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EFFECT OF RECRUITMENT TRAINING ON IMMUNE SYSTEM

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การออกกำลังกายเป็นการส่งเสริมให้มีสุขภาพดี ซึ่งรวมถึงการทำงานของระบบภูมิคุ้มกัน อย่างไรก็ตามมีรายงานมากมายที่แสดงให้เห็นถึงการมีภาวะภูมิคุ้มกันลดลงในนักกีฬาที่ผ่านการฝึกที่หนัก และเป็นเวลาติดต่อกัน ทหารเกณฑ์ที่ต้องผ่านโปรแกรมการฝึกทหารเป็นเวลานานถึง 8 สัปดาห์อาจมีภาวะภูมิคุ้มกันลดลงด้วย ทหารเกณฑ์จำนวน 20 คน (อายุ ระหว่าง 21ถึง 23ปี) ที่ทำการฝึกที่ กองพันทหารราบ โรงเรียนนายร้อยพระจุลจอมเกล้า ได้รับการคัดเลือกเป็นอาสาสมัครในการศึกษานี้ โดยการใส่เครื่องวัดอัตราการเต้นของหัวใจ (Polar Accurex Plus) ตลอดระยะเวลาของการฝึกซ้อม โปรแกรมการฝึกทหารประกอบด้วยกิจกรรมหลัก 4 กิจกรรมดังนี้ คือ การออกกำลังกาย ซึ่งทำให้อัตราการเต้นของหัวใจอยู่ระหว่าง122 ถึง 154 ครั้งต่อนาที ค่าเฉลี่ยเท่ากับ 142.53 ครั้งต่อนาที,การฝึกท่าบุคคลเบื้องต้นอัตราการเต้นของหัวใจอยู่ระหว่าง120 ถึง 160 ครั้งต่อนาที ค่าเฉลี่ยเท่ากับ 145.55 ครั้งต่อนาที, การเดินสวนสนาม อัตราการเต้นของหัวใจอยู่ระหว่าง128 ถึง 160 ครั้งต่อนาที ค่าเฉลี่ยเท่ากับ 146.11 ครั้งต่อนาที และ การฝึกแถวชิด อัตราการเต้นของหัวใจอยู่ระหว่าง120 ถึง 169 ครั้งต่อนาที ค่าเฉลี่ยเท่ากับ 145.48 ครั้งต่อนาที, ทำให้ทราบว่า โปรแกรมการฝึกทหารเกณฑ์เป็นการออกกำลังกายระดับปานกลางถึงระดับหนัก การทำทูเบอร์คูลินสกินเทสต์ ซึ่งเป็นวิธีตรวจกรองการทำงานของระบบภูมิคุ้มกันชนิดผ่านเซลล์นั้นพบว่า มีการลดลงของภาวะภูมิคุ้มกันภายหลังจากทำการฝึก 8 สัปดาห์อย่างมีนัยสำคัญทางสถิติ ($p<0.02$) ปริมาณของซีรั่มอิมมูโนโกลบูลิน จี และเอ็ม ในเลือด ของอาสาสมัครเก็บก่อนเข้าโปรแกรม ,4 สัปดาห์ และ 8 สัปดาห์ ของการฝึก และหลังจบโปรแกรมการฝึก 1 สัปดาห์ มีการลดลงของโปรตีน ทั้งสองชนิด ในช่วงเริ่มต้นเมื่อเปรียบเทียบระหว่างก่อนเข้าโปรแกรมและ 4 สัปดาห์ของการฝึกอย่างมีนัยสำคัญทางสถิติ ($p<0.01$) และจะกลับเข้าสู่ระดับเดิมเมื่อร่างกายสามารถปรับตัวได้ ในเวลา 8 สัปดาห์ของการฝึก โดยสู่ระดับปกติหลังจากการฝึก 8 สัปดาห์การศึกษาชี้ให้เห็นว่า อาสาสมัคร ที่ผ่านโปรแกรมการฝึกทหารสามารถที่จะปรับตัวเองให้มีภาวะภูมิคุ้มกันเพื่อมีสุขภาพที่ดีได้หลังจากการฝึกไปแล้วในระยะปรับตัว การลดลงของภาวะภูมิคุ้มกันในระยะเริ่มต้นอาจแก้ไขได้โดยให้ได้รับอาหารที่เหมาะสมและพักผ่อนเพียงพอ

หลักสูตร…เวชศาสตร์การกีฬา…… ลายมือชื่อนิสิต…….....
สาขาวิชา…เวชศาสตร์การกีฬา…… ลายมือชื่ออาจารย์ที่ปรึกษา …….
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The aim of exercise is to improve body health and enhance immune system function. However, several studies have observed the suppression of immune response in athletes who were trained with strenuous exercise. Recruits who were undertrained in the recruitment training program for 8 weeks long might also have the immune suppression. Twenty males, age ranging from 21 to 23 years in Chulachomklao Royal Military Academy infantry battalion volunteered to participate in this study. They were asked to wear a Polar Accurex Plus Telemetric heart rate monitor during the training period. The training program composed of four types of activities; the exercise, the basic training, the marching and the formation, which have been considering the moderate to high intensity exercise training program. The results have shown as follows; first from the exercise, the heart rate ranging from 122 to 154 bpm with the average of 142.53 bpm, second activity ranging from 120 to 160 bpm with the average of 145.55 bpm, third one ranging from 128 to 154 bpm with the average of 146.11 bpm and the last activity of formation ranging from 120 to 169 bpm with the average of 145.48 bpm, respectively. Tuberculin skin test which is the screening method for cellular immune responses was significantly decreased at post-training compared to the pre-training ($p<0.02$). The levels of serum IgG and IgM at pre-training , 4^{th}weeks , 8^{th}weeks and one week post-training were significantly decreased at the initiation period , which were compared between pre-training and 4^{th}weeks of training ($p<0.01$) , and returned to normal at the 8^{th}weeks of training and one week post-training. This study demonstrated that subjects underwent the recruit-training program could be able to adjust themselves to a good health after the period of time i.e., in the adaptation period. The decrease in the initiation period could be compensated with the proper diet and enough rest.

Department……-…… ……. Student's signature…….

Field of study…Sports Medicine…..…….. Advisor's signature……

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LIST OF ABBREVIATIONS

ACSM	American College of Sports Medicine
bpm	beat per minute
BW	Body weight
CAD	Coronary artery disease
CRMA	Chulachomklao Royal Military Academy
dl	deciliters
ECG	Electrocardiogram
e.g.	Exempli gratia
HDL	High density lipoprotein
HR	Heart rate
HR max	Maximal heart rate
i.e.	Id est
Ig	Immunoglobulin
IL	Interleukin
Kg	Kilograms
KJ	Kilojoule
MET	Metabolic equivalent
mg/dl	Milligram / deciliters
MHC	Major histocompatibility Complex
MI	Myocardial infarction
min	Minute
mm	Millimeters

LIST OF ABBREVIATIONS

PPD	Purified protein derivatives
RPE	Rating of perceive exertion
SD	Standard deviation
URI	Upper respiratory infection
VO_2	Oxygen uptake
VO_2 max	Maximum oxygen uptake
yr	Year

CHAPTER I

INTRODUCTION

Many people have always been told that exercise is good for health. It can decrease the duration of illness as well as prevent illness. Studies have shown that exercise esteems a positive effect on the immune system. Moderate exercise protects against infection and may have beneficial effects on cardiovascular system as well as the psychological well-being of the individual (Roy and Pang 1999). On the other hand, the prolong strenuous exercise will induce many changes in the body such as lactic acid accumulation, muscle injuries, fatigue and increase susceptibility to infection.

Effects of exercise on the immune system are dependent on the level of fitness of subject , the degree of exercise intensity and the duration of exercise (Hoffman-Goetz and Pederson 1994). Several reports have linked long term exercise and heavy training with increased susceptibility to infection. Thus a high incidence of illness such as upper respiratory tract infection and gastrointestinal problems has been reported to occur in athletes after prolong , exhaustive exercise (Benner et al.1994; Hoffman-Goetz and Pederson 1994 ; Neiman 1994). Although a considerable leukocytosis occurs in response to this type of exercise, the number and functions of circulating immune cells can be adversely affected by prolonged, exhaustive exercise (Neiman 1994; Shephard et al. 1994). The high incidence of infections may thus be associated with impaired function of cells of immune system . For example, reductions in natural killer cell activity and decreased T-cell response to mitogenic simulation have been observed after prolonged, excessive exercise (Neiman 1994; Shephard et al. 1994). However some studies have shown that intensity and duration of an exercise bout had various effects on immune system. Other factors which are essential to these results include types of exercise performance exercise and frequency of performance. Regular, moderate exercise enhances

immune function but acute exercise attenuates immune disturbances (Roy and Pang 1999).

Neiman DC in 1994 postulated the relationship between amount of exercise and the risk of upper respiratory tract infection or susceptibility to viral infection as a J-shaped model (Figure 1.1). According to this model , regular moderate physical activity will enhance immune response. In contrast, excessive exercise may increase the suppressive immunity or susceptibility to viral infections.

