EFFECTS OF TABATA WORKOUT ON IMMUNE CELLS RESPONSE IN PHYSICALLY INACTIVE INDIVIDUALS

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by

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CERTIFICATE

This is to certify that the dissertation entitled “Effects of Tabata Workout on Immune Cells Response in Physically Inactive Individuals” is the bona fide record of research work done by Nur Ain Syahira binti Mohd Noor during the period from September 2019 to June 2020 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Health Sciences (Honours) (Exercise and Sports Science).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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LIST OF ABBREVIATIONS AND ACRONYMS

%	Percentage
>	Greater than
<	Less than
ANOVA	Analysis of variance
BMI	Body mass index
bpm	beats per minute
CD	Cluster of differentiation
cm	Centimetre
ESS	Exercise and Sports Science
HIIT	High-intensity interval training
HR	Heart rate
HR_{max}	Maximal heart rate
HR_{peak}	Peak heart rate
HRR	Heart rate reserve
kg	Kilogram
m	Metre
MAV	Maximal aerobic velocity
mL	Millilitre
N	Total sample size

NK cells	Natural killer cells
PAR-Q	Physical activity readiness questionnaire
RCT	Randomised controlled trial
RPE	Rating of perceived exertion
SD	Standard deviation
SIT	Sprint interval training
T_C	T cytotoxic cells
T_H	T helper cells
TLR	Toll-like receptors
URTI	Upper respiratory tract infection
USM	Universiti Sains Malaysia
VO_{2max}	Maximal aerobic capacity
WBC	White blood cells
WHO	World Health Organization

KESAN-KESAN LATIHAN TABATA TERHADAP TINDAKBALAS SEL IMUN DALAM KALANGAN INDIVIDU YANG TIDAK AKTIF SECARA FIZIKAL

ABSTRAK

Latihan berselang berintensiti tinggi (HIIT) sangat terkenal masa kini kerana ia menghasilkan kesan positif yang lebih kurang sama dengan senaman berintensiti sederhana, dan pada masa yang sama, ia menjimatkan masa kerana ia memerlukan tempoh latihan yang lebih pendek. Sehingga kini, tindakbalas sel imun terhadap HIIT tidak didokumentasikan dengan baik. Oleh itu, tujuan kajian ini adalah untuk menentukan kesan-kesan latihan Tabata terhadap tindakbalas sel imun dalam kalangan individu yang tidak aktif secara fizikal. Seramai dua belas individu yang tidak aktif secara fizikal (N= 12, 8 lelaki dan 4 perempuan, umur = 22.0 ± 0.9 tahun) yang terdiri daripada pelajar Kampus Kesihatan Universiti Sains Malaysia mengambil bahagian dalam kajian “one-arm” ini. Program latihan Tabata dijalankan sebanyak 3 kali seminggu untuk tempoh 6 minggu (sebanyak 18 sesi secara keseluruhannya). Semasa setiap sesi senaman, para peserta melakukan pemanasan badan, diikuti dengan 4 set latihan Tabata dan berakhir dengan penyejukan badan. Setiap set latihan Tabata mengandungi 2 jenis senaman (dilakukan selama 20 saat) dengan tempoh rehat antara senaman selama 10 saat. Tempoh rehat antara set adalah selama 1 minit. Kemajuan beban digunakan dalam kajian ini; jumlah pusingan setiap set latihan Tabata meningkat daripada 2 pusingan untuk 2 minggu pertama intervensi kepada 3 pusingan untuk 2 minggu intervensi yang seterusnya, dan 4 pusingan untuk 2 minggu intervensi yang terakhir. Kadar denyutan jantung dan tanggapan daya usaha juga direkodkan pada setiap sesi. Berat badan, indeks jisim badan, peratusan lemak badan dan sampel darah (2 mL) peserta diukur sebelum, semasa dan selepas tempoh ujian. Sampel darah diambil untuk menganalisis jumlah bilangan leukosit, limfosit, neutrofil dan monosit.

Analisis ‘One-way ANOVA with repeated measures’ telah dilakukan untuk mengukur perbezaan antara tiga masa pengukuran tersebut (minggu pertama, minggu ketiga dan minggu keenam ujian). Keputusan menunjukkan bahawa terdapat kenaikan yang signifikan dalam jumlah leukosit ($p = 0.042$) dan neutrofil ($p = 0.039$) selepas menjalani latihan Tabata selama 6 minggu. Corak peningkatan juga dapat dilihat dalam bilangan monosit ($p = 0.065$) tetapi tidak dalam bilangan limfosit ($p = 0.304$) selepas menjalani latihan Tabata selama 6 minggu. Berat badan, indeks jisim badan dan peratusan lemak badan tidak berubah sepanjang tempoh intervensi. Kesimpulannya, kajian ini menunjukkan bahawa latihan berselang berintensiti tinggi yang kronik memberikan kesan positif terhadap tindak balas sel imun dalam kalangan individu yang tidak aktif secara fizikal. Kajian lanjut diperlukan untuk memahami manfaat HIIIT terhadap tindak balas imun secara lebih mendalam.

