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TITLE

THE EFFECTS OF DIFFERENT TYPES OF EXERCISE ON HEALTH INDICATORS

BY

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ABSTRACT

Purpose: To investigate effects of different exercise modes on resting energy expenditure (REE), peak aerobic capacity (VO_{2peak}), body composition and complete blood count (CBC). **Methodology**: Forty-three healthy, untrained and non-smoking volunteer men (age 34.8±7.6 years, body mass index 27.4±4.6, VO_{2peak} 27.8±8.4 ml/kg/min) were randomized into four groups: aerobic-training group (group 1, n=12), resistance-training group (group 2, n=11), combined-training group (aerobic and resistance, group 3, n=11), and control group (group 4, n=9). During weeks 1-8, all participants in groups 1-3 followed specific progressive exercise programs, while participants in the control group followed their normal lifestyle. During weeks 9-16, all participants returned to their normal lifestyle. Throughout the study, each participant completed a dietary and physical activity (PA) diary. All parameters were assessed at baseline and at the end of weeks 8 and 16. Multivariate analysis of convariance (MANCOVA) was used to assess statistical differences between group means and time-point means on the dependent variables after controlling covariates. Independent variables were the group and time-point, dependent variables were REE, VO_{2peak} , body composition and CBC and covariates were the physical activity and dietary intake. **Results**: There was no statistically significant interaction between group and time point of the health parameters (dependent variables) ($p>0.05$). The dependent variables were significantly influenced by the PA, the caffeine intake, and the fat intake (all $p<0.05$). Specifically, PA [$F_{(1,97)}$=7,92, p=0.009] and caffeine intake [$F_{(1,97)}$=6.69, p=0.016] controlled VO_{2peak}, while sugar intake controlled REE [$F_{(1,97)=}$4, p=0.048]. Moreover, PA, caffeine and sugar intake controlled free fat mass ($p<0.05$). Finally, CBC was significantly controlled by PA and dietary intake ($p<0.05$). **Conclusion**: The adopted 8-week exercise protocols did not influence REE, VO_{2peak}, body composition and CBC in healthy untrained men.

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KEY WORDS:

Resting energy expenditure, body composition, aerobic capacity, complete blood count.

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ΠΕΡΙΛΗΨΗ

Σκοπός: Να συγκριθούν οι επιδράσεις διαφορετικών προγραμμάτων άσκησης στο βασικό μεταβολικό ρυθμό (ΒΜΡ), στη μέγιστη πρόσληψη οξυγόνου (VO_{2peak}), στη σωματική σύσταση (ΣΣ) και σε δείκτες γενικής εξέτασης αίματος (ΓΕΑ). **Μέθοδος**: Συμμετείχαν 43 υγιείς, μη-καπνιστές και μη-ασκούμενοι άνδρες (ηλικία 34.8±7.6 έτη, δείκτης μάζας σώματος 27.4±4.6, VO_{2peak} 27.8±8.4 ml/kg/min). Οι εθελοντές χωρίστηκαν τυχαία στις ομάδες αερόβιας προπόνησης (ΟΜΑΔΑ 1, n=12), προπόνησης με αντιστάσεις (ΟΜΑΔΑ 2, n=11), συνδυαστικής προπόνησης (αερόβια+αντιστάσεις, ΟΜΑΔΑ 3, n=11) και ελέγχου (ΟΜΑΔΑ 4, n=9). Κατά τις εβδομάδες 1-8 οι ΟΜΑΔΕΣ 1-3 ακολούθησαν εξειδικευμένα προγράμματα άσκησης ενώ η ΟΜΑΔΑ 4 ακολούθησε το φυσιολογικό γι' αυτή τρόπο ζωής. Κατά τις εβδομάδες 9-16 όλες οι ΟΜΑΔΕΣ ακολούθησαν το φυσιολογικό γι' αυτές τρόπο ζωής. Κατά τη διάρκεια της μελέτης, καταγράφονταν η φυσική δραστηριότητα (ΦΔ) και η διατροφή. Οι μετρήσεις πραγματοποιήθηκαν πριν την φάση παρέμβασης και μετά την ολοκλήρωση των εβδομάδων 8 και 16. Χρησιμοποιήθηκε πολυμεταβλητή ανάλυση συνδιακύμανσης με εξαρτημένες μεταβλητές τις ΒΜΡ, VO_{2peak}, ΣΣ, και ΓΕΑ, ανεξάρτητες μεταβλητές την ΟΜΑΔΑ και τον χρόνο, και μεταβλητές συνδιακύμανσης τη ΦΔ και τα συστατικά διατροφής. **Αποτελέσματα**: Δεν βρέθηκε αλληλεπίδραση ΟΜΑΔΑΣ*χρόνου σε καμία εξαρτημένη μεταβλητή ($p>0.05$). Ο γραμμικός συνδυασμός των εξαρτημένων μεταβλητών ρυθμιζόταν στατιστικώς σημαντικά από τη ΦΔ καθώς και την κατανάλωση καφεΐνης και πρωτεϊνών ($p<0.05$). Συγκεκριμένα, η ΦΔ [$F_{(1,97)}=7,92$, $p=0.009$] και η κατανάλωση καφεΐνης [$F_{(1,97)}=6.69$, $p=0.016$] ρυθμίζουν στατιστικά σημαντικά τη VO_{2peak}, ενώ η κατανάλωση ζάχαρης ρυθμίζει στατιστικά σημαντικά το ΒΜΡ [$F_{(1,97)}=4$, $p=0.048$]. Επιπλέον, η ΦΔ, η ποσότητα καφεΐνης και η ποσότητα ζάχαρης ρυθμίζουν στατιστικά σημαντικά την άλιπη μάζα του σώματος ($p<0.05$). Τέλος, οι δείκτες ΓΕΑ ρυθμίζονται στατιστικά σημαντικά από τη ΦΔ και από διαφορετικούς δείκτες διατροφής ($p<0.05$).

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Συζήτηση-Συμπεράσματα: Τα συγκεκριμένα 2-μηνα προγράμματα άσκησης δεν επηρέασαν το BMP, τη VO_{2peak}, τη ΣΣ και τους δείκτες ΓΕΑ σε υγιείς μη-ασκούμενους άνδρες.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ:

Βασικός μεταβολικός ρυθμός, σύσταση σώματος, αερόβια ικανότητα, γενική εξέταση αίματος.