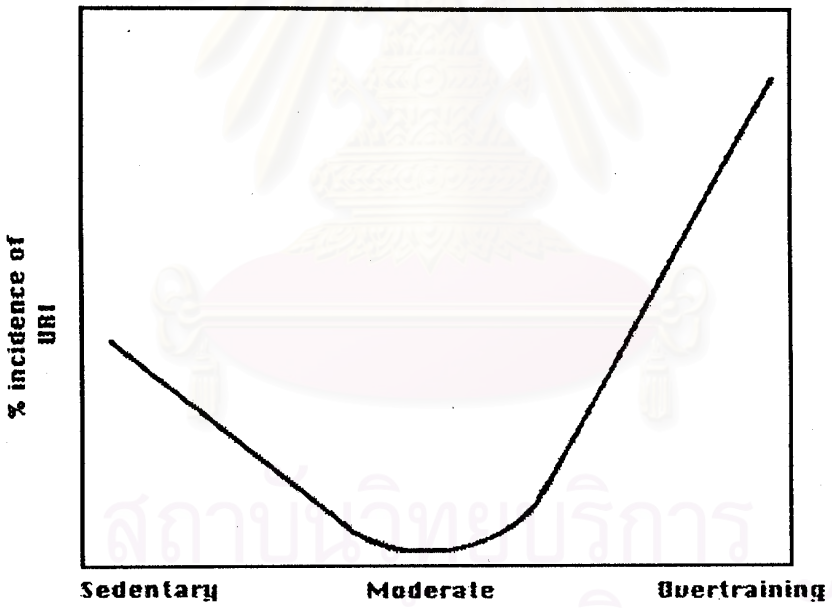

Figure 1.1 A J-shaped relationship between the level of physical activity and immune function has been hypothesized. Regular moderate exercise enhances immune function, and exhaustive exercise cause temporary immunosuppression.

Recruitment training, a physical exercise program, aims to enhance physical performance and endurance. It is considered as a prolonged vigorous exercise which leads to body stress due to limited time. Recruits are scheduled for training from 05:30 Am to 05:30 PM each day, 5 day per week for 8 weeks. With this training program, recruits are expected to have a physical fitness, good health, with a positive response of immune system.

In general, the recruits training program encounters an excessive amounts of exercise for a long period of time up to 8 weeks. This type of training program should have some effects on immune system. Therefore, this research was designed to study the effects of the training on cellular immune response in term of tuberculin skin test, and humoral immune response by measuring the level of serum immunoglobulin G (IgG) and serum immunoglobulin M (IgM) throughout the training period.

1.1 Research Question

Are there any changes in immune response of recruits throughout training program?

1.2 Objectives of this study

The purpose of this study about recruitment training exercise and immune function are as follows.

1. Classification of the recruitment training program according to level of intensity, duration and frequency.
2. Changes of tuberculin skin test in the trained recruits.
3. Effect of recruitment training on serum immunoglobulin i.e., IgG and IgM levels.

1.3 Assumptions

1. The study population were the healthy recruits in infantry battalion in Chulachomklao Royal Military Academy (CRMA) who were the first drafted group of the year 2000 recruitment is a healthy population.
2. Food consumption has no confounder effect on the studies since every one was provided by the same food menu through 8 weeks training session.
3. Daily living activities were under the same condition during the training program.

1.4 Operational Definitions

1. Moderate intensity is defined as the physical activity which gives target heart rate at 60 to 79 % of HRmax and continuous duration for at least 20 minutes.
2. High intensity is defined as the physical activity which gives target heart rate at 80 to 89 % of HRmax and continuous duration at least 5 minutes.
3. Frequency is defined as a number of session per week.
4. Duration is defined as the length of time for each recruitment training session.

1.5 Expected Benefits of the study

1. Classification of recruitment training program in term of exercise.
2. Effect of e recruitment training exercise on immune system will be considered for the improvement of the best benefit from the training program.
3. The results of this study might be useful for recruits preparation before, during and after training.

CHAPTER II

LITERATURE REVIEW

2.1 The Immune System and Its Function

The immune system functions as the body defense against any foreign material particularly pathogens. This system is very complex. It is composed of various cell types and many cellular components. The defense reaction is called the immune response which can be classified into nonspecific and specific immune responses.

The body has essentially two main distinct pathways to fight infection: first is through a nonspecific response, which involves the phagocytes and natural killer cells (NK) ; and second is through a specific response, which involves the B cells and T cells. (Shepherd et al.,1994).

The nonspecific immune response is analogous to the first line of defense against pathogen or foreign materials. The broken body's external protective coat (the skin) the injured internal body tissue and bacterial or viral infection will trigger an inflammatory response to develop. The response is initiated by local release of molecules from the damaged tissue. These events result in increase localized blood flow and phagocytes which are assisted by several mediators, migrate to the damaged tissue as site of infection. Then the complement system becomes activated during inflammation and assists in the rupturing of bacterial cell walls. In addition interferon which is released by immune cells will stimulate other immune cells to improve their resilience to viral infection.

The nonspecific response to infection differs from the B-cell and T-cell responses in three important ways (Mackinnon LT.1992):

1. The phagocytes, complements, and interferon responses are not specific to any given pathogen.
2. No "memory" of the response to a specific pathogen results.
3. The inflammation is localized at the region of initial infection of damage.

Unlike the nonspecific response system, B-cells and T-cells interact to induce a specific, memorized, and systemic response to infection.

The body's ability to mount a specialized immune response is based on specific proteins that exist on the membranes of host cells, and pathogens. One's own body cells are labeled with a special class of proteins recognized by the immune system as "self". These proteins are called major histocompatibility complex (MHC) proteins. Because MHC proteins are different in each individual except in identical twins, they induce an immune response triggered by tissue donation between individuals. This immune response occurs because the recipient's immune system does not recognize the donor MHC protein as safe, but rather interprets it to be a foreign protein (antigen). The B cells and T cells launch the specific response when they recognize the pathogen. Recognition of an antigen will stimulate a humoral immune response as well as a cell-mediated immune response.

In humoral immune responses, the antigen recognition of B cells will lead to B cell activation and proliferation, causing the formation of identical B cell receptive to that antigen. These cells then circulate in the blood and lymph and develop the ability to synthesize and secrete proteins (antibodies) that can bind to the pathogen. These developed B cells are called plasma cells. The duration from infection to maximum circulating levels of antibodies may be as long as 10 days (Marieb, 1995).

Many of the plasma cells remain stored in the lymph node tissue and are released when exposed to their antigen, thereby providing a type of “memory” to the immune response.

Antibodies, which are also called immunoglobulins (Ig), are classified into five classes- IgA, IgD, IgE, IgG and IgM according to structural sizes, complexity and functions. Among these immunoglobulins, IgG is the most abandant and functions in protecting the body against bacteria, virus, and toxins. Interestingly IgE is involved in histamine release in the lung airways during allergic reactions and asthma. The diversity of the antibodies within each class is due to the continual rearrangement of the genes of the B cells. Thus B cell membrane proteins have a natural genetic diversity which is extended into a diversity of plasma cell proteins and the antibodies they manufacture. The stimulated antibody binds to specific antigen to form a circulating antigen-antibody complex. This complex will trigger the function of complement cascade system. The activated complement proteins bind to antigen-antibody complex to initiate the lysis process. The circulating antigens or infected cells might be removed by diverse mechanisms such as agglutination, opsonization and phagocytosis, lysis by complement or neutralization (Fig 2.1) (Van Wynsberghe D. et al. 1995).

In cell-mediated immune response, the majority of cells are T cells. However, unlike the B cells and phagocytes, which act on the antibodies against exposed pathogens, the T cells target the exposed antigens of infectious organisms that penetrate cells to reproduce and interfere with cell functioning.

T cells are divided into three functional classes (helper T cells, cytotoxic T cells and T-suppressor cells). Helper T cells are activated by binding to antigen fragments associated with class I MHC proteins as to B cells attached to antigens associated with class II MHC. After binding T-helper cells release proteins (lymphokines) such as interleukin-2 or IL-2 whereas stimulated phagocytes release interleukin-I orIL-1.

IL-1 stimulates the activity of the bound T cells, thereby increasing the release of IL-2 and creating a positive feedback loop that amplifies the immune response.

Cytotoxic T cells, or killer T cells, are activated by binding to antigen fragments associated with class I MHC proteins. This association occurs when the microorganism (e.g., virus) enters a host cell. When bound to the infected cell, the cytotoxic T cell impregnates the target cell with a chemical that results in cell lysis. This process is enhanced by lymphokines (e.g.,IL-1) stimulation.

Because the body's cell-mediated immune response is based on positive feedback, something must "turn off" the process. This is the function of the T-suppressor cells. As yet it is unclear what guides these cells to down-regulate the T-cell immune response, or how this down-regulation is made to coincide with complete destruction of the pathogens (Figure 2.2) (Van Wynsberghe D. et al. 1995).

The most common method for monitoring development and maintenance of cellular immunity is skin testing, which is used for such antigens as tuberculin, candida or histoplasmin. More specific in vitro tests include that involve phytohemagglutinin (PHA) purified antigens, or other lymphocytes for demonstrating cellular reactivity .

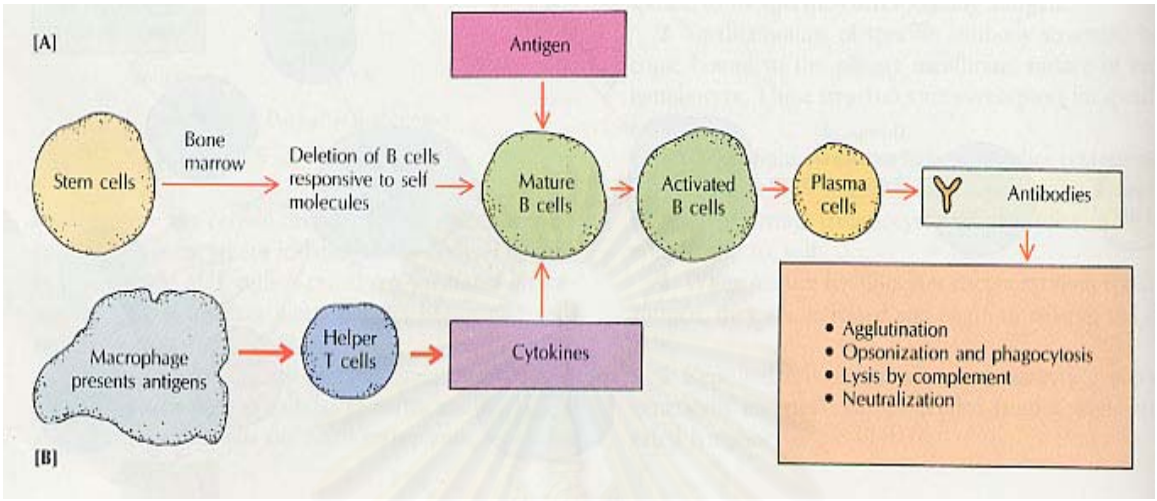

Figure 2.1 Summary of humoral immunity.