EFFECTS OF TABATA WORKOUT ON IMMUNE CELLS RESPONSE IN PHYSICALLY INACTIVE INDIVIDUALS

ABSTRACT

High-intensity interval training (HIIT) is very popular nowadays as it produces similar positive results to moderate-intensity exercise, and at the same time, it is time efficient as it needs shorter exercise duration. To date, immune responses following HIIT is not well documented. Hence, the purpose of this study is to determine the effects of Tabata workout on immune cells responses among physically inactive individuals. A total of twelve physically inactive individuals (N = 12, 8 males and 4 females, age = 22.0 ± 0.9 years) from Health Campus Universiti Sains Malaysia participated in this one-arm experimental study. The Tabata workout programme was carried out 3 times per week for 6 weeks (a total of 18 sessions). During each exercise session, participants performed warming up, followed by 4 sets of Tabata workout and ended with cooling down. Each sets of Tabata workout contain 2 types of exercise (performed for 20 seconds) with rest intervals between exercises of 10 seconds. The rest interval between sets was 1 minute. Progressive load was employed in this study; the number of rounds for each sets of Tabata workout were increased from 2 rounds in the first 2 weeks of intervention to 3 rounds for the next 2 weeks of intervention and 4 rounds for the last 2 weeks of intervention. Heart rate (HR) and rate of perceived exertion (RPE) were recorded during each session. Participants' body weight, body mass index (BMI), body fat percentage, and blood samples (2 mL) were measured at pre-, mid- and post-test. Blood samples were analysed for the total leukocytes, lymphocytes, neutrophils and monocytes count. One-way analysis of variance (ANOVA) with repeated measures was performed to measure significant difference between the three time points of measurement (at week 1, week 3 and week 6 of tests). The results showed that there

were significant increases on total leukocytes ($p = 0.042$) and neutrophils ($p = 0.039$) following six weeks of Tabata workout. An increasing trend was also observed for monocytes count ($p = 0.065$) but not for the lymphocytes count ($p = 0.304$) following six weeks of Tabata workout. Participants' body weight, BMI and body fat percentage were maintained throughout the intervention period. Thus, in conclusion, this study showed that chronic HIIT induced positive immune cells response among physically inactive individuals. Further studies are warranted for in depth understanding of the benefits of HIIT on immune response.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Physical inactivity in adults are rapidly increasing globally. Recent studies show that one out of five adults engaged in low levels of regular physical activity (Dumith *et al.*, 2011) which results in increasing risk factors for mortality. Sedentary or lack of physical activity also results in increased risk for many health problems including cardiovascular diseases, cancer and diabetes (Khaw *et al*., 2008; Cornelissen and Smart, 2013). Leading an active lifestyle by being engaged in physical activity would prevent such pathologies and improve overall health and functional capacity (United States Department of Health and Human Services, 2008). Physical activity is defined by World Health Organization (WHO) as any bodily movement produced by skeletal muscles that requires energy expenditure. It includes exercise as well as other activities which involve bodily movement (WHO, 2019). Exercise is a subcategory of physical activity that is planned, structured, repetitive and aim to improve or maintain of one or more components of physical fitness (WHO, 2019).

The WHO generally recommend adults aged 18 to 64 years to engage in moderate-intensity physical activity for at least 150 min/week or at least 75 min/week of vigorous-intensity physical activity or an equivalent combination of moderate- and vigorous-intensity physical activity. This includes activities such as walking, jogging and cycling or anything that causes a sustained increase in heart rate (~40 – 60 of maximal aerobic capacity [VO_{2max}] or ~55 – 70% maximal heart rate [HR_{max}]) (Francois and Little, 2015). Although various health organisations including WHO emphasize on the importance and beneficial of physical activity, the number of inactive adults remain high (Dumith *et al.*, 2011). Thus, high-intensity interval training (HIIT) which consists of

repeated exercises at high-intensity (≥80% of maximal heart rate [HR_{max}]), relatively with short recovery periods (40 – 50% of HR_{max}) was introduced. It provides an attractive approach by reducing the time commitment for exercise.

High-intensity interval training is classified into two categories: aerobic HIIT and body weight or resistance HIIT (Kilpatrick *et al*., 2014). Both categories involved periods of intense effort followed by recovery segments with the primary difference is the modality of exercise. Aerobic HIIT is an interval training that uses traditional aerobic exercise modalities such as running and cycling (Kilpatrick *et al*., 2014). Meanwhile, body weight or resistance HIIT is an interval training that uses bodily movements, weighted objects, bars or devices for high repetition resistance activities (Kilpatrick *et al*., 2014). HIIT has commonly been used in athletes but is rapidly increasing in popularity in sedentary and clinical populations.

Tabata, a type of high-intensity interval training, was initially developed by Dr. Izumi Tabata among young sedentary/recreationally active individuals (Tabata *et al.,* 1996). This type of interval training commonly consists of seven to eight rounds of 20 seconds of all-out exercise bouts, interspersed by 10 seconds of rest or low intensity where, each workout lasting for 4 minutes. Research showed that Tabata training could enhance anaerobic as well as aerobic capacity (Tabata *et al.,* 1996;1997). This type of exercise became popular probably because it is associated with astonishing results together with an attractive time efficiency. However, to date, no study has reported possible positive benefits of Tabata workout on immune responses.