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LIST OF ABBREVIATIONS

1. TEE (Total Energy Expenditure)
2. REE (Resting Energy Expenditure)
3. TEF (Thermic effect of food)
4. VO_{2peak} (Peak Aerobic Capacity)
5. BMR (Basic metabolic rate)
6. BMI (Body Mass Index)
7. WBC (White Blood Cell)
8. RBC (Red Blood Cell)
9. PLT (Platelets)
10. MCV (Mean corpuscular volume)
11. MCH (Mean corpuscular hemoglobin)
12. MCHC (Mean corpuscular hemoglobin concentration)
13. RDW (Red cell distribution width)
14. Hb (Hemoglobin)
15. Htc (Hematocrit)
16. NO (Nitric oxide)
17. ATP (Adenosine triphosphate)
18. TBW (Total body water)
19. WC (Waist circumference)
20. WHR (Waist to hip ratio)
21. NaBr (Sodium bromide)
22. ECW (Extracellular water)
23. FM (Fat mass)
24. FFM (Free fat mass)

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25. ADP (Air displacement plethysmography)

26. MRI (Magnetic resonance imaging)

27. CT (Computed tomography)

28. DEXA (Dual energy x ray absorptiometry)

29. BIA (Bioelectrical impedance analysis)

30. MON (Monocytes)

31. PCT (Platelet hematocrit)

32. PDW (Platelet distribution width)

33. PA (Physical activity)

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CHAPTER 1: INTRODUCTION

Over the past decades, the living environment has changed and it is now characterized by increased technology systems and industrialization which lead to sedentary lifestyle, household entertainment, and passive transportation, all of which are described as the main causes of physical inactivity (Borsheim, Knardahl, Hostmark, & Bahr, 1998; Denham, Marques, O'Brien, & Charchar, 2014). Epidemiology describes lack of physical activity as the fourth leading cause of death by decreasing energy expenditure and increasing obesity and risk factors for chronic diseases (Dishman, Heath &Washburn, 2004) (Denham et al., 2014; deRuiter & Faulkner, 2006). Physical activity is an important lifestyle aspect that promotes health, wellness and reduces all risks of mortality (deRuiter & Faulkner, 2006). Furthermore, physical activity leads to an important decrease of cardiovascular diseases (Wang, Pratt, Macera, Zheng, & Heath, 2004). Studies have shown that there is a strong correlation between physical activity and reduction in the development of coronary artery disease through increasing cardiovascular functional capacity and decreasing myocardial oxygen demand at any level of physical activity (Fletcher et al., 1996). Being physically active leads to improvements of blood pressure (Schubert, Desbrow, Sabapathy, & Leveritt, 2013) and obesity (Anderssen & Stromme, 2001) which are negatively correlated with the development of chronic diseases (King, Hopkins, Caudwell, Stubbs, & Blundell, 2009) such as type 2 diabetes, osteoporosis (Greendale, Barrett-Connor, Edelstein, Ingles, & Haile, 1995), and cancer (Farrell, Cortese, LaMonte, & Blair, 2007). Physical activity is a strong key to weight loss or weight management influencing energy balance, mainly through an effect on energy expenditure (Schubert et al., 2013). Many studies have investigated the influence of exercise on resting metabolic rate, which is the largest component of the total energy expenditure (see Chapter 2 for further details). Besides the effect on weight loss, exercise is one of the main contributors to changes in body composition such as increasing

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lean fat mass and decreasing adipose tissue. The benefits of physical exercise on the body's functional system depend on the type, duration, frequency and intensity of exercises. Exercise is classified into two main categories:

Aerobic exercise: It is characterized by usage of large muscle groups and depends primarily on oxygen in producing the required energy for muscle contraction (*Your Guide to Physical Activity and Your Heart*, 2006). Aerobic exercise leads to increases in peak aerobic capacity, it induces increases in blood volume, mitochondrial and capillary density, and intramuscular myoglobin, and it enhances the sensitivity of catecholamines (Glowacki et al., 2004; Ozaki, Loenneke, Thiebaud, & Abe, 2013).

Resistance exercise or strength exercise: It is characterized by muscle hypertrophy and functional ability. It has little effect on aerobic capacity, but results in increased muscle force production, glycolytic enzyme activity, and intramuscular ATP/phosphocreatine stores and a possible reduction of muscle mitochondrial and capillary density (Glowacki et al., 2004).

In conclusion, exercise has two main roles in health: (i) it is a potent approach for preventing major health problems, and (ii) it is a strong medical treatment for health issues (Khan et al., 2012). The World Health Organization recommends, for healthy adults and older people, at least 150 minutes per week of moderate-intensity exercise (Lerdal, Celius, & Pedersen, 2013) (Anderssen & Stromme, 2001).

Purpose

The main objective of this study was to investigate the effects of different 8-week exercise protocols on resting energy expenditure, peak aerobic capacity, body composition and complete blood count. A secondary objective of the study was to investigate changes on resting energy expenditure, peak aerobic capacity, body composition and complete blood cell count after a 8 week de-training period.

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Null Hypothesis

- Different protocols of exercise for 8 weeks will not have effect on resting energy expenditure, peak oxygen capacity, body composition and complete blood cell count.
- The 8 week de-training period will not have effect on resting energy expenditure, peak aerobic capacity, body composition and complete blood cell count.

Alternative Hypothesis

- Different protocols of exercise for 8 weeks will have effect on resting energy expenditure, peak oxygen capacity, body composition and complete blood cell count.
- The 8 week de-training period will have effect on resting energy expenditure, peak aerobic capacity, body composition and complete blood cell count.

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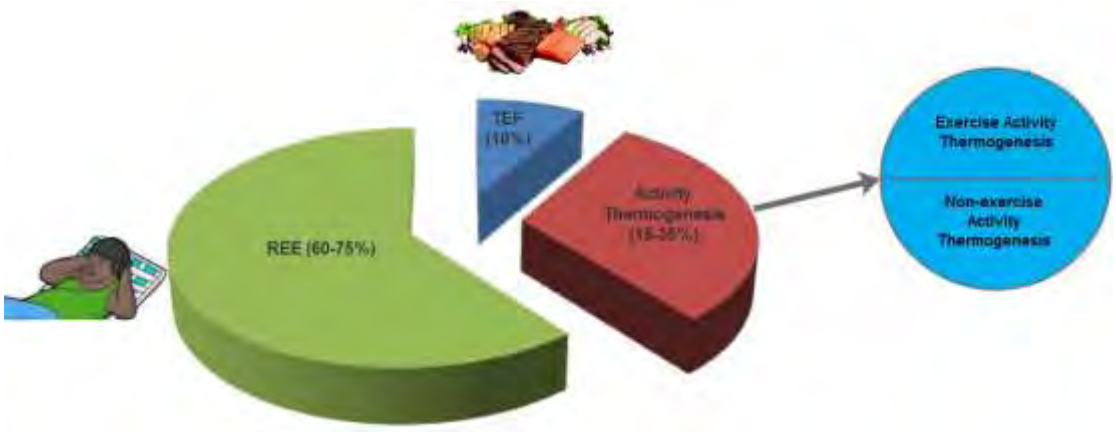
CHAPTER 2: LITERATURE REVIEW

The purpose of the literature review is to provide general information about resting energy expenditure, peak oxygen capacity, body composition and complete blood count. Furthermore, data from previous studies are provided about the effect of different types of exercise on each of the studied health parameters. To achieve the purposes of the present literature review, three databases were included in the searching procedure: Pub Med, Scopus and Science Direct. Keywords for the searching procedure were: exercise, resting energy expenditure, body components/composition, peak aerobic capacity, complete blood cell count.

Resting energy expenditure

Total energy expenditure includes three basic components: resting energy expenditure (REE), thermic effect of food (TEF) and activity thermogenesis [(Figure 1) (Poehlman, 1989)].

Figure 1. The components of total energy expenditure. Note: REE is defined for resting energy expenditure and TEF is defined for thermic effect of food. Activity thermogenesis is separated in two parts: exercise activity thermogenesis and non-exercise activity thermogenesis.



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The thermic effect of food (TEF) is characterized as the cost of energy from the food ingestion and nutrient absorption and storage (Bouchard, Perusse, Deriaz, Despres, & Tremblay, 1993). It is the component with the smallest influence on the TEE accounting for 10% and has a strong correlation with dietary components (Crovetti, Porrini, Santangelo, & Testolin, 1998). It has been recognized by the majority of studies that protein meals increase TEF compared to meals with fat or carbohydrates (Crovetti et al., 1998).

