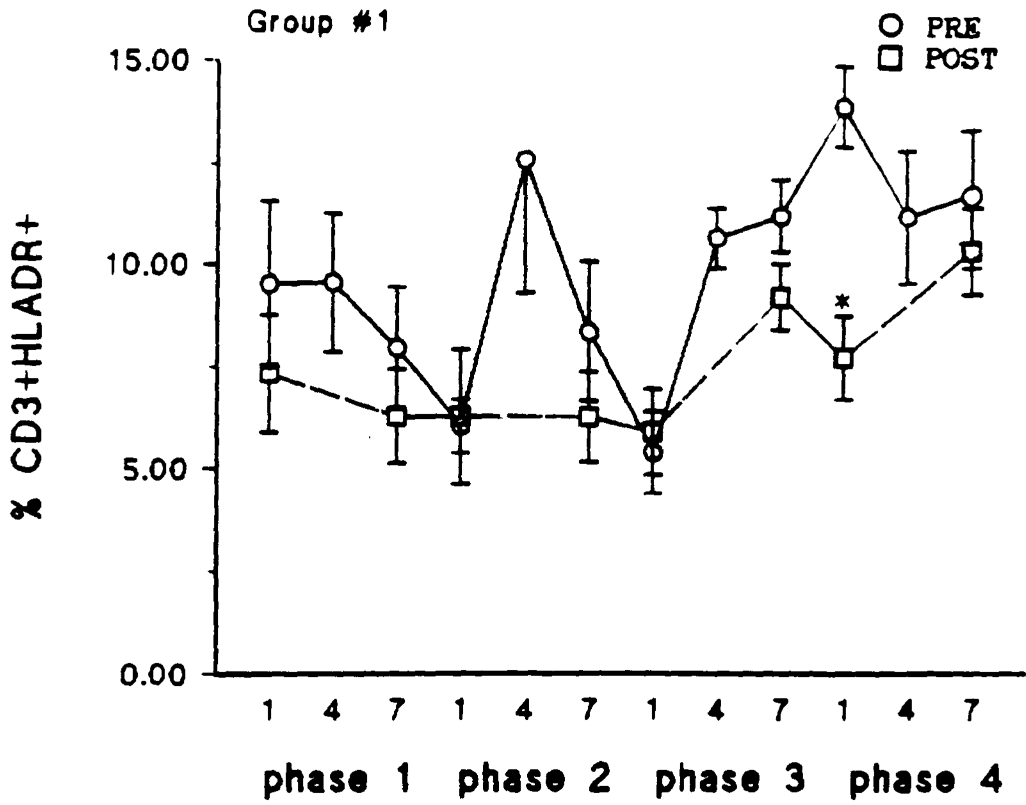

3.0
2.0
1.0
0.0
−1.0
−2.0
−3.0
HLADR+ (pre−post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

F. CD3+HLA-DR+. The pre- and post-exercise CD3+HLA-DR+ percent values for group 1 are illustrated in Figure 12A. Pre- and post-exercise values were only significantly ($p < 0.05$) different from each other during day 1 of phase 4 (HV/HI). The magnitude of pre-post exercise differences in percent CD3+HLA-DR+ values for group 1 are illustrated in Figure 12B. The largest pre-post difference was found on day 1 of phase 4 (HV/HI).

The pre- and post-exercise CD3+HLA-DR+ percent values for group 2 are presented in Figure 13A. Pre-exercise values did not vary significantly over all 4 training phases. Pre-post exercise values were only significantly ($p < 0.05$) different from each other during the first part of phase 2 (HV/HI). The mean magnitude of pre-post exercise differences in CD3+HLA-DR+ percent values for group 2 are presented in Figure 13B. The largest pre-post difference (4.54%) was found on day 1 of phase 2 (HV/HI).

FIGURE 12: A. Group #1 percent CD3+HLA-DR+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.
* $p \leq 0.05$

B. Group #1 magnitude of pre-post CD3+HLA-DR+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.


Group #1
O PRE
□ POST
15.00
10.00
5.00
0.00
% CD3+HLADR+
*
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

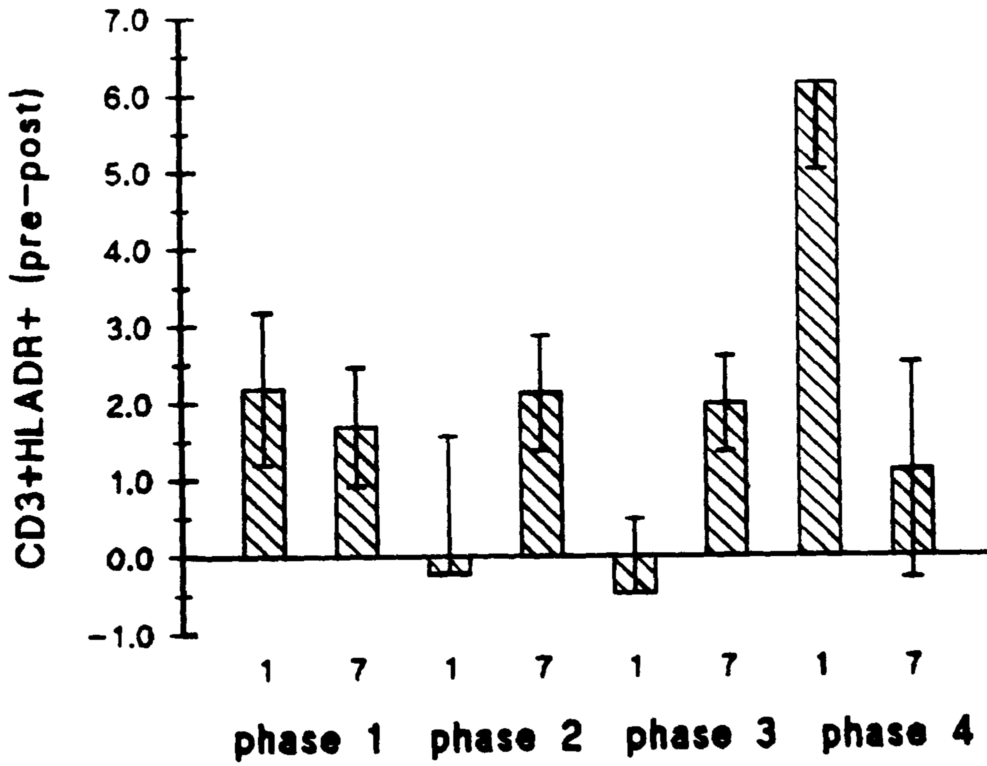
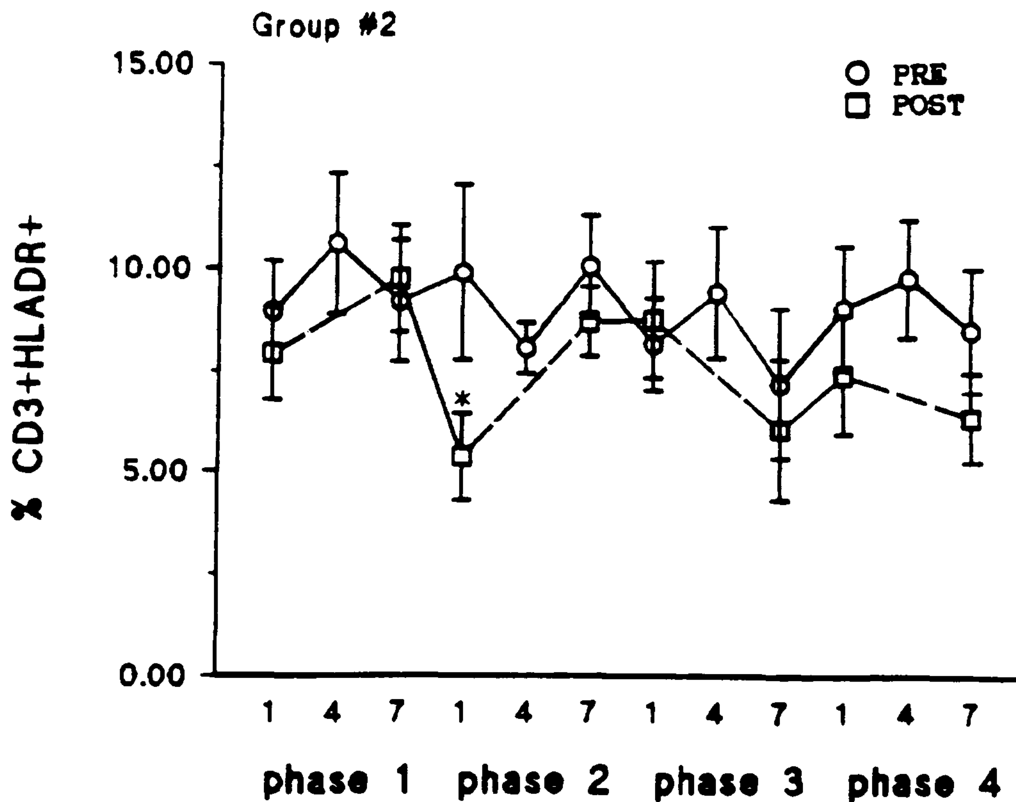
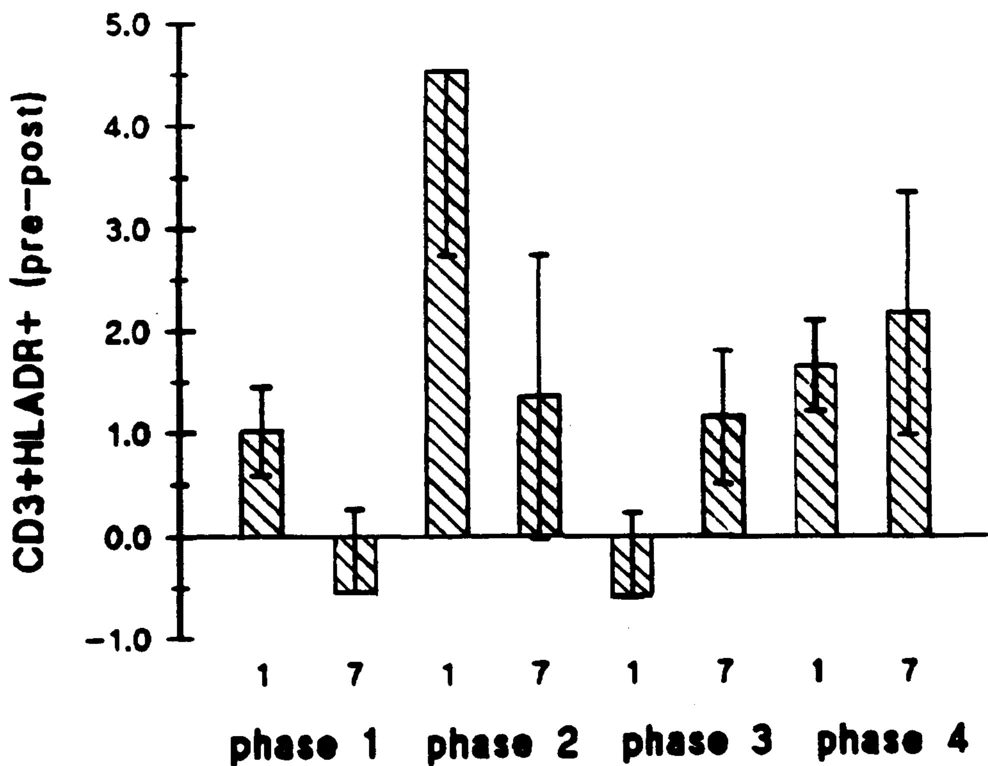

7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
CD3+HLADR+ (pre-post)
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

FIGURE 13: A. Group #2 percent CD3+HLA-DR+ values pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.

B. Group #2 magnitude of pre-post CD3+HLA-DR+ differences on days 1 and 7 of training phases 1 to 4.
n = 6
Values are means ± SE.



Group #2
PRE
POST
% CD3+HLADR+
15.00
10.00
5.00
0.00
*
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4



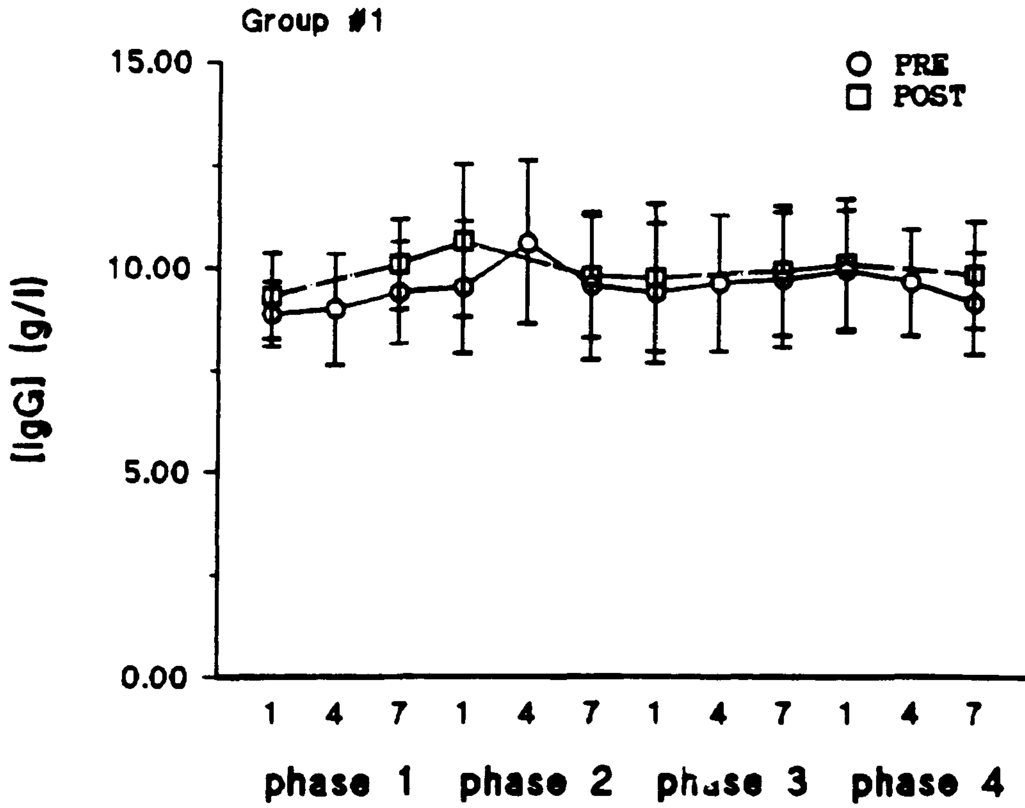
CD3+HLADR+ (pre-post)
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
1 7 1 7 1 7 1 7
phase 1 phase 2 phase 3 phase 4

2.3.3 Immunoglobulins IgG, IgA and IgM.

The pre- and 5 minute post-exercise concentrations (g/l) of immunoglobulins IgG, IgA and IgM as determined by ELISA analysis for groups 1 and 2 are illustrated in Figures 14, 15 and 16. Possibly due to the large variance between subjects, the concentrations of IgG, IgA and IgM did not significantly vary over the 4 training phases nor did pre- and post-exercise values differ significantly.

FIGURE 14: A. Group #1 IgG values (g/l) pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.

B. Group #2 IgG values (g/l) pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.


Group #1
15.00
10.00
5.00
0.00
[IgG] (g/l)
PRE
POST
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

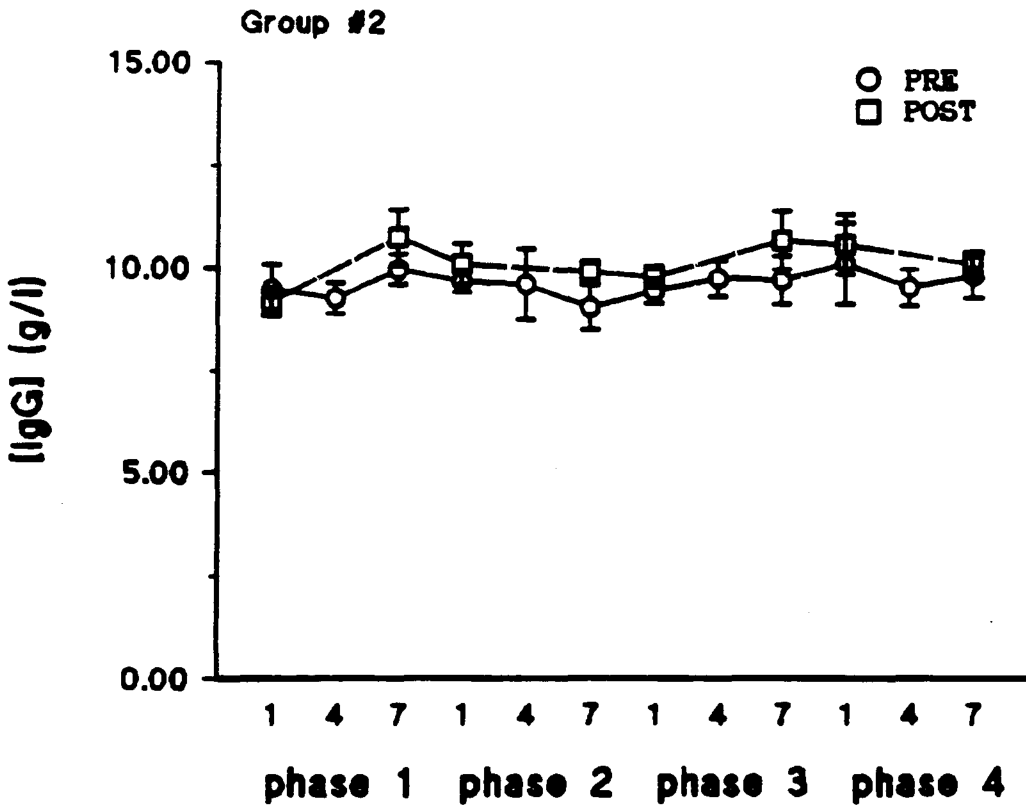
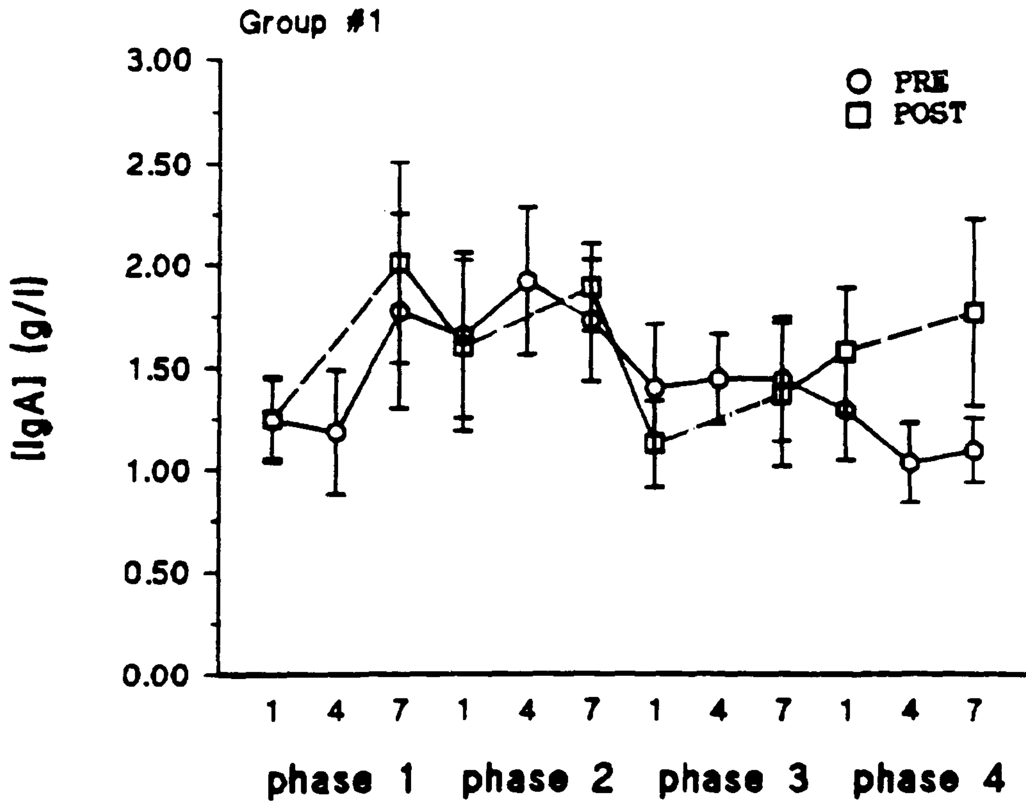

Group #2
15.00
10.00
5.00
0.00
[IgG] (g/l)
PRE
POST
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

FIGURE 15: A. Group #1 IgA values (g/l) pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.

B. Group #2 IgA values (g/l) pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.


Group #1
PRE
POST
[IgA] (g/l)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

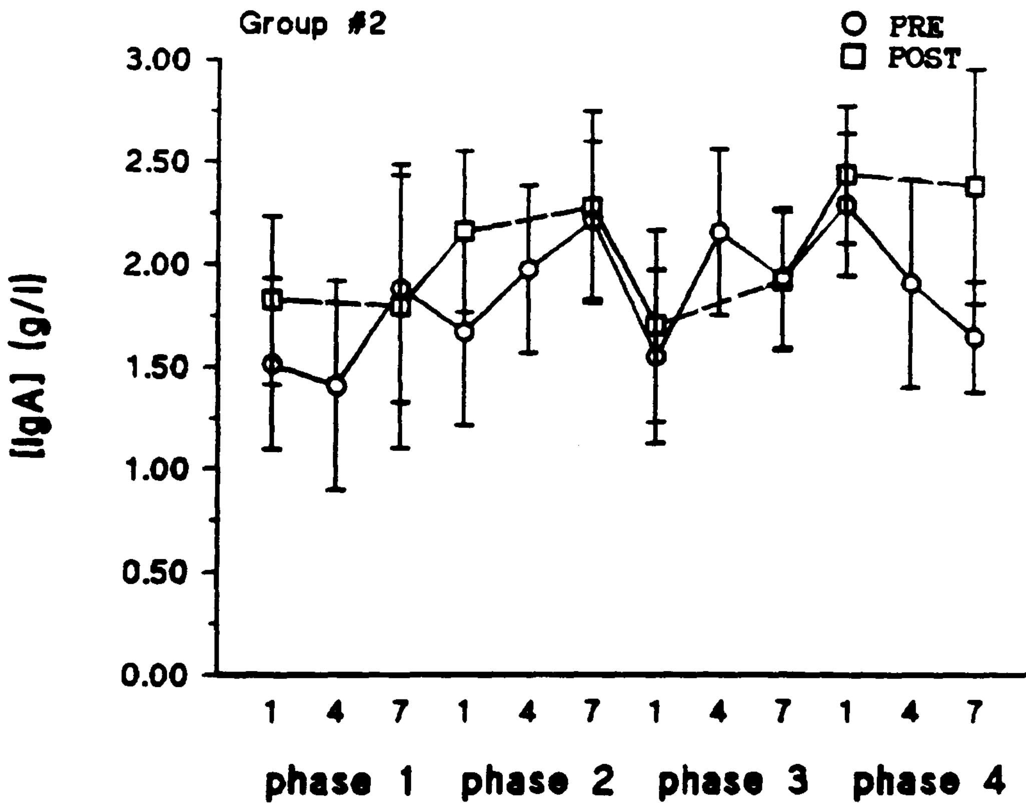
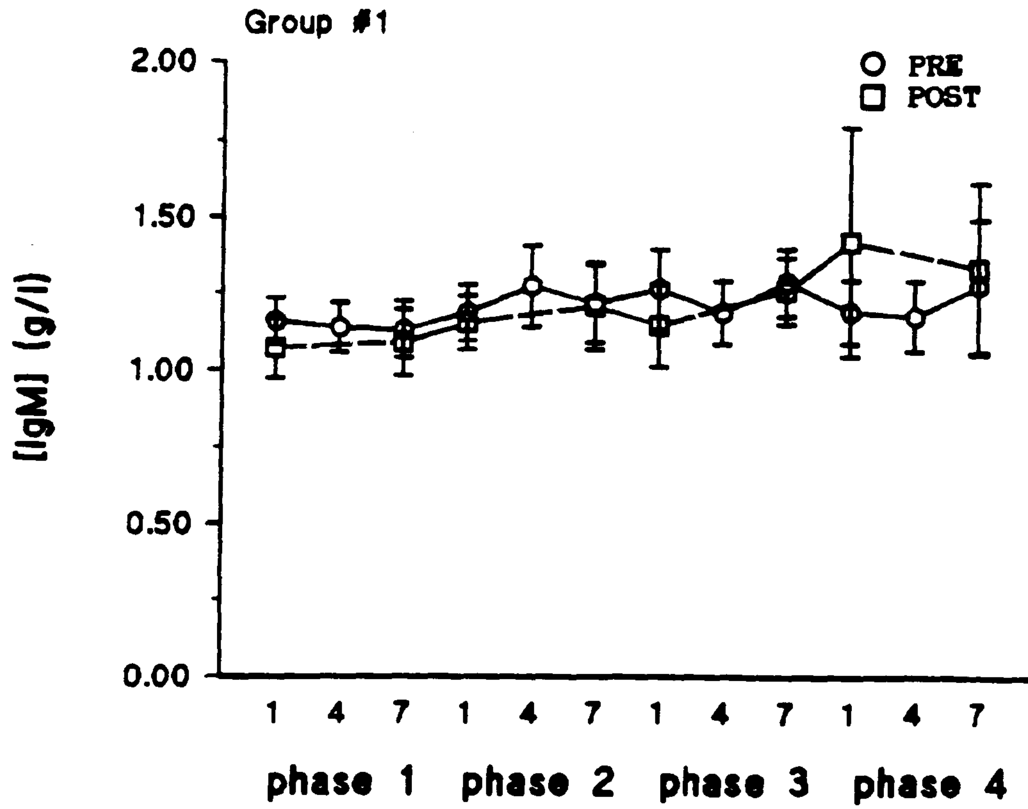
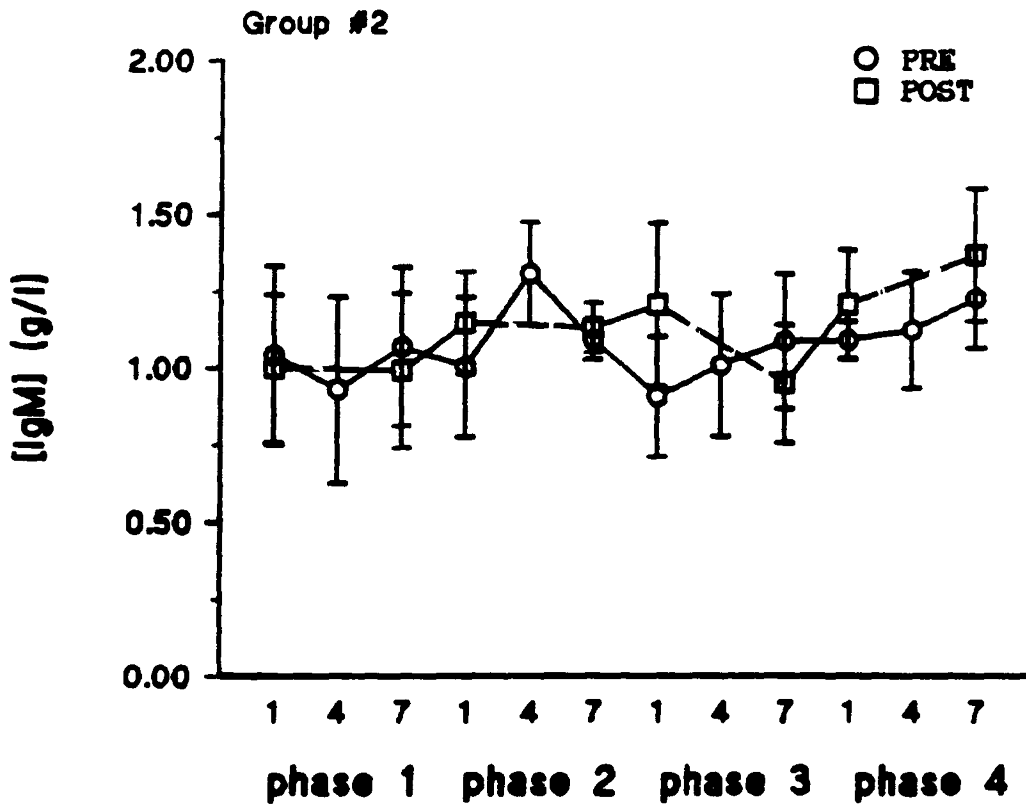

Group #2
PRE
POST
[IgA] (g/l)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

FIGURE 16: A. Group #1 IgM values (g/l) pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.