Immune system is defined as body's defence against disease causing organisms, malfunctioning cells and foreign particles. Immune system is vital to protect against pathogenic microbes and tumours as well as maintaining proper health. Virus, bacteria, fungus, parasites etc. are examples of pathogens refer as biological agents that capable of causing host damage. When host damage reaches a certain threshold, it can manifest itself as a disease. Human immunity acts as protection against infections and will respond to these pathogens either via innate immunity alone or together with acquired/adaptive immunity. Innate immunity provides non-specific, hereditary defence which acts as protection against pathogens/antigens without prior exposure, which is also called as genetic immunity. In contrast, acquired/adaptive immunity provides specific, non-hereditary defence that act as protection after exposure to a specific pathogen/antigen (Kindt *et al*., 2007; Parker *et al*., 2016).

1.2 PROBLEM STATEMENT

Several barriers to exercise exist in physically inactive individuals include lack of time, health status, environmental conditions, lack of exercise knowledge and fear to exercise. However, lack of time seems the most common factor to the prevalence of physical inactivity among adults. Thus, manipulation of exercise in terms of duration, frequency, as well as intensity of exercise is needed. Nowadays, HIIT is a recommended exercise program to overcome this barrier to exercise. However, its relationships with immune response are not widely studied to date. Hence this study was proposed to determine the effects of Tabata workout, a type of HIIT, on immune cell response in physically inactive individuals.

1.3 OBJECTIVES OF THE STUDY

1.3.1 General objective

To determine the effects of Tabata workout on immune cells response in physically inactive individuals.

1.3.2 Specific objectives

1. To assess the changes in total leukocytes count in physically inactive individuals following six weeks of Tabata workout.
2. To assess the changes in neutrophils count in physically inactive individuals following six weeks of Tabata workout.
3. To assess the changes in monocytes count in physically inactive individuals following six weeks of Tabata workout.
4. To assess the changes in lymphocytes count in physically inactive individuals following six weeks of Tabata workout.

1.4 RESEARCH QUESTIONS

1. What are the effects of Tabata workout on total leukocytes count in physically inactive individuals?
2. What are the effects of Tabata workout on neutrophils count in physically inactive individuals?
3. What are the effects of Tabata workout on monocytes count in physically inactive individuals?
4. What are the effects of Tabata workout on lymphocytes count in physically inactive individuals?

1.5 HYPOTHESES OF THE STUDY

H_{O1} : There are no changes in total leukocytes count in physically inactive individuals after 6 weeks of Tabata workout.

H_{A1} : There are changes in total leukocytes count in physically inactive individuals after 6 weeks of Tabata workout.

H_{O2} : There are no changes in neutrophils count in physically inactive individuals after 6 weeks of Tabata workout.

H_{A2} : There are changes in neutrophils count in physically inactive individuals after 6 weeks of Tabata workout.

H_{O3} : There are no changes in monocytes count in physically inactive individuals after 6 weeks of Tabata workout.

H_{A3} : There are changes in monocytes count in physically inactive individuals after 6 weeks of Tabata workout.

H_{O4} : There are no changes in lymphocytes count in physically inactive individuals after 6 weeks of Tabata workout.

H_{A4} : There are changes in lymphocytes count in physically inactive individuals after 6 weeks of Tabata workout.

1.6 SIGNIFICANCE OF THE STUDY

To date, studies on the effects of HIIT on immune responses are scarce. Thus, this study is significant in establishing knowledge regarding the effects of Tabata workout on immune cells response in physically inactive individuals. It is hope that positive findings found in this study can be used to promote exercise among physically inactive individuals to enhance their overall health.

1.7 OPERATIONAL DEFINITIONS

High-intensity interval training (HIIT):

Repeated, brief bouts of high intensity exercise (≥80% of maximal heart rate [HR_{max}]), interspersed by short recovery period either light-intensity exercise (40 – 50 % HR_{max}) or rest.

Tabata workout:

HIIT consisting of varying exercises with each exercise performed for only 20 seconds, interspersed with a brief rest of 10 seconds.

Physically inactive individuals:

Individuals who do not meet the recommended guidelines of regular physical activity by WHO for at least 150 minutes of moderate-intensity exercise throughout the week.

Immune responses:

Response of immune system to stressors, for example, bacterial infection, exercise, etc. It can be determined by measuring changes of the level and function of the immune system components, for example, immune cells count and functions, cytokine levels, etc.

CHAPTER 2: REVIEW OF LITERATURE

Inadequate amount of regular physical activity can lead to severe health and functional problems during aging (Hamer *et al.*, 2014). It has been reported that only one out of five adults engaged in regular physical activity (Dumith *et al.*, 2011). The WHO generally recommends that adults should participate in 150 minutes of moderate-intensity aerobic physical activity per week or 75 minutes of vigorous-intensity aerobic physical activity per week or an equivalent combination of both (Kilpatrick *et al.*, 2014).

Growing evidence shows that physical activity improves strength, flexibility, aerobic capacity, balance, mental well-being and cognition (Hamer *et al*., 2014). However, despite the long list of benefits of exercise reported in the literature, lack of time has been the major factor of the number of inactive individuals remain high (Mullahy and Robert, 2010). Thus, a sustainable alternative and time-efficient approaches known as high-intensity interval training (HIIT) was introduced to increase participation in physical activity as HIIT involves shorter duration but also at the same time is effective to improve fitness and health.