Activity thermogenesis represents approximately the 15-35% of a daily 24-h TEE (Speakman & Selman, 2003) and is the most important and variable fraction of the TEE because of its major impact on increasing the physical activity energy expenditure (Hunter, Weinsier, Bamman, & Larson, 1998). Activity thermogenesis can be separated in two parts: exercise activity thermogenesis and non-exercise activity thermogenesis (Levine, 2004). The latter includes all the non-exercise activities of a daily life, such as sitting, fidgeting, walking, standing, typing, etc. (Levine, Schleusner, & Jensen, 2000).

The BMR constitutes the largest part, 60-75%, of the TEE and is described as the minimum rate of energy that an individual needs just to stay in life (Levine, 2005). The measurement of BMR requires the subject to sleep at the laboratory and immediately after the awakening it is conducted under standardized conditions: at rest in a supine position; after a 12-hour post-absorptive; at least after an 8-hour night sleep; and under thermoneutral conditions (Weibel & Hoppeler, 2005). REE represents the minimum cost of energy maintaining life physiological functions and is within 10% of BMR (Levine, 2005). Unlike with BMR, REE can be measured with the subjects staying at their facilities, being transported to the laboratory in the morning after awakening. REE is conducted under the same standardized conditions mentioned above.

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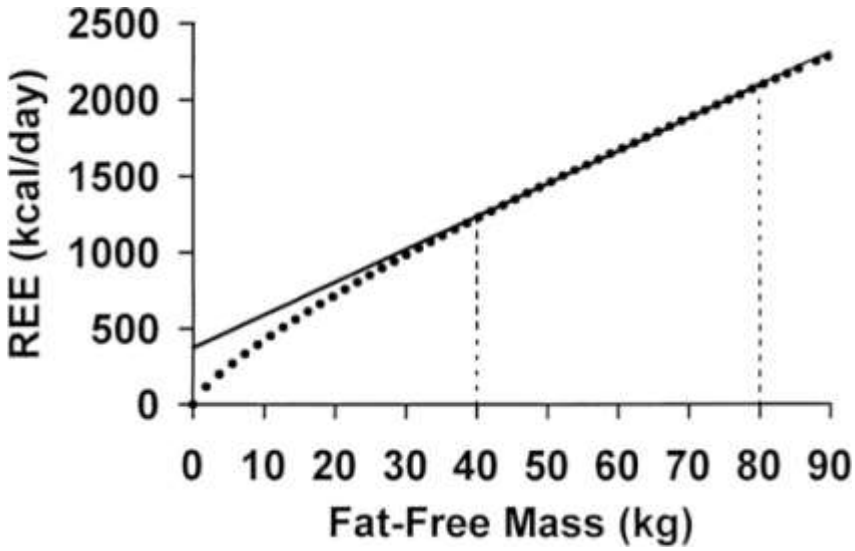
Exercise and REE

One of the most important impacts of exercise on REE is the result of preserving or increasing lean adipose tissue (Speakman & Selman, 2003), because lean adipose tissue is a major determinant of REE variation, approximately about 50-70% [(Figure 2) (Speakman & Selman, 2003)]. Exercise can also increase the function of sympathetic nervous system due to an increase in catecholamine sensitivity and/or by increasing levels of catecholamines (Borsheim et al., 1998). Furthermore, exercise correlates with thyroid status influence on REE are the association between physical activity (Speakman & Selman, 2003) and significant impacts on REE can also be occurred be acute periods of exercise (Dolezal, Potteiger, Jacobsen, & Benedict, 2000; Schuenke, Mikat, & McBride, 2002). These effects are observed immediately after the bout of exercise, with REE being elevated even 24 hours after the bout. These changes after a short period of physical activity have been termed the excess post-exercise O_2 consumption without the time to influence lean tissue mass (Speakman & Selman, 2003).

Figure 2. Relationship between resting energy expenditure (REE) and fat free mass. (Wang Z et al., 2000).



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There is a large body of human studies that investigated the effects of exercise on REE. Many of these studies included either short-term or long-term exercise protocols, others studies examined the combination of exercise treatment and energy restriction, while many investigated the effects of exercise alone on REE. Regarding the studies that performed exercise treatment alone, some of them used a single type of exercise and others examined the influence on REE using more than one type of exercise. A number of studies investigated the influence of different exercise types (aerobic exercise, resistance exercise and combined aerobic and resistance training) on REE and the results are controversial. For instance, 19 sedentary obese women (age: 38.0±0.9 years, percent body fat: 37.5±0.8) were randomized either in the control group, the resistance exercise group or the combined resistance and aerobic (walking) exercise group (Byrne & Wilmore, 2001). Measurements of REE were conducted pre and post the intervention period. This study found a significant increase in REE in the resistance group, but surprisingly failed to find the same increase in the combined exercise group. Instead, the combined group demonstrated a significant decrease in REE (Byrne & Wilmore, 2001). An intervention study of 10 weeks endurance training, resistance training and combined training on 30 healthy men (20.1±1.6 years) showed significant increases in post-REE at the resistance training group and combined training group, in contrast to the endurance group were there was no significant change in REE (Dolezal & Potteiger, 1998).

In a study which investigated the effect of 12 week high-intensity aerobic or resistance exercise training on 47 males (18-35 years), participants were randomly assigned in three groups: control group, resistance group and endurance group. The results did not show a significant change in REE before and after the training session in any groups (Broeder, Burrhus, Svanevik, & Wilmore, 1992). In another study, chronic resistance exercise training for 18 weeks in 26 healthy sedentary men did not have a significant impact

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on REE at the end of the exercise protocol (Van Etten, Westerterp, Verstappen, Boon, & Saris, 1997).

Peak aerobic capacity

Peak aerobic capacity (VO_{2peak}) is defined as the highest rate of oxygen consumption attainable during maximal or exhaustive exercise (Huggett, Connelly, & Overend, 2005). The VO_{2peak} is widely accepted as the gold standard of measuring cardiorespiratory system function and is commonly expressed in milliliters per kilogram of body weight per minute (ml/kg/min). Maximal exercise tests with direct measurement of oxygen consumption are the mostly used method for assessing VO_{2peak}, with treadmill or bicycle ergometer being commonly used (Huggett et al., 2005).