B. Group #2 IgM values (g/l) pre- and 5 minutes post-exercise during training phases 1 to 4.
n = 6
Values are means ± SE.


Group #1
PRE
POST
[IgM] (g/l)
2.00
1.50
1.00
0.50
0.00
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4


Group #2
PRE
POST
[IgM] (g/l)
2.00
1.50
1.00
0.50
0.00
1 4 7 1 4 7 1 4 7 1 4 7
phase 1 phase 2 phase 3 phase 4

2.3.4 Treadmill Performance Test.

The mean performance time (in seconds) for group 1 at the end of each of the 4 training phases are presented in Figure 17. The longest mean treadmill performance time was found at the end of phase 4 (HV/HI). The 2 shortest mean treadmill performance times were found at the end of phases 1 and 3 (baseline). Mean treadmill performance times for group 2 are presented in Figure 18. The longest mean treadmill performance times were found at the end of phase 2 (HV/HI) and phase 4 (HV/LI). The 2 shortest mean treadmill performance times were found at the end of phases 1 and 3 (baseline). The baseline phases 1 and 3 (LV/LI) for both groups were not significantly different. In both groups, the HV/LI and HV/HI phases were significantly different from the baseline phases, though not different from each other. Normalized treadmill performances (as a percentage change from phase 1) are also presented in figures 19 and 20, though this manipulation did not affect the statistical significance.

FIGURE 17: Group #1 treadmill performance test time (s) at the end of each of the 4 training phases.
n = 6
Values are means ± SE.

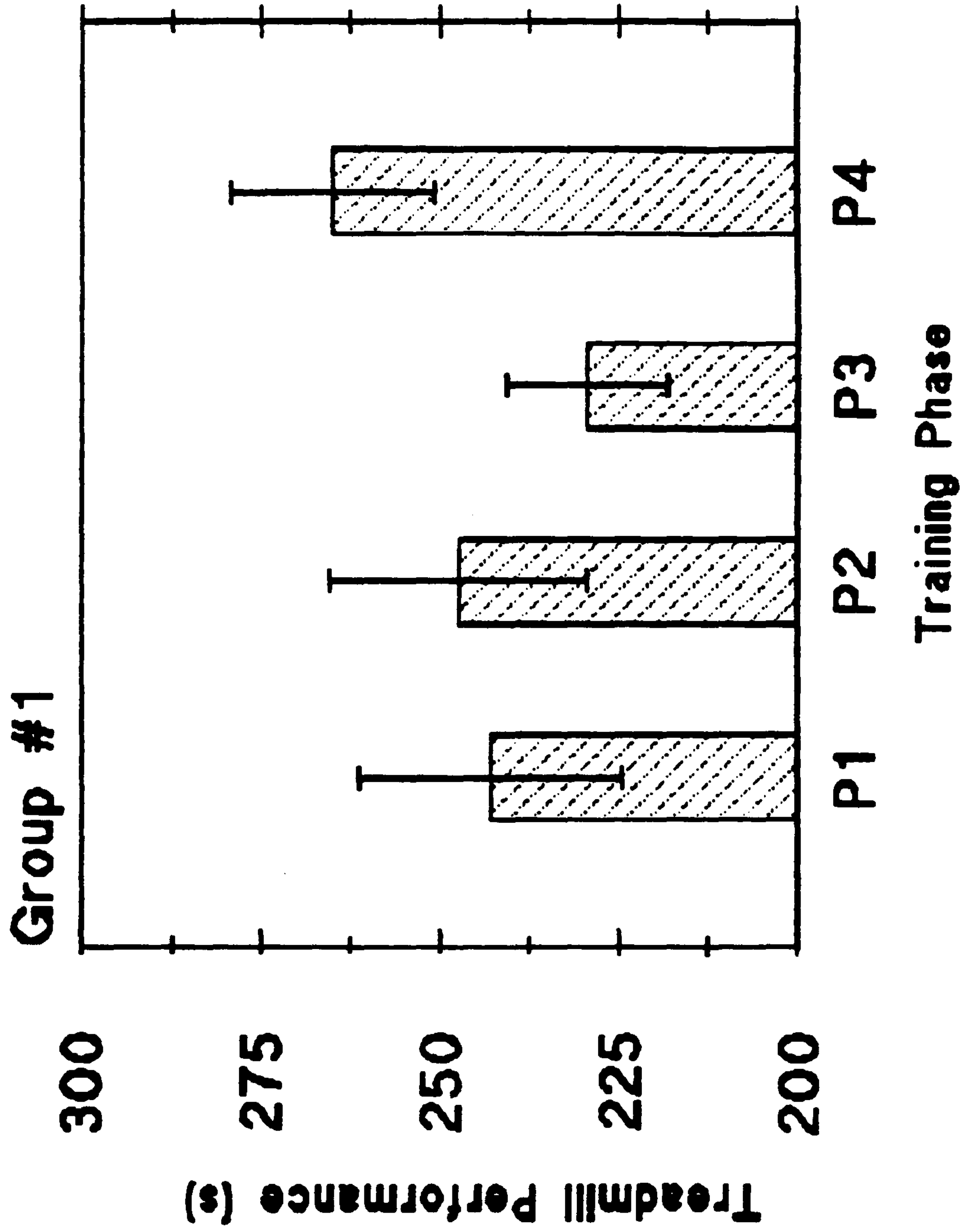

Group #1
Treadmill Performance (s)
300
275
250
225
200
P1
P2
P3
P4
Training Phase

FIGURE 18: Group #2 treadmill performance test time (s) at the end of each of the 4 training phases.
n = 6
Values are means ± SE.

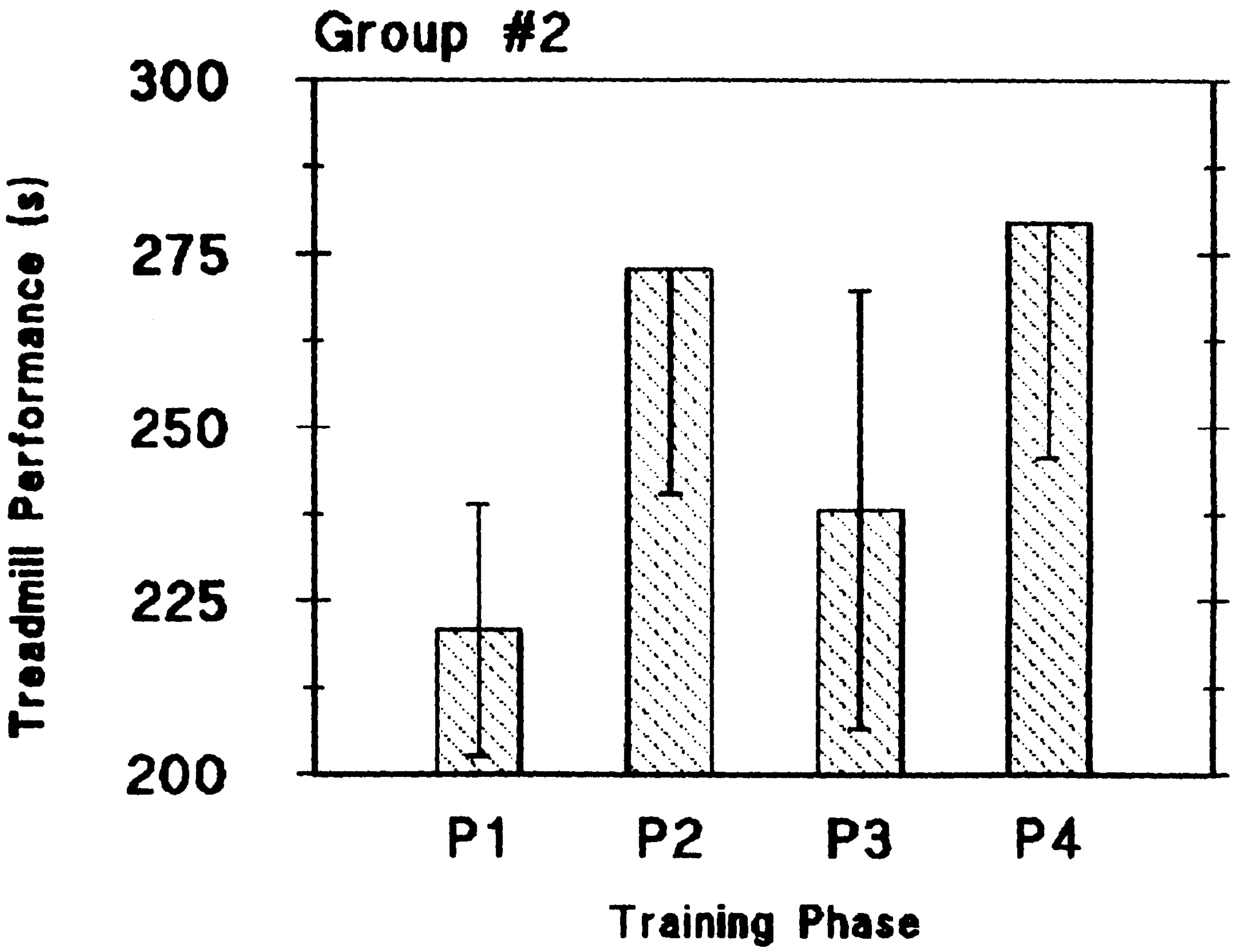
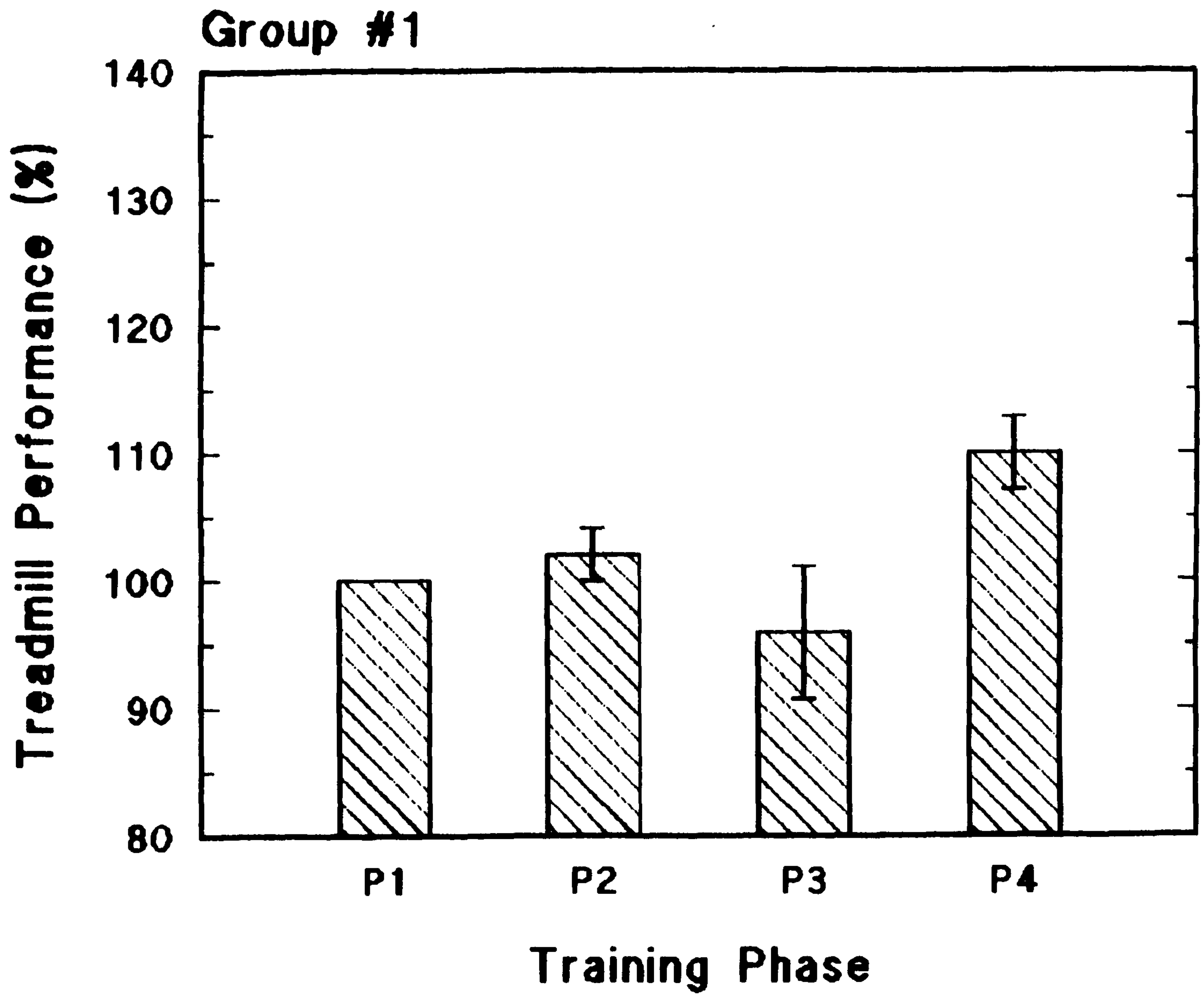
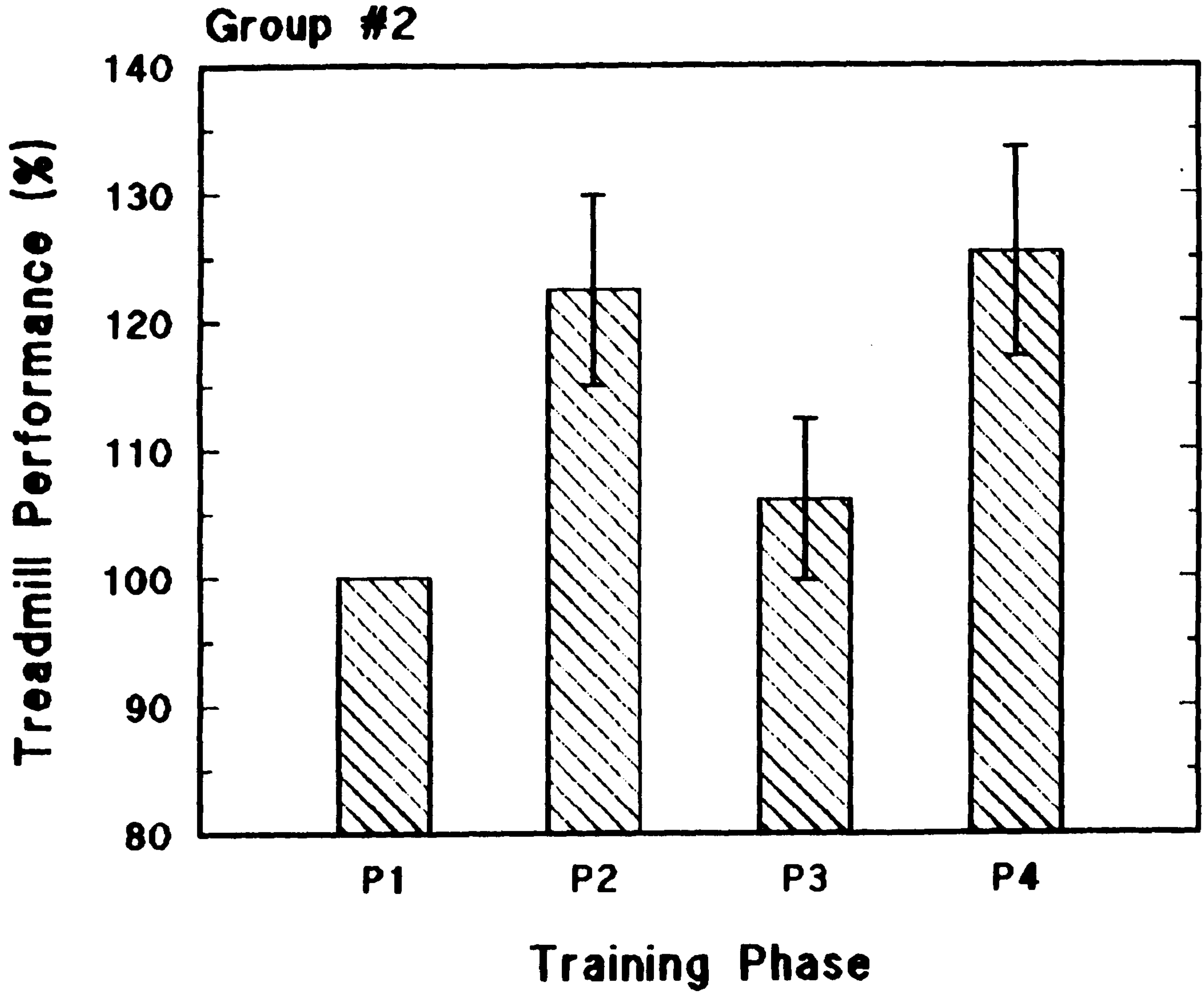

Group #2
Treadmill Performance (s)
300
275
250
225
200
P1
P2
P3
P4
Training Phase

FIGURE 19: Group #1 normalized treadmill performance (%) at the end of each of the 4 training phases.
n = 6
Values are means ± SE.



Group #1
Treadmill Performance (%)
140
130
120
110
100
90
80
P1
P2
P3
P4
Training Phase

FIGURE 20: Group #2 normalized treadmill performance (%) at the end of each of the 4 training phases.
n = 6
Values are means ± SE.


Group #2
Treadmill Performance (%)
140
130
120
110
100
90
80
P1
P2
P3
P4
Training Phase

2.4 DISCUSSION

The present study examined the acute and chronic effects of changes in training volume and intensity on the blood lymphocyte percentages and immunoglobulin concentrations in runners. The T-lymphocyte subpopulations measured were T-cells (CD3+), T-helper cells (CD4+), T-cytotoxic/suppressor cells (CD8+) as well as HLA-DR+ (a marker for B cells and "activated" T-lymphoid cells). All counts were found to be in the lower end of the normal range as cited in the Becton Dickinson Immunocytometry Systems reference manual (CD3+: 73 $\pm$ 6.5; CD4+: 44 $\pm$ 7.6; CD8+: 33 $\pm$ 7.4; CD4/CD8: 1.4 $\pm$ 0.6).

2.4.1 Acute Effects

It was observed that acute bouts of exercise (differing in volume and intensity) produced various perturbations in the percentages of T-lymphocyte subpopulations as measured in venous blood. Since one blood sample was taken 5 minutes post-exercise, the duration of the acute effect is unknown. Other literature indicates a transient range of suppression of 30 minutes (Kendall et al., 1990), 2 hours (Shinkai et al., 1992), and 21 (Nieman et al., 1989) to 24 hours (Espersen et al., 1990; Oshida et al., 1988; Ricken et al., 1990) post-exercise, depending upon the duration and intensity.

A. CD4+ - helper cells. Percentages of CD4+ lymphocytes were reduced immediately (5 minutes) post-exercise. Acute exercise resulted in fairly consistent (though minimal) reductions in the percentages of these cells during the period of LV/LI training. The magnitude of these reductions was influenced more during periods of increased volume and intensity of training. Exercise bouts of high volume/ high intensity (HV/HI) induced greater reductions in CD4+ percentages than exercise of high volume/low intensity (HV/LI). This increase in relative exercise volume and/or intensity may have resulted in larger lymphocyte percentage shifts due in part to the increased stress and subsequent increased release of immunomodulatory hormones in the blood.

These results support findings of similar decreases in T-helper lymphocyte percentages immediately following maximal treadmill exercise (Berk et al., 1986) and various exercise intensities (ranging from 30 to 75% $\dot{V}O2$ max) and durations (30 to 120 minutes) on a cycle ergometer (Brahmi et al., 1985; Hedfors et al., 1983; Kendall et al., 1990; Oshida et al., 1988; Shinkai et al., 1992).

B. CD8+ - suppressor cells. The T-suppressor (CD8+) cell response was very resistant to change immediately after low volume/low intensity exercise. Berk et al. (1986) found significant increases in CD8+ cells after acute maximal

exercise and others have reported relative increases (Landmann et al., 1984; Lewicki et al., 1988; Masuhara et al., 1987) or no net change (Brahmi et al., 1985; Hedfors et al., 1983; Kendall et al., 1990). Previous reports of the effect of exercise on the CD8+ T-suppressor subset have been inconclusive and the effect of repetitive bouts of high intensity exercise on the CD8+ lymphocyte subset was not previously known. This study, however, did investigate the effects of high intensity interval exercise during the HV/HI training phase. Post-exercise CD8+ percentage values rose above the pre-exercise values, for both training groups 1 and 2, during the HV/HI phase, although this increase was not significant. The increase in CD8+ lymphocytes may possibly reflect potential suppression of T-cell and T-cell-dependent B cell function (Cameron et al., 1989).

C. CD4/CD8 - helper/suppressor T cell ratio. In vivo, the ratio of helper cells to suppressor cells (CD4/CD8) is believed to play an important role in immunosurveillance, with a ratio below 1.5 being indicative of an increased susceptibility to infection (Keast et al., 1988). Some studies have found no significant changes in the CD4/CD8 ratio following acute exercise (Kanonchoff et al., 1984; Nieman et al., 1989; Verde et al., 1992) while others have reported a reduction (Berk et al., 1985; Brahmi et al., 1985; Cameron et

al., 1987; Edwards et al., 1984; Hedfors et al., 1983; Landmann et al., 1984; Lewicki et al., 1988; Masuhara et al., 1987; Nguygen et al., 1984; Oshida et al., 1988; Shinkai et al., 1992). In the present study, exercise-induced reductions in the CD4/CD8 ratio were noted following low volume/low intensity exercise and were significantly greater after increases in volume and/or intensity. Interestingly, 4 of the 12 subjects (2 from each group) displayed extremely depressed CD4/CD8 ratios (below 1.0) during the HV/LI and HV/HI phases. One subject had post-exercise CD4/CD8 ratios of 0.88 and 0.92! These are quite low, considering that AIDS patients have been reported to have CD4/CD8 ratios less than 0.9 (Kimball, 1986).

In the present study, the reduction in the CD4/CD8 ratio post-exercise was primarily due to the large decrease in the CD4+ T cell percentage. This finding differs from that of Landmann et al. (1984), who attributed the decrease in the CD4/CD8 ratio to the proportionately larger increase in the CD8+ suppressor cells. The decrease in the CD4/CD8 ratio would appear to indicate a weakened host defence (Landmann et al., 1984; MacKinnon and Tomasi, 1986) and immune response (perhaps providing a temporary "open window" for infectious agents).

D. <u>CD3+ - Total T-lymphocytes.</u> CD3+ T-lymphocyte percentages were also reduced immediately after exercise. The

magnitude of the reduction was further affected by the volume and intensity of exercise. These results are in agreement with previous findings (Brahmi et al., 1985; Deuster et al., 1988; Hoffman-Goetz et al., 1989; Kendall et al., 1990; Lewicki et al, 1988; Oshida et al., 1988; Shinkai et al., 1992; Tvede et al., 1989) who found similar reductions after exercise. Although the percentage of T cells has been reported to decrease, the total number of circulating T cells has been found to increase (Deuster et al., 1988; Lewicki et al., 1988) or remain unchanged (Berk et al., 1986). The reduction in the CD3+ lymphocyte percentages has been attributed to the decrease in the CD4+ subset (Tvede et al., 1989). This may be a valid conjecture because the CD3+ T marker is present on all T-lymphocytes, and since the CD4+ subset was markedly reduced by exercise (CD8+ was very robust), the CD3+ decrease may be due primarily to the reduction in CD4+ levels. In this study, there were similar (though not identical) percentage reductions in the CD3+ and CD4+ levels. The largest reduction in CD3+ T-lymphocyte percentages occurred during phase 2 (the initiation of volume and/or intensity increases) for both groups 1 and 2.

E. HLA-DR+ - B cells and "activated" T-lymphoid cells. Very few studies have examined the effect of exercise upon the HLA-DR+ activation marker. Espersen et al. (1990) have

reported significantly elevated HLA-DR+ levels after a 5 km race in runners compared to control subjects, but suggested that the data should be interpreted with caution due to the small number of subjects which were studied (11). The present study, however, failed to find a persistent activation of lymphoid cells post-exercise, in any of the conditions where volume and/or intensity were manipulated. The large between subject variance in the HLA-DR+ percentages is one possible explanation for the lack of statistical significance.

2.4.2 Chronic Effects.

It was hypothesized that repeated exposure to exercise training (stress) would alter either the resting T-lymphocyte subset level or the magnitude of the pre-post exercise difference as each exercise phase progressed (a comparison of days 1 and 7 of each phase). The literature contains only a few exercise training studies where T-lymphocyte subset populations have been measured (Nehlsen-Cannarella et al., 1991; Soppi et al., 1982; Verde et al., 1992; Watson et al., 1986).

In the present study, no significant change in the resting percentage of total T cells (CD3+) was found throughout the 4 training phases. Watson et al. (1986) reported a significant increase in the percentage of mature

T cells but no changes in lymphocyte percentage in 15 young males (ages 17 to 34 years) who walked/jogged for 15 weeks. The increase in the T cell percentage may have been due to the maturation of NK cells into mature T cells. Total leukocyte number was not reported, thus preventing determination of changes in lymphocyte numbers. Ndon et al. (1992) reported that 28 days of exercise training (cycling, running, swimming and weights) did not alter the resting values or magnitude of the circulating leukocytic response to acute exercise on a cycle ergometer. Nehlsen-Cannarella et al. (1991) reported a significant but mild decrease in the number of lymphocytes (especially T cells) in response to 15 weeks of moderate-walking exercise training in 36 sedentary, mildly obese women. These responses were strongest after 6 weeks of training with some attenuation between 6 and 15 weeks.