2.1 INTERVAL TRAINING

Interval training involves repeated short to long bouts of rather high-intensity exercise (equal or superior to maximal lactate steady-state velocity) interspersed with recovery periods (light exercise or rest) (Billat, 2001). Kilpatrick *et al*. (2014) generally defined interval training as exercise that involves periods of intense work and recovery that vary on intensity and duration of each segment. Interval training commonly refers to high-intensity interval training (HIIT), where work intervals often exceed 90% of heart rate reserve (HRR).

This training can be categorised into aerobic interval training and body weight/resistance interval training. Both involve periods of intense work followed by recovery segments with the major difference being the modality of exercise. Aerobic interval training most often uses running and cycling to deliver the desired intensities by way of activities such as spin classes and track-based running workouts. Meanwhile, resistance/body weight interval training makes use calisthenics, plyometrics and/or loaded lifts in training program like Tabata, CrossFit or other similar classes (Kilpatrick *et al*., 2014). Although there are a lot of different ways to perform interval training, all the programmes are characterised by periods of very heavy effort separated by periods of either complete rest or low intensity recovery.

2.1.1 High Intensity Interval Training (HIIT)

Numerous different HIIT protocols have been employed in research studies involving different numbers, intensity levels and lengths of vigorous-intensity portions and/or recovery periods. According to Francois and Little (2015), this protocol may therefore represent an ideal strategy for implementing vigorous exercise in individuals who are unfit or unaccustomed to vigorous-intensity physical activity. Given the availability of the numerous HIIT protocols, HIIT often classified into aerobic HIIT and anaerobic HIIT (usually known as sprint interval training (SIT)) (Kessler *et al.*, 2012).

High intensity interval training (HIIT) involves multiple (~4 – 10 repetitions) brief bouts (20 seconds – 5 minutes) of high intensity exercise (80 – 100% peak heart rate [HR_{peak}]), interspersed with either rest or low intensity workloads throughout an exercise session. This method is generally referred to as high-volume HIIT as the exercise sessions are typically longer than 30 minutes (Kilpatrick *et al.*, 2014). Generally, this protocol is sometimes performed below the maximal heart rate and therefore not "all-out" exercise. This protocol is effective in individuals who are unfit or unaccustomed to vigorous-intensity physical activity and has been used in both young, healthy participants and clinical populations including those with coronary artery disease, congestive heart failure and metabolic syndrome patients (Rognmo *et al*., 2004; Warbuton *et al*., 2005; WislØff *et al*., 2007; TjØnna *et al*., 2008). Effective and efficient exercise for fit and healthy individuals may involve running or sprint cycling, whereas for overweight individuals with type 2 diabetes may involve brisk or uphill walking (Francois and Little, 2015).

Several studies demonstrated that patients with cardiovascular and metabolic diseases have generally followed a HIIT intervention by using longer work intervals of 4 – 6 minutes at 80 – 95% of HR_{max} for 12 to 16 weeks (WislØff *et al*., 2007; TjØnna *et al*., 2008). These studies found that aerobic interval training (90% of HR_{max}) showed a significant improvement in maximal oxygen consumption as well as significant changes in body composition than continuous moderate-intensity (70% of HR_{max}). On the other hand, other studies have utilised HIIT protocols with shorter work intervals (30 seconds - 1 minute at 100% of HR_{max}) (Whyte *et al*., 2010; Whitehurst., 2012; Sloth *et al*., 2013; Terada *et al*., 2013).

Tabata, a type of HIIT was first studied by Japanese scientist, Izumi Tabata in 1996. Tabata and his colleagues (1996) conducted a study that compared moderate-intensity continuous training (70% of VO_{2max} for 60 minutes) with HIIT (170% of VO_{2max}) among young recreationally active students. High-intensity interval training consisted of seven to eight sets of 20 seconds of “all-out” exercise bouts, followed by 10 seconds of rest for a total of 4 minutes of exercise. Their study found that HIIT resulted in 28% increase in anaerobic capacity as well as improved aerobic capacity compared to moderate-intensity continuous training. McRae *et al*. (2012) in their study found that subjects who trained using one set of 8 × 20 seconds of a single exercise (burpees, jumping jacks, mountain climbers or squat thrusts) with 10 seconds of rest between session for a total of 4 minutes per training, 4 times per week for 4 weeks showed increased VO_{2max} value; similar to the group who performed 30 minutes of traditional endurance training per session.

Another prominent form of interval training is known as supramaximal intensity or sprint interval training (SIT) which generally characterised by 4 – 6 repetitions, 30 seconds maximal sprints (at intensity of over 90% of VO_{2max}) with 3 sessions per week interspersed by 4 minutes of recovery (Kessler *et al.*, 2012). This is generally referred to as low-volume HIIT as the exercise session are extremely brief. Wingate Test is the most common test used in low-volume HIIT, consists of 30 seconds of "all-out" cycling against a high resistance on a specialised cycle ergometer (at an intensity of over 90% of VO_{2max}). This protocol results in only 2 – 3 minutes of exercise at maximum intensity and 15 – 25 minutes of low intensity exercise per session, making it a time efficient method of exercise. "All-out" HIIT protocols are effective but this form of training may not be safe, tolerable or practical for general populations, especially sedentary and obese people (Gillen and Gibala, 2014). This protocol may be more suited for young and healthy participants.