Exercise and maximal aerobic capacity

In the majority of studies, aerobic exercise training leads to an increase of aerobic capacity with the most effective method being at high intensities (i.e., 90-100% of VO_{2peak}). The effectiveness of aerobic exercise on VO_{2peak} is due to improvements in stroke volume, heart rate, and arterial-venous oxygen difference (Wenger & Bell, 1986). Resistance training, is a main stimulus for muscle hypertrophy and strength gain, and its results on VO_{2peak} are controversial with most studies showing no significant effects. Regarding studies that used resistance training >50% of one-repetition maximum (1RM), only 3 of 17 studies with young people have shown an increase in VO_{2peak}. Campos et al (2002) divided 32 untrained men in four groups (one control group and three groups of different intensity of resistance) who participated in a progressive resistance training program for 8 weeks. The results showed no changes in VO_{2peak} regardless of the exercise intensity (Campos et al., 2002). Furthermore, combined exercise training (resistance and aerobic) improves both cardiorespiratory system

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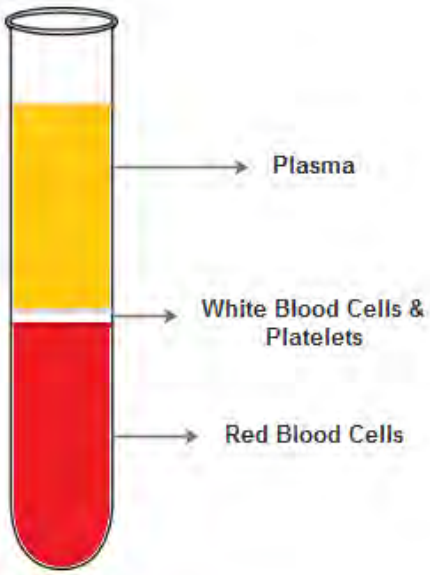
and muscular system. A 12 weeks study of 23 healthy men (65±4 years) examined the effect of different types of exercise on VO_{2peak} (Cadore et al., 2011). The participants were divided into three groups: resistance, aerobic and concurrent resistance and aerobic and trained three times per week. The measurements were obtained at the baseline and at the end of the training period. Significant increase in VO_{2peak} was observed only in the aerobic group and the combined group (Cadore et al., 2011).

Complete blood cell count

Humans vascular system has about 5 to 6 liters of blood (Dean, 2005) fluid due to its important functions. Approximately 40% of the blood volume is consisted by cells: red blood cell (RBC), white blood cells (WBC) and platelets (PLT) and the other 60% is called plasma (Figure 3).

Figure 3. Blood components.



The complete blood cell count is a typical test in the clinical and medical area which counts different types of cells including RBC, WBC and PLT and their concentrations in

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blood. It also provides information about the total amount of hemoglobin (Hb) in the blood and the fraction of the blood composed of red blood cells (hematocrit) (Dean, 2005).

Red blood cells

Red blood cells (RBCs), also called "erythrocytes", are the most common type of cells in the bloodstream (Dean, 2005; Peter Klinken, 2002). They originate from haemopoietic stem cells in the bone marrow every second (2-3 million) (Dean, 2005; Peter Klinken, 2002). RBCs are small flexible biconcave (Peter Klinken, 2002) cells (6μm diameter) and they are the carriers of oxygen and carbon dioxide. The characteristic of flexibility gives them the ability to circulate even through the smallest blood vessels, while the biconcave shape gives them the suitable surface for the gas exchange (Gordon-Smith, 2009). The main function of the RBCs is to carry Hb around the body, so that to accomplish the transport of oxygen from the lungs to the tissues and carbon dioxide back from the tissues to the lungs (Peter Klinken, 2002). RBCs lifespan is 120 days from release from the bone marrow to final destruction in the reticuloendothelial system (Gordon-Smith, 2009).

Measuring the RBC count we can have information for the called "red blood indices": mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These values are important for estimating the reasons for anemia, but can be measured only if hematocrit, Hb and the amount of RBC are known (Kenneth, 2015).

- *Mean corpuscular volume (MCV):* defines the average size of the red cells.
- *Mean corpuscular hemoglobin (MCH):* quantifies the average amount of hemoglobin per red blood cell.
- *Mean corpuscular hemoglobin concentration (MCHC):* quantifies the concentration of hemoglobin per unit volume.

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Finally, RBC count calculation provides data about the variation of the red cell size (anisocytosis) (Kenneth, 2015) and is expressed as red cell distribution width (RDW) or as red cell morphology index.

Hemoglobin

Hemoglobin (Hb) is the main protein of all red blood cells, carrying oxygen around the body through the cardiovascular system (Mairbaurl, 2013). It constitutes 99% of the cytosolic protein in mature RBCs. Hb is the reason for the characteristic red color of erythrocytes. Hb is a tetrameter of two a and two b chains each of one enclosing a core haem-moiety (Gordon-Smith, 2009; Peter Klinken, 2002). At the center of the haem is the iron which it responsible for the gas transport (Peter Klinken, 2002).

Hematocrit

Hematocrit (Hct) measures the fraction of the blood composed of red blood cells (Kenneth, 2015). It reflects the combination of the total number of RBCs, and the volume that they occupy (Dean, 2005). Abnormal values of Hct reflect an impaired correlation between the blood volume and the erythrocytes synthesis and poses impacts on health.

Low values of Hct suggest that there is a decrease in the production of RBC from the bone marrow. Reasons for this decrease can be:

- A disease related to the bone marrow (cancer, toxins).
- Reduction of erythropoietin synthesis, the hormone that provides RBCs production.
- Decreased lifespan of the RBCs.
- Pregnancy (due to plasma fluid excess, with no changes in the RBCs production).

Increased values of Hct are correlated with an increase in RBCs production and release from the bone marrow. Reasons for this increase can be:

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- Decreased blood plasma levels.
- Increased production of RBCs from the bone marrow, which can be a result of a tumor called polycythemia rubra vera, a myeloproliferative disorder (Dean, 2005; Zubieta-Calleja, Paulev, Zubieta-Calleja, & Zubieta-Castillo, 2007).

White blood cells

White blood cells (WBC) or leukocytes or leucocytes are a heterogeneous group of nucleated cells, circulating through the blood fluid and lymphatic system, (Chung, Ou, Kulkarni, & Yang, 2015) with a main role in the immune system and phagocytosis protecting the body from infections. WBC are classified into granulocytes, lymphocytes, and monocytes. Granulocytes owe their name to the presence of distinct cytoplasmic granulation. Three varieties of granulocytes are recognized: *neutrophils* (or polymorphonuclear granulocytes) which are responsible for the phagocytosis of bacteria, *eosinophils*, and *basophils* (Kenneth, 2015). Both eosinophils and basophils develop a function that is not very clear. Eosinoplils main action is to protect against parasites (Klion & Nutman, 2004) and develop limits of inflammation. On the other side, basophils are the smallest population of granulocytes (1%-2%) and the play role in immunity and allergic disorders (Geurts et al., 2015).

Lymphocytes are round cells that contain a single, large round nucleus and account for the 20-45% of WBC and are separated into bone marrow-lymphocytes (B) and thymus-dependent lymphocytes (T) (Kenneth, 2015). Both types of lymphocytes have an immune response but in different pathways. B-lymphocytes produce specific antibodies, while T-lymphocytes produce specific chemicals which activate other immune cells, regulating and coordinating the whole defense against infections (Dean, 2005).

Monocytes are young WBCs in the bloodstream, when they leave the circulation and migrate into tissues they differentiate into macrophages. They are the main phagocytic cells

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because of their functional to digest pathogens before any other type of WBCs (Gordon-Smith, 2009; Kenneth, 2015). Monocytes are also “antigen-presenting cells”, helping other immune cells to detect the antigens, activating and coordinating an immune response (Dean, 2005).

Platelets

Platelets (PLTs) are anuclear fragments of cell, born from stems cell in the bone marrow (Kenneth, 2015). The stem cells develop into platelet precursors called megakaryocytes, 3 to 4 μm in diameter with limited synthetic ability. PLTs circulate in the bloodstream for 9 days and during this period they are either activated to form a blood clot or at the end of their life they are removed by the spleen (Dean, 2005). PLTs clotting is activated from an interruption to the normal vascular system (Kenneth, 2015).