The resting CD4/CD8 ratio has been found to either decrease after training (Kinderman et al., 1990; Verde et al., 1992) or remain unchanged (Nehlsen-Cannarella et al., 1991). In the present study, the CD4/CD8 ratio at rest fluctuated somewhat during all 4 phases, yet did not change significantly over the 6 weeks of the study.

Hoffman-Goetz et al. (1990) reported that exercise-induced changes in T-lymphocyte subset numbers (absolute and relative) disappeared by day 5 of a 5-day cycle ergometry protocol (blood samples were taken on days 1, 3 and 5). They

suggested that immediate post-exercise lymphocyte changes may represent a general acute stress response rather than the specific response to exercise. In the present study, the pre-post T-lymphocyte percentage changes (CD3+, CD4+, CD8+ and CD4/CD8 ratio) did not disappear by even day 7 of each phase. In fact, the pre-post exercise lymphocyte differences increased slightly throughout each phase, particularly during the LV/LI "baseline" phases. The HV/LI and HV/HI phases did display a slightly decreased exercise- induced lymphocyte change by day 7 of the phase, but the exercise effect was still apparent. The extent of this reduction during the HV/HI phase was also dependent upon the training phase order (phase order 2 displayed less of a reduction by day 7) and generally less reduced by the 7th day in comparison to the HV/LI phase. The measurements on day 4 enabled observations of the effects of intensive training performed on the previous day. The pre-exercise T-lymphocyte subset measurements were not significantly different on day 4 (when athletes had trained intensively the previous day). Since intervals were performed on days 1, 3, 5 and 7 of the HV/HI phase, the pre-exercise (rest) measurements taken on day 4 of this phase were unlike the pre-exercise values on days 1 and 7.

2.4.3 Training Order Effect.

In group 1, (training phase order of LV/LI, HV/LI, LV/LI, HV/HI) the subjects were exposed to the increase in volume (HV/LI) before the increase in intensity (HV/HI). The HV/LI phase caused the expected reduction in the CD4+ lymphocyte subpopulation and CD4/CD8 ratio, but the subsequent HV/HI phase resulted in an even greater reduction. In group 2, (training phase order of LV/LI, HV/HI, LV/LI, HV/LI) the subjects were exposed to the HV/HI phase (greater intensity) before the HV/LI phase. The HV/HI phase resulted in an even greater reduction than it had in group 1 (where it occurred after the HV/LI phase) and this post-exercise lymphocyte subset response was still evident by day 7 of this phase. The immunosuppressive effect of the subsequent HV/LI phase was practically negated (exhibiting essentially the same response as the baseline LV/LI phases) by the previous HV/HI phase. The fact that the HV/LI phase was not significantly immunosuppressive when preceded by the HI phase suggests an order effect. This would appear to indicate that the extent of the T-lymphocyte subset reduction is more dependent upon previous increases in training intensity than in volume, and the order of exposure to the high-intensity stimulus may determine the magnitude of subsequent lymphocyte subset percentage reductions.

2.4.4 Stress/Mood States.

In an attempt to further determine the effects of altered training loads, a stress/mood state rating scale was arbitrarily devised. As expected, subjects reported feeling "tired" during the pre-exercise measurements of the HV/LI and HV/HI phases to a greater extent than during the LV/LI "baseline" phases. Post-exercise ratings revealed that HV/HI exercise was more "tiring" than HV/LI exercise. Furthermore, low volume/low intensity exercise resulted in an improved stress/mood state (ie: the subject reported feeling "better" or "more refreshed") after exercise. This observation further supports the relationship between moderate activity and improved psychological outlook and physical well-being (Heath et al., 1992; Nash, 1986).

2.4.5 Changes in Sleep Patterns.

A disrupted sleep pattern has been a commonly cited symptom of "overtraining" in athletes (Ryan et al., 1983). Although none of the athletes in this study documented disturbed or abbreviated sleep patterns in their training diaries, 3 of the 12 mentioned that they felt that they required more sleep or afternoon naps during the HV/LI and HV/HI phases. It is thus possible that although their

training volume was doubled, they may have been capable of tolerating even greater increases in training load and thus provoke an even greater reduction in various immune system indicies. Such a manipulation may have more closely approximated the "overreaching" condition experienced by competitive athletes during periods of high-level training and competition. In the present study, it was not considered to be ethical to force the athletes into a state of chronic fatigue or exhaustion. Furthermore, any further increase in training load would probably have threatened subject compliance and may have increased the incidence of musculo-skeletal injuries.

2.4.6 Clinical Symptoms.

The incidence of musculo-skeletal injury, infection and illness was also documented during each training phase by the subjects and the investigator. It was anticipated that the heavy training stimulus would increase the incidence of these clinical symptoms that are well documented in the literature as being the consequence of "overtraining" (Hackney et al., 1990; Kuipers and Keizer, 1988; Fry et al., 1991). Five of the 12 subjects (2 from group 1 and 3 from group 2) reported muscle/joint pain during the HV/LI phase. Seven of the 12 subjects (3 from group 1 and 4 from group 2) experienced

muscle/joint pain during the HV/HI phase. Five and 3 of the 12 subjects reported cough/sore throats during the HV/LI and HV/HI phases, respectively. Three and 2 of the 12 subjects reported full manifestations of the common cold during the HV/LI and HV/HI phase, respectively. No incidence of the muscle/joint pain, cough/sore throat or colds was reported during the LV/LI "baseline" phases! It is likely that the increased training volume and intensity equally contributed to the incidence of musculo-skeletal injuries and possibly the incidence of infections and illnesses. In addition, all of these symptoms were reported midway throughout the HV/LI and HV/HI phases, reducing the possibility that they resulted exclusively from the previous baseline (LV/LI) phase.

2.4.7 Treadmill Test and $\dot{V}O2$ Max.

The treadmill performance times for the subjects in groups 1 and 2 increased by the end of the HV/LI and HV/HI phases. The baseline phases 1 and 3 (LV/LI) for both groups were not significantly different. In both groups, HV/LI and HV/HI phases were significantly different from the baseline phases, though not different from each other and thus there was no training effect that would decrease the relative intensity of the HV/LI phase of group 2. Each athlete was tested at a different treadmill velocity (based upon

individual 1500m times) which may have affected the duration of their treadmill performance. The performance times were probably also affected by the incidence of numerous clinical symptoms during the HV/LI and HV/HI phases. Seven of the 12 subjects reported joint pain, muscle tightness and fatigue in their lower legs during the HV/HI phase treadmill test, which may have affected their treadmill performance. It was expected that the interval training sessions during the HV/HI phase would contribute to improved performance test times due to the specific similarity of the training velocities of the 1000 m intervals and the simulated 1500 m treadmill performance test. Unfortunately, this high-intensity training may have also predisposed the athletes to musculo-skeletal injuries. Although the athletes were given a rest day before each treadmill test, this probably was not sufficient. In addition, there were 5 reports of colds during the HV/HI and HV/LI phases which interfered with normal respiratory function during the treadmill performance tests.

$\dot{V}O_2$ max did not change significantly over the 4 training phases but this is not uncommon within an already trained population. These results (refer to table 10 in Appendix II) may also have been affected by various musculo-skeletal injuries and illnesses which prevented the subjects from exerting themselves maximally. Increases of 8 to 16% in $\dot{V}O_2$ max following training have been cited in the literature

(Ekblom et al., 1968; Clausen, 1977; Houston, 1987; Pechar et al., 1974; Watson et al., 1986). A significant reduction in $\dot{V}O2$ max following an intensive period of seasonal training was found in speed skaters (Foster et al., 1982). Similarly, Kinderman (1986) observed lower $\dot{V}O2$ max values in "overtrained" athletes. In contrast, Costill (1987) did not observe any change in the $\dot{V}O2$ max of a single "overtrained" middle-distance runner. It was noticed, however, that the runner required an additional 10% of his $\dot{V}O2$ max to run at a given submaximal pace. This increase in the required O2 cost of running at a 3:44 min/km pace moved up from 70 to 80% $\dot{V}O2$ max by season end. Each subject in the present study had a similar body mass before and after training, indicating that any change in O2 uptake (relative to body mass) was not due to alterations in body mass.

2.4.8 Immunoglobulins.

The circulating immunoglobulin (IgG, IgA and IgM) concentrations were unaffected by exercise training in both groups. These findings are in agreement with previous studies reporting no significant alterations in immunoglobulins during 2 weeks of daily intensive training (Eberhardt, 1971) and several other acute exercise studies (Hanson and Flaherty, 1981; Nieman et al., 1989; McDowell et al., 1991; Housh et

al., 1991). Acute increases in immunoglobulins following maximal exercise have been accounted for by exercise induced plasma volume shifts (Stevenson et al., 1985). In this study, however, mean hematocrit levels (see table 11 in Appendix II) were resistant to change and were not expected to affect the data.

The literature regarding changes in serum immunoglobulin levels in response to exercise is conflicting. From the few studies available, heavy short-term submaximal or graded-maximal exercise is associated with increases in serum immunoglobulins due to the plasma volume changes (Nieman et al., 1989; Poortmans, 1970; Stevenson et al., 1985). In contrast, immunoglobulin increases have been reported despite no changes in plasma volume (Nehlsen-Cannarella et al., 1991) or slightly greater increases than could be explained by the increase in plasma volume (Poortmans, 1970). In these instances, it was speculated that part of the serum immunoglobulin increase following acute short-term exercise may also result from mobilization from extravascular plasma protein pools and the lymph (Poortmans, 1970; Nieman and Nehlsen-Cannarella, 1991). The state of dynamic equilibrium between the intra- and extravascular protein pools and acute exercise may slightly increase the total influx of various proteins (including globulins) into the intravascular pool. The lymph flow (which contains immunoglobulins) is also

increased into the vascular compartment as a result of acute exercise. B cells may also secrete immunoglobulins in response to nonspecific stimulation (lymphokine signals) from T cells. Minor and/or statistically insignificant changes in serum immunoglobulins have been reported following endurance exercise (MacKinnon et al., 1987; Nieman and Nehlsen-Cannarella, 1991).

Once the expanded plasma volumes of athletes have been adjusted for (Rocker et al., 1976), most reports have revealed resting serum immunoglobulin levels of trained athletes to be similar to sedentary controls and within the normal reference range (Green et al., 1981; Nieman and Nehlsen-Cannarella, 1991; Nieman et al., 1989).

The majority of chronic exercise studies suggest that exercise training has a minimal effect on serum immunoglobulin levels. Immunoglobulin synthesis is probably not affected by exercise training. Some studies of _in vivo_ antibody production in animals have shown that exercise training does not affect the primary antibody response to antigen (Davis et al., 1986; Douglass, 1974; Keast et al., 1988). Furthermore, both _in vitro_ and _in vivo_ immunoglobulin production, have been shown to be unaffected by a bout of endurance exercise (Eskola et al., 1978; MacKinnon et al., 1987).

2.4.9 Mechanisms.

The mechanisms which might account for the immediate but transient changes in T-lymphocyte subset percentages with exercise are not known. Mobilization of cells from tissue reservoirs (eg., lung and spleen) and the movement of cells into and out of the circulation may account for alterations in the lymphoid cell number. Increased cardiac output (rather than the effects of catecholamines) and subsequent demargination of leukocytes from endothelial surfaces have been reported to be major factors in exercise-induced leukocytosis (Foster et al., 1986). Large increases in blood flow could dislodge T-lymphocytes and NK cells from capillaries in the alveoli, resulting in overall increases in mononuclear cells. This could result in increased or decreased lymphocyte subsets in the circulation contingent upon lymphocytes or size and cell adhesion characteristics.

The effects of catecholamines on the immune system are complex. Increased levels of epinephrine (or adrenaline, regarded as an immunosuppressive hormone) have been associated with decreased lymphocyte function and reduced antibody production (MacKinnon and Tomasi, 1989). Administration of adrenaline induces an increase in lymphocytes (specifically T cytotoxic/suppressor and NK cells) (Gader and Cash, 1975), a decrease in T-lymphocytes (Eriksson and Hedfors, 1977) and a

reduction in T helper cells and the CD4/CD8 ratio (Crary et al., 1983). During acute endurance exercise, there is a rise in catecholamines and an associated mild leukocytosis but rapid and strong lymphocytosis (probably from various storage sites and high-endothelial venules) (Crary et al., 1983). High-intensity or long-term submaximal exercise has been shown to increase cortisol levels, which induces leukocytosis while reducing circulatory lymphocytes by redirecting them to peripheral tissues (Davis et al., 1986; McCarthy and Dale, 1988; Nieman et al., 1989).

A number of mechanisms have been postulated by which elevated cortisol levels may reduce immune activity. In brief, cortisol reduces circulating lymphocytes by temporarily sequestering helper T cells in the bone marrow (Fauci, 1975) and also impairs the release of interleukin-1 (IL-1) which in turn impairs the release of IL-2 (necessary for lymphocyte proliferation) by the activated lymphocyte (Berk et al., 1990; Snyder and Uanue, 1982).

Catecholamines and cortisol also cause redistribution of blood mononuclear cells (Kappel et al., 1991). Altered blood mononuclear cell composition has been theorized to effect functional lymphocyte changes. Increased monocyte concentrations are believed to be responsible for the downregulation of NK and B cell (and immunoglobulin secretion) functions. After exercise, more monocytes have been shown to

be activated (as expressed by the higher levels of HLA-DR+ and HLA-DQ+) supporting their post-exercise immunosuppressive role. It is not clear, however, whether monocytes are redistributed (to the blood) or activated in relation to exercise, nor are the mechanisms for this (Pedersen, 1991).

While the present study did not investigate the time course of the lymphocyte subset count recovery, biphasic exercise-induced changes in peripheral blood leukocyte subset counts have been reported (Hansen et al., 1991; Shinkai et al., 1992). Shinkai et al. have hypothesized that adrenaline was largely responsible for redistribution of immunocompetent cells during exercise (as it is released into and cleared from the circulation quickly) while cortisol has a greater effect later in exercise and during recovery (since it accumulates and peaks in the blood later, depending on the type and intensity of exercise).

Although an attempt was made to lessen anticipatory stress by having the subjects relax for 20 minutes before blood sampling, it is possible that psychological factors (anticipation or anxiety) may have affected catecholamine levels. Psychological stress is known to activate the sympathetic nervous system and to affect the immune system (Blalock et al., 1984). This would probably have a considerable effect during the beginning of the first training phase where the subjects would be under more stress due to the

sudden onset of a training and testing regime. The remaining 3 exercise phases (with possibly the exception of the HV/HI phase) were not highly stressful because the runs were not competitive in nature. Stressful athletic competitions may have influenced immunological reductions in the studies by Tomasi et al. (1982) and Tharp and Barnes (1990). The athlete's fitness level (e.g., moderately fit versus highly trained) may also contribute to the immune system's response to the exercise stressor.

Glutamine has also been hypothesized to be a critical fuel for macrophages, tumor cells, and lymphocyte cell replication (Szondy and Newsholme, 1989). Overtraining may impair the release of sufficient glutamine from muscle tissue and subsequently depress immune system function (Newsholme, 1990). In this study, it is unlikely that this depletion occurred since the chronic levels of the various immune components measured were resistant to change.

Secretion of opioid neuropeptides (such as B-endorphin and B-lipotrophin) have been stimulated by exercise (Carr et al., 1981; Colt et al., 1981; Howlett et al., 1984; Landmann et al., 1984; Yu et al., 1977) but little is known about the effects on lymphocytes. Peripheral blood monocytes, granulocytes and lymphocytes have been shown to possesss opiate and specific nonopiate receptors, making them targets for circulating peripheral opioid peptides (Hazum et al.,

1979; Wybran et al., 1979; Mehrishi and Mills, 1983). In addition, plasma levels of interferon alpha, interleukin-1, B-endorphin and met-enkephalin all increase with exercise (Fitzgerald, 1988). A number of studies have reported increased B-endorphin levels in peripheral blood after incremental graded exercise (De Meirleir et al., 1986; Donevan and Andrew, 1987; Goldfarb et al., 1987; Rahkila et al., 1987) as well as anaerobic exercise (Brooks et al., 1988; Farrell et al., 1987). Endurance exercise, however, has resulted in either unchanged (DeMeirleir et al., 1986; Elias et al., 1986; Langenfeld et al., 1987) or increased (Farrell et al., 1982; Schwarz and Kindermann, 1989) B-endorphin levels.

Obviously many other factors are involved in exercise-induced enhancement and suppression of immune function and in the potential causation of infection. Numerous extraneous confounders such as cardiovascular and respiratory fitness, diet, lifestyle, the individual's psychological and physiological stress levels, circadian rhythms, seasonal variations, unknown or uncontrolled exposure to pathogenic viruses, and the individual's ability to elicit specific immune and non-immune responses further complicate the issue (Cannon, 1993; Solomon et al., 1974). In addition, day to day variations in these factors may contribute to the complexity of the etiology of immunosuppression or enhancement following exercise (Galbo, 1983; Keast et al., 1988).

2.5 Conclusions.

In summary, the results of this study indicate that the percentages of several T-lymphocyte subsets may be affected by increases in the intensity and volume of exercise. In addition, the present findings show that immunological changes are transient and that repeated intensive exercise within the space of a few days (as habitually performed by high-level athletes) does not appear to suppress resting immune status. A transient decrease was observed in T-lymphocyte percentages of CD3+, CD4+ and the CD4/CD8 ratio post-exercise which was significant ($p < 0.05$) during the HV/LI and HV/HI phases. Adaptation of these lymphocyte subpopulations occurred with repeated exposure to increases in the volume and intensity of exercise. A training order effect was also suggested, whereby an initial HI phase was more immunosuppressive and appeared to negate the effects of subsequent HV phases. The results indicate that the degree of the transient acute lymphocyte subset reduction is more dependent upon training intensity than volume, and the order of exposure to the high intensity stimulus may determine the magnitude of subsequent responses.

This study has also revealed a remarkable stability of humoral (antibody) immune components during and after training runs of various intensities and volumes in athletes. Immunoglobulin (IgG, IgA and IgM) levels were not

significantly affected by alterations in volume or intensity over the 4 training phases. This finding disputes the postulate of transient modification of immune host defences due to decreased concentrations of circulating immunoglobulins.

Many highly motivated athletes have the work ethic that "more is better" and that the need for rest is a sign of weakness. Coaches who push too hard, too quickly or add too much competition with insufficient recovery may have a similar effect. These attitudes, along with the stressful demands of training and competition may provoke excessive fatigue and induce temporary immunological suppression, making the athlete more susceptible to infections and other illnesses.

During peak periods of training or sudden increases in training load (intensity and/or duration), athletes should be advised to avoid exposure to sick individuals and crowds immediately following exercise and particularly after the initial intense training session. Athletes should also be advised against strenous exercise when fever and other symptoms of systemic infection are present (the intensity aspect of training seems to be the most damaging to the immune system). Training should be resumed at a moderate level, progressing gradually. It would appear that there are certain optimal levels of exercise for each individual's immune system (eg, triathalons may be too strenuous for some) and those

athletes that train several times per day could induce a severe state of immunodeficiency, resulting in an increased susceptibility to infection.

Further research is necessary to investigate the mechanisms and the various immunomodulators involved and to determine the optimal combinations of exercise intensity and duration most beneficial to the immune status of high-level athletes. Additional larger longitudinal training studies involving children, sedentary and active individuals are necessary in order to establish whether habitual exercise training alters immune function. Furthermore, epidemiological training studies on older, competitive (master's level) athletes may be of interest, to verify whether decades of "assault" through intensive training will damage or enhance the immune system's resistance to viral infections and cancer.

REFERENCES

Abbas, A.K., Lichtman, A.H., and Pober, J.S., Cellular and Molecular Immunology, by W.B. Saunders Company, USA, 1991.

Andersen, K.L. Leucocyte response to brief, severe exercise. J. Appl. Physiol., 7: 671-674, 1955.

Anderson, O. A run a day keeps the doctor away. Runner's World, (Jan): 54-57, 1989.

Andreef, M., Clinical Cytometry, Vol 468, June 15. Annals of the New York academy of Sciences, 1986.

Ardawi, M.S., and Newsholme E.A. Metabolism in lymphocytes and it's importance in the immune response. Essays in Biochem., 21: 1-43, 1985.

Balow, J.E., Hurley, D.L., and Fauci, A.S. Immunosuppressive effects of glucocorticosteroids: Differential effects of acute vs. chronic administration on cell-mediated immunity. J. Immunol. 114: 1072-1076, 1975.

Baron, R.C., Hatch, M.H., Kleeman, K., and MacCormack, J.N. Aseptic meningitis among members of a high-school football team. J. Amer. Med. Assoc., 248: 1724-1727, 1982.

Barron, J.L., Noakes, T.D., Levy, W., Smith, C., and Millar, R.P. Hypothalamic dysfunction in overtrained athletes. J. Clin. Endocrin. Metabol. 60: 803-806, 1985.

Bellanti, J.A. (Ed). Immunology III, W.B. Saunders Co., Philadelphia, 1985.

Benjamini, E. and Leskowitz, S. Immunology: a short course. Alan R. Liss, Inc., New York, 1988.

Berk, L.S., Nieman, D.C., Tan, S.A., Nehlsen-Cannarella, S., Kramer, J., Eby, W.C., and Owens, M. Lymphocyte subset changes during acute maximal exercise. (Abs.): Med Sci. Sports Ex. 18: 706, 1986.

Berk, L.S., Nieman, D.C., Youngberg, W.S., Arabatzis, K., Simpson-Westberg, M., et al. The effect of long endurance running on natural killer cells in marathoners. Med. Sci. Sports Ex. 22: 207-212, 1990.

Berk, L.S., Tan, S.A., Nieman, D.C., and Eby, N.C. The suppressive effect of stress from acute exhaustive exercise on T lymphocyte helper/suppresive cell ratio in athletes and non-athletes. Abstract: Med. Sci. Sport Ex. 17: 492, 1985.

Blalock, J.E. The relationship between neuroendocrine

hormones and lymphokines. Lymphokines. 9: 1-13, 1984.

Bloom, S.R., Johnson, R.H., Park, D.M., Rennie, M.J., and Sulaiman, W.R. Differences in the metabolic and hormonal response to exercise between racing cyclists and untrained individuals. J. Physiol. 258: 1-18, 1976.

Bonavida, B., and Wright, S.C. Multistage model of natural killer cell mediated cytotoxicity involving NKCF as soluble cytotoxic mediators. Adv. Cancer Res. 49: 169-187, 1987.

Bongers, V., and Bertrams, J. The influence of common variables on T cell subset analysis by monoclonal antibodies. J. Immunol. Meth. 67: 243-253, 1984.

Bosenberg, A.T., Brock-Utne, J.G., Gaffin, S.L., Wells, M.T.B., and Blake, G.T.W. Strenuous exercise causes endotoximia. J. Appl. Physiol. 65: 106-108, 1988.

Brahmi, Z., Thomas, J.E., Park, M., Park, M., and Dowdeswell, I.R.G. The effect of acute exercise on natural killer cell activity of trained and sedentary human subjects. J. Clin. Immunol. 5: 321-328, 1985.

Brooks, S., Burrin, J., Cheetham, M.E., Hall, G.M., Yeo, T., and Williams, C. The responses of the catecholamines and B-

endorphins to brief maximal exercise in man. Eur. J. Appl. Physiol. 57: 230-234, 1988.

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

Burton, D.R., and Gregory, L. Structure and function of immunoglobulins. In: French MAH (ed.) Immunoglobulins in health and disease. MIP Press Limited, Boston, 1986.

Busse, W.W., Anderson, C.L., Hanson, P.G., and Folts, J.D. The effect of exercise on the granulocyte response to isoproterenol in the trained athlete and unconditioned individual. J. Allergy and Clin. Immunol. 65: 358-364, 1980.

Butler, J., O'Brien, M., O'Malley, K., and Kelly, J.G. Relationship of B-adrenoceptor density to fitness in athletes. Nature, 298: 60-63, 1982.

Cameron, K., Morton A., and Keast, D. Hematological profiles and polyclonal lymphocyte function in continuous and intermittent exercise. M.Sc. thesis, University of Western Australia, 1987 as cited in Cameron et al., 1989.

Cameron, K., Morton, A., and Keast, D. T-cell sub-populations and polyclonal lymphocyte function in continuous and

intermittent exercise. Aust. J. Sci. Med. Sport. 21(4): 15-19, 1989.

Cannon, J.G., and Kluger, M.J. Endogenous pyrogen activity in human plasma after exercise. Science 220: 617-619, 1983.

Cannon, J.G. Exercise and resistance to infection. J. Appl. Physiol. 74(3): 973-981, 1993.

Carr, D.B., Bullen, B.A., Skrinnar, G.S., et al. Physical conditioning facilitates the exercise-induced secretion of B-endorphin and B-lipotropin in women. N. Engl. J. Med. 305(10): 560-562, 1981.

Clausen, J.P. Effect of physical training on cardiovascular adjustments to exercise in man. Physiol. Rev. 57: 779-815, 1977.

Colt, E.W.D., Wardlaw, S.L., and Franz, A.G. The effect of running on plasma B-endorphin. Life Sci. 28: 1637-1640, 1981.

Cooper, B.T., Douglas S.A., Firth, L.A., Hannagon, J.A., and Chadwick, V.S. Erosive gastritis and gastrointestinal bleeding in a female runner. Gastroenterology 92: 2019-2023, 1987.

Costill, D.L. Physiological Alterations During Periods of Training Overload. - presented at Symposium "Overtraining: Physiological and Psychological Effects of Sudden Increase in Training". Med. Sci. Sports Ex. 19(2) Suppl: S77, 1987.

Crary, B., Hauser, S.L., Borysenko, M.,Kutz, I., and Hoban, C. Epinephrine-induced changes in the distribution of lymphocyte subsets in peripheral blood of humans. J. Immunol. 1: 1178-1181, 1983.

Cupps, T.R. and Fauci, A.S. Corticosteroid-mediated immunoregulation in man. Immunol. Rev. 65: 133-155, 1982.

Davidson, R.J.L., Robertson, J.D., Galea, G., and Maughan, R.J. Hematological changes associated with marathon running. Int J. Sports Med. 8: 19-25, 1987.

Davies, C.T.M., Few, J., Foster, K.G., and Sargent, A.J. Plasma catecholamine function during dynamic exercise involving different muscle groups. Eur. J. Appl. Physiol. 32: 195-206, 1974.

Davies, A.O., and Lefkowitz, R.J. Corticosteroid-induced differential regulation of B-adrenergic receptors in circulating human polymorphonuclear leucocytes and mononuclear leucocytes. J. Clin. Endocrinol. Metab. 51: 599-605, 1980.

Davis, M.A., Kendrick, Z.V., and Paslone, M. The measurement of antibody response to influenza virus vaccine in aerobically trained mice. Med. Sci. Sports Ex. 18: S9, 1986.

DeMeirleir, K., Naaktgeboren, N., Steirteghem, V., Gorus, F., Albrecht, J., and Block, P. Beta-endorphin and ACTH levels in peripheral blood during and after aerobic and anaerobic exercise. Eur. J. Appl. Physiol. 55:5-8, 1986.