Despite these concerns, several studies with clinical populations (obese and type 2 diabetes patients) have implemented SIT protocols which lead to significant improvements in cardiorespiratory fitness and insulin sensitivity with minimal adverse effects (Whyte *et al*., 2010; Little *et al*., 2011). This protocol with fewer and shorter intervals was also found to increase VO_{2max} among young sedentary/recreationally active individuals (Allemeier *et al*., 1994; Hazell *et al*., 2010; Zelt *et al*., 2014; Gillen and Gibala, 2014; Ijichi *et al*., 2015). In addition, Metcalfe and his colleagues (2011) in their study also reported that a mean increased of 14% of VO_{2max} can be achieved by performing just two sets of 20 seconds of "all-out" sprints, 3 days a week for 6 weeks. Furthermore, another study also demonstrated that high intensity bouts as short as 10 seconds which is less than 30 seconds bouts of Wingate test could induce substantial change in VO_{2max} value (Hazell *et al.,* 2010).

2.1.2 Health Benefits of HIIT

Numerous studies have been conducted on the effects of HIIT on fitness and health among physically inactive individuals. For example, it was reported that aerobic HIIT had a significant improvement on cardiovascular health, metabolic capacity and aerobic fitness (Kilpatrick *et al.*, 2014). Other benefits of aerobic HIIT include improved serum lipid profiles, blood pressure and inflammatory markers as well as reduced risk of stroke, acute coronary syndrome and overall cardiovascular mortality (Manson *et al*., 2002; Tanasescu *et al*., 2002; Lee *et al*., 2003; Fagard and Cornelissen, 2007; Johnson *et al*., 2009; Swadfager *et al*., 2012; Rossi *et al*., 2012). Furthermore, HIIT has been shown to significantly reduce subcutaneous fat, especially abdominal fat (Boutcher, 2011), as well as total body mass (Perry *et al*., 2008; TjØnna *et al*., 2008) and to improve VO_{2max} (Helgerud *et al*., 2007), and insulin sensitivity (Trapp *et al*., 2008).

Franch *et al*. (1998) in their study investigated the effects of continuous and high-intensity training in recreational runners (n = 36; VO_{2max} = 54.8 ± 3.0 ml/kg/min). The participants were equally stratified into 3 groups; either in short HIT (30 to 40 repetitions × 15 seconds at 20.4 km/h, 15 seconds rest), long HIT (4 to 6 repetitions × 4 minutes at 16.6 km/h, 2 minutes rest) or continuous running (15 km/h, ~26 minutes). All groups trained 3 times per week (2.2 hours/week) for 6 weeks at a mean intensity of ~65% HR_{max}. Both the continuous running and the long HIT groups significantly improved their VO_{2max} more than short HIT group (6% vs 3%; $p < 0.05$). Similarly, Macfarlane and his colleagues (2006) reported that 5 sets of 6 minutes of light-to-moderate physical activities per day, 4 – 5 days per week for 8 weeks improved VO_{2max} of sedentary participants.

In a recent study, Khammassi *et al*. (2018) reported the effects of 12 weeks of HIIT programme without caloric restriction on body composition and lipid profile among sedentary healthy overweight/obese youth (N = 20). They were randomly allocated into two groups: HIIT group (3 sessions per week, 30 seconds of work at 100% maximal aerobic velocity (MAV) interspersed by 30 seconds of recovery at 50% of MAV) and control group. This study showed a significant decrease in waist circumference, body mass index ($p < 0.01$) and fat mass percent ($p < 0.05$) as well as improvement in MAV and VO_{2max}.

In another study with untrained individuals (Billat, 2001), high intensity training (5 sets of 4 minutes of exercise at 100% VO_{2max}, 2 minutes of rest; n = 13) was found to enhance the oxidative capacity (succinate dehydrogenase and cytochrome oxidase) of type II fibres ($p<0.05$), when compared with a continuous exercise training group (n = 8), which performed exercise of a similar duration at the same average intensity (79% VO_{2max}). However, despite many health benefits of HIIT reported to date, its effects on immune responses among physically inactive individuals are yet to be explored.

2.2 IMMUNE FUNCTION

Immunity which refers to the protection from infectious disease has both less specific and more specific components. The less specific component known as innate immunity, provides the first line of defence against infection. Most components of innate immunity are present before the onset of the infection that are ready for immediate activation prior to attack by a pathogen. The innate immune system includes both soluble factors and cells. Soluble factors include protein lysozyme, the interferon proteins and components of the complement system. Meanwhile, major cells of the innate immune system include neutrophils, macrophages, monocytes, natural killer cells and dendritic cells (Kindt *et al*., 2007).

The second form of immunity, known as adaptive/acquired immunity develops in response to infection and adapts to recognize, eliminate and remember the invading pathogens. It provides a second, comprehensive line of defence that eliminates pathogens that evade the innate responses. B cells and T cells are the two major components of adaptive immunity (Parker *et al*., 2016). Most of the studies involving exercise as physical stressor has examined mononuclear immune cells (lymphocytes, monocytes and natural killer cells) responses. However, only a few studies have examined the polymorphonuclear phagocytic cells (neutrophils, eosinophils and basophils) (Pyne, 1994). Normal range of immune cells in healthy adults are as shown in the Table 2.1 (Kindt *et al*., 2007).