Exercise and complete blood cell count

Exercise is a reason for alterations in complete blood cell count, providing changes in the circulating amounts of cells and their functions. Exercise, results in an increased supply of oxygen due to the increased muscle blood flow. RBCs release nitric oxide and ATP which improves the vasodilation and blood flow to the working muscles (Mairbaurl, 2013). Exercise causes tissue damage, results in production of stress hormones, and changes the number and function of immune cells, including WBC (Natale et al., 2003). During exercise, Hct can be increased due to the reduction of plasma volume, which is caused by sweating, and transport of water plasma to the extracellular space due to osmotic metabolites. However, long term effects of exercise on Hct not consistent. In a review of studies with training periods from 4 to 12 months the results showed no differences in RBC and Hct (Sawka, Convertino, Eichner,

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Schnieder, & Young, 2000). A study showed that total Hb did not increase in a training period less than eleven days, but had a markedly increase when the training lasted for 21 days (Sawka et al., 2000). In the same study, Hct remained decreased for several days following the training periods, but RBC started to increase after several days of training. Regarding studies that investigated the effects of exercise on WBC, moderate exercise training for 15 weeks showed that there was no association with an improvement in lymphocyte function (Nehlsen-Cannarella et al., 1991). However, significant decreases in circulating numbers of lymphocytes, especially the T cell subpopulation, were found mostly at the 6-weeks time-point (Nehlsen-Cannarella et al., 1991). Finally, a study of 34 young women who were divided into three weight-dependent groups (underweight, normal, overweight) showed no significant differences in complete blood cell count indices after a 12-week aerobic training program (Kostrzewa-Nowak et al., 2015).

Body composition

Human body composition is an active research area for over 100 years and can be defined as the percentage of muscles, fat, bones, water, and tissues in the body. Estimation of body composition can be achieved via many methods, varying in techniques, difficulty, equipment, required expertise, cost, and accuracy (Wells & Fewtrell, 2006). The purpose of body composition measurement is to estimate either excesses or deficiencies of a component which is thought or known to be related with health risks (Lee & Gallagher, 2008). Researchers have developed models by dividing the body in levels/components and measurements of the human body are based on those. Some of the most used models for measuring body composition are:

- **The five-level model**. An organizational level, in which the body can be characterized at five levels, each of one having clearly components that comprise total body weight. The 5 levels include: atomic level (1), molecular level (2), cellular (3), tissue system

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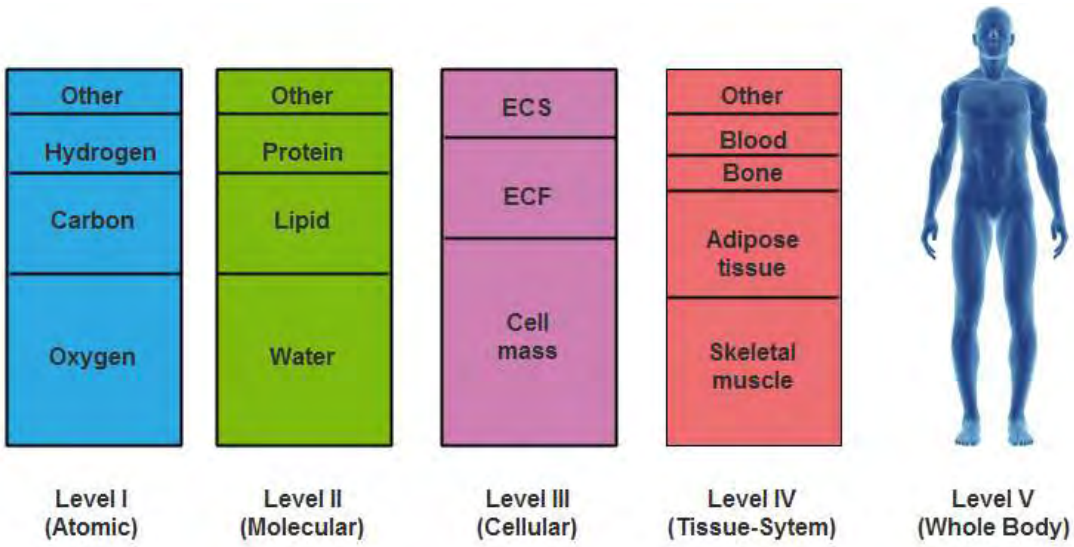
level (4) and whole body level (5) [(Figure 4) (Wang Z.M., Pierson, & Heymsfield, 1992)].

Figure 4. Illustration of the 5-level model. Note: ECS: extracellular fluid and ECS: extracellular solids.



- **<u>The two components (2C) model.</u>** It is the most widely used model for the body composition assessment which divides the body into the fat mass (FM) and the free-fat mass (FFM) (Wang Z.M. et al., 1992).
- **<u>The four compartment (4C) model</u>**. It is the most accurate method for estimating body composition, measuring the body mass or weight, the total body volume, the total body water (TBW) and the bone mineral (Wang Z.M. et al., 1992). Body composition measurements

Anthropometric measurements for total body composition include skinfold thickness, limb circumferences, and bone dimensions and through specialized equations body fat and bone density can be predicted. There are many regression equations developed for predicting body

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fat and bone dimension. Most of them use skinfold thickness and others use the combination of skinfold thickness and circumferences (Wells & Fewtrell, 2006).

Skinfold thickness

Skinfold thickness measurement is one of the mostly used and common techniques for the calculation and prediction of body composition in research. The main purpose of this method is to estimate the relative "fatness" and size of subcutaneous adipose tissue (Wells & Fewtrell, 2006). It is considered as a safe, noninvasive, and pain-free method. The skinfold caliper is a widelyused equipment for measuring subcutaneous adipose tissue in various sites of the body. It is inexpensive, easy to use, and, most importantly, it is portable which gives the ability to use in field testing besides the laboratory (Brodie, Moscrip, & Hutcheon, 1998). Measurements are quick and simple to perform in most age groups including children, elderly, and infants (Wells & Fewtrell, 2006). Sources of errors during the measurement are the caliper selection and reliability and experience of the person performing the measurement (Brodie et al., 1998).

Body mass index

Body mass index (BMI) is calculated as body weight in kilograms divided by the square of body height in meters. BMI has been suggested as a measure for body density similar to that of skinfold thickness (Deurenberg, 1996). BMI is an indicator of heaviness rather than fatness and it cannot differentiate FM from FFM (Brodie et al., 1998).

Waist circumference and waist to hip ratio

Waist circumference (WC) and waist to hip ratio (WHR) are simple measurements of central fatness and have a strong relationship with adipose tissue indicators and morbidity of health problems such as type 2 diabetes, lipidemia, etc (Brodie et al., 1998; Wells & Fewtrell,

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2006). WHR is calculated by dividing the WC to the hip circumference. WC is measured using a tape measure at the mid-level between the costal margin and iliac crest, and hip circumference was assessed at the widest level above the trochanter (Chan, Watts, Barrett, & Burke, 2003). Studies have shown that WC correlates well (r index in the range of 0.5 to 0.8) with the measurement for abdominal fat depots of magnetic resonance imaging (MRI), while correlations of abdominal fatness and WHR are controversial between studies (Wells & Fewtrell, 2006).