Deuster, P.A., Curiale, A.M., Cowan, M.L., and Finkelman, F.D. Exercise-induced changes in populations of peripheral blood mononuclear cells. Med. Sci. Sports Ex. 20: 276-280, 1988.

Dickson, D.N., Wilkinson, R.L., and Noakes, T.D. Effects of ultra-marathon training and racing on hematologic parameters and serum ferritin levels in well-trained athletes. Int. J. Sports Med. 3: 111-117, 1982.

Donevan, R.H., and Andrew, G.M. Plasma B-endorphin immunoreactivity during graded cycle ergometry. Med. Sci. Sports Exerc. 19: 229-233, 1987.

Dorner, H., Heinold, D., and Hilmer, W. Exercise-induced leucocytosis- Its dependence on physical capability. Int. J. Sports Med. 8: 152, 1987.

Douglas, D.J., and Hanson, P.J. Upper respiratory infections in the conditioned athlete. Med. Sci. Sports Ex. 10: 55, 1978.

Douglass, J.H. The effects of physical training on the immunological response in mice. J. Sports Med. 14: 48-54, 1974.

Dressendorfer, R.H., Wade, C.E., and Amsterdam, E.A. Development of pseudoanemia in marathon runners during a 20 day road race. J. Am. Med. Assoc. 246: 1215-1218, 1981.

Eberhardt, A. Influence of motor activity on some serologic mechanisms of non-specific immunity of the organism. Acta Physiol. Polonica 22: 201-212, 1971.

Edwards,, A.J., Bacon, T.H., Elms, C.A., Verardi, R., Felder, M., and Knight, S.C. Changes in the populations of lymphoid cells in human peripheral blood following physical exercise. Clin. Exp. Immunol. 58: 420-427, 1984.

Eichner, E.R. Infection, immunity and exercise. Phys. and Sports Med. 21(1), Jan., 125-135, 1993.

Ekblom, B., Astrand, P.-O., Saltin, B., Stenberg, J. and Wallstrom, B. Effect of training on circulatory response to exercise. J. Appl. Physiol. 24(4): 518-528, 1968.

Elias, A.N., Iyer, K., Pandian, M.R., Weatherbee, P., Stone, S., Tobis, J. B-endorphin/B-lipotropin release and gonadotropin secretion after acute exercise in normal males. J. Appl. Physiol. 61: 2045-2049, 1986.

Eriksson, B., and Hedfors, E. The effect of adrenaline, insulin and hydrocortisone on human peripheral blood lymphocytes studied by cell surface markers. Scand. J. Haematol. 18: 121-128, 1977.

Eskola, J., Ruuskanen, O., Soppi, E., Viljanen, M.K., Jarvinen, M., Toivonen, H., and Kouvalainen, K. Effect of sport stress on lymphocyte transformation and antibody formation. Clin. Exper. Immunol. 3: 339-345, 1978.

Espersen, G.T., Elbaek, A., Ernst, E., Toft, E., Kaalund, S., Jersild, C., and Grunnet, N. Effect of physical exercise on cytokines and lymphocyte subpopulations in human peripheral blood. APMIS. 98: 395-400, 1990.

Estes, G., Solomon, S., and Norton, W.L. Inhibition of lymphocyte stimulation by cyclic and non-cyclic nucleotides. J. Immunol. 107: 1489-1492, 1971.

Evans, W.J., Meredith, C.N., Canon, D.J., Dinarello, C.A., Frontera, W.R., Hughes, V.A., Joness, B.H. and Knuttgen, H.G.

Metabolic changes following eccentric exercise in trained and untrained men. J. Appl. Physiol. 61: 1864-1868, 1986.

Farrar, W.L., Johnson, H.M., and Farrar, J.J. Regulation of the production of immune Interferon and cytotoxic T lymphocytes by interleukin-2. J. Immunol. 126: 1120-1225, 1981.

Farrell, P.A., Kjaer, M., Bach, F.W., and Galbo, H. Beta-endorphin and adrenocorticotropin response to supramaximal treadmill exercise in trained and untrained males. Acta Physiol. Scand. 130: 619-625, 1987.

Fauci, A.S. and Dale, D.C. The effect of in vivo hydrocortisone on subpopulations of human lymphocytes, J. Clin. Invest. 53: 240-246, 1974.

Fauci, A.S. Mechanisms of corticosteroids on lymphocyte subpopulations. I. Redistribution of circulating T and B-lymphocytes to the bone marrow, Immunology, 28: 669-679, 1975.

Fauci, A.S. Mechanisms of corticosteroid action on lymphocyte subpopulations II. Differential effect of in vivo hydrocortisone, prednisone and dexamethasone on in vitro expression of lymphocyte function. Clin. Exper. Immunol. 24: 54-62, 1976.

Fernandes, G., Jeng, G., and Baker, D. Effect of exercise during middle age on immune function in mice maintained on normal and/or restricted calorie intake. Fed. Proc. 44: 767, 1985.

Ferry, A. Influences de l'exercice musculaire sur le systeme immunitaire: Exemples d'immunomodulation. Science and Sports 4: 25-40, 1989.

Few, J.D. Effect of exercise on the secretion and metabolism of cortisol in man. J. Endocrinol. 62: 342-353, 1974.

Fitzgerald, L. Exercise and the immune system. Immunol. Today, 9: 337-339, 1988.

Foster, N.K., Martyn, J.B., Rango, R.E., Hogg, J.C., and Pardy, R.L. Leukocytosis of exercise: role of cardiac output and catecholamines. J. Appl. Physiol. 61(6): 2218-2223, 1986.

Foster, C., Pollock, M., Farrell, P., Maksud, M., Anholm, J., and Hare, J. Training responses of speed skaters during a competitive season. Research Quart. Exerc. Sport. 53: 243-246, 1982.

Fox, G.N. Revisiting the revisited football team hepatitis outbreak. Correspondence. J. Am. Med. Assoc. 254: 317, 1985.

Fraser, J.N., Robertson, D., and Wood, A.J.J. Regulation of human leucocyte beta receptors by endogenous catecholamines: Relationship of leucocyte beta receptor density to the cardiac sensitivity to isoproterenol. J. Clin. Invest. 67: 1777-1784, 1981.

Friedman, L.S., O'Brien, T.F., and Morse, L.J. Revisiting the Holy Cross football team outbreak (1969) by serological analysis. J. Am. Med. Assoc. 254: 774-776, 1985.

Frohlich, J., Simon, G., Schmidt, A., Hitschhold, T., and Bierther, M. Disposition to infections of athletes during treatment with immunoglobulins. Int. J. Sports Med. 8: 119, 1987.

Fry, R.W., Morton, A.R., and Keast, D. Acute intensive interval training and T-lymphocyte function. Med. Sci. Sports Ex. 24(3): 339-345, 1992.

Fry, R.W., Morton, A.R., and Keast, D. Overtraining in athletes- an update. Sports Med. 12: 32-65, 1991.

Gader, A.M.A., and Cash, J.D. The effect of adrenaline, noradrenaline, isoprenoline and salbutamol on the resting levels of white blood cells in man. Scand. J.Haematol. 14: 5-10, 1975.

Galbo, H. Hormonal and Metabolic Adaptation to Exercise, George Thieme Verlag, Stuttgart, 1983.

Galbo. H., Christensen N.J., and Holst, J.J. Glucose-induced decrease in glucagon and epinephrine responses to exercise in man. J. Appl. Physiol. 42: 525-530, 1977.

Galbo H., Holst, J.J. and Christensen, N.J. Glucagon and plasma catecholamine responses to graded and prolonged exercise in man. J. Appl. Physiol. 38: 70-76, 1973.

Galun, E., Burstein, R., Assia, E., Tur-Kaspa, I., Rosenblum, J., and Epstein, Y. Changes of white blood cell count during prolonged exercise. Int. J. Sports Med. 8: 253-255, 1987.

Gillis, S., Crabtree, G.R. and Smith, K.A. Glucorticoid-induced inhibition of T-cell growth factor production I. The effect of mitogen-induced lymphocyte proliferation. J. Immunol. 123: 1624-1631, 1979.

Gimenez, M., Mohan-Kumar, T., Humbert, J.C., De Talance, N., and Teboul, M. Training and leucocyte, lymphocyte and platelet response to dynamic exercise. J. Sports Med. 27: 172-177, 1987.

Gisler, R.H. Stress and the hormonal regulation of the immune

response in mice. Psychother. Psychosom. 23: 197-208, 1974.

Goldfarb, A.H., Hatfield, B.D., Sforzo, G.A., and Flynn, M.G. Serum B-endorphin levels during a graded exercise test to exhaustion. Med. Sci. Sports Ex. 19(2): 78-82, 1987.

Golub, E.S., Green, D.R. Immunology: A Synthesis, 2nd Ed. Sinauer Associates, Inc., Massachusets, 01375, USA, 1991.

Goodwin, J.S., Messner, R. P., and Williams, R.C. Inhibitors of T-cell mitogenesis: effect of mitogen dose. Cellular Immunol. 45: 303-308, 1979.

Goodwin, J.S. and Webb, D.R. Regulation of the immune response by prostaglandins. Clin. Immunol. Immunopathol. 15: 106-122, 1980.

Goodman, J.W. Immunoglobulins I: structure and function. In: Stites, D.P. et al. (Eds) Basic and Clinical Immunology 6th ed., Appleton and Lange, Los Altos CA, 1987.

Gordon, D. and Nouri, A.M.E. Comparison of the inhibition by glucocorticosteroids and cyclosporin A of mitogen-stimulated human lymphocyte proliferation. Clin. Exper. Immunol. 44: 287-294, 1981.

Gray, A.B., Smart, Y.C., Telford, R.D., Weidemann, M.J., and Roberts, T.K. Anaerobic exercise causes transient changes in leukocyte subsets and IL-2R expression. Med. Sci. Sports Exerc. 24(12), 1332-1338, 1992.

Green, R.L., Kaplan, S.S., Rabin, B.S., Stanitski, C.L. and Zdziarski, U. Immune function in marathon runners. Ann. Allergy 47: 73-75, 1981.

Guyton, A.C. Textbook of Medical Physiology. Sixth Edition, W.B. Saunders, Toronto. p. 919, 1986.

Hackney, A.C., Pearman, S.N., and Nowacki, J.M. Physiological profiles of overtrained and stale athletes- a review. Appl. Sport Psych. 2: 21-33, 1990 as cited in Fry et al., 1991.

Hadden, J.W., Hadden, E.M., and Middleton, Jr., E. Lymphocyte blast transformation. I. Demonstration of adrenergic receptors in human peripheral lymphocytes. Cellular Immunol. 1: 583-595, 1970.

Hadden, J.W., Hadden, E.M., Middleton, Jr., E. and Good, R.A. Lymphocyte blast transformation. II. The mechanism of action of alpha adrenergic receptor effects. Int. Archives of Allergy 40: 526-539, 1971.

Hagberg, J.M., McLane, J.A., Ehsani, A.A., and Winder, W.W. Disappearance of norepinephrine from the circulation following strenuous exercise. J. Appl. Physiol. 47: 1311-1314, 1979.

Hansen, J.B., Wilsgard, L., and Osterud, B. Biphasic changes in leukocytes induced by strenous exercise. Eur. J. Appl. Physiol. 62: 157-161, 1991.

Hanson, P.G. and Flaherty, D.K. Immunological responses to training in conditioned runners. Clin. Sci. 60: 225-228, 1981.

Haq, A., Al-Hussein, K., Lee, J., and Al-Sedairy, S. Changes in peripheral blood lymphocyte subsets associated with marathon running. Med. Sci. Sports Exerc. 25(2): 186-190, 1993.

Haralambie, G., and Keul, J. Serum glycoprotein levels in athletes in training. Experientia 26: 959-960, 1970.

Hartley, L.H., Manson, J.W., Hogan, R.P., Jones, L.G., Kotchen, T.A., Mongex, E.H., Wherry, F.E., Pennington, L.L., and Ricketts, P.T. Multiple hormonal response to graded exercise in relation to physical training. J. Appl. Physiol. 33: 602-606, 1972.

Hazum, E., Chang, K.J., and Cuatrecasus, B.A. Specific nonopiate receptors for B-endorphins. Science. 205: 1033-1035, 1979.

Heath, G.W., Macera, C.A., and Nieman, D.C. Exercise and upper respiratory tract infections. Sports Med. 14(6): 353-365, 1992.

Hedfors, E., Biberfeld, P., and Wahren, J. Mobilization to the blood of human non- T and K lymphocytes during prolonged exercise. J. Clin. Lab. Immunol. 1: 159-162, 1978.

Hedfors, E., Holm, G., Ivansen, M., and Wahren, J. Physiological variation of blood lymphocyte reactivity: T-cell subsets, immunoglobulin production, and mixed lymphocyte reactivity. Clin. Immunol. Immunopharmacol. 27: 9-14, 1983.

Hedfors, E., Holm, G., and Ohnell, B. Variations of blood lymphocytes during work studied by cell surface markers, DNA synthesis and cytotoxicity. Clin. Exper. Immunol. 24: 328-335, 1976.

Hefeneider, S.H., Conlon, P.J., Henney, C.S., and Gillis, S. In vivo Interleukin-2 administration augments the generation of alloreactive cytolytic T lymphocytes and resident natural killer cells. J. Immunol. 130: 222-227, 1983.

Heilman, D.H., Gambrill, M.R., and Leichner, J.P. The effect of hydrocortisone on the incorporation of tritiated thymidine by human blood lymphocytes cultured with phytohaemagglutinin and pokeweed mitogen. Clin. Exp. Immunol. 15: 203-212, 1973.

Hoffman-Goetz, L., Simpson, J.R., Cipp, N., Arumugam, Y., and Houston, M.E. Lymphocyte subset responses to repeated submaximal exercise in men. J. Appl. Physiol. 68: 1069-1074, 1990.

Hoffman-Goetz, L., Thorne, R.J., Simpson, J.A.R., and Arumugam, Y. Exercise stress alters murine lymphocyte subset distribution in spleen, lymph nodes and thymus. Clin Exp. Immunol. 76: 307-310, 1989.

Housh, T.J., Johnson, G.O., Housh, D.J., Evans, S.L., and Tharp, G.D. The effect of exercise at various temperatures on salivary levels of immunoglobulin A. Int. J. Sports. Med., 12(5): 498-500, 1991.

Houston, H.A. The Effect of Acute and Chronic Exercise on Immune Function in Man. M.Sc. Thesis, Department of Health Studies, University of Waterloo, Ontario, Canada, 1987.

Howlett, T.A., Tomlin, S., Ngahfoong, L., Rees, L.H., Bullen, B.A., Skrinar, G.S., and McArthur, J.W. Release of B-

endorphin and met-enkephalin during exercise in normal women: response to training. Br. Med. J. 288: 1950-1952, 1984.

Ilfeld, D.N., Krakauer, R.S., and Blaese, R.M. Suppression of the human autologous mixed lymphocyte reaction by physiologic concentrations of hydrocortisone. J. Immunol. 119: 428-434, 1977.

Israel, S., Buhl, B., Krause, M., and Neumann, G. Die Konzentration der immunoglouline A,G und M im serum bie trainierten und untrainerten sowie nach verschiedenen sportlicken Ausdauerleistungen. Med. und Sport 22: 225-231,1982, as cited in Nieman and Nehlsen-Cannarella, 1991.

Johanssen C. Individually programmed training and prevention of injuries in elite orienteers. Abstracts of the XXIII FIMS World Congress of Sports Medicine. 1986, as cited in Fry et al., 1991.

Jokl, E. The immunological status of athletes. J. Sports Med. 14: 165-172, 1974.

Kanonchoff, A.D., Cavanaugh, D.J., Mehl, V.L., Bartels, R.L., and Penn, G.M. Changes in lymphocyte subpopulations during acute exercise. (Abs.) Med. Sci. Sport Ex. 16: 175, 1984.

Kappel, M., Tvede, N., Galbo H., Haahr, P.M., Kjaer, M., et al. Epinephrine can account for the effect of physical exercise on natural killer cell activity J. Appl. Physiol., 70(6): 2530-2534, 1991.

Katz, P. and Fauci, A.S. Autologous and allogeneic intercellular interactions: modulation by inherent cells, irradiation and in vitro and in vivo corticosteroids. J. Immunol. 123: 2270-2277, 1979.

Keast, D. A simple runting index for the measurement of the runting syndrome and its use in the study of the influence of the gut flora in its production. Immunology 15: 237-245, 1968.

Keast, D. The urine runting syndrome and neoplasia. Immunology 16: 693-697, 1969.

Keast, D., Cameron, K., and Morton, A.R. Exercise and the immune response. Sports Med. 5: 248-267, 1988.

Keller, R.H., and Calvanico, N.J. Suppressor macromolecules. CRC Critical Reviews in Immunology. 5:149-199, 1982.

Keller, S.E., Weiss, J.M., Schleifer, S.J., Miller, N.E., and Stein, M. Suppression of immunity by stress: Effect of a graded series of stressors on lymphocyte stimulation in the

rat. Science 213: 1397-1400, 1981.

Kendall, A., Hoffman-Goetz, L., Houston, M., MacNeil, B., Arumugam, Y. Exercise and blood lymphocyte subset responses: intensity, duration and subject fitness effects. J. Appl. Physiol. 69(1): 251-260, 1990.

Khan, M.M., Easoni, P. Silverman, E.D., Engelman, E.G., and Melmon, K.L. β-Adrenergic receptors on human suppressor, helper and cytotoxic lymphocytes. Biochem. Pharmacol. 35: 1137-1142, 1986.

Kimball, J.W. Introduction to Immunology, 2nd Ed. MacMillan publishing Co. New York, p 499, 1986.

Kinderman, W. Das Uebertraining - Ausdruk einer vegetativen Fehlsteuerung. Deutsche Zeitschrift fur Sportmedizin. H8: 138-145, 1986 as cited in Kuipers and Keizer, 1988.

Kindermann, W., Shanabel, A., Schmitt, W.M., Biro, G., Cassens, J., and Weber, F. Catecholamines, growth hormone, cortisol, insulin, and sex hormones in anaerobic and aerobic exercise. Eur. J. Appl. Physiol. 49: 389-399, 1982.

Kinderman, W., Urhausen, A., Ricken, K.H. Einfluss von mehrtagigen intensivem training nachfolgender regeneration

lymphozyten subpopulationen. Dtsch. Z fur Sportmedizin. 40(11): 30-41, 1990 as cited in Shephard et al., 1991.

Kono, I., Matsuda, H.K.M., Haga, S., Fukushima, H., and Kashiwagi, H. Weight reduction in athletes may adversely affect the phagocytic function of monocytes. Phys. Sportsmed. 16(7): 56-65, 1988.

Kuipers, H. and Keizer, H.A. Overtraining in elite athletes: Review and directions for the future. Sports Med. 6: 79-92, 1988.

Kuipers, H. Physiological mechanism involved in adaptation: training and overtraining. - presented at Symposium "Overtraining Physiological and Psychological Effects of a Sudden Increase in Training". Med. Sci. Sports Ex. 19(2): S77, 1987.

Landmann, R.M.A., Muller, F.B., Perini, C.H., Wesp, M., Erne, P., and Buhler, F.R. Changes of immunoregulatory cells induced by psychological and physical stress: relationship to catecholamines. Clin. Exp. Immunol. 58: 127-135, 1984.

Lagenfeld, M.E., Hart, L.S., and Kao, P.C. Plasma B-endorphin responses to one-hour bicycling and running at 60% VO2 max. Med. Sci. Sports Exerc. 19: 83-86, 1987.

LaPerriere, A., Fletcher, M.A., Antoni, M.H., Klimas, N.G., and Schneiderman, N. Aerobic exercise training in an AIDS risk group. Int. J. Sports Med. 12: S53-S57, 1991.

Lewicki, R., Tchorzewski, H., Majewska, E., Nowak, Z., and Baj, Z. Effect of maximal physical exercise on T-lymphocyte subpopulations and on interleukin-1 (IL-1) and interleukin-2 (IL-2) production in vitro. Int. J. Sports Med. 9: 114-117, 1988.

Lewis, D.E., Gilbert, B.E., and Knight, V. Influenza virus infection induces functional alterations in peripheral blood lymphocytes. J. Immunol. 137: 3777-3781, 1986.

Liesen, H., Fisher, W., Dufaux, B. and Hollmann, W. Effects of endurance training on changes in serum-glycoproteins and immunoglobulins in 55 to 70 year-old men. Proc. Int. Congress Phys. Activity Sci., Quebec: 189, 1976.

Lydyard, P., and Grossi, C. Cells involved in the immune response. In: Roit et al. (Eds) Immunology 2nd ed., Grower Medical Publishing, New York, 1989.

MacKinnon, L.T., Chick, T.W., van As, A., and Tomasi, T.B. The effect of exercise on secretory and natural immunity. Adv. Exp. Med. Biol. 216A: 869-876, 1987.

MacKinnon, L.T., and Tomasi, T.B. Immunology of exercise. Ann. Sports Med. 3(1): 1-4, 1986.

MacKinnon, L.T., and Tomasi, T.B. Immunology of exercise. In: Appenzeller, O. and Atkinson, R.A., (eds.), Sports Medicine (Fitness, Training, Injuries) (3rd ed.). pp. 273-289. Baltimore: Urban and Schwarzenberg, 1989.

Masuhara, M., Kami, K., Umobayasi, K., and Tatsumi, N. Influences of exercise on leucocyte count and size. J. Sports Med. Phys. Fitness 27: 285-290, 1987.

Mathur, D.N., Toriola, A.L., and Dada, O.A. Serum cortisol and testosterone levels in conditioned male distance runners and non- athletes after maximal exercise. J. Sports Med. 26: 245-250, 1986.

Mayall, B. H. Cytometry in the Clinical Laboratory: Quo Vadis? In: Clinical Cytometry edited by M. Andreef. Annals of the New York Academy of Sciences Vol 468., 1986.

McCarthy, D.A., and Dale, M.M. The leucocytosis of exercise: a review and model. Sports Med. 6: 333-363, 1988.

McDowell, S.L., Chaloa, K., Housh, T.J., Tharp, G.D., and Johnson, G.O. The effect of exercise intensity and duration

on salivary immunoglobulin A. Eur. J. Appl. Physiol. 63: 108-111, 1991.

Mehrishi, J.N., and Mills, I.H. Opiate receptors on lymphocytes and platelets in man. Clin. Immunol. Immunopathol. 27: 240-249, 1983.

Mishler J.M. and Sharp, A.A. Adrenaline: further discussion of its role in mobilization of neutrophils. Scand. J. Haematol. 17: 78-80, 1976.

Monjan A.A., and Collector, M.I. Stress-induced modulation of the immmune response. Science 196: 307-308, 1977.

Moorthy, A.V. and Zimmerman, S.W. Human leucocyte response to an endurance race. Eur. J. Appl. Physiol. 38: 271-276, 1978.

Morse, L.J., Bryan, J.A. and Murie, J.P. Holy Cross football team hepatitis outbreak. J. Amer. Med. Assoc. 219: 706-708, 1972.

Muir, A.L., Cruz, M., and Martin, B.A. Leucocyte kinetics in the human lung: role of exercise and catecholamines. J. Appl. Physiol. 57: 711-719, 1984.

Nash, H.L. Can exercise make us immune to disease? Phys. and Sportsmed. 14(3): 250-253, 1986.

Ndon, J.A., Snyder, A.C., Foster, C., and Wehrenberg, W.B. Effects of chronic intense exercise training on the leukocyte response to acute exercise. Int. J. Sports Med. 13(2), 176-182, 1992.

Nehlsen-Cannarella, S.L., Nieman, D.C., Balk-Lamberton, A.J., Markoff, P.A., Chritton, D.B.W., Gusewitch, G., and Lee, J.W. The effects of moderate exercise training on immune response. Med. Sci. Sports Ex. 23(1), 64-70, 1991.

Neifield, J.P., and Tormey, D.C. Effects of steroid hormones on phytohaemaglutinin stimulated human peripheral blood lymphocytes. Transplantation 27: 309-314, 1979.

Newsholme, E.A. Psychoimmunology and cellular nutrition: an alternative hypothesis. Biol. Psychiatry 27: 1-3, 1990.

Nguyen, T.B., Bradley, J., and Roberts-Thompson, P. The effects of physical exercise on circulating white cells and lymphocyte subpopulations. Programme and Abstracts of the 14th Annual Meeting of the Australian Society for Immunology 100, 1984, as cited in Keast et al., 1988.

Nieman, D.C., and Nehlsen-Cannarella, S.L. The effects of acute and chronic exercise on immunoglobulins. Sports Med. 11(3): 183-201, 1991.

Nieman, D.C., Berk, L.S., Simpson-Westerberg, M., Arabatzis, K., Youngberg, W.S., Tan, S.A., Lee, J.W., and Eby, W.C. Effects of long endurance running on immune system parameters and lymphocyte function in experienced marathoners. Int. J. Sports Med. 10: 317-323, 1989.

Nieman, D.C., Henson, D.A., Johnson, R., LeBeck, L., Davis, J.M., and Nehlsen-Cannarella, S.L. Effects of brief, heavy exertion on circulating lymphocyte subpopulations and proliferative response. Med. Sci. Sports Exerc. 24(12), 1339-1345, 1992.

Nieman, D.C., Johansen, L.M., Lee, L.W., and Arabutzis, K. Infectious episodes in runners before and after the Los Angeles Marathon. Med. Sci. Sports Ex. 20: S42, 1988.

Nowell, P.C. Inhibition of human leucocyte mitosis by prednisolone in vitro. Cancer Res. 21: 1518-1521, 1961.

Oshida, Y., Yamanouchi, K., Hayamizu, S., and Sato, Y. Effect of acute physical exercise on lymphocyte subpopulations in trained and untrained subjects. Int. J. Sports Med. 9: 137-

140, 1988.

Parry-Billings, M., Budgett, R., Koutedakis, Y., Blumstrand, E., Brooks, S., Williams, C., Calder, P.C., Pilling, S., Baigrie, R., Newsholme, E.A. Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. Med. Sci. Sports Exerc., 24(12), 1353-1358, 1992.

Parry-Billings, M., Evans, J., Calder, P.C. and Newsholme, E.A. Does glutamine contribute to immunosuppression after burns? Lancet, 336: 523-525, 1990.

Parving, H.H., Nielsen, S.L., and Lassen, N.A. Increased trans-capillary escape rate of albumin, IgG and IgM during Angiotensin II induced hypertension in man. Scan. J. Clin. Lab. Invest. 34: 111-118, 1974.

Pechar, G.S., McArdle, W.D., Katch, F.I., Magel, J.R., and DeLuca, J. Specificity of cardiorespiratory adaptation in bicycle and treadmill training. J. Appl. Physiol. 36(6): 753-756, 1974.

Pedersen, B.K. Influence of physical activity on the cellular immune system: Mechanisms of action. Int. J. Sports Med. 12(1), S23-S29, 1991.

Pedersen, B.K., Tvede, N., Christensen, L.D., Klarlund, K., Kragbak, S., and Halkjaer-Kristensen, J. Natural killer cell activity in peripheral blood of highly trained and untrained persons. Int. J. Sports Med. 10: 129-131, 1989.