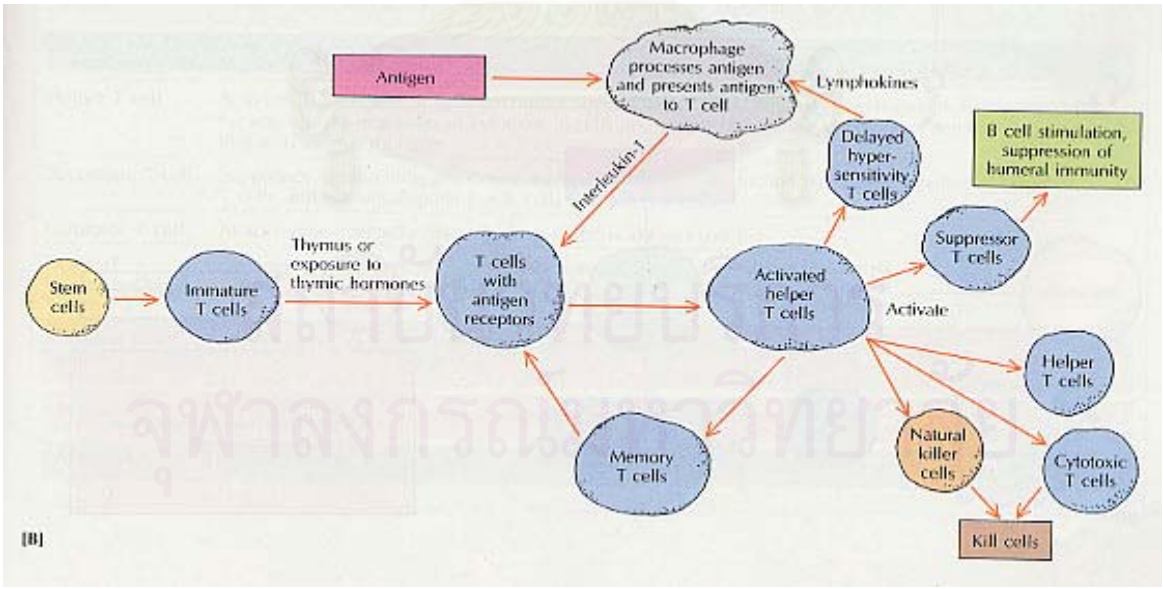

Figure 2.2 Summary of cell-mediated immunity.

2.2 Factors Influencing Immune Response (Roy AND Pang, 1999)

2.2.1 Pathogen Exposure

For athletes, the local of competition and any requisite air travel may cause exposure to a greater range of infected persons and unfamiliar microorganisms. This alone can increase the incidence of viral infection, even if inherent susceptibility remains unchanged.

2.2.2 Nutrition

Clinical malnutrition is a well-recognized of impaired immune function. Most athletes show only small deficiencies of essential nutrients. Nevertheless, they may lack the glutamine needed for lymphocyte proliferation. Deficiencies in arginine, L-carnitine, essential fatty acids, vitamin B_6, folic acid, vitamin E, and trace elements may also contribute to reduced immune function in athletes.

2.2.2 Environmental Influences

Because greater physical activity is associated with a higher socioeconomic status and such status is associated with less exposure to environmental stressors, moderately active people may have less exposure to stressors than sedentary persons . However, an international competitor encounters many environmental stressors during training and competition. Extremes of heat and cold , high and low ambient pressures, inhalation of polluted air and a resultant acute-phase response in the alveolar macrophages, sleep deprivation, time zone shifts, and exposure to high and low gravitational forces can all influence immune function adversely. Environmental stress can also affect immune function through neurohormonal response, such as the catecholamines secreted in response to severe cold exposure or the cortisol, IL-1, and IL-6 secretion associated with a

rise in core temperature. Finally, an individual in an unfamiliar environment may experience psychological stress and result in effecting on immune function.

2.2.3 Psychological Influences

Links between the hypothalamus and the immune system allow psychological factors to modulate the impact of acute and chronic physical activity on immune function. The effects of psychological stress and vigorous physical activity tends to be additive, since heavy exercise induces many of the same neurohormonal response, including substantial secretions of epinephine, norepinephine, cortisol, and growth hormone. Athletes plainly face more severe psychological challenges than laboratory subjects who exercise at a similar relative intensity, so they have a greater potential for immune disturbances. For example, as much as a quarter of the variance in virus shedding among individuals inoculated with a rhinovirus can be traced to restricted social contacts and spending too much time in cold-directed activities; such as lifestyle has become almost inevitable for athletes who are preparing for international competition.

2.2.4 Muscle Microtrauma

Cumulative microtrauma from exercise causes local and systemic acute phase reactions. In the short term, the resulting release of C- reactive protein stimulates monocyte phagocytosis. However, the migration of leukocytes to injured muscle may reduce immune function in other parts of the body. The generation of oxidant free radicals during the repair process may suppress immune function in manner analogous to clinical sepsis. Perhaps for this reason, antioxidants such as vitamin C were used for reducing the risk of exercise-induced infections.

2.3 Effect of Exercise on Immune system

Pyne DB and Gleeson M(1998) studied effect of intensive exercise training on immunity in athletes and showed that serum IgG were lower than control . Suppression of mucosal immune parameters has been associated with the risk of upper respiratory tract infection (URTI). Swimmers with a lower pre-season salivary IgA and / or lower pre-exercise salivary IgA level were more likely to contract an illness during a 7 month training period. In a shorter 12 week study, infected swimmers had a mean salivary IgA concentration that dropped more sharply after a single session.

Rowbottom et al.(1995) compared ten subjects diagnosed as suffering from overtraining syndrome and found no significant differences between the normal value for an age-matched subject group and the mean values for ten subjects, for any of the measured lymphocyte subset parameters (T-cells, T-helper/inducer cells, cytotoxic/suppressor cells, natural killer cells). The only parameter measured which deviated significantly from the established normal range was the plasma concentration of glutamine (Rowbottom et al. 1995). This finding is in agreement with previous studies which reported lower plasma glutamine concentration in athletes who were suffering from overtraining (Lehmann et al.1993; Urhausen et al.1995; Sharp and Koutedakis 1992;Parry-Billings et al.1992)

Fry et al. (1991) demonstrated that subjects overtrained for 10 days which revealed an elevation in the expression of lymphocyte activation antigens combined with a reduced response of T- lymphocytes to mitogenic challenge, and a depressed number of natural killer cells in the peripheral circulation. These changes occurred in the apparent absence of infection and are analogous with immunological findings in patients suffering the chronic fatigue syndrome (CFS) (Fry et al. 1991). In CFS sufferers there is an increased expression of lymphocyte activation antigens, increased production of cytokines, depressed T- cell response to mitogenic challenge, and decreased numbers of circulating

natural killer cells (Fry et al. 1991). It has been suggested that fatique associated with CFS may, to some degree, be the result of a deranged immune system. Fry et al. (1991) concluded that this may have contributed to the fatigue expressed by the subjects in the study.

The increase in circulating leukocytes (phagocytes and lymphocytes) that occured in response to moderate to intense exercise has been well established (McCarthy and Dale, 1988). This increase was greater for more prolonged exercise and returns to normal resting values within the first 2 hours of recovery (Fry et al1991, Morton and Kest, 1992, McCarthy and Dale, 1988).

The fact that exercise-induced leukocytosis is related separately to both the intensity and the duration of exercise indicates a possible connection of the hormonal response to exercise. In fact, this connection has been documented (Shephed et al.1994) with increased circulating catecholamines being responsible for the relationship between leukocyte counts and exercise intensity. The site of action of the catecholamines are believed to be a combination of systemic effects plus direct sympathetic innervation of the spleen and pulmonary and systemic blood vessels (McCarthy and Dale, 1988). The increase in cortisol release during prolong exercise is believed to cause the rapid increase in leukocytes after 2 hours, attributable to an increased release of leukocytes from bone marrow (McCarthy and Dale, 1988).

Obviously, simply measuring total leukocyte concentrations is a poor reflection of the specific functions of the phagocytes, NK cells, B cells, and T cells. Also, an increase in either the phagocyte or lymphocyte category may not necessarily reflect an alternation of immune function (Shephed et al. 1991).

Research that has specifically measured the phagocyte and lymphocyte classes has shown that prolong exercise that increase serum cortisol primarily increase eosinophils (Fauci and Dale, 1975). Futhermore, individuals who are more cardiorespiratory

endurance trained (higher VO_2max) have a larger increase in neutrophils during a given exercise intensity, whereas less trained individuals have a greater increase in lymphocytes (Dorner, Heinold and Hilmer, 1987).

Unlike what might be a positive response of the immune system to acute exercise, long- term exposure to exercise actually causes a suppression of immune function. Studies generally have been consistent in finding lower total leukocyte counts, lymphocyte counts, and immunoglobulin in well trained athletes at rest and during exercise (Shephed et al. 1991). Longitudinal training studies have provided inconsistent finding compared to the previous cross-sectional data, probably because of the poorly controlled influence of the previous bout of training on immune function.

Well-trained athletes often report a higher incidence of upper respiratory infections than more than sedentary counterparts. This has been explained as being a result of a mechanism that suppresses immune response (Shephed et al. 1991); however, no experimental evidence of such a mechanism exists.