Dilution techniques

An important component of the body is water and any changes in the amount of TBW can affect body composition (Wells & Fewtrell, 2006). Dilution allows the estimation of TBW and consequently FM and FFM, assuming that the hydration FFM value is stable (Wells & Fewtrell, 2006). The isotope dilution for measuring TBW can be conducted by deuterated (^{2}H), tritaded (^{3}H) or oxygen-labeled (^{18}O) water. The tracer sodium bromide (NaBr) is used for assessing extracellular water (ECW). After the enrichment of the body with an isotope dilution, samples are collected (saliva, blood, urine) and analyzed by isotope ratio mass spectrometry (Lee & Gallagher, 2008; Wells & Fewtrell, 2006). Fat mass is calculated as body weight divided by FFM. Although it is a relatively easy method, it is not practical for all age groups, especially little children and newborns, and for large-scale studies. Furthermore, FFM hydration value can be influenced by health problems, affecting the measurement for FM and FFM (Lee & Gallagher, 2008).

Air displacement plethysmography

Air displacement plethysmography (ADP) is used for assessing human body composition based on Boyle's law that the volume inversely related to pressure (McCrory, Mole, Gomez, Dewey, & Bernauer, 1998). There is one available and widely used system for ADP known as BOD POD (Life Measurement, Inc, Concord, CA) which measures the volume of the air

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forced out by the body volume of the subject inside a chamber (plethysmograph) (Lee & Gallagher, 2008). As with every technique, BOD POD has its advantages and disadvantages. The advantages of BOD POD include that it is noninvasive, quick, comfortable, available for all individuals (e.g. children, elderly, obese), automated, safe, and includes no radiation exposure. On the other hand, the disadvantages of the BOD POD can be its controversial results of studies for its reliability, validity and practicality in various populations (Fields, Goran, & McCrory, 2002).

Magnetic resonance imaging and computed tomography

MRI and computed tomography (CT) are imaging methods and considered to be accurate approaches for visualization and quantification of body composition components (adipose tissue, skeletal muscle, organs etc) (Lee & Gallagher, 2008). MRI has been accepted as an accurate measuring technique of adipose tissue in vivo quantifying it to visceral, subcutaneous and recently intermuscular depots and estimating the relationship of the depots with cardiovascular diseases (Lee & Gallagher, 2008). MRI is a promising method due to its ability of imaging the adipose tissue and its lack of radiation exposure. However, MRI is very costly and it is not suitable for large persons who cannot fit within the field-of-view (48x48 cm). Also it is not suggested for claustrophobic individuals (Lee & Gallagher, 2008).

CT is another imaging method of whole body composition and assesses different compartments of body fat. It allows the estimation of internal and subcutaneous fat parts (Thomas et al., 1998). Although the cost of CT is lower compared to MRI, the CT is severely compromised by the exposure to ionizing radiation, making this method unsuitable for many studies (Thomas et al., 1998). As with MRI, CT is not possible for large people (BMI $>40 kg/m^2$) (Thomas et al., 1998).

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Dual energy x ray absorptiometry

Dual energy x ray absorptiometry (DEXA) was developed for measuring bone mineral mass and is the gold standard diagnostic measurement for osteoporosis and osteopenia (Brodie et al., 1998; Lee & Gallagher, 2008). However, due to the estimation of three main body composition components (bone mineral mass, bone-free FFM, and fat mass), DEXA has become a potential criterion also for assessing body composition (Brodie et al., 1998; Lee & Gallagher, 2008). DEXA is recognized as a non-invasive technique and acceptable for all ages, including infants (Wells & Fewtrell, 2006). It uses ionizing radiation, ranged from 0.04 to 0.86 which is about 1-10% of a chest radiograph (Wells & Fewtrell, 2006). Ease of use, availability, reproducibility, good accuracy and low radiation exposure belong in DEXAs advantage's group. Limitations of DEXA are related to the body weight and the body weight (Lee & Gallagher, 2008).

Bioelectrical impedance analysis (BIA)

BIA is a body-composition method, based on a two compartment model, which measures impedance of the body to a small electric current (approximately 50Hz) (Wells & Fewtrell, 2006). This type of analysis perceives the body as a single cylinder and measurers the current through disposable electrodes placed on two of the limbs (on a wrist or the ankle). The BIA allows the estimation of TBW, fluid volumes (extracellular fluid and intracellular fluid), body cell mass, and FFM (Heymsfield, Wang, Visser, Gallagher, & Pierson, 1996). The BIA method is easy to use, safe, non-invasive, and has high accuracy and precision. However, it requires standardized conditions and it is relatively expensive. A negative limitation of using BIA is the hydration factor, especially in obese individuals (Deurenberg, 1996). New approaches to BIA include foot to foot impedance systems with stand-on platforms and hand to hand impedance systems with hand–hold devices (Oppliger & Bartok, 2002).

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Exercise and body composition

Human body composition can be affected by factors such as, exercise, diet, diseases, and genetic reasons (Donges, Duffield, & Drinkwater, 2010). The majority of studies have shown that exercise generally influences body components, however, the changes in body composition is related to the type, duration, frequency and intensity of exercise (Donges et al., 2010). A study investigated the effect of a 10-week resistance or aerobic exercise training in 102 sedentary subjects and found that the aerobic group had a significant reduction in intra-abdominal FM and body mass, while the resistance group had a significant improvement in total body fat (Donges et al., 2010). In another study, 45 untrained healthy men were recruited in three groups: endurance training group, resistance training group and combined training group, during a 12-week period (Glowacki et al., 2004). Combined and resistance training increased significantly the weight and FFM, while the percentage of body fat was decreased significantly in the endurance and combined training group (Glowacki et al., 2004).

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CHAPTER 3: MATERIALS AND METHODS

Participants

With an ethics approval by the University of Thessaly Ethics Review Board (see Appendix), a total of 43 male participants (age 34.8±7.6, BMI 27.4±4.6) were recruited for participation through newspaper, wall posters, social media blogs and word of mouth. Participants were non-smokers, with no history of chronic health problems, they were under no medication, and reported being physical inactive. All participants were informed verbally about the procedures of the study including possible risks and discomforts. Following their agreement to participate, they signed a written informed consent and completed a medical history questionnaire, a family history questionnaire and a physical activity questionnaire.

Experimental Protocol

Participants were randomly placed in one of the four following groups: aerobic training group, resistance training group, combined (aerobic and resistance) training group and control group. Participants in the latter group were asked to maintain their daily lifestyle. The duration of the study was 16 weeks and included two periods: the intervention period (week 1-8) and the de-training period (week 9-16). At the intervention period all participants in the training groups followed a specific progressive exercise program while the subjects in the control group followed their normal sedentary lifestyle. At the de-training period all subjects of all groups maintained their daily lifestyle.