Pedersen, B.K., Tvede, N., Hansen, F.R., Andersen, V., Bendix, T., Bendixen, G., Bentzen, K., Galbo, H., Haahr, P.M., Klarlund, K., Sylvest, J., Thomsen, B.S., and Halkjaer-Kristensen, J. Modulation of natural killer cell activity in peripheral blood by exercise. Scand. J. Immunol. 27: 673-678, 1988.

Peters, E.M., and Bateman, E.D. Respiratory tract infections: An epidemiological survey. S. Afr. Med J. 64: 582-584, 1983, as cited in Keast et al., 1988.

Plaut, M. Lymphocyte hormone receptors. Ann. Rev. Immunol. 5: 621-669, 1987.

Playfair, J.H.L. Immunology at a Glance. 5th Ed. Blackwell Scientific Publications, Oxford, 1992.

Poortmans, J.R. Serum protein determination during short exhaustive physical activity. J. Appl. Physiol. 30: 190-192, 1970.

Poortmans, J.R., and Haralambie, G. Biochemical changes in a 100 km. run: Proteins in serum and urine. Eur. J. Appl. Physiol. 40: 245-254, 1979.

Portz, W. Running from infection. Running World. 19: 78-79, 1984.

Rahkila, P., Hakala, E., Salminen, K., and Laatikainen, T. Response of plasma endorphins to running exercise in male and female endurance athletes. Med. Sci. Sports Exerc. 19: 451-455, 1987.

Reid, R.A., Fricker, P.A., Kestermann, O., and Shakespear, P. A profile of female rowers injuries and illnesses at the Australian Institute of Sport. Excel 5: 17-20, June 1989, as cited in Fry et al., 1991.

Reilly, T., and Rothwell, J. Correlates of injury and illness in female distance runners. British Association of Sport and Medicine Congress. University of Liverpool, 1987, as cited in Fry et al., 1991.

Reynolds, H.Y. Identification and role of immunoglobulins in respiratory secretions. Eur. J. Resp. Diseases. 71(Suppl.): 103-116, 1987.

Ricken, K.-H., Rieder, T., Hauck, G., and Kindermann. Changes in lymphocyte subpopulations after prolonged exercise. Int. J. Sports Med., 11(2), 132-135, 1990.

Robertson, A.J., Ramesar, K.C., Potts, R.C., Gibbs, J.H., Browning, M.C., Brown, R.A., Hayes, P.C., and Swanson-Buck, J. The effect of strenuous physical exercise on circulating blood lymphocytes and serum cortisol levels. J. Clin. Lab. Immunol 5: 53-57, 1981.

Rocha, B., Lembezat, M.P., Freitas, A., and Banderia, A. Interleukin-2 receptor expression and interleukin -2 production in exponentially growing T-cells: Major differences between in vivo and in vitro proliferating T lymphocytes. Eur. J. Immunol. 19: 1137-1145, 1989.

Rocker, L., Kirsch, K.A., and Stoboy, H. Plasma volume, albumin and globulin concentrations and their intravascular masses. A comparative study in endurance athletes and sedentary subjects . Eur. J. Appl. Physiol. 36: 57-64, 1976.

Roit, I.M. Essential Immunology, Blackwell Scientific Publications, Osney Mead, Oxford, 1988.

Ryan, A.J. Overtraining in athletes: a round table. Phys. and Sportsmed. 11: 93-110, 1983.

Saxon, A., Stevens, R.H., Ramer, S.L., Clements, P.J., and Yu, D.Y. Glucocorticoids administered in vivo inhibit human suppressor T-lymphocyte function and diminish B-lymphocyte responsiveness in vitro immunoglobulin synthesis. J. Clin. Invest. 61: 922-930, 1978.

Schaeffer, R. M. Kokot, K., Heidland, A., and Plass, R. Jogger's leucocytes. N. Eng. J. Med. 316: 223-224, 1987.

Schwarz, L., and Kindermann, W. B-endorphin, catecholamines and cortisol during exhaustive endurance exercise. Int. J. Sports Med. 10(5), 324-328, 1989.

Selye, H. The Stress of Life. New York: McGraw Hill. 1956.

Shapiro, H.M. Multistation multiparameter flow cytometry: A critical review and rationale. Cytometry, 3(4):227-243, 1983.

Shapiro, H.M. The little laser that could: Applications of low power lasers in clinical flow cytometry. In: Clin. Cytometry M. Andreef. Annals of the New York Academy of Sciences. Vol 468, 1986.

Shephard, R.J., Verde, T.J., Thomas, S.G., and Shek, P. Physical activity and the immune system. Can J. Sport Sci.

16:3, 163-185, 1991.

Shephard, R.J. and Sidney, K.H. Effects of physical exercise on plasma growth hormone and cortisol levels in human subjects. Ex. Sport Sci. Rev. 3: 1-30, 1975.

Shimokata, K., Totani, Y., Tsunekawa, H., Sason, H., and Miyachi, T. Serologic study of febrile upper respiratory tract infections in adults. Jap. J. Med. 27: 286-290, 1988.

Shinkai, S., Shore, S., Skek, P.N., Shephard, R.J. Acute exercise and immune function - relationship between lymphocyte activity and changes in subset counts. Int. J. Sports Med. 13(6), 452-461, 1992.

Simon, H.B. The immunology of exercise: A brief review. J. Am. Med. Assoc. 252(19): 2735-2738, 1984.

Simon, H.B. Exercise and infection. Physician Sportsmed. 15(10): 134-141, 1987.

Smith, K.A., Crabtree, G.R., Kenned, S.J., and Munck, A.V. Glucocorticoid receptors and glucocorticoid sensitivity of mitogen stimulated and unstimulated human lymphocytes. Nature 267: 523-526, 1977.

Snyder, D., and Unanue, E.R., Corticosteroids inhibit murine macrophage: Ia expression and interleukin I production. J. Immunol. 129: 1803-1807, 1982.

Solomon, G.F., Amkraft, A.A., Kasper, P. Immunity, emotions and stress. Ann. Clin. Res. 6: 313-322, 1974.

Soppi, E., Varjo, P., Eskola, J., and Laitinen, P.A. Effect of strenuous physical stress on circulating lymphocyte function before and after training. J. Clin. Lab. Immunol. 8: 43-46, 1982.

Steel, C.M., Evans, J., and Smith, M.A. Physiological variation in circulating B-cell: T-cell ratio in man. Nature 247: 387-388, 1964.

Steel, C.M., French, E.B., and Aitchison, W.R.C. Studies on adrenaline-induced leucocytosis in normal man. I. The role of the spleen and the thoracic duct. Br. J. Haematol. 21: 413-421,1971.

Stephenson, L.A., Kolka, M.A., and Wilkerson, J.E. Effect of exercise and passive heat exposure on immunoglobulin and leucocyte concentrations. In: Exercise Physiology: Current Selected Research, C.O. Dotson and J.H. Humphrey (Eds.). New York: AMS Press, Inc., pp. 145-157, 1985.

Stites, D.P., Stobo, J.D., and Wells, J.V. Basic and Clinical Immunology 6th Ed., Appleton and Lange, Prentice-Hall, Norwalk, Connecticut, 1987.

Szondy, Z. and Newsholme, E.A. The effect of glutamine concentration on the activity of carbamoyl-phosphate synthase II and on the incorporation of [3H]-thymidine into DNA in rat mesentaric lymphocytes stimulated by phytohaemagluttinin. Biochem J. 261: 979-983, 1989.

Tharp, G.P., and Barnes, M. Reduction of immunoglobulin-A by swim training. Eur. J. Appl. Physiol. 60: 61-64, 1990.

Tharp, G. Basketball exercise and secretory immunoglobulin-A. Med. Sci. Sports Exerc. 22(2): S125 (Abs.), 1990.

Tomasi, T.B., Trudeau, F.B., Czerwinski, D., and Erredge, S. Immune parameters in athletes before and after strenuous exercise. J. Clin. Immunol. 2: 173-178, 1982.

Turgeon, M.L. Immunology and Serology in Laboratory Medicine by the C.V. Mosby Company, St. Louis, Missouri, USA, 1990.

Tvede, N., Pedersen, N.K., Hansen, F.R., Bendix, T., Christensen, L.D., Galbo, H., and Halkjaer-Kristensen, J. Effect of physical exercise on blood mononuclear cell

subpopulations and in vitro proliferative responses. Scand. J. Immunol. 29: 383-389, 1989.

VanHelder, W.P., Radonski, M.W., Goode, R.C., and Casey, K. Hormonal and metabolic response to three types of exercise of equal duration and external work output. Eur. J. Appl. Physiol. 54: 337-342, 1985.

Verde, T.J. The effects of acute exercise and heavy training on immune function in elite athletes. University of Toronto Graduate Dept. of Community Health, Ph.D. Dissertation, 1990.

Verde, T.J., Thomas, S.G., Moore, R.W., Shek, P., and Shephard, R.J. Immune responses and increased training of the elite athlete. J. Appl. Physiol. 73(4): 1494-1499, 1992.

Verde, T.J., Thomas, S.G. and Shephard R.J. Effects of acute exercise and heavy training on immune function in elite athletes. Med. Sci. Sports Exerc. 22(2):(Abs.) S125-S125, 1990.

Vishnu-Moorthy, A., and Zimmerman, S.W. Human leucocyte response to an endurance race. Eur. J. Appl. Physiol. 38: 271-276, 1978.

Watson, J. The involvement of cyclic nucleotide metabolism in

the initiation of lymphocyte proliferation induced by mitogens. J. Immunol. 117: 1656-1663, 1976.

Watson, R.R., Moriguchi, S., Jackson, J.C., Werner, l., Wilmore, J.H., and Freund, B.J. Modification of cellular immune functions in humans by endurance training during B-adrenergic blockade with atenolol or propanolol. Med. Sci. Sports Ex. 18: 95-100, 1986.

Webel, M.L., and Ritts, R.E. The effects of corticosteroid concentrations on lymphocyte blastogenesis. Cellular Immunol. 32: 287-292, 1977.

Weinstein, L. Poliomyelitis- a persistent problem. N. Engl. J. Med. 288: 370-372, 1973.

Welliver, R.C., and Ogra, P.L. Immunology of respiratory viral infections. Ann. Rev. Med. 39: 147-162, 1988.

Wells, C.L., Stern, J.R., and Hecht, L.H. Hematological changes following a marathon race in male and female runners. Eur. J. Appl. Physiol. 48: 41-49, 1982.

Wybran, J., Appelboom, T., Farmaey, J.P., and Govaerts, A. Suggestive evidence for receptors for morphine and methionine-enkephalin on normal and human blood T lymphocytes. J.

Immunol. 123: 1068-1070, 1979.

Yakovlev, N.N, and Viru, A.A. Adrenergic regulation of adaptation to muscular activity. Int. J. Sports Med. 6: 255-265, 1985.

Yu, D.T.Y., Clements, P.J., Paulus, H.E., Peter, J.B., Levy, J., and Barnett, E.V. Human lymphocyte subpopulations. Effect of corticosteroids. J. Clin. Invest. 53: 565-571, 1974.

Yu, D.T.Y., Clements, P.J., and Pearson, C.M. Effects of corticosteroids on exercise-induced lymphocytosis. Clin. Exp. Immunol. 28: 326-331, 1977.

APPENDICES

APPENDIX I

CD4+ ACROSS TRAINING PHASES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	15.170	1	15.170	.071	
ERROR	2151.609	10	215.161		
WITHIN BLOCKS/SUBJECTS					
PHASE	558.922	3	186.307	7.273	.001
GROUP PHASE	62.695	3	20.898	.816	
ERROR	768.548	30	25.618		
DAY	63.652	1	63.652	3.491	.088
GROUP DAY	.319	1	.319	.017	
ERROR	182.312	10	18.231		
PHASE DAY	90.857	3	30.286	2.395	.087
GROUP PHASE DAY	42.545	3	14.182	1.121	.356
ERROR	379.369	30	12.646		
PRE/PO	2354.170	1	2354.170	54.184	<.001
GROUP PRE/PO	22.860	1	22.860	.526	
ERROR	434.477	10	43.448		
PHASE PRE/PO	100.269	3	33.423	4.836	.007
GROUP PHASE PRE/PO	20.084	3	6.695	.969	
ERROR	207.366	30	6.912		
DAY PRE/PO	7.415	1	7.415	.981	
GROUP DAY PRE/PO	40.379	1	40.379	5.345	.041
ERROR	75.555	10	7.555		
PHASE DAY PRE/PO	73.146	3	24.382	3.173	.037
GROUP PHASE DAY PRE/PO	16.701	3	5.567	.725	
ERROR	230.504	30	7.683		
TOTAL	7898.924	191			
(RESIDUAL)	2278.131	150			

CD4/CD8 ACROSS TRAINING PHASES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	.379	1	.379	.234	
ERROR	16.171	10	1.617		
WITHIN BLOCKS/SUBJECTS					
PHASE	.582	3	.194	2.553	.073
GROUP PHASE	.711	3	.237	3.118	.040
ERROR	2.288	30	.076		
DAY	.338	1	.338	7.042	.023
GROUP DAY	.147	1	.147	3.063	.108
ERROR	.476	10	.048		
PHASE DAY	1.123	3	.374	5.268	.005
GROUP PHASE DAY	.474	3	.158	2.225	.104
ERROR	2.143	30	.071		
PRE/PO	5.034	1	5.034	47.491	<.001
GROUP PRE/PO	.129	1	.129	1.217	.296
ERROR	1.059	10	.106		
PHASE PRE/PO	.768	3	.256	8.000	<.001
GROUP PHASE PRE/PO	.117	3	.039	1.219	.319
ERROR	.950	30	.032		
DAY PRE/PO	3.79264355E-04.000	1	0.000		
GROUP DAY PRE/PO	.125	1	.125	7.353	.021
ERROR	.171	10	.017		
PHASE DAY PRE/PO	.134	3	.045	1.406	.259
GROUP PHASE DAY PRE/PO	.096	3	.032	1.000	
ERROR	.949	30	.032		
TOTAL	34.362	191			
(RESIDUAL)	8.035	150			

CD3+ ACROSS TRAINING PHASES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	55.718	1	55.718	.248	
ERROR	2250.227	10	225.023		
WITHIN BLOCKS/SUBJECTS					
PHASE	1351.457	3	450.486	9.681	<.001
GROUP PHASE	353.967	3	117.989	2.536	.074
ERROR	1395.932	30	46.531		
DAY	5.185	1	5.185	.087	
GROUP DAY	3.088	1	3.088	.052	
ERROR	596.085	10	59.608		
PHASE DAY	643.163	3	214.388	3.275	.034
GROUP PHASE DAY	1101.523	3	367.174	5.610	.003
ERROR	1963.621	30	65.454		
PRE/PO	1034.118	1	1034.118	30.773	<.001
GROUP PRE/PO	16.316	1	16.316	.486	
ERROR	336.055	10	33.605		
PHASE PRE/PO	263.831	3	87.944	4.265	.012
GROUP PHASE PRE/PO	71.151	3	23.717	1.150	.345
ERROR	618.646	30	20.622		
DAY PRE/PO	21.554	1	21.554	.881	
GROUP DAY PRE/PO	1.453	1	1.453	.059	
ERROR	244.602	10	24.460		
PHASE DAY PRE/PO	19.618	3	6.539	.353	
GROUP PHASE DAY PRE/PO	9.405	3	3.135	.169	
ERROR	556.478	30	18.549		
TOTAL	12913.192	191			
(RESIDUAL)	5711.419	150			

NORMALIZED TREADMILL TIMES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	1810.360	1	1810.360	3.887	.074
ERROR	4657.710	10	465.771		
WITHIN BLOCKS/SUBJECTS					
PHASE	1527.264	2	763.632	13.554	<.001
GROUP PHASE	380.640	2	190.320	3.378	.053
ERROR	1126.846	20	56.342		
TOTAL	9502.820	35			

	P2	P3	P4	
G1	6	6	6	103.82
	102.17	97.69	111.59	
	5.11	11.62	5.21	
	130.36	674.69	135.49	
G2	6	6	6	118.00
	125.44	106.08	122.48	
	19.94	15.51	18.18	
	1988.21	1203.25	1652.56	
	113.80	101.89	117.03	

TREADMILL TIMES ACROSS PHASES

SOURCE	SS	DF	MS	F	P
BETWEEN BLOCKS/SUBJECTS					
GROUP	517.582	1	517.582	.042	
ERROR	121847.246	10	12184.725		
WITHIN BLOCKS/SUBJECTS					
PHASE	13550.386	3	4516.795	10.206	<.001
GROUP PHASE	4434.215	3	1478.072	3.340	.031
ERROR	13277.274	30	442.576		
TOTAL	153626.704	47			

	P1	P2	P3	P4	
G1	6	6	6	6	
	242.89	247.41	229.40	264.99	246.17
	44.89	44.01	27.74	35.01	
	10073.40	9686.47	3848.54	6128.91	
G2	6	6	6	6	
	220.75	279.45	238.07	272.69	252.74
	44.62	82.90	77.20	79.08	
	9956.09	34364.83	29799.16	31267.11	
	P1	P4	P3	P2	
	231.82	263.43	233.73	268.84	

$q @_{.05,30} = 2.89$

$q @_{.01,30} = 3.84$

$$cv = q\sqrt{\frac{442.576}{6}}$$

$$= 2.89\sqrt{73.76}$$

$$= 24.8$$

G1 P1 P2 P3 P4
G2 P1 P4 P3 P2

APPENDIX II

TABLE 3. FLOW CYTOMETRY DATA - MEAN CD4+, CD8+ AND CD4/CD8 RATIO FOR GROUPS #1 AND #2.

	P1					P2					P3					P4				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Group #1.																				
CD4	42.43	37.98	43.36	41.53	36.16	42.44	33.17	39.95	43.89	38.69	43.88	39.57	43.85	44.37	39.57	42.97	30.50	45.00	41.62	36.98
	±2.56	±1.45	±2.42	±1.71	±2.05	±1.20	±1.82	±2.98	±1.45	±1.46	±1.52	±1.94	±1.91	±1.76	±1.73	±2.06	±1.72	±1.95	±2.29	±2.06
CD8	23.66	24.01	22.27	22.17	23.31	24.38	25.65	24.40	26.92	26.44	25.24	22.64	26.55	25.11	24.47	25.07	25.75	25.38	20.38	20.89
	±1.86	±1.64	±1.50	±1.55	±1.96	±1.50	±1.52	±2.70	±1.73	±2.39	±2.25	±1.58	±1.07	±1.32	±1.65	±1.44	±1.25	±0.93	±2.16	±2.60
RATIO	1.83	1.61	1.99	1.93	1.61	1.78	1.33	1.69	1.66	1.57	1.81	1.71	1.66	1.79	1.65	1.73	1.19	1.79	2.12	1.79
	±0.12	±0.10	±0.16	±0.18	±0.16	±0.13	±0.15	±0.11	±0.11	±0.06	±0.18	±0.13	±0.06	±0.09	±0.11	±0.10	±0.05	±0.09	±0.15	±0.22
Group #2.																				
CD4	44.40	38.42	45.26	44.20	36.66	41.68	30.75	43.78	42.01	32.42	43.93	37.98	44.32	48.17	39.57	43.38	37.56	42.01	45.38	38.24
	±2.48	±3.14	±2.42	±2.77	±3.23	±1.74	±1.94	±2.03	±2.99	±2.18	±1.89	±2.05	±2.52	±2.38	±2.76	±1.97	±1.87	±2.74	±2.24	±2.44
CD8	22.46	21.29	22.74	24.63	24.97	23.47	25.01	23.18	21.67	24.44	22.80	22.90	24.36	23.14	23.20	23.10	23.26	23.97	22.91	22.73
	±1.39	±1.08	±1.31	±1.69	±1.70	±1.72	±1.34	±1.64	±2.11	±1.11	±1.40	±0.90	±1.32	±1.06	±1.10	±1.07	±1.14	±1.45	±1.33	±1.41
RATIO	2.03	1.83	2.04	1.87	1.53	1.82	1.25	1.95	2.06	1.34	1.97	1.68	1.85	2.07	1.74	1.91	1.65	1.82	2.04	1.74
	±0.18	±0.19	±0.19	±0.24	±0.24	±0.15	±0.13	±0.18	±0.27	±0.10	±0.16	±0.14	±0.15	±0.15	±0.17	±0.15	±0.14	±0.24	±0.19	±0.20

Values are means ± SE

TABLE 4. DIFFERENCES IN PRE AND POST-EXERCISE VALUES FOR CD4+, CD8+ AND CD4/CD8 RATIO FOR GROUPS 1 AND 2.

Group 1.

	P1		P2		P3		P4	
	1	7	1	7	1	7	1	7
CD4 (pre-post)								
S1	6.74	4.75	8.45	8.29	5.9	7.88	14.59	7.37
S2	10.26	10.23	15.69	9.43	6.33	11.18	11.66	12.44
S3	0.90	0.64	-0.32	0.20	-1.47	2.45	8.91	4.01
S4	-0.15	5.04	5.83	0.62	4.29	2.13	24.78	-2.37
S5	5.75	9.61	14.07	10.37	8.22	3.01	8.37	1.70
S6	3.28	1.95	11.88	2.33	2.63	2.12	6.46	4.64
$\bar{X}$	4.46	5.37	9.27	5.21	4.32	4.80	12.46	4.63
	±1.59	±1.59	±2.42	±1.90	±1.39	±1.56	±2.72	±2.05
CD8 (pre-post).								
S1	0.66	-1.05	1.02	-4.22	0.09	2.32	3.05	0.94
S2	-4.32	0.79	-3.29	1.73	2.98	2.31	1.29	-0.86
S3	-1.93	-4.31	-1.09	2.30	5.71	-1.44	-3.5	-1.73
S4	-0.18	1.19	1.52	1.36	0.62	1.25	-1.07	-0.09
S5	3.04	-0.51	-5.05	0.24	-0.04	0.86	-2.93	1.08
S6	0.61	-2.92	-0.73	1.50	0.21	-1.45	-0.90	-2.41
$\bar{X}$	-0.35	-1.14	-1.27	0.49	1.60	0.64	-0.68	-0.51
	±1.03	±0.87	±1.03	±0.98	±0.94	±0.70	±1.02	±0.58
CD4/CD8 (pre-post).								
S1	0.21	0.24	0.26	0.00	0.20	0.14	0.36	0.25
S2	0.75	0.50	0.84	0.28	0.06	0.31	0.42	0.66
S3	0.25	0.40	0.06	-0.12	-0.35	0.19	0.51	0.30
S4	0.00	0.14	0.14	-0.08	0.15	0.02	1.11	-0.15
S5	0.00	0.44	0.96	0.44	0.47	0.07	0.60	0.61
S6	0.08	0.21	0.41	0.01	0.08	0.14	0.25	0.28
$\bar{X}$	0.22	0.32	0.45	0.09	0.10	0.15	0.54	0.33
	±0.11	±0.06	±0.15	±0.09	±0.11	±0.04	±0.12	±0.12

Group 2.

	P1		P2		P3		P4	
	1	7	1	7	1	7	1	7
CD4 (pre-post)								
S1	1.47	1.06	6.65	5.83	0.42	1.07	4.28	5.01
S2	3.27	10.54	18.33	15.31	3.04	5.25	5.04	1.53
S3	6.78	10.51	14.20	6.36	12.38	6.64	4.55	9.07
S4	9.73	5.73	4.84	5.48	5.91	9.73	2.71	10.74
S5	11.10	12.91	12.09	17.68	8.49	18.43	9.69	11.25
S6	3.51	4.46	9.5	6.9	5.45	4.49	7.63	5.24
$\bar{X}$	5.98	7.54	10.94	9.59	5.95	7.60	5.65	7.14
	±1.58	±1.84	±2.04	±2.21	±1.70	±2.45	±1.04	±1.56
CD8 (pre-post).								
S1	0.97	-0.92	-1.48	-8.63	-3.73	1.02	0.63	1.07
S2	-0.11	-0.07	-3.16	-1.16	1.77	0.02	1.53	0.92
S3	-0.97	-2.06	-2.65	-3.77	-2.02	-1.94	-2.71	-3.05
S4	2.58	-3.11	-0.45	-2.32	0.05	-0.98	-1.14	0.68
S5	1.87	0.65	-3.38	-0.90	0.79	0.37	0.35	-0.43
S6	2.43	3.46	1.93	0.12	2.50	1.22	0.36	1.89
$\bar{X}$	1.13	-0.34	-1.54	-2.77	-0.11	-0.05	-0.16	0.18
	±0.59	±0.94	±0.83	±1.29	±0.96	±0.50	±0.62	±0.71
CD4/CD8 (pre-post).								
S1	-0.05	0.18	0.48	1.61	0.50	-0.07	0.14	0.14
S2	0.18	0.45	0.97	0.75	-0.02	0.23	0.10	0.00
S3	0.39	0.52	0.62	0.51	0.65	0.46	0.45	0.61
S4	0.28	0.31	0.22	0.30	0.20	0.44	0.16	0.38
S5	0.36	0.54	0.89	0.91	0.32	0.85	0.42	0.56
S6	0.00	0.00	0.24	0.25	0.05	0.09	0.27	0.08
$\bar{X}$	0.19	0.33	0.57	0.72	0.28	0.33	0.26	0.30
	±0.08	±0.09	±0.13	±0.21	±0.11	±0.13	±0.06	±0.11

Values are means ± SE

TABLE 5. FLOW CYTOMETRY DATA - MEAN CD3+, HLA-DR+ AND CD3+HLA-DR+ PERCENTAGES FOR GROUPS #1 AND #2.