Table 2.1 Effect of Training on Resting immune Function (Roy and Pang, 1999)

Author	Variable	Subjects	Findings
Baslund et al. 1993	NK activity	18 rheumatiod arthritis patient	No change over 8 weeks training
Campbell et el.1988	T cell function	Beagle dogs 8T*,8UT*,	No change with training
Ferry et el. 1991	T cell function	Young rats	Impaired by training
Jensen 1989	T cell function	Pigs	No change with Training
Kajiura et el. 1993	Lymphocyte subset	12 runners	Immunosupressant
Mitchell et el.1993	Ig production lymphocyte proliferation	11T 10 control	No change of lymphocyte proliferation or Ig production over 12 weeks moderate T
Wit 1990	IgA,IgG,IgM	217 Athletes	Decrease with peak T
Verde et el.1993	Lymphocyte counts Mitogen stimulated Cell proliferation And Ig synthesis	10 Runner	Decrease IgG synthesis with heavy training

*T = trained, UT = untrained

2.4 Measurement of Exercise Intensity

Intensity of exercise determines the total caloric expenditure during a training session, and are integrally related. Training induced physiological changes depends on the intensity of the overload. There are at least seven ways to express exercise intensity (ACSM'S Guidelines 1995 and McARDLE et al, 1995).

1. As calories expended per unit time (for example, 9 kcal'min^{-1} or 37.8KJ min^{-1}).
2. As particular absolute exercise level or power output (for example, 180 kg-mmin^{-1}or 29.4 W).
3. As particular relative metabolic level expressed as a percentage of VO_2max (for example, 58 % VO_2max).
4. As a level of exercise below, at, or above the lactate threshold (i.e, 4 millimole lactate).
5. As particular exercise heart rate or percentage of maximum heart rate (for example, 180 beats per minute or 80% HRmax).
6. As multiple of resting metabolic rate (MET is metabolic equivalent, a unit used to estimate the metabolic cost (energy expenditure as reflected by oxygen consumption) of physical activity, One MET equates the resting metabolic rate, which is approximately 3.5 milliliters of oxygen per kilogram body weight per minute. MET are used to compare the energy costs of different activities).
7. As some rating of perceived exertion (RPE) from 6 (very, very light) to19 (very, very hard).

Heart rate (HR) methods (McArdle W D. et el 1996)

HR is used as a guide to exercise intensity since it gave relatively linear relationship between HR and VO_2. Because maximal HR (HRmax) declines with age and has large inter-individual difference, it is best to measure HRmax during a maximal graded exercise test whenever possible. When prescribing exercise intensity based on HR, consideration must be given to potential influences.

Although establishing training intensity from measures of oxygen uptake is resonably accurate, it is impractical without sophisticated equipment. An effective alternative is to use heart rate to classify exercise for relative intensity and then establish the training protocol. This practice is possible because the percent VO_2max and percent HRmax are related in a predictable way regardless of gender, fitness level, or age. Selected values for percent VO_2max and corresponding percentages of HRmax obtained from several sources are presented in Table 2.2. The error in estimating percent VO_2max from percent HRmax , or vice versa, is about (8%). Because of this intrinsic relationship, it is only necessary to monitor heart rate to estimate the exercise stress or percent VO_2max. The relationship between percent HRmax and percent VO_2max is essentially the same for arm or leg exercise among healthy subjects, normal and obese groups, cardiac pateins, and people with spinal cord injuries. The important point is that HRmax is significantly lower in arm compared to leg exercise, and this difference must be considered when formulating the exercise prescription for different exercise modes.

Table 2.2 Classification of Intensity of Exercise based on 30 to 60 minutes of Endurance Training

Classification of intensity	%HRmax	% VO_2max	RPE
Very Light	<35	<30	<9
Light	35-39	30-49	9-10
Moderate	60-79	50-74	11-12
High	80-89	75-84	13-16
Very high	>90	>85	>16

Modified from ACSM'S Guidelines 1995.

2.5 Exercise duration (McArdle W D. et el 1996)

The time constrains imposed on the individual influence both the duration and frequency of exercise sessions. Although improvements in cardiorespiratory endurance have been demonstrated with 5 to 10 minutes of very high intensity (>90% VO_2max) exercise, the risk benefit tradeoff of this format argues against its consideration. Typically, caloric goal can be best met in sessions lasting 20 to 30 minutes, excluding time spent for warming up and cooling down. ACMS recommends 20 to 60 minutes of continuous aerobic activity. Initial goals should be set reasonably so that individuals can reach preset goals with exercise sessions of moderate duration (20 to 30 minutes). For severely deconditioned individuals, multiple sessions of short duration (10 minutes) may be necessary.

2.6 Exercise frequency (McArdle W D. et el 1996)

Frequency is interrelated with both intensity and duration of exercise and therefore depends on those two variables. However, functional capacity is of key importance. Patients with functional capacities of <3 METs benefit from multiple short daily exercise sessions; one to two sessions/day are most appropriate for three to five MET capacities; and 3 to 5 sessions/week are recommended for individuals with functional capacities >5 METs. Clearly, the number of exercise sessions per week will vary given caloric goals, participant preferences, and limitations imposed by the participant's lifestyle.

CHAPTER III

RESEARCH METHODOLOGY

3.1 Research Design

The design of this study can be classified as an observational study with longitudinal method. The study protocol was approved by the local ethics committee, Faculty of Medicine, Chulalongkorn University. Subjects were recruited from Civilian selection to be active army for the first shift in the year 2000, stationing at Nakhon Nayok province. Before entering the program, all subjects gave written informed consent to participate in the study.

3.2 Population and sample

3.2.1 Population

Target Population

The target population were defined as all recruits from civilian selection to be active army in each year.

Sample Population

Sample population were recruited for the first shift in the year 2000 and were training at Nakhon Nayok province.

3.2.2 Eligibility Criteria

Inclusion criteria

1. All participants are recruits in infantry battalion in CRMA shift 1/2000.
2. They were volunteered to participate in this study.

Exclusion criteria

1. Volunteer who cannot be trained up to 8 weeks

3.3 Materials

3.3.1 A weight Scale

3.3.2 A Scale for height measurement

3.3.3 Cardiotachometer (Polar Accurex Plus, Polar electro, Finland)

3.3.4 Electroimmunodiffusion

3.3.5 Rulers

3.3.6 Insulin syringe

3.3.7 Disposable syringes and needles

3.3.8 Ice box

3.3.9 Vaccutainer tubes

3.3.10 Purified Protein Derivatives (PPD)

3.3.11 Stethoscope

3.3.12 Electronic bicycle ergometer (Cateye Ergociser model EC-1000)

3.3.13 Ball pen

3.3.14 70% Alcohol

3.3.15 Cotton

3.3.16 Plaster

3.3.17 Software Analysis (Polar Precision Performance 2.0, Italy)

3.4 Measurement

3.4.1 Measurement of Oxygen Consumption (VO_2max)

The oxygen consumption of each subject was measured using the electronic bicycle ergometer (Cateye ergociser model EC-1000). The subjects were interviewed for exercise behavior whether they performed regularly or not to get the information was to used for setting up the workload in the Astrand – Rythming Step Test which was the

manual program. The irregular exercise subjects were set for workload at 1.5 kgm (78 Watt) while the regular exercise subjects were set at 2.0 kgm (100 Watt).

Subjects were asked to be warm up by riding the bicycle ergometer with free load for 2 minutes while the researcher was loading the personal data such as sex, age and workload into the program.

After warming up, subjects performed the test for 6 minutes. The heart rate at 5 and 6 minutes were recorded by the ergometer and the researcher in order to be used for VO_2max calculation.

3.4.2 Measurement of Heart Rates During the Training Program by Electrocardiographic Recorder (ECG)

The ECG of each subject was recorded at 1 minute interval during training using Polar Accurex Plus (Polar electro, Finland). The equipment consisted of electrodes belt with a transmitter which record ECG and send radio signal to receiving watch, incorporating a microprocessor which is stored in the watch was later transferred to computer by computer interface. The exercise intensity was calculated as a percentage of the maximum heart rate.

3.4.3 Measurement of Cellular Immune Response by Tuberculin Skin Test

The cellular immune response was measured by using PPD of tuberculin skin test form Thai Red Cross Society (Lot. 98, EPD. 9/00) before and after training. This test is a screening method for determining cellular immune response. The researcher was trained under supervision of trained personal for injection and measurement of induration at Division of Science, Thai Red Cross Society prior to start the study.

3.4.3.1 Procedure of Tuberculin skin test

Extreme precaution must be taken underminded in order to avoid unexpected fatal error.

Site of injection

It is usually on the anterior aspect of the proximal one-third of the forearm using an area of healthy skin away from obvious blood vessels and if possible free hair.

Position of Subject

The subject is preferably seated or stand with the tested arm on which the test is to be done resting on a table. The arm on which the test is to be carried out slightly flexed (Figure 3.1).

Skin Preparation

The test site is firmly swabbed with cleaning agent (70% alcohol) and is then allowed to dry swabbed.

Mantoux (intradermal) Test

0.1 ml of dilute tuberculin is injected into the superficial layers of the skin. The injection is made slowly into the skin and requires considerable pressure. With the bevel uppermost and the syringe almost parallel to the forearm the needle is introduced into the superficial layer of the skin until the bevel is completely covered. Both hands should be free to carry out the test, one being used to hold and operate the syringe and the other to make the skin taut while the needle is being inserted. The needle –syringe junction is held firmly while making the injection to ensure that the pressure generated dose not dislodge the needle from the syringe. Care must be taken that the complete volume of 0.1 ml is injected intradermally. And that none reaches the subcutaneous tissues. When the needle is correctly placed the injected volume should immediately produce a small wheal.