Measurements included anthropometric data (weight, height, BMI, WHR), body composition, complete blood cell count, VO_{2peak} and REE. All parameters were assessed in three different time periods: baseline, at the end of week 8 and at the end of week 16 (Figure 5). All measurements were conducted under the same conditions, in the morning between 06:00 to 09:00 following a 12-hour fast and after participants had abstained for 24 hours from caffeine

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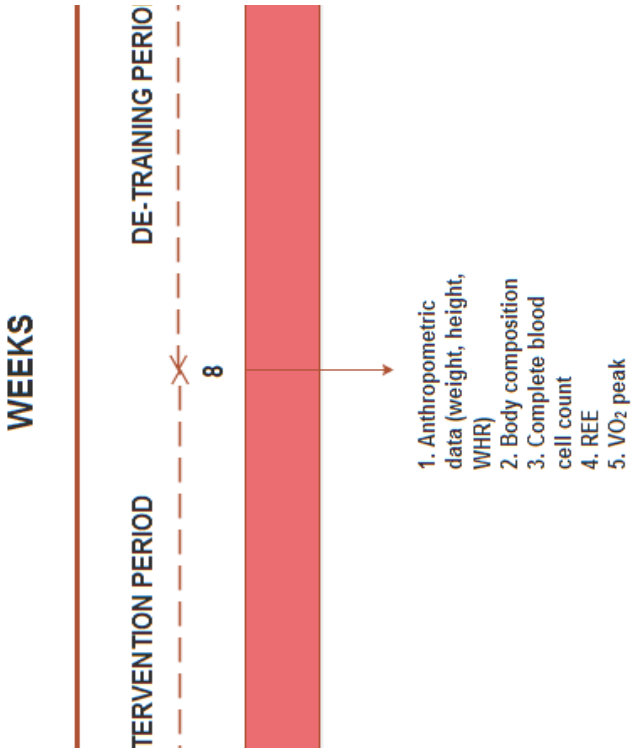
and alcohol intake and 72 hours from strenuous physical activity. Participants were also asked to be hydrated by drinking a glass of water 30min before visiting the laboratory.

Figure 5. Experimental protocol illustrating the study timeline as well as the measurements conducted. Note: WHR: waist to hip ratio, REE: resting energy expenditure, VO2peak: peak aerobic capacity.



During the 16-week experimental protocol, each subject completed a dietary and a physical activity recall. Both dietary and physical activity record were obtained three days per week, two weekdays and one weekend day. Physical activity was obtained through a pedometer (DIGI-WALKER, SW-200, Yamax, Japan). Participants were randomly called on their phone at the end of the day (approximately at 22:00) and were asked about their diet intake that day and were asked to put on the pedometer the next day.

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Measurements

Weight and height. Body weight was measured in light clothing and shoeless (Hu, Jousilahti, Antikainen, Katzmarzyk, & Tuomilehto, 2010) using an operating instruction platform scale (KERN & Sohn GmbH, Version 5.3). Subjects were asked to hold their breath for 8 seconds and stay still for a most accurate measurement. Height was measured with a stadiometer with subjects being also shoeless. BMI was calculated as weight in kilograms divided to the square of height in meters (Hu et al., 2010; Hu, Tuomilehto, Silventoinen, Barengo, & Jousilahti, 2004).

Waist to hip ratio. The WHR was calculated by dividing the waist circumference to the hip circumference. WC was measured using a tape measure at the mid-level between the costal margin and iliac crest, and hip circumference was assessed at the widest level above the trochanter (Chan et al., 2003).

Body composition. Body composition was measured via BIA (Body Composition Monitor, Fresenius Medical Care AG & Co., KGaA D-61346 Bad Homburg, Germany) with participants being in a supine position. Subjects were asked to remain still for a few minutes until the end of the measurement.

Complete blood cell count. Under aseptic techniques, fasting blood sampling (5ml blood samples) was carried out by an experienced phlebotomist in the exercise physiology laboratory of the University of Thessaly and collected in anticoagulant-free plastic tubes. Complete blood cell count, including Hct, Hb, WBC, RBC, MON, MCV, MCH, MCHC, RDW, PLT, mean platelet volume (MPV), platelet hematocrit (PCT) and platelet distribution width (PDW) and was performed by the automatic analyzer Mythic 18 (Orphée S.A., Geneva, Switzerland).

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Peak aerobic capacity (VO_{2peak}). Peak aerobic capacity was estimated via a previously-used maximal exercise protocol using a cycle ergometer (Monark Ergomedic 839E, Vansbro, Sweden) (Flouris et al., 2012). The protocol started with a 3 minute warm-up period with the participants cycling at 60 W, followed by an increase of resistance of 30 W per minute, until exhaustion, maintaining their speed at 60 rpm according to a screen on the cycle (Flouris et al., 2012). For safety reasons during the protocol participants heart rate was evaluated, using a polar (polar RS800CX, Electro Oy, Finland). VO_2 output was obtained through breath by breath sampling and expressed every 20 seconds via the open-circuit method using a metabolic cart (VO_2 analyzer, Care Fusion Germany 234 GmbH, Germany). VO_{2peak} value was estimated as the participant's highest oxygen uptake during the cycle protocol.

Resting energy expenditure. REE was obtained early in the morning, between 06:00 and 09:00, within the first 30 minutes from waking up, following a 12h overnight fast and having refrained from strenuous physical activity for 72h. Measurements took place in a semi-darkened and thermoregulated room (24-25°C) with external distractions minimized. The REE was obtained with subjects in a supine position in a comfortable bed and were asked not to sleep or hyperventilate during the procedure. Breath-by-breath data were collected and averaged every 20 seconds for 30 minutes via the open-circuit method using a metabolic cart (VO_2 analyzer, Care Fusion Germany 234 GmbH, Germany) that was calibrated before each test using standard gases of known concentrations. The REE was calculated via the Weir equation (Weir, 1949) using values from 20 min (excluding the first and last 5 min) and was expressed per 24h.

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Exercise protocols

Participants recruited in one of the training groups: aerobic training group, resistance training group and combined (aerobic and resistance) training group, followed a specific progressive exercise program for 8 weeks (week 1- week 8) using information provided by a recent study that examined the effects of exercise on the expression of PGC-1a4 in human skeletal muscle (Ruas et al., 2012). A detailed description for each training protocol is available in Tables 7-20 (Ruas et al., 2012):

Table 1. Detailed description of the aerobic exercise training protocol.

AEROBIC TRAINING GROUP			
	Frequency	**Intensity**	**Type of exercise**
Week 1	3 days	65% VO_{2peak}	30 min cycling
Week 2	3 days	65% VO_{2peak}	45 min cycling
Week 3	4 days	65% VO_{2peak}	45 min cycling
Week 4-8	5 days	65% VO_{2peak}	60 min cycling

Table 2. Detailed description of the resistance exercise training protocol.
Note: IDBP=Inclined dumbbell bench press.

RESISTANCE TRAINING GROUP				
Intensity	**Day 1**	**Day 2**	**Day 3**	**Day 4**

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Week 1	Low resistance	Lunges Leg Press Leg extension Leg curls Toe raises Crunches	Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	Lunges Leg Press Leg extension Leg curls Toe raises Crunches	Chest press IDBP press Latpulldowns Seated row Lateral raise Biceps curls Triceps extension
Week 2	65% of 1 RM 2 set of 8-10 repetitions	Lunges Leg Press Leg extension Leg curls Toe raises Crunches	Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	Lunges Leg Press Leg extension Leg curls Toe raises Crunches	Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension
Week 3	65% of 1 RM 3 set of 8-10 repetitions	Lunges Leg Press Leg extension Leg curls Toe raises Abs	Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	Lunges Leg Press Leg extension Leg curls Toe raises Crunches	Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension
Week 4-8	65% of 1 RM 4 set of 8-10 repetitions	Lunges Leg Press Leg extension Leg curls Toe raises Crunches	Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	Lunges Leg Press Leg extension Leg curls Toe raises Crunches	Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension

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Table 3. Detailed description of the combined aerobic and resistance exercise training protocol.
Note: IDBP=Inclined dumbbell bench press.