	P1					P2				
	1	2	3	4	5	1	2	3	4	5
Group #1.										
CD3+	78.89	75.66	81.07	66.03	64.78	62.37	53.07	63.16	70.58	62.33
	±2.80	±2.97	±4.09	±6.13	±5.26	±2.42	±2.02	±3.38	±2.62	±2.03
HLA-DR+	4.58	3.82	4.16	7.36	7.89	10.64	10.98	10.33	8.70	9.67
	±1.11	±0.89	±1.72	±1.77	±2.13	±1.25	±1.79	±1.68	±1.53	±0.96
CD3+HLA-DR+	9.52	7.34	9.58	7.98	6.30	6.06	6.29	12.61	8.38	6.28
	±2.04	±1.45	±1.70	±1.50	±1.65	±0.66	±1.11	±3.25	±1.71	±1.11
Group #2										
CD3+	71.65	70.73	73.31	73.33	73.47	70.71	60.22	70.43	69.68	63.65
	±2.11	±2.12	±2.68	±2.22	±1.58	±3.20	±3.09	±3.58	±3.37	±2.89
HLA-DR+	5.44	5.41	6.27	6.49	3.98	6.61	8.62	8.48	7.45	7.19
	±0.76	±0.48	±1.19	±0.97	±0.44	±1.40	±1.47	±1.72	±1.59	±1.67
CD3+HLA-DR+	8.96	7.93	10.62	9.21	9.77	9.92	5.38	8.09	10.10	8.75
	±1.23	±1.13	±1.72	±1.49	±1.30	±2.15	±1.09	±0.62	±1.25	±0.85

	P3					P4				
	1	2	3	4	5	1	2	3	4	5
Group #1.										
CD3+	68.47	63.35	77.50	74.94	71.29	71.58	64.96	71.98	66.68	62.32
	±1.28	±2.71	±2.59	±1.85	±1.67	±2.46	±4.55	±1.14	±4.00	±3.73
HLA-DR+	12.01	10.43	5.00	5.56	4.94	6.92	7.63	6.37	6.18	5.13
	±1.20	±0.64	±1.62	±0.78	±0.59	±1.15	±2.18	±0.91	±0.99	±0.58
CD3+HLA-DR+	5.41	5.91	10.67	11.20	9.24	13.90	7.75	11.21	11.44	10.36
	±1.01	±1.07	±0.74	±0.88	±0.80	±0.99	±1.03	±1.63	±1.93	±1.06
Group #2										
CD3+	69.55	67.37	71.77	68.60	65.34	69.67	65.04	73.52	70.30	65.21
	±2.04	±1.39	±2.77	±2.00	±1.65	±1.87	±1.69	±1.88	±2.56	±3.02
HLA-DR+	8.21	6.44	7.33	10.18	8.01	7.74	7.57	5.68	8.47	7.12
	±0.96	±0.90	±1.18	±1.31	±0.67	±1.14	±1.02	±0.87	±1.13	±1.07
CD3+HLA-DR+	8.19	8.79	9.46	7.23	6.08	9.09	7.46	9.81	8.54	6.39
	±1.14	±1.42	±1.60	±1.86	±1.76	±1.51	±1.48	±1.45	±1.50	±1.12

Values are means ± SE

TABLE 6. DIFFERENCES IN PRE AND POST-EXERCISE VALUES FOR CD3+, HLA-DR+ AND CD3+HLA-DR+ FOR GROUPS 1 AND 2.

	Group 1.								Group 2.							
	P1		P2		P3		P4		P1		P2		P3		P4	
	1	7	1	7	1	7	1	7	1	7	1	7	1	7	1	7
CD3+ (pre-post).																
S1	5.23	7.58	2.27	10.34	3.91	6.42	1.46	-14.0	0.40	-1.45	4.31	12.92	7.36	1.56	3.95	10.08
S2	8.40	3.80	12.08	5.37	5.96	4.59	18.89	4.74	3.92	4.21	14.80	10.42	-1.68	4.76	0.52	1.81
S3	0.59	-2.54	2.52	13.95	3.25	2.18	7.67	0.47	0.15	-2.71	25.14	7.38	6.44	5.51	3.71	4.66
S4	2.95	6.09	5.12	10.48	3.30	4.02	6.83	25.24	0.08	-2.74	6.74	-5.74	-3.96	-5.37	-1.27	6.63
S5	4.05	14.00	10.18	8.75	13.63	4.28	1.87	9.76	-0.21	-1.66	4.99	9.12	5.54	5.14	5.09	5.67
S6	-1.87	-21.42	23.64	0.61	0.69	0.46	3.03	-0.04	1.34	3.53	6.96	2.07	-0.64	7.97	15.79	1.73
$\bar{X}$	3.23	1.25	9.30	8.25	5.13	3.66	6.63	4.36	0.95	-0.14	10.49	6.03	2.18	3.26	4.63	5.10
	±1.46	±5.04	±3.30	±1.90	±1.83	±0.85	±2.67	±5.28	±0.63	±1.29	±3.31	±2.78	±1.97	±1.92	±2.43	±1.29
HLA-DR+ (pre-post).																
S1	-0.13	-2.74	-0.13	-2.53	1.03	0.00	1.35	-1.33	0.94	1.55	0.72	-1.19	-1.56	-1.77	-3.65	1.97
S2	1.69	0.34	-1.89	1.18	1.74	0.97	-6.43	3.10	0.27	0.39	0.20	-1.36	2.50	3.51	2.49	1.84
S3	1.00	3.90	-1.29	-9.04	1.61	0.36	-0.86	1.51	0.83	4.11	-8.08	-0.73	1.04	1.37	-0.02	0.00
S4	1.34	0.26	2.91	-0.79	-0.79	0.88	2.18	0.62	-0.31	3.32	-5.83	3.56	5.01	6.95	3.33	1.49
S5	-0.44	-0.75	1.41	2.17	0.96	-0.07	-0.13	1.06	0.05	5.75	0.79	0.78	0.32	3.36	0.91	1.53
S6	1.10	4.21	-3.07	3.17	4.87	1.56	-0.39	1.38	-1.61	-0.07	0.14	0.46	3.30	-0.41	-2.07	1.27
$\bar{X}$	0.76	0.87	-0.34	-0.97	-1.57	0.62	-0.71	1.06	0.03	2.51	-2.01	0.25	1.77	2.17	0.17	1.35
	±0.35	±1.11	±0.90	±1.82	±0.76	±0.26	±1.24	±0.59	±0.38	±0.93	±1.59	±0.75	±0.95	±1.28	±1.09	±0.29
CD3+HLA-DR+ (pre-post).																
S1	1.55	3.54	1.29	1.34	-0.98	1.60	5.96	-4.95	1.47	0.98	3.08	6.19	0.35	4.21	2.11	3.56
S2	1.20	2.80	1.93	-0.60	-1.39	2.98	11.11	0.17	2.10	2.21	5.87	3.66	1.52	-0.28	-0.55	-1.93
S3	1.56	-0.56	1.72	4.69	2.13	3.65	6.73	2.17	-0.22	-2.29	6.28	-3.38	-2.05	0.98	1.94	1.81
S4	2.28	1.30	-9.14	2.55	1.89	-0.70	3.38	4.35	2.23	-1.65	11.73	0.49	-3.39	0.38	2.21	4.64
S5	-0.32	3.54	1.91	1.34	-4.28	2.53	3.78	0.55	-0.09	-2.86	0.67	2.08	1.47	1.22	1.73	5.37
S6	6.82	-0.52	0.87	3.32	-0.41	1.70	5.86	4.19	0.65	0.29	-0.39	-0.91	-1.52	0.35	2.38	-0.53
$\bar{X}$	2.18	1.68	-0.24	2.11	-0.51	1.96	6.13	1.08	1.02	-0.55	4.54	1.36	-0.60	1.14	1.64	2.15
	±0.99	±0.78	±1.79	±0.75	±0.97	±0.62	±1.13	±1.40	±0.44	±0.82	±1.81	±1.38	±0.82	±0.65	±0.45	±1.19

Values are means ± SE

TABLE #7. MEAN ELISA IgG, IgA, AND IgM CONCENTRATIONS (g/l) FOR GROUP #1 AND #2.

	Group #1			Group #2		
	IgG	IgA	IgM	IgG	IgA	IgM
P1 1	8.87 ± 0.79	1.25 ± 0.21	1.16 ± 0.07	9.48 ± 0.59	1.51 ± 0.42	1.04 ± 0.29
2	9.32 ± 1.04	1.25 ± 0.20	1.07 ± 0.10	9.18 ± 0.35	1.83 ± 0.41	1.00 ± 0.24
3	9.00 ± 1.36	1.19 ± 0.31	1.14 ± 0.08	9.27 ± 0.59	1.41 ± 0.51	0.93 ± 0.30
4	9.42 ± 1.26	1.78 ± 0.48	1.14 ± 0.09	9.96 ± 0.27	1.88 ± 0.55	1.07 ± 0.26
5	10.12 ± 1.11	2.02 ± 0.49	1.09 ± 0.11	10.74 ± 0.68	1.80 ± 0.69	0.99 ± 0.25
P2 1	9.56 ± 1.63	1.66 ± 0.40	1.19 ± 0.09	9.67 ± 0.27	1.67 ± 0.45	1.01 ± 0.23
2	10.69 ± 1.87	1.61 ± 0.42	1.16 ± 0.09	10.09 ± 0.51	2.16 ± 0.39	1.15 ± 0.17
3	10.66 ± 2.00	1.93 ± 0.36	1.28 ± 0.13	9.61 ± 0.86	1.98 ± 0.41	1.31 ± 0.17
4	9.59 ± 1.82	1.74 ± 0.30	1.22 ± 0.13	9.05 ± 0.54	2.22 ± 0.39	1.10 ± 0.06
5	9.82 ± 1.50	1.90 ± 0.21	1.22 ± 0.14	9.91 ± 0.27	2.28 ± 0.47	1.14 ± 0.08
P3 1	9.42 ± 1.70	1.41 ± 0.31	1.27 ± 0.13	9.44 ± 0.29	1.55 ± 0.42	0.91 ± 0.20
2	9.78 ± 1.80	1.13 ± 0.22	1.15 ± 0.14	9.79 ± 0.26	1.70 ± 0.47	1.21 ± 0.27
3	9.66 ± 1.67	1.45 ± 0.22	1.19 ± 0.10	9.76 ± 0.43	2.16 ± 0.41	1.01 ± 0.23
4	9.75 ± 1.67	1.45 ± 0.30	1.29 ± 0.11	9.71 ± 0.58	1.94 ± 0.34	1.09 ± 0.22
5	9.96 ± 1.60	1.38 ± 0.35	1.26 ± 0.11	10.68 ± 0.72	1.93 ± 0.34	0.95 ± 0.19
P4 1	9.97 ± 1.49	1.30 ± 0.24	1.20 ± 0.10	10.12 ± 0.99	2.30 ± 0.35	1.09 ± 0.06
2	10.13 ± 1.61	1.59 ± 0.31	1.43 ± 0.37	10.58 ± 0.74	2.44 ± 0.33	1.21 ± 0.18
3	9.66 ± 1.31	1.04 ± 0.20	1.18 ± 0.11	9.52 ± 0.45	2.08 ± 0.50	1.12 ± 0.19
4	9.15 ± 1.25	1.10 ± 0.16	1.28 ± 0.22	9.80 ± 0.53	1.65 ± 0.27	1.23 ± 0.17
5	9.84 ± 1.30	1.77 ± 0.46	1.34 ± 0.28	10.08 ± 0.32	2.39 ± 0.57	1.37 ± 0.22

Values are means ± SE

TABLE 8. SUBJECT'S TREADMILL PERFORMANCE TEST TIME (s)

Group 1 (Training Order #1)	Training Phase P1 (LV/LI)	P2 (HV/LI)	P3 (LV/LI)	P4 (HV/HI)
S1	219.15	231.93	241.50	247.21
S2	242.12	256.37	246.40	279.28
S3	189.51	186.31	200.14	213.79
S4	317.52	321.53	270.60	318.54
S5	267.81	251.87	205.96	273.27
S6	221.23	236.47	211.79	257.85
$\bar{X}$	242.89	247.41	229.40	264.99
	±18.32	±17.97	±11.33	±14.29

Group 2 (Training Order #2)	P1 (LV/LI)	P2 (HV/HI)	P3 (LV/LI)	P4 (HV/LI)
S1	208.57	199.48	216.64	203.96
S2	300.90	392.06	363.54	396.46
S3	183.46	216.67	193.74	220.35
S4	191.90	209.58	150.37	210.36
S5	195.77	278.58	209.94	284.33
S6	243.92	339.79	294.19	361.21
$\bar{X}$	220.75	272.69	238.07	279.45
	±18.22	±32.28	±31.52	±33.85

Values are means ± SE

TABLE 9. NORMALIZED TREADMILL PERFORMANCE DATA
(As a percent improvement from P1)

Group 1 (Training Order #1)	Training Phase P1 (LV/LI)	P2 (HV/LI)	P3 (LV/LI)	P4 (HV/HI)
S1	100.00	105.83	110.20	112.80
S2	100.00	105.89	101.77	115.35
S3	100.00	98.31	105.61	112.81
S4	100.00	94.05	76.91	102.04
S5	100.00	106.89	95.73	116.55
S6	100.00	102.04	95.91	109.98
$\bar{X}$	100.00 ± 0.00	102.04 ± 2.09	95.91 ± 5.19	109.98 ± 2.85

Group 2 (Training Order #2)	P1 (LV/LI)	P2 (HV/HI)	P3 (LV/LI)	P4 (HV/LI)
S1	100.00	95.64	103.87	97.79
S2	100.00	130.30	120.82	131.76
S3	100.00	118.10	105.60	120.11
S4	100.00	109.21	78.36	109.62
S5	100.00	142.30	107.24	145.24
S6	100.00	139.30	120.61	148.09
$\bar{X}$	100.00 ± 0.00	122.48 ± 7.42	106.08 ± 6.33	125.44 ± 8.14

Values are means ± SE

TABLE 10. TREADMILL VO2 DATA FOR GROUPS 1 AND 2.

	VO2 (ml/kg/min)			
	P1	P2	P3	P4
Group 1				
S1 JR	66.03	63.93	68.38	67.85
S2 MTM	66.90	68.40	64.23	67.96
S3 CM	71.15	72.57	71.21	70.34
S4 KL	64.39	65.42	65.55	66.02
S5 JB	71.10	66.53	68.61	67.99
S6 MM	59.88	52.77	53.72	54.36
$\bar{X}$	66.58	64.94	65.28	65.75
$\pm$	4.28	6.66	6.18	5.75
Group 2				
S1 DK	66.56	61.68	67.73	70.06
S2 GB	65.29	55.45	70.37	64.74
S3 IB	61.74	62.52	61.17	59.99
S4 SC	53.65	54.81	51.07	52.28
S5 SR	55.83	60.83	54.15	57.47
S6 BA	57.70	55.75	53.92	55.29
$\bar{X}$	60.13	58.51	59.74	59.97
$\pm$	5.23	3.53	7.99	6.51

Values are means $\pm$ SE

TABLE 11. HEMATOCRIT VALUES (AVERAGED TRIPLICATES) FOR GROUPS 1 AND 2.

	P1					P2					P3					P4				
	D1		D4	D7		D1		D4	D7		D1		D4	D7		D1		D4	D7	
GROUP 1																				
1)	44.7	48.8	44.6	48.0	47.5	44.6	48.4	45.1	47.0	49.3	47.8	50.0	45.8	46.7	48.5	46.7	48.6	46.1	44.0	47.3
2)	49.8	50.6	45.1	46.8	48.2	42.7	46.4	44.2	43.8	46.0	47.9	50.0	46.2	46.8	47.9	44.6	45.3	46.1	48.5	48.1
3)	41.9	40.1	41.4	41.3	42.7	45.2	46.2	43.0	43.6	45.5	44.0	43.5	44.9	42.5	43.9	43.1	46.7	44.8	44.0	44.9
4)	44.0	45.5	43.9	44.6	43.8	43.2	45.1	46.7	43.1	45.2	46.5	46.6	44.7	44.4	45.3	43.5	46.5	46.6	44.9	48.2
5)	43.6	45.1	41.7	41.8	40.7	39.2	40.2	42.1	42.7	42.2	39.3	43.0	44.1	42.5	42.0	42.4	43.9	44.1	43.9	45.2
6)	39.0	39.6	46.3	40.2	41.7	37.9	42.5	41.2	41.1	45.4	42.3	43.6	41.3	41.5	42.4	42.5	44.8	45.9	45.8	46.3
$\bar{X}$	43.8	45.0	43.8	43.8	44.1	42.1	44.8	43.7	43.6	45.6	44.6	46.1	44.5	44.1	45.0	43.8	46.0	45.6	45.2	46.7
$\pm$	3.56	4.45	1.94	3.18	3.09	2.95	2.97	2.03	1.94	2.26	3.42	3.26	1.74	2.28	2.75	1.63	1.66	0.95	1.78	1.43
GROUP 2																				
1)	43.4	43.7	44.2	41.6	43.8	41.7	46.4	44.3	42.7	46.4	43.9	44.4	43.1	42.5	45.0	43.3	44.5	43.9	44.9	42.8
2)	50.7	50.1	50.7	47.5	45.8	47.0	47.9	46.3	50.1	48.8	47.2	48.5	49.4	46.8	49.3	48.0	46.8	47.4	46.6	48.2
3)	45.7	45.0	45.1	46.9	47.2	47.2	47.4	47.3	46.7	47.6	46.8	46.5	44.7	42.5	42.5	43.6	43.9	40.6	42.1	43.4
4)	47.6	47.5	45.6	47.0	47.5	47.5	48.1	47.7	46.1	46.8	45.5	44.6	44.8	44.6	45.0	48.3	46.1	48.2	45.1	46.2
5)	50.7	52.6	50.6	50.7	53.2	51.3	53.3	50.4	51.9	53.1	51.4	51.5	51.3	51.1	51.0	49.8	51.9	52.2	53.5	55.3
6)	50.0	51.5	50.9	52.5	52.3	52.5	52.7	49.0	51.5	52.2	47.2	47.9	50.1	49.0	52.2	47.1	49.3	49.9	49.4	51.2
$\bar{X}$	48.0	48.4	47.9	47.7	48.3	47.9	49.3	47.5	48.2	49.2	47.0	47.2	47.2	46.1	47.5	46.7	47.1	47.0	46.9	47.9
$\pm$	3.01	3.59	3.19	3.75	3.70	3.81	2.93	2.12	3.61	2.85	2.50	2.67	3.43	3.52	3.88	2.65	3.03	4.19	4.00	4.79

Values are means $\pm$ S.D

TABLE 12. CLINICAL SYMPTOMS REPORTED BY SUBJECTS DURING EACH PHASE.
· numbers in parentheses represent the day that the symptom was present.

Group #1.

	Muscle/joint pain	Cough/sore throat	Cold
P1 (LI/LV)			
P2 (LI/HV)	KL (ankles) (4) JR (legs, hamstrings)(5)	CM (chest congest)(3,4) MM (1) KL (5,7)	CM (3,4) MTM (6)
P3 (LI/LV)			
P4 (HI/HV)	JR (legs, (2,3,4) soleus (6,7) CM (calfs) (1 post, 2-7) MM (knees, quads)(2-4)	KL (1-3) MTM (2,4)	KL (4-7)

Group #2.

	Muscle/joint pain	Cough/sore throat	Cold
P1 (LI/LV)			
P2 (HI/HV)	DK (ribs, legs) (4,7,9) GB (calves (4-9), hamstrings (2,3) SC (r. calf) (2-7) SR (r. achilles)(9)	SR (2-9)	SR (7)
P3 (LI/LV)			
P4 (LI/HV)	GB (r. per. longus (6,7) hip (4,5) SC (r. knee) (1 post-5) SR (stress fract. r.toe) (9)	SR (1-9) DK (7-9)	SR (4)

TABLE 13. TRAINING DISTANCE (km) AND TIME (min) COVERED BY EACH SUBJECT IN GROUP #1.

	Day	S1 JR Dist/Time	S2 MTM Dist/Time	S3 CM Dist/Time	S4 KL Dist/Time	S5 JB Dist/Time	S6 MM Dist/Time
P1	1	4/18	7.5/30	7/35	3/16	5/22	3/19
(LI/LV)	2	4/18	8/35	7/30	3/16	5/21	3/20
	3	4/17	7.5/30	7/30	3/16	6/22	3/20
	4	4/17.5	7.5/29	7/30	3/16	5/21	3/20
	5	4/18	8/32	7/30	3/15	6/22	3/20
	6	4/18	7.5/30	7/30	3/15	5/21	3/20
	7	4/17	7.5/30	7/30	3/15	5/21	3/20
P2	1	8/37	14/57	15/65	6/34	10/49	6/40
(LI/HV)	2	9/36	15/60	14/60	6/32	9/40	6/40
	3	9/33	14/60	14/60	7/37	10/41	6/45
	4	9/33	14/60	14/60	6/34	11/49	6/40
	5	9/33	15/65	14/60	7/38	10/43	6/42
	6	10/37	14/60	14/60	7/38	10/42	6/40
	7	11/41	15/62	14/60	7/40	10/45	6/40
P3	1	4.5/18	7.5/30	7/30	3/20	5/20	3/20
(LI/LV)	2	4.5/18	7.5/30	7/30	3/15	5/19	3/20
	3	5/19	8/32	7/30	3/15	5/21	3/20
	4	5/19	7.5/28	7/30	3/20	5/20	3/20
	5	5/19	8/32	7/30	3/15	5/21	3/20
	6	4.5/18	8/32	7/30	3/14	5/20	3/20
	7	4.5/17	7.5/30	7/30	3/20	5/21	3/20
P4	1	9 x 1km	10 x 1km	10 x 1km	6 x 1km	8 x 1km	6 x 1km
(HI/HV)	2	9/30	14/60	14/60	5/38	10/41	6/40
	3	10 x 1km	11 x 1km	11 x 1km	6 x 1km	10 x 1km	6 x 1km
	4	9.5/34	15/65	14/60	6/40	10/41	6/40
	5	8 x 1km	10 x 1km	10 x 1km	7 x 1km	9 x 1km	6 x 1km
	6	9/32	14/60	14/60	5/39	10/40	6/40
	7	8 x 1km	9 x 1km	9 x 1km	6 x 1km	8 x 1km	6 x 1km

TABLE 14. TRAINING DISTANCE (km) AND TIME (min) COVERED BY EACH SUBJECT IN GROUP #2.

	Day	S1 DK Dist/Time	S2 GB Dist/Time	S3 IB Dist/Time	S4 SC Dist/Time	S5 SR Dist/Time	S6 BA Dist/Time
P1 (LI/LV)	1	7/26	3.7/20	3/20	3/20	3/20	3/20
	2	7/28	3.7/19	3/20	3/18	3/18	3/17
	3	7/26	3.7/23	3/23	3/19	3/15	3/19
	4	7/29	3.7/20	3/21	3/16	3/20	3/18
	5	7/29	3.7/20	3/19	3/18	3/15	3/20
	6	7/29	3.7/20	3/20	3/20	3/20	3/17
	7	7/26	3.7/18	3/20	3/15	3/18	3/20
P2 (HI/HV)	1	9 X 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km
	2	15/60	7/36	7/45	6/40	7/50	6/43
	3	10 x 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km
	4	15/60	7/36	6/39	6/43	7/50	6/42
	5	9 x 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km
	6	15/60	7/36	7/43	6/38	5/37	6/44
	7	9 x 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km	6 x 1km
P3 (LI/LV)	1	7/29	3.7/20	3/21	3/20	3/20	3/20
	2	7/29	3.7/21	3/20	3/20	3/15	3/19
	3	7/29	3.7/18	3/16	3/21	3/20	3/20
	4	7/29	3.7/20	3/18	3/18	3/15	3/20
	5	7/29	3.7/21	3/18	3/18	3/18	3/25
	6	7/28	3.7/22	3/20	3/18	3/22	3/18
	7	7/28	3.7/18	3/20	3/17	3/20	3/20
P4 (LI/HV)	1	15/60	10/46	8/48	13/62	6/45	6/45
	2	15/60	11/47	9/53	9/48	6/40	6/47
	3	14/59	13/60	12/65	9/45	5/35	6/37
	4	15/60	7/35	8.9/45	9/47	6/45	6/40
	5	15/60	13/60	12/60	9/46	6/45	7/43
	6	15/60	8/38	8/50	8/41	6/40	6/37
	7	16/60	11/50	8/50	8/41	6/45	6/45

TABLE #15. STRESS/MOOD STATES AND SLEEP PATTERNS ON BLOOD SAMPLING AND TREADMILL TEST DAYS.

	Stress/mood state pre and post-exercise (0=tired, 3=OK, 5=good)							Previous night's sleep (hours)						
Group #1	S1 JR	S2 MTM	S3 CM	S4 KL	S5 JB	S6 MM	$\bar{X}$	S1 JR	S2 MTM	S3 CM	S4 KL	S5 JB	S6 MM	$\bar{X}$
DAY														
P1 1	3,4	3,4	3,4	3,4	3,3	3,4	3,3.8	8	8	10	7	7	7	7.83
4	4	3	3	3	4	3	3.3	6	9	10	6.5	7	6	7.42
7	3,4	2,3	3,3	2,3.5	4,5	3,4	2.8,3.8	7.5	8	8	6	7.5	6	7.17
9	2,1	4,1	3,1	4,1	4,1	4,1	3.5,1	7	7.5	7	5	7	6	6.58
P2 1	3.5,3.5	4,3	3,2	3,4	4,4	3,2	3.4,3.1	5	7	9	5	7.5	6	6.58
4	4	3	2	3	4	3	3.2	8	8	9	5	7.5	6	7.25
7	2,1	2.5,1	2,1	4,2	4,4	2,2	2.75,1.8	7	9	10	5	7.5	6.5	7.5
9	4,1	4,1	2,1	4,1	3,1	2,1	3.2,1	7.5	8	6	6	7.5	6	6.83
P3 1	2,3	4,4	4,4	4,4	4,4	3,4	3.5,3.8	6.5	7	8	5.5	6.5	6	6.58
4	3	4	4	4	4	3	3.7	8	8	10	7.5	7.5	6.5	7.92
7	4,4	4,3	4,4	4,4	4,4	4,4	4,3.8	8	7	9	7	7	7	7.5
9	4,1	4,1	3,1	4,1	3,1	2,1	3.3,1	7.5	7	8	7.5	5	6	6.83
P4 1	4,1	3,1	3,2	3,1	3,1	2,1	3,1	5	7	9	7	7.5	8	7.25
4	2	3	3	3	3	3	2.8	4	10	9	7.5	8	6.5	7.5
7	2,1	3,1	3,1	2.5,0	3,2	1,1	2.4,1	8	9	12	8.5	8	5.5	8.5
9	2,1	3,2	3,2	2,1	3,1	3,1	2.7,1.3	7.5	8	10	7.5	7.5	5	7.58
Group #2	S1 DK	S2 GB	S3 IB	S4 SC	S5 SR	S6 BA	$\bar{X}$	S1 DK	S2 GB	S3 IB	S4 SC	S5 SR	S6 BA	$\bar{X}$
DAY														
P1 1	3,4	2,5	2,4	2,3	4,4	3,4	2.7.4	5	7	4	6	7	8.5	6.25
4	3	4	4	4	3	2.5	3.4	6	7	6	7.5	3	8	6.25
7	3,5	3,4	4,4	3.5,4	4,4	4,4	3.6,4.2	7	8	7.5	7.5	7	8	7.50
9	4,1	3.5,1	4,1	4,1	4,1	4,1	3.9,1	5	8	8	8	7.5	8	7.42
P2 1	3,2	4,2	4,1.5	3,1	3,2	3,1	3.3,1.6	5	6	7	6	4	8.75	6.13
4	3	3	2.5	3	3	3.5	3	7	8.5	7	7.5	7.5	7.75	7.54
7	4,1	3,1	3,2	3,1.5	3,2	1,1	2.8,1.4	7	5	6	6.5	7	5.5	6.17
9	1,0	4,1	3,1	3,1	2,1	3,1	2.7,0.8	6.5	6	5.5	8.5	6	8	6.75
P3 1	4,4	5,5	4,4	4.5,4	4,4.5	2,3	3.9,4.1	8	6	7	8	6.5	6	6.92
4	5	4	3.5	2.5	4	2	3.5	8	5.5	6	7.5	3.5	4	5.75
7	4,5	2,4	3,4	3,3	4,4	4,4	3.3,4	8	5	6	8	7.5	8	7.08
9	4,2	3,1	3,1	3,1	3,1	4,1	3.3,1.2	7	7	5	8	7	7	6.83
P4 1	2,2	2,3	3.5,3	3,3	3,3	3,3	2.8,2.8	6	8	7	7	7	6	6.83
4	3	4	3	4	3.5	3.5	3.5	6	8	6	8	11	8	7.83
7	2,2	2,3.5	2.5,2.5	2.5,3	3.5,3	3.5,3	2.7,2.8	5	6.5	6	3	2	9	5.25
9	3,1	3,1	3,1	3,1	3,1	3,1	3,1	8	7	5	6	8	7	6.83

TABLE 16. FLOW CYTOMETRY DATA - PERCENT CD4+, CD8+ AND CD4/CD8 RATIO FOR SUBJECTS IN GROUP #1.