Reading the Test and Interpretation

All test should be read between 48 and 72 hours after injection.

When performed the measurement, the researcher should focus only on the induration but not erythema. This was determined by using ball pen to draw straight line to the circumference of induration verticaly and horizontaly (Figure 3.2 and 3.3). The distance between both edges of the indication was measured. The reading were taken by three measures at each time. Data were average of three reading for each subject.

Positive reaction is the presence of raised area with erythema measuring at least 10 mm. in diameter.

Negative reaction is the absence of raised area and erythema.

Figure 3.1 Photograph of injection site and small wheal at one-third of forearm.

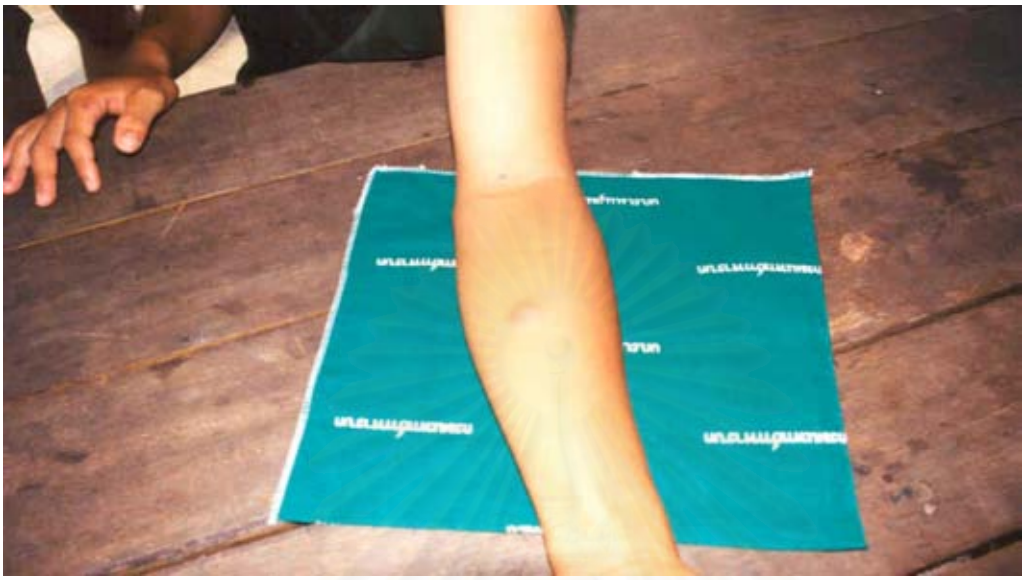

Figure 3.2 Photograph of induration after 48 hours injection.

Figure 3.3 Photograph of measurement induration by using ball pen draw straight line to the circumference of induration vertical and horizontal.

3.4.4 Measurement of Humoral Immune Responses by Serum Immunoglobulin

Humoral immune response was represented by serum immunoglobulin levels. Blood samples were drawn from an anticubital vein by venepuncture early in the morning (06:00 AM) before training in week 0, 4, 8 and one week post-training. Five ml of clotted blood were used for determining the Ig level at immunology laboratory, Faculty of Medicine, Chulalongkorn Memorial Hospital by the immunoeletrophoresis technique.

3.5 Data Collection

The recruitment training program was eight weeks long. In order to study the effect of exercise on the immune system of each subject, the data collection schedule was shown in diagram.

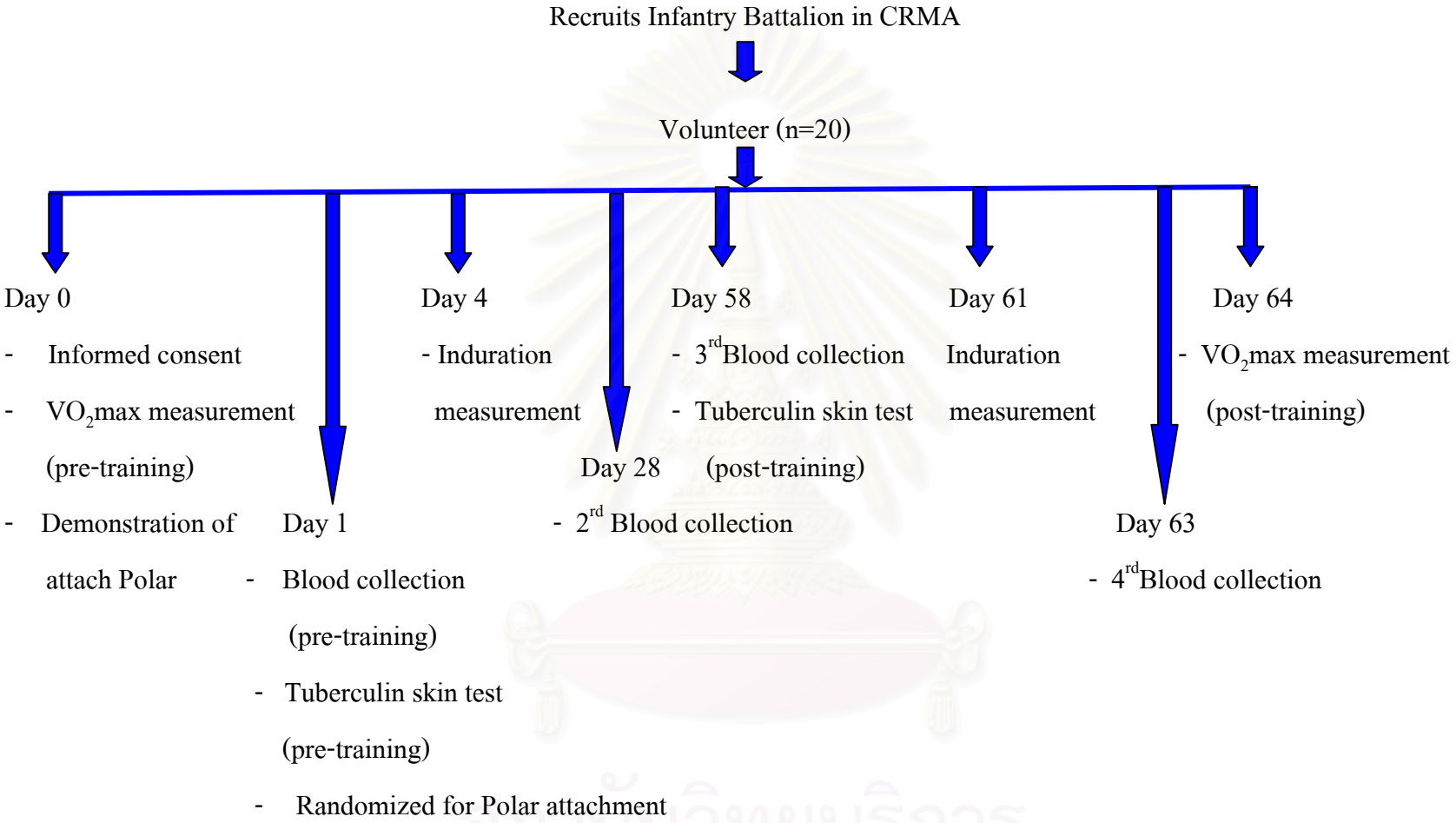

Figure 3.1 Diagram of data collection program

3.6 DATA ANALYSIS

Average values and standard deviations were computed. Paired t-test was used to test for significant differences between pre and post-training program of VO_2max, diameter of indication and comparison of immunoglobulin (IgG and IgM) in an initial period (pre and 4thweek). Whereas ANOVA was used for comparison of immunoglobulin levels among pre-training and 4thweek, 8thweek of training and one week post-training.

All data were analyzed using the Statistical Package for the Social (SPSS). Differences at significance level of $p<0.05$ were considered to be significant.

CHAPTER IV

RESULTS

A total of 20 subjects were enrolled and completed the study. Subject characteristics were summarized in Table 4.1. Their ages were ranged from 21 to 23 years with the average at 21.1(0.44) years. Their height were between 160 to 176 centimeters with the average at 166.45 (5.58) centimeters and their weight were between 45 to 72 kilograms with the average at 59.35 (8.0) kilograms. The VO_2max after the recruitment training program demonstrated a significantly increase at the level of $p<0.001$.

4.1 Classification of Exercise Intensity of the Recruitment training Program

The exercise intensity of recruitment training program was measured in subjects throughout the training program. During the training activity, heart rate were monitored by a cardiotachmeter (Polar Accurex Plus monitor) for measurement of the one minute interval heart rate from 5:30 AM to 6:30 PM. The collective heart rate data were used for analyzing intensity of exercise throughout the program. The results were summarized in Figure 4.1 and 4.2. This training program included four main activities as 1) exercise (running at least 2 kilometers, pull-up and push-up ect.), 2) basic training (individual drill with and without weapons), 3) marching (walking with regular, steady steps of equal length, usually in a group or military formation), 4) formation (an arrangement or positioning in a group) (see details in the Appendix C). During the training program the heart rate (HR) were ranged from 122 to 154 beats/minute with the average at 142.53 beats/minute in exercise , basic training HR were ranged from 120 to 160 beats/minute with the average at 145.55 beats/minute, marching HR were ranged 128 to 160 beats/minute with the average at 146.11beats/minute and formation HR were ranged 120

to 169 beats/minute with the average at 145.48 beats/minute. In comparison with the HR peak attained from the training program, important finding is discerned, mean HR during the steady-state performance approximate 72 percent of HR peak. Therefore , this recruitment training program is considered to be endurance training with moderate to high intensity.

4.2 Effect of Recruitment training on Tuberculin skin test

The effect of recruitment training on tuberculin skin test was determined in all subjects at pre and post-training. Nineteen subjects showed the response in pre-training induration. One subject (no.2) has no reaction in pre-training and post-training. Diameter of induration in pre-training range between 0 to 18.66 millimeters and post-training range between 0 to 17.5 millimeters. There were statistically significant decreased in induration average at 12.82 (4.58) to 10.37 (5.46) millimeters in comparison between pre-training and post-training ($p< 0.02$) (Table 4.3).