Leukocytes also known as white blood cells have a primary role in immune function and consist of 4×10^9 per litre of total blood in the body. Types of leukocytes are granulocytes (neutrophils, eosinophils and basophils) and agranulocytes (monocytes and lymphocytes - B cells and T cells) depending on the presence of

granules in their cells. Neutrophils are the most abundant cellular component of the human immune system, consisting of 60 – 70% of the circulating leukocytes. They act as first line of defence cells in the plasma that use phagocytosis either alone or in cooperation with antigen-specific defence to eliminate infectious agents. Neutrophils contain multi-lobed nucleus with finely purple granules and are often referred to as polymorphonuclear cells (Kindt et al., 2007; Parker *et al*., 2016).

Lymphocytes are the second most type of leukocyte, consisting of 20 – 30 % of all leukocytes and play an important role in immune response. The three major groups of lymphocytes include natural killer (NK) cells, B cells and T cells. B cells mature in the bone marrow and are responsible for the production of glycoproteins known as antibodies or immunoglobulins. Antibodies are involved in the body's defence against pathogens and toxins in the extracellular environment. Mechanism of adaptive specific immunity that involve B cells and antibody production are referred to as humoral immunity. Meanwhile, the maturation of T cells occurs in the thymus. T cells plays an important role in immune response and are responsible for destruction of cells infected with intracellular pathogens. This type of cell is generally classified into two groups either cell surface cluster of differentiation (CD) 4 or CD8 receptors. The CD8 T cells are most commonly known as cytotoxic T cells (T_C) and their function is to kill target cells while the CD4 T cells are commonly referred to as T helper cells (T_H) because they play an important role in contributing to the cytokine response. The targeting and destruction of intracellular pathogens by T cells is called cell-mediated immunity or cellular immunity (Parker *et al*., 2016).

Monocytes are circulating mononuclear phagocytes of the innate immune system which consist of 3 – 8% of the circulating leukocytes. They are the largest type of leukocyte which formed in the bone marrow and are released into peripheral blood, where they circulate for several days. This type of cells performs important functions including phagocytosis, antigen presentation and the secretion of anti- and pro-inflammatory cytokines (Medvedev *et al*., 2006).

Table 2.1: Normal range of immune cells in healthy adults (Kindt *et al.,* 2007)

Cell type	Cells count	Total leukocyte (%)
Red blood cells	5.0×10^6	
Platelets	2.5×10^5	
White blood cells (Leukocytes)	7.3×10^3	
Neutrophil	$3.7 - 5.1 \times 10^3$	50-70
Lymphocyte	$1.5 - 3.0 \times 10^3$	20-40
Monocyte	$1.0 - 4.4 \times 10^2$	1-6
Eosinophil	$1.0 - 2.2 \times 10^2$	1-3
Basophil	$< 1.3 \times 10^2$	<1

2.2.1 Exercise and Immune Function

Generally, exercise appears to affect immune system whereby the effects are depending on the intensity and duration of the exercise (Nieman *et al*., 2007). Immune cells respond to the effects of acute exercise either in term of numbers and functions (Gabriel *et al.*, 1992; Nieman and Nehlsen-Cannarella, 1994; Pyne, 1994). Nieman (1994) had proposed a J-curve model which explain the relationship between exercise load and infection risk. According to this model, moderate exercise may lower the risk of upper respiratory tract infections (URTI) while excessive amounts of exercise may increase the risk.

This model has been supported by Matthews and his colleagues (2002) which reported that regular performance about 2 hours of moderate exercise per day is associated with a 29% reduction in risk of getting URTI compared with a sedentary lifestyle. In contrast, there is a 100 – 500% increase in risk of getting an infection in the weeks following a competitive ultra-endurance running event (Nieman *et al*., 1990; Peters *et al*., 1993; 1996). Since URTI risk is inversely related to immune function, thus this model suggests that regular moderate-intensity exercise augments immune function while prolonged high-intensity exercise may depress immune function. Nevertheless, reports regarding effects of short period of high intensity exercise like HIIT on immune function is yet to be established.

Several studies reported a relation between exercise training and immune function. In previous studies on the influence of moderate exercise training on host protection and immune function, it was found that, near-daily brisk walking reduced the number of sickness days by half over a 12 to 15 weeks' period without any significant changes in resting immune function when compared with inactivity (Nieman *et al*., 1990;

1993; 1998). Rhind *et al*. (1996) also stated that moderate endurance training for 12 weeks was associated with sustained alterations in immune function, both at rest and when exercising.

Regarding immune cell responses, an increase in leukocytes has been reported during and after short intense and longer submaximal exercise and resistance exercise (Brenner *et al.*, 1999; Natale *et al.*, 2003; Nieman *et al.*, 2007), which may also trigger the increase in several cytokines (Nieman *et al.*, 2006). Exercise-induced leukocytosis is characterised by a biphasic response in which the rise of leukocytes during and immediately after exercise is mainly due to an increase in both neutrophils and lymphocytes, while the delayed rise peaking several hours at post-exercise is mainly due to circulating neutrophils (Hansen *et al*., 1991; McCarthy *et al*., 1991; Rowbottom and Green, 2000; Natale *et al*., 2003). Thus, the magnitude of the response seems to be exercise intensity and duration-related (Hansen *et al.*, 1991; Rowbottom and Green, 2000).