	P1					P2					P3					P4				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Group #1.																				
S1 CD4	45.77	39.03	39.33	47.57	42.82	44.55	36.10	45.15	47.57	39.28	49.09	43.19	51.53	51.18	43.30	49.41	34.82	52.03	46.81	39.44
CD8	28.87	28.21	21.80	26.44	27.49	26.99	25.97	29.54	33.00	37.22	28.54	22.45	30.18	28.74	26.42	29.11	26.06	28.89	22.53	21.59
RATIO	1.59	1.38	1.80	1.80	1.56	1.65	1.39	1.52	1.44	1.44	1.72	1.52	1.71	1.78	1.64	1.70	1.34	1.80	2.08	1.83
S2 CD4	50.44	40.18	36.46	45.80	35.57	42.34	26.65	39.01	42.18	32.75	43.71	37.38	43.66	44.58	33.40	40.35	28.69	39.30	43.13	30.69
CD8	22.39	26.71	17.64	16.84	16.05	22.80	26.09	23.52	24.51	22.78	25.63	22.65	23.73	24.19	21.88	23.82	22.53	23.94	20.87	21.73
RATIO	2.25	1.50	2.09	2.72	2.22	1.86	1.02	1.66	1.72	1.44	1.71	1.65	1.84	1.84	1.53	1.69	1.27	1.64	2.07	1.41
S3 CD4	32.16	31.26	43.08	39.34	38.70	37.11	37.43	47.13	44.08	43.88	42.44	43.91	46.24	46.59	44.14	43.08	34.17	45.28	47.47	43.46
CD8	16.78	18.71	23.50	19.40	23.71	24.82	25.91	26.06	29.63	27.33	32.05	26.34	29.03	25.58	27.02	25.27	28.77	26.23	23.40	25.13
RATIO	1.92	1.67	1.83	2.03	1.63	1.50	1.44	1.81	1.49	1.61	1.32	1.67	1.59	1.82	1.63	1.70	1.19	1.72	2.03	1.73
S4 CD4	40.69	40.84	50.44	40.63	35.59	43.17	37.34	40.09	39.15	38.53	47.54	43.25	43.11	44.20	42.07	48.55	23.77	46.96	37.18	39.55
CD8	21.36	21.54	19.56	20.97	19.78	20.63	19.11	23.49	23.13	21.77	19.66	19.04	24.15	21.66	20.41	23.21	24.28	23.30	14.16	14.25
RATIO	1.90	1.90	2.58	1.94	1.80	2.09	1.95	1.71	1.69	1.77	2.42	2.27	1.79	2.04	2.06	2.09	0.98	2.02	2.63	2.78
S5 CD4	45.23	39.57	50.57	37.17	27.56	45.54	31.47	26.41	48.38	38.01	40.07	31.85	38.24	40.47	37.46	37.15	28.78	46.53	32.99	31.29
CD8	24.25	21.21	22.81	23.30	23.81	20.99	26.04	12.53	22.35	22.11	17.72	17.76	25.49	21.63	20.77	20.00	22.93	23.20	14.06	12.98
RATIO	1.87	1.87	2.22	1.60	1.16	2.17	1.21	2.11	2.16	1.72	2.26	1.79	1.50	1.87	1.80	1.86	1.26	2.04	2.35	1.74
S6 CD4	40.29	37.01	40.29	38.66	36.71	41.91	30.03	41.92	42.00	39.67	40.44	37.81	40.30	39.19	37.07	39.25	32.79	39.87	42.11	37.42
CD8	28.31	27.70	28.31	26.08	29.00	30.05	30.78	31.27	28.91	27.41	27.83	27.62	26.74	28.87	30.32	29.01	29.91	26.72	27.26	29.67
RATIO	1.42	1.34	1.42	1.48	1.27	1.39	0.98	1.34	1.45	1.44	1.45	1.37	1.51	1.36	1.22	1.35	1.10	1.49	1.54	1.26

TABLE 17. FLOW CYTOMETRY DATA - PERCENT CD4+, CD8+ AND CD4/CD8 RATIO FOR SUBJECTS IN GROUP #2.

		P1					P2					P3					P4			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Group #2.																				
S1 CD4	50.15	48.68	52.36	51.96	50.90	45.37	38.72	42.69	38.55	32.72	43.92	43.50	53.13	48.83	47.76	46.39	42.11	53.76	46.19	41.18
CD8	20.46	19.49	19.86	18.46	19.38	19.59	21.07	17.71	12.09	20.72	16.84	20.57	23.16	20.74	19.77	20.32	19.69	18.27	17.32	16.25
RATIO	2.45	2.50	2.64	2.81	2.63	2.32	1.84	2.41	3.19	1.58	2.61	2.11	2.29	2.35	2.42	2.28	2.14	2.94	2.67	2.53
S2 CD4	40.58	37.31	48.08	45.74	35.20	44.47	26.14	48.74	46.38	31.07	44.25	41.21	40.83	47.00	41.75	44.73	38.69	42.42	41.80	40.27
CD8	19.55	19.66	23.00	23.54	23.61	22.35	25.51	20.78	22.76	23.92	22.69	20.92	21.98	22.74	22.72	23.81	22.28	23.10	24.06	23.14
RATIO	2.08	1.90	2.09	1.94	1.49	1.99	1.02	2.34	2.04	1.29	1.95	1.97	1.86	2.07	1.84	1.88	1.78	1.84	1.74	1.74
S3 CD4	49.59	42.81	45.15	48.12	37.61	43.62	29.42	45.65	38.94	32.58	48.42	36.04	45.84	51.42	44.78	47.51	42.96	43.18	48.55	39.48
CD8	20.91	21.58	23.15	25.71	27.77	27.03	29.68	23.93	22.18	25.95	23.66	25.68	23.76	22.24	24.18	20.94	23.65	24.75	22.61	25.66
RATIO	2.37	1.98	1.95	1.87	1.35	1.61	0.99	1.91	1.76	1.25	2.05	1.40	1.93	2.31	1.85	2.27	1.82	1.74	2.15	1.54
S4 CD4	35.11	25.38	36.05	32.60	26.87	34.24	29.40	35.89	30.44	24.96	35.09	29.18	34.73	40.23	30.50	34.00	31.29	33.34	36.85	26.11
CD8	24.20	21.62	22.36	27.48	30.59	24.53	24.98	25.13	24.90	27.22	24.06	24.01	25.54	24.78	25.76	25.29	26.43	24.53	25.91	25.23
RATIO	1.45	1.17	1.61	1.19	0.88	1.40	1.18	1.43	1.22	0.92	1.46	1.22	1.36	1.62	1.18	1.34	1.18	1.36	1.42	1.04
S5 CD4	48.74	37.64	48.97	46.27	33.36	38.97	26.88	48.74	49.24	31.56	46.42	37.93	46.85	57.14	32.71	43.75	34.06	39.77	52.66	41.41
CD8	21.03	19.16	19.60	22.29	21.64	18.26	21.64	22.05	20.96	21.86	22.18	21.39	21.45	20.85	20.48	21.41	21.06	23.84	21.50	21.93
RATIO	2.32	1.96	2.50	2.08	1.47	2.13	1.24	2.21	2.35	1.44	2.09	1.77	2.18	2.45	1.60	2.04	1.62	1.67	2.45	1.89
S6 CD4	42.21	38.70	40.92	40.49	36.03	43.42	33.92	40.95	48.51	41.61	45.46	40.01	44.53	44.39	39.90	43.87	36.24	39.61	46.24	41.00
CD8	28.63	26.20	28.49	30.27	26.81	29.08	27.15	29.45	27.10	26.98	27.34	24.84	30.24	27.49	26.27	26.83	26.47	29.31	26.04	24.15
RATIO	1.48	1.48	1.44	1.34	1.34	1.49	1.25	1.39	1.79	1.54	1.66	1.61	1.47	1.61	1.52	1.64	1.37	1.35	1.78	1.70

TABLE 18. FLOW CYTOMETRY DATA - PERCENT CD3+, HLA-DR+ AND CD3+HLA-DR+ FOR SUBJECTS IN GROUP #1.

	P1					P2					P3					P4				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Group #1.																				
S1 CD3+	79.97	74.74	90.59	87.20	79.62	55.66	53.39	49.43	78.40	68.06	70.04	66.13	86.72	81.71	75.29	78.06	76.60	70.31	47.41	61.41
HLADR+	3.85	3.98	0.02	0.16	2.90	6.30	6.43	5.35	6.18	8.71	9.57	8.54	1.69	3.70	3.70	4.60	3.25	4.49	3.33	4.66
CD3+HLADR+	5.61	4.06	8.96	11.69	8.15	4.17	2.88	27.26	5.56	4.22	2.80	3.78	8.43	9.70	8.10	12.90	6.94	6.39	3.88	8.83
S2 CD3+	74.00	65.60	72.25	72.83	69.03	58.85	46.77	58.77	60.77	55.40	62.99	57.03	73.48	73.98	69.39	63.77	44.88	71.42	68.04	63.30
HLADR+	6.79	5.1	7.31	4.65	4.31	12.95	14.84	14.64	11.30	10.12	13.75	12.01	5.57	5.82	4.85	9.63	16.06	6.31	9.29	6.19
CD3+HLADR+	6.01	4.81	8.21	11.44	8.64	6.06	4.13	11.68	2.86	3.46	2.57	3.96	13.25	11.79	8.81	15.22	4.11	11.90	10.93	10.76
S3 CD3+	85.10	84.51	81.60	71.78	74.32	61.98	59.46	64.49	76.33	62.38	70.90	67.65	78.99	77.10	74.92	69.04	61.37	73.64	73.49	73.02
HLADR+	3.84	2.84	3.63	7.82	3.92	8.36	9.65	15.01	4.42	13.46	12.42	10.81	5.14	5.89	5.53	9.10	9.96	5.34	6.80	5.29
CD3+HLADR+	5.44	3.88	11.21	9.01	9.57	5.98	4.26	7.31	12.49	7.80	6.29	4.16	11.70	12.68	9.03	16.22	9.49	17.33	14.47	12.30
S4 CD3+	77.33	74.38	94.99	63.56	57.47	63.25	58.13	68.68	71.95	61.47	71.09	67.79	82.38	76.31	72.29	78.97	72.14	76.61	73.23	47.99
HLADR+	4.22	2.88	0.00	8.74	8.48	9.57	6.66	6.64	5.81	6.60	7.71	8.45	1.84	4.41	3.53	4.52	2.34	6.40	4.44	3.82
CD3+HLADR+	10.93	8.65	2.40	4.54	3.24	4.92	14.06	5.36	7.14	4.59	7.01	5.12	10.20	8.96	9.66	9.48	6.10	7.18	10.85	6.50
S5 CD3+	87.63	83.58	77.08	57.65	43.65	61.33	51.15	64.37	67.59	58.84	66.57	52.94	73.50	68.52	64.24	67.48	65.61	68.67	67.12	57.36
HLADR+	0.44	0.88	3.24	10.81	11.56	12.37	10.96	11.91	10.58	8.41	12.70	11.74	3.36	4.45	4.52	3.95	4.08	5.01	4.62	3.56
CD3+HLADR+	10.85	11.17	12.38	8.52	4.98	8.91	7.00	8.76	8.29	6.95	4.86	9.14	9.01	9.59	7.06	14.91	11.13	12.44	10.46	9.91
S6 CD3+	69.30	71.17	69.91	43.17	64.59	73.14	49.50	73.21	68.44	67.83	69.22	68.53	69.92	72.04	71.58	72.18	69.15	71.25	70.77	70.81
HLADR+	8.32	7.22	10.75	11.96	16.17	14.28	17.35	8.44	13.91	10.74	15.89	11.02	12.40	9.06	7.50	9.71	10.10	10.64	8.61	7.23
CD3+HLADR+	18.3	11.48	14.29	2.67	3.19	6.29	5.42	15.27	13.96	10.64	8.91	9.32	11.41	14.48	12.78	14.57	8.71	11.99	18.06	13.87

TABLE 19. FLOW CYTOMETRY DATA - PERCENT CD3+, HLA-DR+ AND CD3+HLA-DR+ FOR SUBJECTS IN GROUP #2.

			P1					P2					P3					P4		
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Group #2.																				
S1 CD3+	73.77	73.37	79.08	73.41	74.86	60.72	56.41	60.98	67.11	54.19	71.21	63.85	81.12	74.94	73.38	69.88	65.93	73.95	72.19	62.11
HLADR+	8.53	7.59	2.93	6.90	5.35	12.28	11.56	12.83	13.51	14.70	8.71	10.27	3.06	6.69	8.46	8.23	11.88	7.16	10.00	8.03
CD3+HLADR+	6.89	5.42	15.87	14.66	13.68	5.87	2.79	10.45	12.79	6.60	6.45	6.10	13.31	10.40	6.19	15.18	13.07	13.23	9.30	5.74
S2 CD3+	71.41	67.49	75.89	76.83	72.62	67.55	52.75	72.93	73.14	62.72	68.43	70.11	65.91	68.53	63.77	71.81	71.29	74.76	68.35	66.54
HLADR+	5.18	4.91	7.88	4.94	4.55	8.23	8.03	7.73	4.97	6.33	7.99	5.49	7.81	12.63	9.12	8.10	5.61	5.28	9.07	7.23
CD3+HLADR+	10.21	8.11	8.52	10.36	8.15	8.93	3.06	7.10	11.28	7.62	8.80	7.28	8.72	4.35	4.63	7.53	8.08	9.55	5.70	7.63
S3 CD3+	71.52	71.37	75.24	75.45	78.16	80.13	54.99	72.90	71.02	63.64	73.63	67.19	71.94	68.77	63.26	71.38	67.67	78.68	72.35	67.69
HLADR+	5.80	4.97	4.60	6.66	2.55	2.78	10.86	7.37	6.94	7.67	5.97	4.93	7.35	11.67	10.30	7.60	7.62	3.54	7.43	7.43
CD3+HLADR+	7.71	7.93	10.63	5.01	7.30	10.24	3.96	7.79	7.13	10.51	7.83	9.88	8.53	3.00	2.02	6.59	4.65	8.14	8.39	6.58
S4 CD3+	61.84	61.92	61.01	64.49	67.23	65.44	58.70	58.61	54.47	60.21	59.95	63.91	63.88	60.08	65.45	62.45	63.72	65.19	58.61	51.98
HLADR+	4.05	4.36	8.49	7.95	4.63	6.53	12.36	14.14	10.77	7.21	12.58	7.57	10.80	13.25	6.30	12.26	8.93	9.20	12.65	11.16
CD3+HLADR+	13.19	10.96	15.09	11.59	13.24	19.71	7.98	8.42	10.83	10.34	11.60	14.99	14.71	14.61	14.23	11.98	9.77	14.75	15.27	10.63
S5 CD3+	75.69	75.90	71.25	70.04	71.70	70.70	65.71	76.30	74.56	65.44	72.29	66.75	69.54	68.13	62.99	66.74	61.65	72.43	76.11	70.44
HLADR+	5.96	5.91	10.04	9.68	3.93	6.34	5.55	5.50	4.89	4.11	6.57	6.25	9.82	11.19	7.83	6.64	5.73	4.68	7.12	5.59
CD3+HLADR+	10.86	10.95	8.75	7.40	10.26	9.88	9.21	8.73	13.04	10.96	10.52	9.05	7.42	7.37	6.15	7.72	5.99	7.79	7.65	2.28
S6 CD3+	75.68	74.34	77.39	79.78	76.25	79.69	72.73	80.87	77.79	75.72	71.78	72.42	78.21	71.16	63.19	75.76	59.97	76.08	74.21	72.48
HLADR+	3.11	4.72	3.69	2.82	2.89	3.52	3.38	3.28	3.59	3.13	7.42	4.12	5.15	5.66	6.07	3.59	5.66	4.20	4.57	3.30
CD3+HLADR+	4.87	4.22	4.86	6.26	5.97	4.89	5.28	6.03	5.57	6.48	3.92	5.44	4.07	3.62	3.27	5.56	3.18	5.37	4.92	5.45

TABLE #20. ELISA IgG CONCENTRATIONS (g/l) FOR SUBJECTS IN GROUP #1 AND #2.

		Group #1						Group #2					
		S1 JR	S2 MTM	S3 CM	S4 KL	S5 JB	S6 MM	S1 DK	S2 GB	S3 IB	S4 SC	S5 SR	S6 BA
P1	1	11.35	7.30	6.85	10.75	9.55	7.45	7.40	10.00	9.60	11.80	9.35	8.75
	2	12.90	7.25	7.10	12.15	8.65	7.85	7.90	9.30	9.00	10.60	9.25	9.00
	3	13.75	5.75	7.25	12.65	7.10	7.50	8.90	8.70	9.65	10.85	9.30	8.20
	4	13.70	7.40	6.40	12.80	7.35	8.85	9.35	10.00	9.63	11.25	10.75	8.75
	5	14.00	8.70	7.50	13.10	8.25	9.15	9.60	10.10	9.95	14.00	11.00	9.78
P2	1	16.25	6.40	6.35	12.15	9.45	6.75	10.30	10.10	9.20	10.30	9.35	8.76
	2	18.20	8.60	7.10	14.50	8.55	7.20	11.25	8.40	9.61	11.80	9.60	9.85
	3	19.85	9.40	6.10	12.05	7.90	8.65	13.35	8.40	9.65	10.45	8.25	7.55
	4	18.10	8.75	6.40	10.40	7.65	6.25	9.75	9.50	8.60	10.95	8.40	7.10
	5	16.25	8.75	7.25	12.15	7.10	7.45	9.75	9.30	9.40	10.45	11.00	9.55
P3	1	17.20	8.65	6.35	10.75	7.40	6.15	9.25	9.90	9.66	10.40	9.15	8.30
	2	18.00	8.10	7.10	11.65	7.25	6.60	10.45	9.30	9.63	10.60	9.00	9.78
	3	16.70	9.00	6.40	12.15	7.65	6.06	11.25	8.60	9.90	10.70	9.30	8.78
	4	17.10	9.05	6.00	11.25	8.75	6.35	12.00	9.40	9.40	10.65	8.90	7.90
	5	16.40	9.35	6.25	12.70	8.55	6.53	12.75	9.90	9.95	12.25	11.25	8.00
P4	1	15.65	9.85	5.85	12.85	7.90	7.70	14.85	8.40	9.64	10.30	9.00	8.50
	2	15.75	9.65	6.40	14.25	7.35	7.35	11.95	9.20	9.95	13.65	9.55	9.20
	3	14.35	10.15	6.35	12.35	6.90	7.85	10.30	9.00	9.89	11.05	8.80	8.08
	4	13.80	8.05	6.10	11.75	6.35	8.85	11.95	9.10	9.62	10.65	9.20	8.29
	5	15.25	9.30	6.25	11.25	7.35	9.65	11.30	9.10	9.75	10.60	9.60	10.10

TABLE #21. ELISA IgM CONCENTRATIONS (g/l) FOR SUBJECTS IN GROUP #1 AND #2.

	Group #1						Group #2					
	S1 JR	S2 MTM	S3 CM	S4 KL	S5 JB	S6 MM	S1 DK	S2 GB	S3 IB	S4 SC	S5 SR	S6 BA
P1 1	1.24	1.25	0.97	1.18	0.92	1.40	1.98	0.97	0.38	0.97	0.19	1.76
2	1.18	0.88	0.92	1.13	0.85	1.48	1.48	1.01	0.38	0.86	0.40	1.86
3.	1.30	0.90	1.12	1.22	0.92	1.38	1.61	1.18	0.14	0.63	0.14	1.88
4	1.35	1.15	0.91	1.21	0.82	1.37	1.59	1.18	0.40	1.43	0.18	1.65
5	1.40	0.89	0.82	1.23	0.87	1.35	1.52	1.13	0.32	0.94	0.27	1.78
P2 1	1.30	1.20	1.14	1.15	0.84	1.52	1.53	0.98	0.33	0.78	0.62	1.79
2	1.30	1.25	0.98	1.00	0.95	1.47	1.51	1.03	0.62	0.94	1.75	1.04
3	1.41	1.58	1.07	1.10	0.84	1.67	1.73	0.96	0.97	0.89	1.65	1.67
4	1.35	1.58	0.95	1.04	0.87	1.55	1.40	0.97	1.04	0.99	1.05	1.12
5	1.45	1.71	0.92	1.05	0.81	1.35	1.38	0.98	1.32	0.90	1.01	1.23
P3 1	1.25	1.69	1.08	1.04	0.92	1.63	1.52	1.17	0.31	1.09	0.35	1.02
2	1.24	1.19	1.02	1.00	0.73	1.72	1.56	1.03	0.48	1.89	0.45	1.85
3	1.25	1.24	1.09	1.13	0.84	1.60	1.67	1.02	0.62	0.91	0.22	1.64
4	1.47	1.53	1.16	1.10	0.91	1.58	1.75	1.00	0.60	0.87	0.57	1.76
5	1.45	1.57	1.07	1.09	0.93	1.47	1.72	1.01	0.83	0.72	0.31	1.13
P4 1	1.35	1.25	1.14	1.08	0.81	1.54	1.32	0.95	1.00	1.10	0.96	1.23
2	1.15	3.25	0.91	1.08	0.78	1.38	1.44	0.92	0.55	1.17	1.42	1.77
3	1.39	1.19	1.03	1.10	0.79	1.58	1.51	1.02	0.88	0.86	0.63	1.86
4	1.00	2.15	0.90	1.07	0.84	1.72	1.45	1.04	0.98	0.66	1.50	1.76
5	1.02	2.55	0.97	1.00	0.74	1.74	1.65	0.96	0.83	0.91	1.90	1.98

TABLE #22. ELISA IgA CONCENTRATIONS (g/l) FOR SUBJECTS IN GROUP #1 AND #2.

		Group #1						Group #2					
		S1 JR	S2 MTM	S3 CM	S4 KL	S5 JB	S6 MM	S1 DK	S2 GB	S3 IB	S4 SC	S5 SR	S6 BA
P1	1	1.93	0.73	1.37	1.08	0.68	1.70	1.00	2.28	3.25	0.70	1.05	0.80
	2	1.78	0.81	0.85	1.53	0.80	1.75	1.76	2.40	3.50	1.40	1.20	0.69
	3	2.06	0.60	0.73	2.10	0.38	1.25	0.50	2.29	3.45	1.40	0.52	0.30
	4	3.20	0.82	0.85	2.40	0.58	2.85	1.42	2.48	4.25	0.30	1.25	1.60
	5	3.40	1.00	1.55	2.40	0.49	3.28	1.50	1.84	4.85	0.29	0.20	2.10
P2	1	1.93	0.97	1.12	2.40	0.46	3.10	1.00	2.35	3.65	0.90	0.93	1.20
	2	2.10	0.84	0.82	2.50	0.49	2.93	1.55	2.29	3.85	1.00	1.99	2.29
	3	3.38	1.48	1.24	2.40	1.00	2.09	1.53	2.43	3.00	1.40	0.51	3.00
	4	2.03	1.75	1.42	2.05	0.51	2.67	1.80	2.70	2.85	0.98	1.50	3.48
	5	2.12	2.05	1.81	2.30	0.89	2.23	1.96	2.35	3.60	0.30	2.30	3.19
P3	1	1.65	0.81	1.02	2.05	0.48	2.44	1.07	2.00	3.45	0.90	0.70	1.20
	2	1.65	0.68	0.85	1.25	0.54	1.82	1.25	2.12	3.30	2.50	0.20	0.85
	3	1.93	1.48	1.38	2.13	0.62	1.18	1.42	2.72	3.65	2.60	1.03	1.55
	4	1.82	0.80	1.36	2.60	0.53	1.60	1.55	2.48	3.30	0.90	1.60	1.80
	5	1.71	0.60	1.01	2.76	0.45	1.73	1.90	2.30	3.25	0.91	1.20	2.00
P4	1	1.10	1.37	1.25	2.40	0.62	1.05	0.70	2.30	3.25	2.52	2.30	2.70
	2	2.82	1.30	1.75	1.90	0.60	1.15	1.75	3.20	3.65	1.80	1.80	2.45
	3	1.87	0.80	0.85	1.20	0.45	1.05	0.72	3.40	3.40	0.91	2.00	1.03
	4	1.64	0.87	1.02	1.19	0.52	1.34	1.24	2.43	2.50	1.29	1.50	0.93
	5	1.82	1.12	1.12	2.23	0.62	3.73	2.10	2.28	4.60	0.29	2.05	2.99

APPENDIX III

CALCULATION OF TREADMILL TEST VELOCITY (miles/hour) BASED ON EACH SUBJECT'S BEST 1500m TIME.

eg, $\frac{1.5\ km}{5.0\ min} \times \frac{60\ min}{1\ hr} \times \frac{1.0\ mile}{1.609\ km} = 11.2$ mile/hr

Subject	Best 1500m time (min:sec)	Decimal	Treadmill speed (miles/hr)
BA	5:20	5.33	10.5
SR	5:20	5.33	10.5
KL	5:05	5.09	11.0
MM	4:52	4.85	11.5
IB	4:52	4.85	11.5
SC	4:52	4.85	11.5
GB	4:52	4.85	11.5
JB	4:40	4.66	12.0
JR	4:35	4.58	12.2
MTM	4:35	4.58	12.2
DK	4:14	4.24	13.2
CM	4:09	4.14	13.5

APPENDIX IV

LEUKOCYTE PREPARATION (PRE-FLOW CYTOMETRY).

Note: The following must be performed in the fumehood with gloves and protective clothing.

Antibody Dilutions:

i) IgG (control)

30ul of PBS
10ul of MsIgG-RD1
10ul of MsIgG-FITC

ii) T4T8

30ul of PBS
10ul of T4RD1
10ul of T8 FITC

1) Three plastic test tubes (12 x 75 mm) must be labelled: i)IgG (control), ii) CD3HLADR, iii) T4T8 per blood sample.

2) After gentle mixing, 100ul of anticoagulated whole blood is pipetted carefully into the bottom of each testtube (avoiding sides of tube).

3) Add the following diluted monoclonal antibodies to each labelled test tube:
i) IgG (control) - 5ul of IgG

ii) CD3HLADR - 5 ul of T3RD1
+ 20 ul of HLADR

iii) T4T8 - 5ul of T4T8

-pipets must be rinsed with distilled H_2O (twice) after adding each diluted antibody.