4.3 Effect of Recruitment training on Serum immunoglobulin

4.3.1 Immune response at initiation period

Comparison of serum immunoglobulin G (IgG) and immunoglobulin M (IgM) between pre-training and 4^{th} weeks was studied. The results demonstrated that immune responses at initiation period were affected by recruitment training (Table 4.7A). Serum immunoglobulin G were significantly decrease between pre-training and 4^{th} weeks ($p=0.004$). Serum immunoglobulin M showed the significantly decrease between pre-training and post-training at 4^{th} weeks ($p=0.007$).

4.3.2 Immune response at adaptation period

Comparison of serum IgG and IgM level at 0,4,8, weeks and one week post training showed that there was no change during the adaptation period (Table 4.7 B).

The normal range of serum IgG is between 700 to 1,600 mg/dl. The average serum Ig G level was higher than normal in the pre-training average at 1,850 (255.35) mg/dl and one week post-training at 1,613.1 (237.52) mg/dl respectively. The statistical analysis by ANOVA method showed no significant difference.

Serum immunoglobulin M or IgM have the similar fluctuation pattern. Although every IgM results fell into the normal range (40 to 230 mg/dl), the slightly difference in the means were still observed. They were 127.36 (39.52), 112.72 (38.59), 126.16 (44.06) and 125.31(45.21) mg/dl at 0, 4^{th} , 8^{th} weeks and one week post-training respectively.

4.4 Effect of Recruitment training on Oxygen Consumption (VO_2max)

Effect of recruitment training on oxygen consumption was determined at pre-training and post-training in all subjects. The results showed the significant improvement of oxygen consumption at 17 per cent from 40.22 (8.71) to 47.10 (4.78) ml/kg/min ($p<0.001$) (Table 4.1 and 4.2).

Table 4.1 Comparison of pre-training characteristics and post-training characteristics of 20 subjects who were participated in the recruitment training program. Data are expressed as mean (SD).

Characteristics	Pre-training	Post-training
Weight (kg)	59.60 (7.8)	58.9 (6.49)
VO_2max (ml/kg/min)	40.22 (8.7)	47.09 (4.78)*
BMI (kg/h^2)	21.60 (2.49)	21.34 (1.83)

* Post-training VO_2max was significantly increased from pre-training at the level of $p<0.001$.

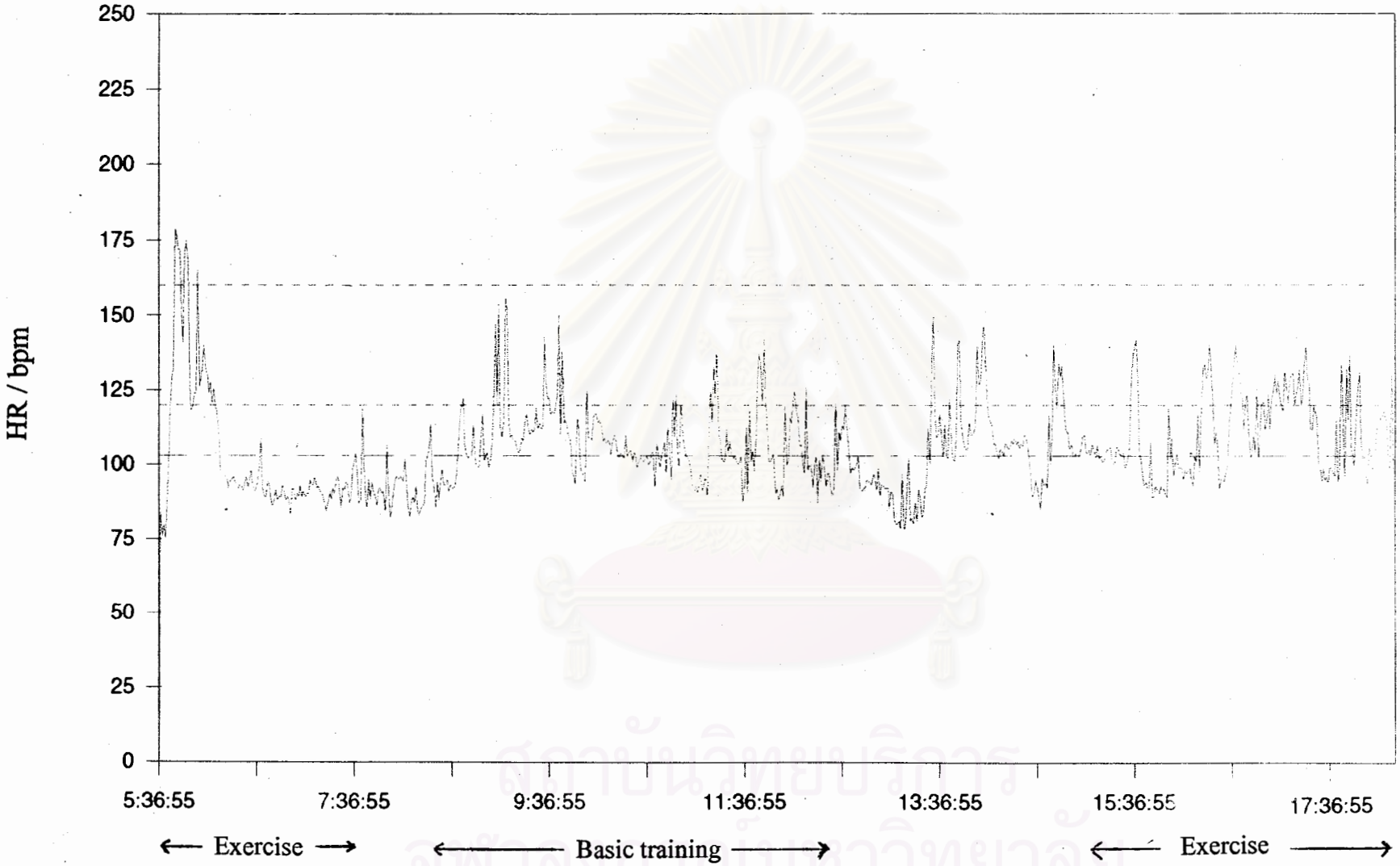

Figure 4.1 The heart rate response of one subject during perform training program in one day. Subject heart rates were recorded throughout the day. This training program included exercise and basic training.

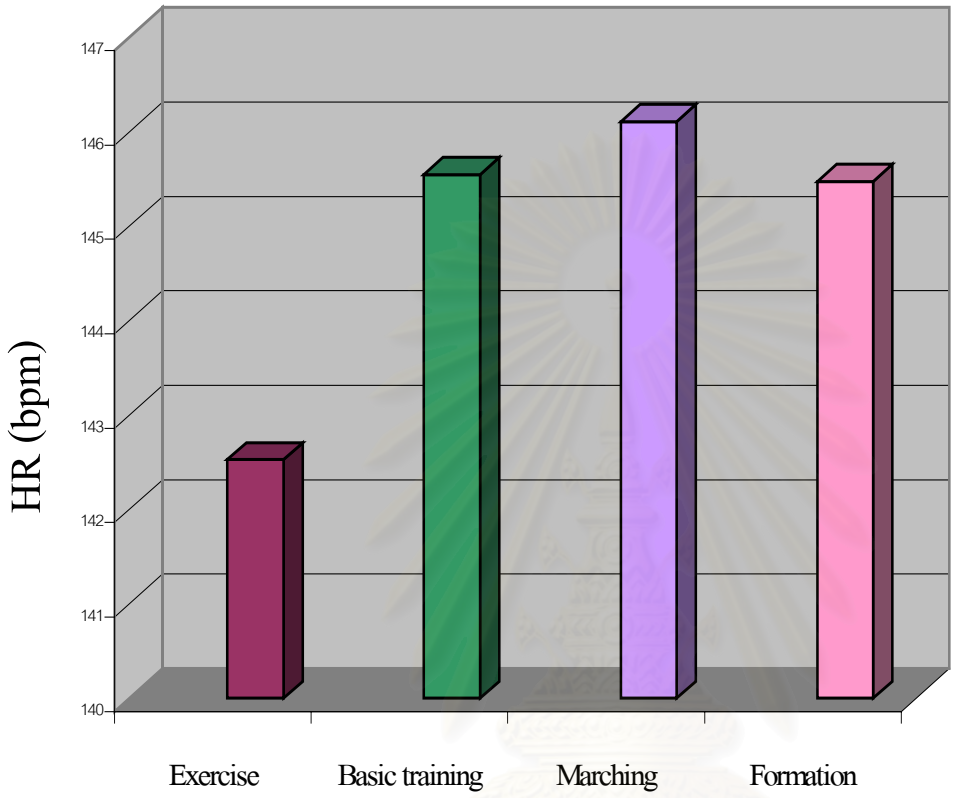

Figure 4.2 The heart rate response of subjects during perform training program (values are expressed as mean). This training program included four main activities as exercise, basic training , marching and formation.

Table 4.2 Effect of recruitment training on oxygen consumption in each subjects (ml/kg/min).

Subject No.	Pre-training	Post-training
1	43.75	45.11
2	28.99	46.46
3	33.64	47.71
4	35.94	53.78
5	41.82	44.44
6	36.00	44.00
7	31.73	40.00
8	41.82	42.50
9	47.22	54.55
10	28.17	41.67
11	52.63	54.39
12	36.84	50.45
13	39.68	41.94
14	55.32	53.19
15	45.61	51.82
16	53.33	45.90
17	53.33	47.96
18	36.11	39.73
19	29.23	46.83
20	33.33	49.55
Mean	40.22	47.10
SD	8.71	4.78
Min	28.17	39.73
Max	55.33	54.55

Table 4.3 Pre and post-training diameter of induration (mm) of tuberculin skin test in each subject.