Similarly, it was found that acute effect of high intensity exercise (HIE) on leukocyte counts was lymphocyte- and monocyte-dependent (Neves *et al*., 2015). High intensity exercise is effective in altering the count of the leukocytes and their subsets (i.e., neutrophils, monocytes and lymphocytes) immediately after exercise and after 2 hours without any interpersonal variation. Moreover, there is a significant rise in the lymphocyte apoptosis ratio when the exercise intensity is increased (Navalta *et al*., 2007), and the elevated apoptosis in monocytes and lymphocytes after exercise (85% VO_2max) (Tuan *et al*., 2008).

A previous study by Natale *et al*. (2003) reported that aerobic training and resistance training induce changes in leukocyte counts in moderately fit individuals, whereas Nieman *et al*. (2007) did not find significant effects of continuous or intermittent cycling on white blood cells (WBC) in well-trained cyclists. These contradictory observations, as stated by Kendall *et al.* (1990), is due to different intensity, type, and duration of exercise as well as fitness level of the participants in both studies. Nevertheless, studies investigating effects of Tabata workout (a type of HIIT) on immune cells count among physically inactive individuals is scarce to date. Hence this study was proposed and carried out.

CHAPTER 3: METHODOLOGY

3.1 STUDY DESIGN

This is a one arm experimental study with pre-, mid- and post-test measurements (Figure 3.1). Duration of participants' involvement in this study was up to six weeks. Participants were recruited based on the inclusion and exclusion criteria that were set for this study. All outcome measurements were taken thrice; before (baseline), during (at week 3) and after (end of week 6) the supervised intervention periods. This study proposal has been approved (Appendix A) by Human Research Ethics Committee (HREC), Health Campus of Universiti Sains Malaysia (USM/JEPEM/19020134). This study had no conflict of interest.

3.2 STUDY LOCATION

The data collection was conducted at Exercise and Sport Science Laboratory of School of Health Sciences, Health Campus, USM, Kubang Kerian, Kelantan. All the Tabata workout sessions were performed at the Grey Square (a spacious area suitable to conduct the exercise sessions) in USM.

3.3 SAMPLE SIZE CALCULATION

By using G*Power software version 3.1.9.2, the sample size of this study was calculated with the effect size of 0.40. The power of the study was set at 80% with a confidence interval of 95% that can tolerate with a margin error of 0.05 (5%). The calculated sample size was 11 participants. However, considering 10% of dropout rate, a total of 12 participants were recruited (N = 12).