4) Immediately vortex samples for 5 seconds and use a Q-tip to remove any blood which may have adhered to the sides of the test tube.

5) Incubate for 10 minutes in the dark at room temperature.

6) Save blood samples for re-testing if necessary.

7) Turn 'on' COULTER Q-PREP Epics immunology work station. Check for adequate amounts of Immunoprep reagents A, B

and C and 'prime' the system by filling appropriately labelled test tubes with these reagents.

8) For low or high-affinity antibodies, place incubated test tube into the work station and press the '35 second cycle' button and close chamber door to "start" process.

9) When cycle is over, ('ready' light is on) remove test tube and cap.

10) Prepared samples may be stored at room temperature if flow cytometric analysis will occur within 2 hours. Otherwise, capped samples must be refrigerated in the dark at 2-8°C. These refrigerated samples should be analysed within 24 hours.

<u>Reagents:</u>

MsIgG-RD1 - Coulter #6603482 Coulter Clone
-Liquid monoclonal antibody reagent.
-mfr: COULTER IMMUNOLOGY a division of Coulter Corporation Hialeah, Florida 33010.

MsIgG-FITC - Coulter #6602431 Coulter Clone
-Lyophilized monoclonal antibody reagent.
-reconstituted to 500ul with distilled water

T4RD1 - Coulter #6602864 Coulter clone
-100 Test (0.5ml)
-Liquid murine monoclonal antibody.

T8FITC - Coulter #6602385 Coulter Clone
-100 Test (0.5ml)
-Lyophilized monoclonal antibody.
-reconstituted with 500ul distilled water.

T3RD1 - Coulter #6602952 Coulter Clone
-100 Test (0.5ml)
-Liquid murine monoclonal antibody.

Anti-human HLA-DR FITC
-Becton Dickinson Immunocytometry Systems.
San Jose, CA 95131-1807 USA.
B-9440 Erembodegem - Belgium.

Immunoprep A - an erythrocyte lytic agent
Formic Acid ....1.2 ml/l
Stabilizer

Immunoprep B - a leukocyte stabilizer.
Sodium Carbonate.............6.0 g/l
Sodium Chloride.............14.5 g/l
Sodium Sulfate..............31.3 g/l
Stabilizer

Immunoprep C - a cell membrane fixative.
Paraformaldehyde.............10.0 g/l
Buffers.

APPENDIX V

HUMAN SERUM ANTIBODY MEASUREMENT BY ELISA USING EXTRAVIDIN-ALKALINE PHOSPHATASE.

Outline: The method described is for the measurement of antibody in human serum, using an ELISA based on alkaline phosphatase enzyme covalently bound to ExtrAvidin (a tetrameric protein prepared from egg white avidin, containing 4 high affinity binding sites for biotin yet does not exhibit the unwanted non-specific binding reported for eggwhite avidin at physiological pH) and anti-human Ig antibody substituted with biotin. The antigen of interest must be bound to the plastic wells of microtitre plates. The procedure presumes that the antigen is a soluble protein. If the antigen is in detergent extract, special procedures may have to be devised for dilution of antigen, washing, or various incubations in the presence of detergent. The assay may require overnight incubation.

Reagents:

- -Extra Avidin conjugate (Sigma)
- -Goat anti human Ig: various isotypes (Southern Biotech assoc.)
- -BSA
- -96 well microtitre (200 ul) plates (NUNC or IMMUNLON) - polystyrene (hard)
- -TBS (.01M, pH 7.4, with azide (.1ml/500ml))
- -TBS-BSA: TBS containing 1 mg/ml BSA
- -BBS (Borate) (.01M, pH 8.5 with NaOH, with azide (1 % NaN3 and 2.5 M NaCl)).
- -WS (wash solution): TBS plus .05% Tween (Sigma) (250ul/50ml)
- -Alk/Phos Substrate: (Sigma)
- -Substrate Buffer: (for 1L) - refrigerate
    - -97 ml diethanolamine
    - -200 mg azide
    - -100 mg MgCl(H2O)6
    - -pH to 9.8 with HCl

Procedures:

1) Coat Plates:

The antigen (Ag) of interest should be diluted (as determined) in BBS, with no other protein as carrier. Plate out at 100 ul per well. For most protein antigens, 1 hr at 37 °C, 3 hrs (allowable range 2.5 to 4 hours) at room temperature (R/T), overnight (O/N) at 4°C will also suffice. In most cases, for proper analysis an Ag-negative well should be coated with an appropriate "sham" Ag, or BSA. In the case of BSA, simple coating during "blocking" (see below) will suffice. These Ag-negative wells control for variability of non-specific sticking of human Ig to the wells, a problem when the serum is not very dilute. Usually any dilution of more than 1/300 does not show this problem and the use of Ag-negative wells can be avoided.

2) Block:

After antigen coating of wells, aspirate excess antigen (washing is not necessary). Usually 10 mg/ml BSA in TBS is added at 200 ul per well, and incubated at R/T for 1 hour to "block" any remaining binding sites of plastic.

3) Serum incubation:

Blocking BSA is removed by flicking out (usually no washing necessary). Sera are dilute in TBS-BSA (1mg/ml), and plated at 100 ul per well. Incubation can be as short as 1 hour at R/T, in most cases it is convenient to use O/N at 4°C. This also ensures that low affinity interactions can equilibrate.

4) Biotin conjugated anti-human Ig reagent incubation:

Wash all wells 3 times with 200 ul of WS per well, flicking out before washing. This is the most important wash step, especially if the serum samples are not very dilute. For more caution, aspirate wells (rather than flick) before first wash. Pat plates dry on a paper towel. Add 100 ul of Biotin anti-human Ig reagent at a predetermined dilution in TBS-BSA (1mg/ml). Incubation at R/T for 3 hours is usually sufficient.

5) Extravidin- Alk/Phos incubation:

Wash 2-3 times with wash solution and pat plates dry on paper towels. Add 100 ul of Extravidin-Alk/Phos (at a predetermined dilution in TBS-BSA) and incubate for 1 hour at R/T.

6) Alk/Phos reagent incubation:

Wash 2-3 times with wash solution and pat plates dry on paper towels. Add 100 ul of substrate dilute in substrate buffer (1M diethenolamine) (usually 1 small tablet in 5 ml). Allow 20 to 30 minutes and check positive wells for colour. Reaction may be stopped with 20 ul of 10 N HCl per well, but if substrate is added at the appropriate time intervals, plates may be read at intervals with no stopping of reaction. Usually developing of action beyond 1 hour does not improve signal/noise. Care should be taken so as not to develop positive signals beyond O.D.(optical density wave length = 410nm) of 1.5, since most ELISA microplate readers give non-linear signals beyond 1.5.

An Example of the 96-well Plate Set Up for ELISA Analysis of IgM and IgA.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	5	13	1	9	17	5	13	S1	S3
B	2	10	18	6	14	2	10	18	6	14	S2	S4
C	3	11	19	7	15	3	11	19	7	15	S3	S5
D	4	12	20	8	16	4	12	20	8	16	S4	S6
E	5	13	1	9	17	5	13	1	9	17	S5	C-S
F	6	14	2	10	18	6	14	2	10	18	S6	C-NS
G	7	15	3	11	19	7	15	3	11	19	S1	NC-S
H	8	16	4	12	20	8	16	4	12	20	S2	NC-NS
	Subject #1 (duplicate)					Subject #2 (duplicate)					Standards & Controls	

Sample dilutions: 1 through 20 are serum samples per subject, diluted 1/100 with TBS-BSA (1mg/ml).

Standard dilutions:
S1 = 1/100
S2 = 1/200
S3 = 1/400
S4 = 1/800
S5 = 1/1600
S6 = 1/3200

C-S = coated, serum added
C-NS = coated, no serum added
NC-S = non-coated, serum added
NC-NS = non-coated, no serum added

An Example of the 96-well Plate Set Up for ELISA Analysis of IgG.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	5	13	1	9	17	5	13	S1	S3
B	2	10	18	6	14	2	10	18	6	14	S2	S4
C	3	11	19	7	15	3	11	19	7	15	S3	S5
D	4	12	20	8	16	4	12	20	8	16	S4	S6
E	5	13	1	9	17	5	13	1	9	17	S5	C-S
F	6	14	2	10	18	6	14	2	10	18	S6	C-NS
G	7	15	3	11	19	7	15	3	11	19	S1	NC-S
H	8	16	4	12	20	8	16	4	12	20	S2	NC-NS
	Subject #1 (duplicate)					Subject #2 (duplicate)					Standards & Controls	

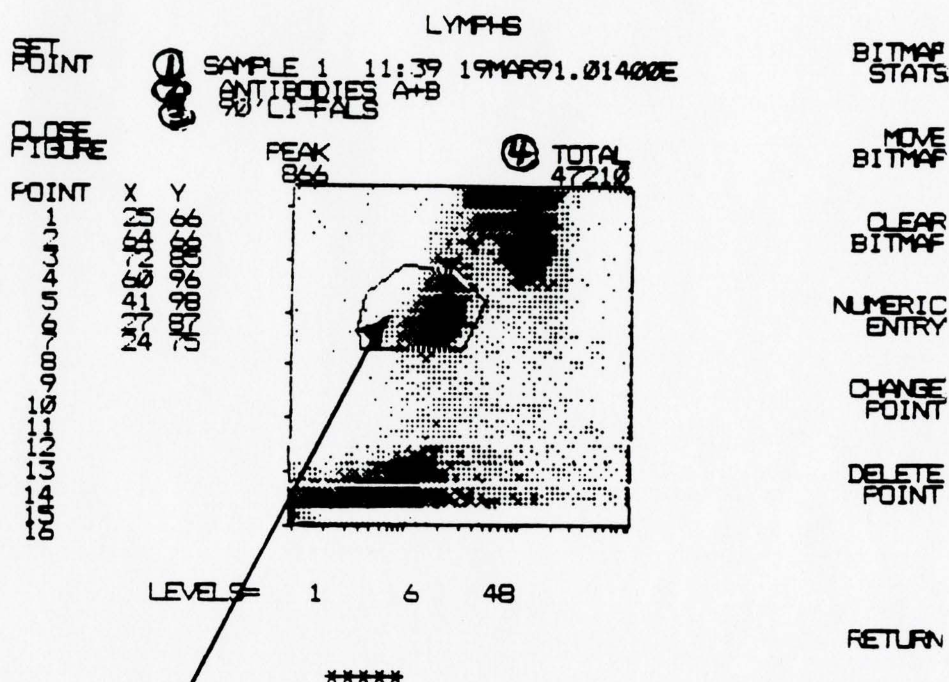
Sample dilutions: 1 through 20 are serum samples per subject, diluted 1/1000 with TBS-BSA (1mg/ml).

Standard dilutions:
S1 = 1/500
S2 = 1/1000
S3 = 1/2000
S4 = 1/4000
S5 = 1/8000
S6 = 1/16000

C-S = coated, serum added
C-NS = coated, no serum added
NC-S = non-coated, serum added
NC-NS = non-coated, no serum added

APPENDIX VI

SAMPLE FAC COMPUTER OUTPUT



BITMAP -bitmaps define certain groups of cells.
This group is lymphocytes in a peripheral blood preparation.

1) Sample identification

2) Type of antibodies used, if any.

3) The parameters plotted on the histogram.
The first one listed is the X-axis, the second is the the Y-axis.
There are 4 possible parameters:
FALS = Foward Angle Light Scatter (an indicator of cell size)
90' LI = 90 degree light scatter (an indicator of cell complexity)
LIGFL = Log Integrated Green Fluorescence
LIRFL = Log Integrated Red Fluorescence

4) The total number of events on the histogram.

SAMPLE CD4+ AND CD8+ HISTOGRAM

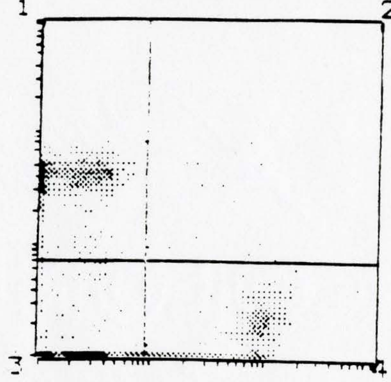
QUADRANT STATISTICS

1) STEV3/18 1Ø:13 19MAR92.Ø13Ø1E
2) POST: CD4 CD8
3) LIGFL-LIRFL/FALS ,9Ø'LI,MAP1

EXTENDED ANALYSIS

PRINT

4) TOTAL= 51Ø7



5) LIGFL 2Ø
LIRFL 18

LOWEST LEVEL
1

QUAD	PERCENT	PEAK POS	PEAK HT	AREA
1	41.41	Ø, 34	17Ø	2115
2	Ø.39	25, 33	2	2Ø
3	36.26	Ø, Ø	349	1852
4	21.93	39, Ø	36	112Ø

*****.ADJUST CURSOR

RETURN

1) Sample identification
2) Antibodies used
3) Parameters plotted on histogram.
Everything following the "/" mark, indicates the "gating" restriction and that <u>only</u> the events occurring within the map 1 restriction are plotted here.
4) The total number of events on the histogram, in this case, within map 1.
5) Co-ordinates of the intersection of the vertical cursor (LIGFL) and the horizontal cursor (LIRFL).

This is a histogram of green fluorescence (CD8+) versus red fluorescence (CD4+):

Red Fluorescence (CD4+)			
	+	**Quadrant 1** **Red +** **Green -** **(CD4+= 41.41%)**	**Quadrant 2** **Red +** **Green +** **(0.39 %)**
	-	**Quadrant 3** **Red -** **Green -** **(36.26 %)**	**Quadrant 4** **Red -** **Green +** **(CD8+= 21.93%)**
		-	**+**

Green Fluorescence (CD8+)

SAMPLE CD3+, HLA-DR+ AND CD3+HLA-DR+ HISTOGRAM

QUADRANT STATISTICS

1) STEV3/18 10:16 19MAR92.01401E

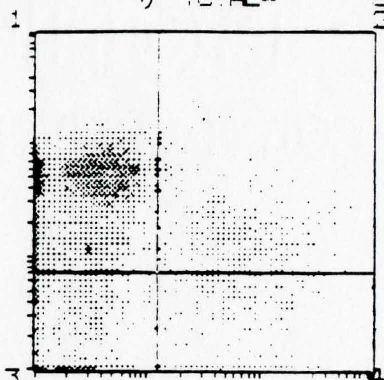
2) POST: CD3 HLADR

3) LIGFL-LIRFL/FALS ,90'LI,MAP1

4) TOTAL= 5081

EXTENDED ANALYSIS

PRINT



5) LIGFL 23
LIRFL 18

LOWEST LEVEL
1

QUAD	PERCENT	PEAK POS	PEAK HT	AREA
1	70.44	0, 36	79	3579
2	7.28	34, 24	5	370
3	16.69	0, 0	233	848
4	5.59	35, 0	9	284

*****.ADJUST CURSOR

RETURN

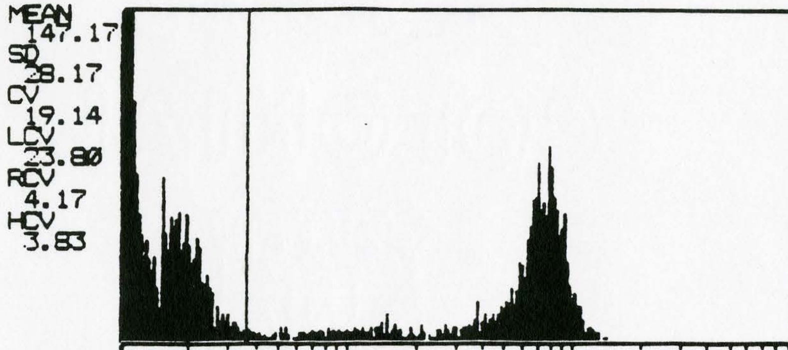
1) Sample identification
2) Antibodies used
3) Parameters plotted on histogram.
   Everything following the "/" mark, indicates the "gating" restriction and that <u>only</u> the events occurring within the map 1 restriction are plotted here.
4) The total number of events on the histogram, in this case, within map 1.
5) Co-ordinates of the intersection of the vertical cursor (LIGFL) and the horizontal cursor (LIRFL).

This is a histogram of green fluorescence (HLA-DR+) versus red fluorescence (CD3+):

Red Fluorescence (CD3+)		Green Fluorescence (HLA-DR+) −	Green Fluorescence (HLA-DR+) +
	+	Quadrant 1 Red + Green - (CD3+= 70.44 %)	Quadrant 2 Red + Green + (CD3+HLA-DR+= 7.28%)
	-	Quadrant 3 Red - Green - (16.69 %)	Quadrant 4 Red - Green + (HLA-DR+= 5.59 %)

STATISTICS

SCALE= 128 TOTAL= 5611



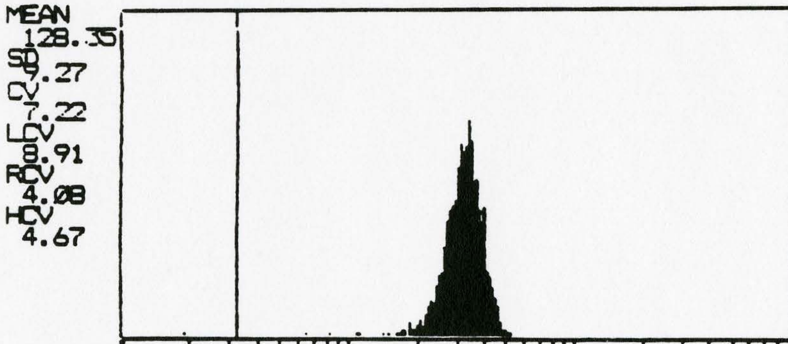
SAMPLE 1 11:39 19MAR91.01402C
ANTIBODIES A+B
LIGFL/FALS ,90'LI,MAP1

(1) CHANNEL 48 TO 255 INTEGRAL 1475
(2) PEAK 74 AT 162 % IN INTERVAL 26.29

*****.ADJUST LOWER CURSOR

STATISTICS

SCALE= 256 TOTAL= 5611



SAMPLE 1 11:39 19MAR91.01403C
ANTIBODIES A+B
LIRFL/FALS ,90'LI,MAP1

CHANNEL 44 TO 254 INTEGRAL 2385
PEAK 167 AT 131 % IN INTERVAL 42.51

*****.ADJUST UPPER CURSOR

The histograms on the previous page are derived from the same sample.

One is the green fluorescence histogram (top), the other is the red fluorescence histogram (bottom).

The vertical axis represents the number of cells.

Line 1) The horizontal axis represents LIGFL and is divided into units called channels. The channels are numbered from 1 to 255. It states here that the cursor starts ta channel 48 and ends at channel 255. The number of events between the 2 cursors (within the interval) is 1475. The total number of events on the histogram is seen as 5611.

Line 2) The peak channel (the channel with the most events in it within the interval) is in channel 162, with 74 events in the channel. The % of events occurring in the interval is 26.29 % of total number of events in the histogram: 1475/5611 = 26.29 %

APPENDIX VII

McMASTER UNIVERSITY
HAMILTON, ONTARIO, CANADA

COMMITTEE ON
THE ETHICS OF RESEARCH ON HUMAN SUBJECTS

TO: Duncan Mac Dougall

RE: Jason Kajiura's M.Sc. research

TITLE: Immunological response to changes in training intensity and volume in highly trained runners

The above named applicant has submitted an application to the Committee on Ethics of Research on Human Subjects.

The Committee has reviewed this request and finds that it meets our criteria of acceptability on ethical grounds. The review has been conducted with a view toward insuring that the rights and privacy of the subject have been adequately protected; that the risks of the investigation do not outweigh the anticipated gain; and that informed consent will be appropriately obtained.

We concur in all necessary endorsements of the application.

Digby Elliott

Date: Oct 15/91

For the Committee on the Ethics of Research on Human Subjects

C.K. Bart, Associate Professor, Business
T. Beckett, Judge, Unified Family Court
I.M. Begg, Professor, Psychology
B. Donst, Ecumenical Chaplain, Chaplains' Office
D. Elliott, Associate Professor, Physical Education and Athletics (Chair)
R. Howard, Professor, Sociology
T. Kroeker, Lecturer, Religious Studies
R.J. Preston, Professor, Anthropology
J. Synge, Associate Professor, Sociology

MCMASTER UNIVERSITY
DEPARTMENT OF PHYSICAL EDUCATION

SUBJECT DATA SHEET: IMMUNOLOGICAL RESPONSE TO CHANGES IN TRAINING INTENSITY AND VOLUME IN HIGHLY TRAINED RUNNERS.

NAME:__________________________ DATE:_____________

ADDRESS:_____________________________ Tel #:____________

AGE:______

HEIGHT:_______

WEIGHT:_______kg

ALLERGIES/MEDS:_____________________

PB's: 800m:________
1500m:________
2K:________
5K:________
10K:________
Other:________

AVERAGE TRAINING DISTANCE PER WEEK:___________km.

MCMASTER UNIVERSITY
DEPARTMENT OF PHYSICAL EDUCATION
RESEARCH PROJECT: IMMUNE SYSTEM RESPONSE TO CHANGES IN TRAINING INTENSITY AND VOLUME IN RUNNERS.

INFORMATION AND CONSENT FORM

A. PURPOSE:

The purpose of this study is to investigate the immunological response (as indicated by certain cells in your blood) to intensity and volume changes in your training program.

B. PROTOCOL:

You will be monitored over 40 days of training, during which intensity and volume will be manipulated. On 20 occasions throughout this period, venous blood samples will be taken. A treadmill performance run will also be given on 4 occasions.

C. MEASUREMENTS:

1. Immunological tests:
On 20 occasions approximately 5 to 10 ml of blood will be withdrawn from a vein at your elbow. Sterile procedures are used so that there is minimal risk of infection however you may notice next-day "bruising" at the site of the puncture.

2. Treadmill test:
At the end of each of 4 training phases of the study you will be asked to run on a treadmill at a velocity equivalent to your best 1500m time. You will do so until you feel that you can no longer maintain that pace. When you grasp the safety rail the test ends and the investigator will stop the treadmill (or you can do so yourself by pushing the red safety button). If you fall during the test the consequences would be similar to falling on the track during a workout.

D. TIME COMMITMENT:
You will be required to perform approximately one hour of training (running at various intensities and distances) each day for a period of 40 days. You will be required to have four treadmill $\dot{V}O2$ max tests, and 20 blood samples taken over the 40 day period.

E. REMUNERATION:
Volunteers who agree to serve as subjects for the study

may receive an honorarium for their services. These services include strict adherence to all measurements and training schedules.

F. WITHDRAWAL:

You are free to withdraw from the study at any time, however doing so disallows you from receiving the financial award. No partial payment will be made.

G. USE OF DATA:

The data collected will be used in the preparation of scientific reports that will be presented at conferences and published in scientific journals. Subjects will not be identified by name in presentations and reports. Data obtained from subjects who have withdrawn prematurely from the study will be destroyed.

H. CONSENT TO PARTICIPATE:

If, after reading the above information, you are interested in participating as a subject, you should read the statement below and sign in the space provided.

I have read and understand the above explanation of the purpose and procedures of the project, and the conditions under which I shall participate, and agree to participate.

\-------------------------- \-----------------------

Signature Witness

\--------------------------

Date

DESIGN OF STUDY: 4 x 10-day phases over a 40-day training period.

```
* = venous blood sample (taken pre- and 5 minutes post-ex.)
T = training day
R = rest day
P = treadmill performance test
```

```
            PHASE 1     PHASE 2     PHASE 3     PHASE 4

Group 1:       A           B           C           D
                     |*  *  *    |           |*  *  *    |
                     |TTTTTTTRPR |           |TTTTTTTRPR |
                     |*     *    |           |*     *    |
          *  *  *    |           |*  *  *    |           |
          TTTTTTTRPR |           |TTTTTTTRPR |           |
          *     *    |           |*     *    |           |

          Baseline   High volume  Baseline   High volume
                     Low intensity           High intensity
           (LV/LI)     (HV/LI)    (LV/LI)     (HV/HI)

Group 2:       A           D           C           B
                     |*  *  *    |           |*  *  *    |
                     |TTTTTTTRPR |           |TTTTTTTRPR |
                     |*     *    |           |*     *    |
          *  *  *    |           |*  *  *    |           |
          TTTTTTTRPR |           |TTTTTTTRPR |           |
          *     *    |           |*     *    |           |

          Baseline   High volume  Baseline   High volume
                     High intensity          Low intensity
           (LV/LI)     (HV/HI)    (LV/LI)     (HV/LI)
```

10-DAY PHASE BREAKDOWN:

```
      Day:  1    2    3    4    5    6    7    8    9    10
eg: Group 1
Phase A = 5km  5km  5km  5km  5km  5km  5km  rest Test rest } 60-70%
      B = 10km 10km 10km 10km 10km 10km 10km rest Test rest } VO2 max.
      C = same as A
      D = same distance as B but on alternate days (day 1,3,5 and 7)
          the 10km is interval training (ie: 10 x 1000m at 95-100 %
          VO2 max. with 3 minutes recovery).
```

Immunological measurements:

Immunoglobulins: IgM, IgA, IgG.
Lymphocyte subsets: CD3+ (total T cells), CD4+ (T4-helper), CD8+ (T8-suppressor/cytotoxic), CD4/CD8 (helper/suppressor T cell ratio), HLA-DR+ (B cell and activated T lymphoid cell-marker).

McMASTER UNIVERSITY
DEPARTMENT OF PHYSICAL EDUCATION
IMMUNOLOGICAL STUDY: TRAINING/CLINICAL DIARY

NAME: ______________ TRAINING ORDER: 1) ☐ DAILY DIST: min: ____ km
GROUP: ______ TIME: ____ 2) ☐ max: ____ km

	DIST/ TIME	Wt. (kg)	SLEEP (hrs)	STRESS/MOOD (How you feel) Tired OK Good					COMMENTS Phys. probs? (any pain?)	VIRUS EXPOSURE (family, friends roommates sick?		Hct (triplicate)			T (°C)
				1	2	3	4	5		NO	YES (who?)				
P1 1pre															
post															
4pre															
7pre															
post															
10pre															
post															
12Test															
P2 1pre															
post															
4pre															
7pre															
post															
9Test															
P3 1pre															
post															
4pre															
7pre															
post															
9Test															
P4 1pre															
post															
4pre															
7pre															
post															
9Test															

McMASTER UNIVERSITY
DEPARTMENT OF PHYSICAL EDUCATION
IMMUNOLOGICAL STUDY: QUADRANT STATISTICS-HISTOGRAM DATA (%).

NAME:____________________ TRAINING ORDER: 1) ☐ DAILY DIST: min=____km
GROUP:________ TIME:______ 2) ☐ max=____km

	DATE	Q3 IgG	Q1 CD4 /	Q4 CD8 = RATIO	Q1 CD3+	Q4 HLADR+	Q2 CD3+HLADR+
P1 1pre							
post							
4pre							
7pre							
post							
9pre							
post							
P2 1pre							
post							
4pre							
7pre							
post							
P3 1pre							
post							
4pre							
7pre							
post							
P4 1pre							
post							
4pre							
7pre							
post							