Subject No.	Pre-training	Post-training
1	9.33	0
2	0	0
3	15.88	15
4	14.66	12.5
5	14.5	12
6	15.5	15
7	15.83	17.5
8	9.83	10.5
9	16.16	19
10	11.83	10
11	15.83	10.5
12	15.16	13.5
13	10.66	6
14	15.5	0
15	7.83	9
16	15.33	13
17	18.66	13.5
18	18	13.5
19	6.5	7
20	9.5	10
Mean	12.82	10.37
SD	4.58	5.46
Min	0	0
Max	18.66	17.5

Table 4.4 Comparison of pre and post-training of induration of tuberculin skin test. Data are expressed as mean (SD).

Induration (mm)		t-statistic	p-value
Pre-training	Post-training		
12.82 (4.58)	10.37 (5.46)	2.58	0.018*

* Post-training induration was significantly decreased from pre-training at the level of $p<0.02$.

Table 4.5 Effect of recruitment training on serum immunoglobulin G (mg/dl) in each subjects.

Subject No.	Pre-training	4th	8th	Post-training
1	1,803	1,588	1,477	1,638
2	1,829	1,880	1,947	1,880
3	1,624	1,651	1,516	1,672
4	1,572	1,297	1,364	1,588
5	1,252	1,265	1,168	1,435
6	1,413	1,145	1,218	1,293
7	1,460	1,532	1,462	1,504
8	1,535	1,214	1,211	1,383
9	1,378	1,242	1,540	1,536
10	1,550	1,469	1,469	1,659
11	1,508	1,383	1,336	1,564
12	1,565	1,601	1,548	1,593
13	1,399	1,175	1,258	1,398
14	1,374	1,398	1,485	1,672
15	1,636	1,454	1,601	1,638
16	1,424	1,473	1,379	1,383
17	2,299	2,132	2,122	2,329
18	1,794	1,791	1,943	1,992
19	1,428	1,258	1,416	1,454
20	1,428	1,258	1,416	1,454
Mean	1,850.20	1,483.10	1,508.10	1,613.10
SD	233.24	247.90	255.35	237.52
Min	1,252	1,145	1,168	1,293
Max	2,299	2,132	2,122	2,329

Table 4.6 Effect of recruitment training on serum immunoglobulin M (mg/dl) in each subjects.

Subject No.	Pre-training	4th	8th	Post-training
1	67.6	73.50	66	58.20
2	147.40	170	170	156.1
3	149.70	139.30	130.70	150.40
4	136.9	106.6	161.30	163.20
5	89.20	116.60	122.30	133.90
6	126.50	98.50	120.50	113.50
7	96.80	80.10	111.40	100.20
8	104.50	84.20	89.30	88.80
9	100.80	93.90	92.20	91.40
10	103.70	90.90	99.40	101.10
11	60.60	55.30	57.40	64.80
12	142.60	111.40	133.40	118.80
13	139.90	102.30	103.60	117.90
14	211.90	226.20	230.20	254.90
15	90.80	80.90	75.10	78.40
16	198.10	157	176.50	167.10
17	174.60	114	133.40	132.50
18	134.70	119.60	187.60	176
19	125.7	101.90	104.50	108.80
20	145.20	132.50	158.50	130.30
Mean	127.36	112.72	126.16	125.31
SD	39.52	38.59	44.06	45.21
Min	60.60	55.30	57.40	58.20
Max	211.90	226.20	230.20	254.90

Table 4.7 (A) Comparison of the mean (SD) of IgG and IgM levels (mg/dl) between pre-training (Day 0) and 4th week training (Day 28). Data are expressed as mean (SD).

Ig	Pre-training	4th week training	t-statistic	p-value
	mean	mean		
IgG	1850.2 (233.24)	1483.1 (247.90)	3.256	0.004*
IgM	127.36 (39.52)	112.72 (38.59)	3.045	0.007*

* Significant difference between pre-training and 4th week training ($p<0.01$).

Table 4.7 (B) Effect of recruitment training on serum immunoglobulin (mg/dl).

Data are expressed as mean (SD).

Ig	Pre-training	4th training	8th training	One week Post-training	p-value
IgG	1,850.20 (233.24)	1,483.10 (247.90)	1,508.10 (255.35)	1,613.10 (237.52)	0.332
IgM	127.36 (39.52)	112.72 (38.59)	126.16 (44.06)	125.31 (45.21)	0.432

CHAPTER V

DISCUSSION

The current study attempts to classify recruitment training program according to exercise intensity and to demonstrate effects of training program on immune system by determining serum immunoglobulin G and M and tuberculin skin test.

Twenty subjects who were volunteered in this study were drafted for the first shift of the year 2000. .

They were underwent the recruit training program for 8 week long , 5 days per week, from 5:30 AM to 5:30 PM each day of training. All subjects were kept and trained under the same environment. i.e , pattern of daily life and diet. The training program was aimed to increase the physical fitness, the military discipline and the military skill. The training program divided into four practices: exercise, basic training marching and formation. After eight weeks of training, the physical activities in term of VO_2max were improved (Table 4.1and 4.2)

Classification of Exercise Intensity

Although this training program was considered to be the intensive program, the analysis of this training program according to the exercise intensity by using mean heart rate, duration and frequency classification showed that it was the moderate to high intensity exercise program. The training program included four main activities as exercise , basic training , marching and formation. Their HR ranged from 122 to 154 (142.53) beats/minute, 120 to 160 (145.55) beats/minute, 128 to 160 (146.11) beats/minute and 120 to 169 (145.48) beats/minute respectively (Figure 4.2). According to the ACSM, the following criteria for an endurance exercise are recommended: 1) the mode of activity should use large muscle groups, be maintained for a prolonged period; 2) the intensity of activity should correspond to 55/65 to 90 percent of maximum heart rate, 3) exercise duration should be 20 to 60 minutes of

aerobic activity; and 4) exercise frequency should be 3 to 5 days per weeks. Even so, many health benefits from physical activity can be achieved at lower intensity of exercise if frequency and duration of training are increased appropriately (ACSM,1998).

In this study, the recruits performed five days per week. The exercise intensity during training program exceeded 60 to 80 percent of the HR at peak exercise. These results imply that the recruitment training program is moderate to high intensity exercise. All these characteristics fulfilled the criteria as recommend by ACSM (1995 and 1998).

Effect of Recruitment Training on Cell-mediated Immune Response

The cellular immune response in the recruits was studied by using PPD method for tuberculin skin test from Thai Red Cross Society. It was used as the screening method for determining cellular immune response. One subject had no reaction from both injections. This subject received the booster dose one week after the injection and there was still no responses. There were statistically significant decreased in average induration from 12.82 (4.58) millimeter in pre-training to 10.37 (5.46) millimeters in post-training. Although the results gave the signal of immunosuppression to the tuberculin skin test. When analyse as a group of volunteer this training program was not excessive enough to show the reduction in every subject. It was so because they were rest for 2 days after 5 training days per week which might help the body immune system to keep up their response. The further study by T-cell proliferation assay might be needed since it is a more specific and sensitive method for the assay of cell-mediated immune responses. This technique was used by Fly et al (1991) to observe the cellular immunosuppression in overtrained athletes.

Effect of Recruitment Training on Humoral Immune Response

Humoral immune responses were presented by serum immunoglobulin levels. The results showed that the IgG levels were higher than normal value both at pre-training with th average at 1,850 (255.35) mg/dl and post-training with the average at 1613 (237.51)mg/dl. The statistical analysis in initiation period showed significant decrease and in adaptation period showed no significant changes.

Nehlsen Cannarella SL et al.(1991) reported the reduction of serum IgG, IgA and IgM levels in 12 woman who walked for 45 min at 60% VO_2 max in laboratory setting. However, the reduction was seen immediately after exercise and returned to baseline within 1.5 hours later. This observation suggested that the changes in serum immunoglobulin was a transient phenomenon and the body's immune system could overcome the changes within a period of time.

In this study, blood specimen were taken from subjects at 6:00 o'clock in the morning after an overnight rest. If a change of immunoglobulin level is a transient phenomenon as suggested by Nehlsen et al. (1991), the slightly decrease of serum immunoglobulin will be difficult to be observed.

Therefore, the decrease levels of serum IgG and IgM at 4^{th} week of training compared to pre-training observed in this study should be a strong suggestion for humoral immunosuppression. This suppression would be overcome by healthier physical fitness of the subject (as shown by VO_2 max) in the post-training which was considered as an adaptation period.

Gleeson M. et al.(1995) found the significantly decreased in serum levels of IgA, IgG and IgM and salivary IgA concentration in 15 males and 11 females athletes who undertaken 20-25 hours of pool training and 5 hours of dry – land training per week in preparation for the World Championship Trials. The reduction of the serum IgG, IgM, IgA and salivary IgA was seen at post-training compared with controls.

To emphasize on our objective of recruitment training suppressed the immune response, the control group should be included in the further study.

There are many confounder factors which effect the function of immune system such as nutritional status, psychological condition and biological environment. Although our subjects were treated under the same condition, they might not be at the optimal status in nutrition, psychological and biological condition. These will be able to contribute to the reduction of immune responses. Further studies concerning these factors should be taken into account.

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APPENDICES

APPENDIX B

LIQUID TUBERCULIN (PURIFIED PROTEIN DERIVATIVE)
FOR MANTOUX TEST FROM SCIENCE DIVISION THAI RED CROSS SOCIETY

Description/Composition : Tuberculin PPD is a preparation obtained from culture filtrates of Mycobacterium tuberculosis sterilized by a chemical –physical process and purified by treatment with Trichoracetic acid and Ammonium sulphate.