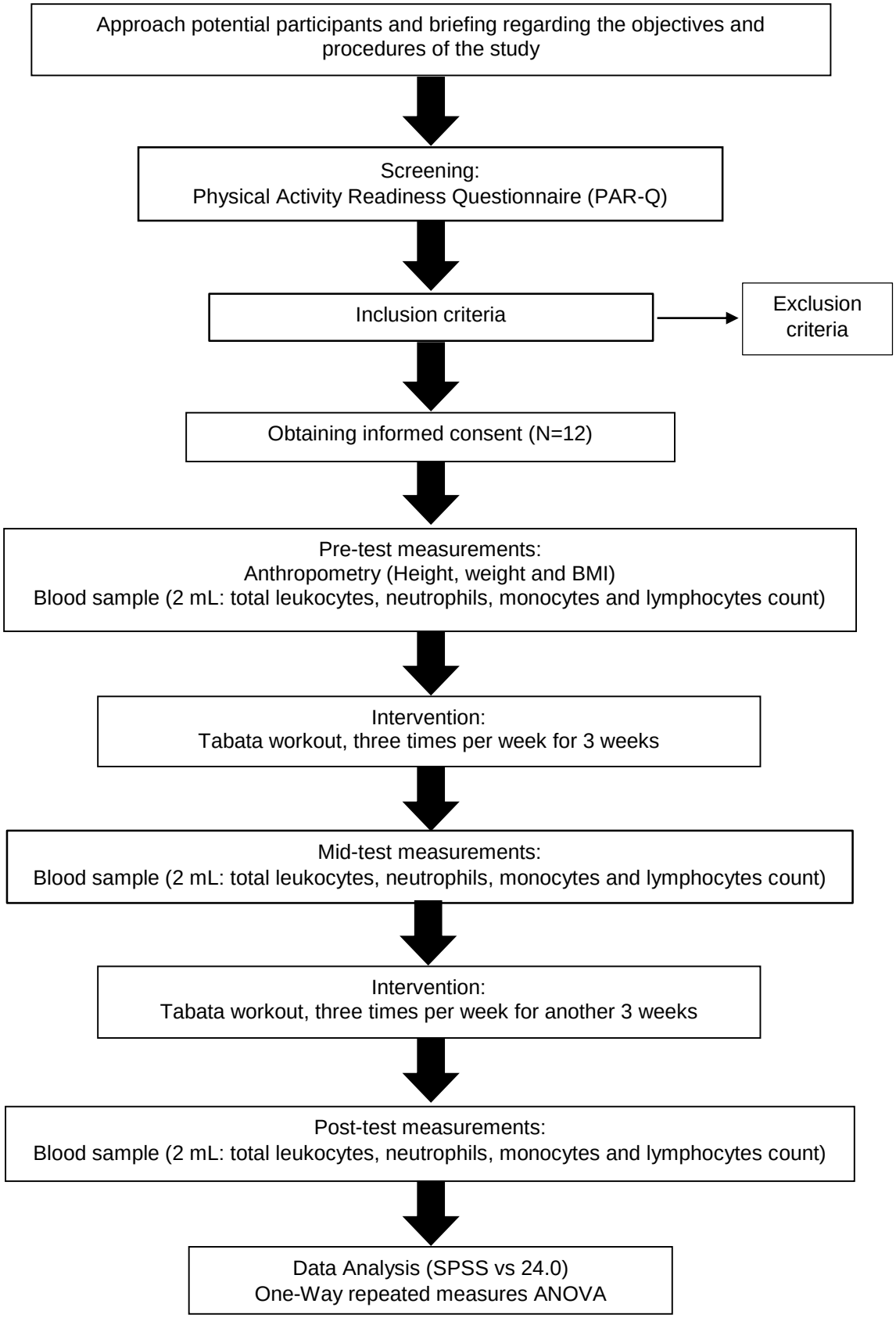

Figure 3.1: Flow chart of the study procedures

3.4 PARTICIPANTS

A total of twelve healthy physically inactive male and female were recruited among students of USM via poster advertisement (Appendix B) placed in and around USM. The participants gave informed consent form after being advised of all possible risks and discomforts associated with the procedures used in the study. The selection criteria were as follows:

Table 3.1: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Male and female adults	Smokers
Aged between 20 to 25 years old	Having illness
Healthy	On medication
Physically inactive (individuals who do not meet the recommended guidelines of regular physical activity by WHO for at least 150 minutes of moderate-intensity exercise throughout the week)	Taking supplements that are known for boosting immune function

3.5 STUDY PROTOCOLS

During the recruitment process, potential participants were approached by researcher and they were thoroughly explained regarding the objectives, procedures, possible harm/risk and benefits of the research study. Potential participants were requested to complete a Physical Activity Readiness Questionnaire (PAR-Q) (Appendix C) to assess their level of physical fitness and their ability to engage in any physical activity. Those who were voluntarily to participate and met the study participant were enrolled for the study. All participants completed the informed consent form (Appendix D). Following that, pre-test measurements were carried out in the Exercise and Sports Science Laboratory of USM.

Participants came to the laboratory at 8:00 am after an overnight fast from 10:00 pm for pre-test measurement. Participants were permitted to drink plain water during this fasting period. Upon arrival, participants' body weight and height were measured by a body composition analyser (Tanita, Japan) and a stadiometer (Seca, China) respectively. Then, calculation of body mass index was done as follows:

$$\text{BMI } (kg.m^{-2}) = \text{weight (kg)} / \text{height (m)} \times \text{height (m)}$$

After that, 2 mL of blood were collected by the laboratory technologies in the Sports Science Laboratory into an EDTA tube to measure total leukocytes, neutrophils, monocytes and lymphocytes count by using haematology analyser (Sysmex XE-5000, America). Following that, participants performed and completed Tabata workout as much as 3 sessions per week for a total of six weeks. Participants were asked to maintain their normal lifestyle throughout this period. As shown in the Table 3.2, each exercise session was started with warming up and ended with cooling down. Four sets of Tabata workout were used during each training session. Tabata is an interval workout with 20 seconds of intense effort followed by 10 seconds of recovery with several rounds per set. Participants were explained by the researcher how to do each Tabata workout exercise properly (Table 3.3).

Progressive load was employed in this study whereby the number of rounds per set were increased from 2 rounds in the first 2 weeks of intervention to 3 rounds for the next 2 weeks of intervention and so on. Between each Tabata workout set, participants rest for 1 minute. In total, exercise session was ranging between 15 to 30 minutes. Participant's heart rate (HR) and rating of perceived exertion (RPE – Appendix E) was recorded during each exercise session to ensure that the participants trained at the correct pre-determined intensity (70 – 85% HR_{max}; Borg scale = 15 – 20). Each

participant completed Tabata workout session at 90 - 95% of their age predicted HR_{max}. This workout protocol has been shown as a feasible and safe to be conducted in physically inactive individual in previous research (Emberts *et al.*, 2013). Participants were taught and shown how to perform the exercise correctly to reduce the risk of musculoskeletal injury. In addition, all the exercise sessions began with warm-up for at least 5 minutes and ended with cool-down. All the exercise sessions were conducted at the Grey Square (a spacious area suitable to conduct the exercise sessions – Appendix F) in USM and each session were supervised by the researcher.

At the end of the study, 12 participants completed the intervention. Tabata workout was carried out 3 times per week for 6 weeks (a total of 18 sessions). The training sessions were conducted on Sunday, Tuesday and Wednesday. After 3 weeks (mid-test) and 6 weeks (post-test) of intervention period, blood sample collection (2 mL) from all participants were carried out for blood count analysis (total leukocytes, neutrophils, monocytes and lymphocytes count). All the blood samples were measured on the same day (within 1 hour) and any left-over blood were disposed in a proper way. All the data obtained were recorded in the data collection form (Appendix G).