One IU. of PPD will have the equivalent activity of 0.00002 mg .PPD of the Reference Standard.

PPD solution consists of Isotonic Phosphate buffer saline, 0.5% W/V Phenol and 0.005% Tween 80.

Indications : PPD for Mantoux test is used for the identificatuin of individuals with delayed sensitivity to tuberculin.

Warning : This liquid PPD must be used intradermally. Some viral infections and immunosuppressive therapy may depress reactivity to tuberculin.

Administration : For the intradermal test, clean the injection site and inject 0.1 ml of the solution carefully (0.1 ml contains 0.00002 mg.PPD). The formation of a wheal will confirm that the intradermal injection has been correctly performed.

Reading and interpretation : The reaction should be read 48 hours after injection and measured in millimeters (mm).

Positive reaction is the presence of a raised area with erythema measuring at least 10 mm. in diameter.

Negative reaction is the absence of a raised area with erythema.

Store : Stored at 4 $^{\circ}$C, the liquid product will retain the potency for 6 month after preparation of the solution.

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Store : Stored at 4 $^{\circ}$C, the liquid product will retain the potency for 6 month after preparation of the solution.

Caution : Protect from light . Opened vials should not be kept in order to avoid secondary infections which could falsify results.

APPENDIX C

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
May	Mon. 1st		Exercise	Receive recruit from Prachinburi Province				Personal recording, Providing personal belonging, hair cut, Bring to sleeping hall					Seminar
				Remaining trainer prepare to receive at traiing camp									
	Tue. 2nd		Basic training	Assign recruit to permanent platoon				Documentation, Distribution and Weapon & Field accessories			Trainer introduction and basic training		Seminar
1st week	Wed. 3rd		Exercise	See routine daily training	Attention, At ease, Parade rest	Rest, Fall out, Dismissed	Right face, Left face, About face		Present arms, Eyes rught, Eyes left	Removal-wearing hat	Crawling and stading up	Exercise	Seminar
	Thu. 4th		Exercise	See routine daily training	Site step, Back step	Marching			Marching		Exercise		Seminar
	Fri. 5th		Exercise										Seminar on Military Ethics
	Sat. 6th		Physical tests	Toilet & barrack cleaning									
	Sun. 7th												
	Mon. 8th		Exercise	See routine daily training	Change step while marching walking	Shuffling, Stop from shuffing, March from shuffing, Shuffing from Marching	Left turn whild marching		Right turn while marching	About face while marching	Double time, Stop form double time		Commander meet recruit
	Tue. 9th		Exercise	See routine daily training	Change step while double time	Change to marching from double time	Change to double time from marching		Left turn while double time	Right turn while double time	About face while double time	Pysical training	Seminar on Military Ethics

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
2nd week	Wed. 10th	Exercise		See routine daily training	Order arms, rest (per regulation)	Rest : normal, relax, rest in formation, formation over	Arms indlination	Arms gathering	Right tuen, Left turn, About face	Haft left turn, Haft right turn	Exercise		Seminar on Thai history
	Thu. 11th	Exercise		See routine daily training	Arms carrying	Arms holding	Salute arms, Left salute arms, Right salute arms	Ready arms	Salute with ready arms	Removal-wearing hat	Exercise		Seminar on security unit
	Fri. 12th	Exercise		See routine daily training	Sash wearing, Sash adjusting	Wearing arms sash	Arms sash inclination	Physical Examination			Sports		Seminar on the Democracy
	Sat. 13th	Exercise			Weapons and Hall cleaning								Seminar on honoring and rayalty to the company
	Sun. 14th												
	Mon. 15th												
	Tue. 16th	Exercise		See routine daily training	Weapons checking			Attaching-Detaching a sword	Crawling and standing up	Review previous uncovered training	Exercise		Seminar on military's mannes

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
3rd week	Wed. 17th	Exercise			First platoon visit 100 yrs memorail Building and see slide And second platoon	Third platoon visit 100 yrs memorail Building and see slide And Fourth platoon		Buddhist laity		Rehearsing of Recruit Training Opening Ceremony	Exercise		Seminar on how to respect people
	Thu. 18th	Exercise		Recruit Training Opening Ceremony	Assistant trainer shows, Recruit shows, Recruit see relatives, Lunch			Review training			Exercise		Seminar on military's ranks
	Fri. 19th	Exercise		First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5				First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5			Exercise		Seminar on military uniform
	Sat. 20th	Exercise			Weapons and Hall cleaning	Commanders time		Recruit time (self training)			Exercise		Seminar on when not to salute
	Sun. 21st			Second platoon goes to station 5 and relax									
	Mon. 22nd	Exercise		First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2				First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2			Formation		Seminar on military uniform
	Tue. 23rd			First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3				First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3			Formation		Seminar on military discipline

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
4th week	Wed. 24th	Exercise		First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4				First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4			Formation		Seminar on 9 military disciplines
	Thu. 25th	Exercise		First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5				First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5			Formation		Seminar on military units from a squad to battalian
	Fri. 26th	Exercise		First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2				First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2			Formation		Seminar on Commander's name list
	Sat. 27th	Physical test			Weapons and Hall cleaning		Commanders time	Recruit time (self training)			Exercise		Seminar on the Greats of Thailand
	Sun. 28th										Exercise		Seminar on modern democracy
	Mon. 29th	Exercise		First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3				First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3			Formation		Seminar on history of King Naresuan the great
	Tue. 30th			First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4				First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4			Formation		Seminar on how to behave when not in the unit

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
	Wed. 31st	Exercise		First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5				First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5			Formation		Seminar on military salary and allowance
5th week June	Thu. 1st	Exercise		First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2				First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2			Formation		Seminar on type of leave letter and a leave on other occasion
	Fri. 2nd	Exercise		First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3				First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3			Formation		Seminar on CRMA organization
	Sat. 3rd	Physical test		Weapons and Hall cleaning			Commanders time	Recruit time (self training)			Exercise		Seminar on Guard duties and obiigations
	Sun. 4th										Exercise		Seminar on modern democracy
	Mon. 5th	Exercise		First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4				First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4			Formation		Seminar on good citizen's duties
	Tue. 6th	Exercise		First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5				First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5			Formation		Seminar on how to do when captured by police

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
6th week	Wed. 7th	Exercise		First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2				First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2			Formation		Seminar on military's manes
	Thu. 8th	Exercise		First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3				First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3			Formation		Book reciting in station 5
	Fri. 9th	Exercise		First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4				First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4			Formation		Book reciting in station 3
	Sat. 10th	Physical test		Weapons and Hall cleaning		Commanders time		Recruit time (self training)			Exercise		Seminar on when not to salute
	Sun. 11th										Exercise		Book reciting in station 5
	Mon. 12th	Exercise		First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5				First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5			Formation		Book reciting in station 3
	Tue. 13th	Exercise		First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2				First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2			Formation		Book reciting in station 5

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
7th week	Wed. 14th	Exercise		First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3				First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3			Formation		Book reciting in station 3
	Thu. 15th	Exercise		First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4				First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4			Formation		Book reciting in station 5
	Fri. 16th	Exercise		First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5				First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5			Formation		Book reciting in station 5
	Sat. 17th	Physical test		Weapons and Hall cleaning		Commanders time		Recruit time (self training)			Exercise		Book reciting in station 5
	Sun. 18th										Exercise		Book reciting in station 3
	Mon. 19th	Exercise		First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2				First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2			Formation		Book reciting in station 5
	Tue. 20th	Exercise		First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3				First platoon goes to station 4 Second platoon goes to station 5 Third platoon goes to station 2 Fourth platoon goes to station 3			Formation		Book reciting in station 3

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
8th week	Wed. 21st	Exercise		First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4				First platoon goes to station 5 Second platoon goes to station 2 Third platoon goes to station 3 Fourth platoon goes to station 4			Formation		Book reciting in station 5
	Thu. 22nd	Exercise		First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5				First platoon goes to station 2 Second platoon goes to station 3 Third platoon goes to station 4 Fourth platoon goes to station 5			Formation		Book reciting in station 5
	Fri. 23rd	Exercise		Long distance traveling and staying overmight in the field				Long distance traveling and staying overmight in the field			Weapons and Hall cleaning		Party
	Sat. 24th	Exercise			Weapons and Hall cleaning		Commanders time	Recruit time (self training)			Exercise		Book reciting in station 3
	Sun. 25th										Exercise		Seminar moder democracy, Seminar on type of leave letter a leave on other occasion
	Mon. 26th	Exercise		First examination				First examination			Exercise		Book reciting in station 5
	Tue. 27th	Exercise		First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2				First platoon goes to station 3 Second platoon goes to station 4 Third platoon goes to station 5 Fourth platoon goes to station 2			Formation		Book reciting in station 3

Recruitment Training Program : First Shift of 2000

Month	Date	5:00 - 6:00	6:00 - 7:00	8:00 - 9:00	9:00 - 10:00	10:00 - 11:00	11:00 - 12:00	13:00 - 14:00	14:00 - 15:00	15:00 - 16:00	16:00 - 17:00	17:00 - 18:00	19:00 - 20:00
9th week	Wed. 28th	Exercise		Preparation for an exmination on each station								Sports	Book reciting in station 5
	Thu. 29th			Examination on formation	Examination at station 2, 3 and 5						Sports		Party
	Fri. 30th			Real bullet shooting examination						Recruit Training Closing Ceremony	Store all training equipment		Party
July	Sat. 1st	Returning weapons and field equipment to the company, Sending recruit to the company and other military units											

BIOGRAPHY

Capt. Praparat Chuntavan was born on October 17,1966 in Chiyaphum province, Thailand. She graduated with her Bachelor of Nursing Science from Khon Kean University in 1991. She is working at CRMA Hospital in Nakhon Nayok province.