COMBINED TRAINING GROUP					
	Intensity	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Week 1	65% VO_{2peak} Low resistance	20 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	20 min cycling Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	20 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	None
Week 2	65% VO_{2peak} 65% of 1 RM 1 set of 8-10 repetitions	30 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	30 min cycling Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	30 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	None
Week 3	65% VO_{2peak} 65% of 1 RM 2 set of 8-10 repetitions	30 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	30 min cycling Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	30 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	30 min cycling Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension
Week 4-8	65% VO_{2peak} 65% of 1 RM 3 set of 8-10 repetitions	30 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	30 min cycling Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension	30 min cycling Lunges Leg Press Leg extension Leg curls Toe raises Abs	30 min cycling Chest press IDBP Latpulldowns Seated row Lateral raise Biceps curls Triceps extension

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Statistical Analysis

Given the prolonged duration of the study, it was deemed logical to assume that PA and calorie/nutrient intake may change and affect the results. Independent t-tests were conducted to assess differences in calorie intake for all groups between the three time points (baseline, at the end of 8th week and at the end of 16th week). Therefore, multivariate analysis of convariance (MANCOVA) was used to assess statistical differences between group means and time-point means on multiple continuous dependent variables (REE, VO_{2peak}, body composition and complete blood cell count) while controlling for those covariates (PA and calorie-nutrient intake). Independent variables were the group and time-point. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, Ver. 22.0, International Business Machines Corporation, New York, U.S.) with significance set at $p<0.05$.

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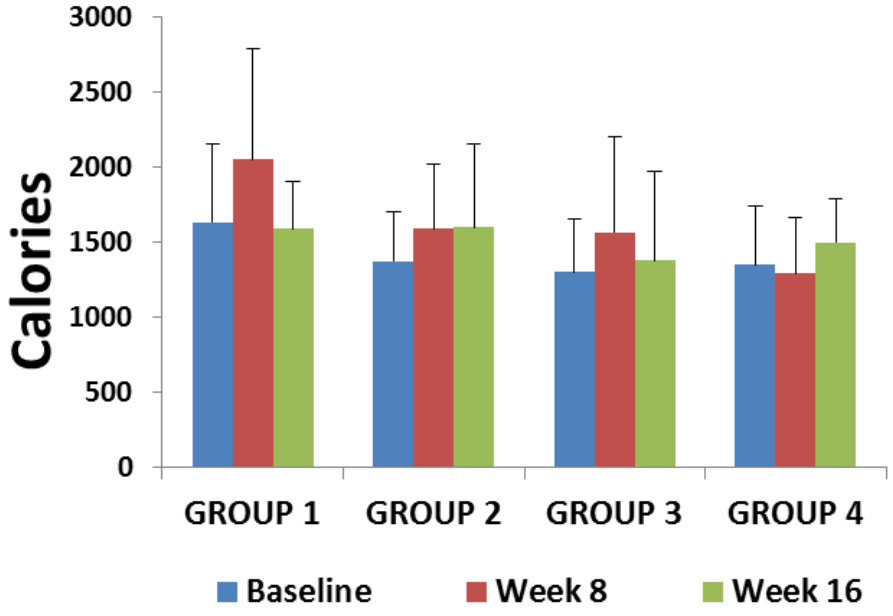
CHAPTER 4: RESULTS

Independent t-tests were conducted to assess differences in calorie intake for all groups between the three time points (baseline, at the end of 8th week and at the end of 16th week). The results revealed no statistical significant differences in any of the groups ($p>0.05$) (Figure 6).

Figure 6. Calorie intake per day in all groups.



Due to the fact that the sample size of each group was small, the participants were then divided in two general groups: control group and exercise group. The aforementioned independent t-test was conducted once more, demonstrating significant statistical difference in calorie intake only in the exercise group between baseline (1401.77±424.02) and week 8 (1681.25±617.96), $p<0.05$ (Figure 7).

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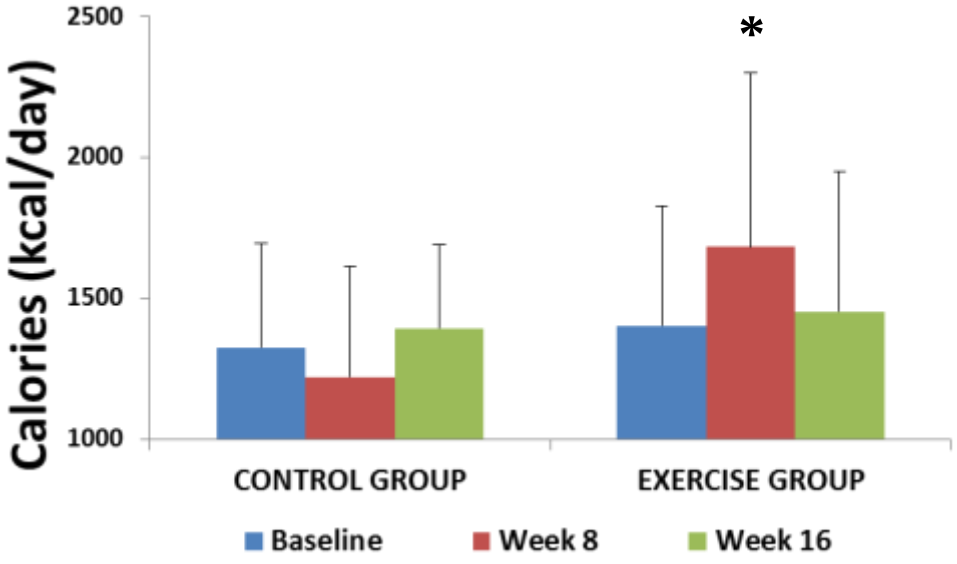
Figure 7. Calorie intake per day in the control and exercise groups.



* p<0.05. Significantly differences in calorie intake

MANCOVA was used to determine statistical mean differences in all health parameters amongst all groups and time points. Diet intake and physical activity were covariates in the analysis. The results showed that the linear combination of the health parameters (dependent variables) was significantly influenced by PA ($p<0.05$), the caffeine intake ($p<0.05$), and the fat intake ($p<0.05$) (Table 4). After controlling for PA and diet intake, statistically significant differences were found between groups ($p<0.05$) but not between time-point ($p>0.05$). There was no statistically significant interaction between group and time-point for any of the dependent variables ($p>0.05$) (Table 4), suggesting no significant changes at any time-point for all parameters measured.

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Table 4. Effects of covariates on dependent variables linear combination.
Note: f=degrees of freedom and p=significant set at <0.05.

Effect	Wilks' L	F	p
Weight of food	0.468	1.478	0.092
Calories	0.448	1.600	0.056
Physical activity	0.391	2.025	0.009
Protein	0.459	1.531	0.074
Carbohydrates	0.521	1.193	0.273
Caffeine	0.421	1.791	0.024
Sugar	0.547	1.075	0.399
Fat	0.426	1.754	0.028
Group	0.047	2.325	0.000
Time	0.289	1.117	0.297
Group*time	0.089	0.664	1.000

Covariate analysis

Covariate analysis was used to examine the covariates' influence in the dependent variables.

Weight of food

The analysis indicated a statistically significant influence of weight of food on the following variables: *OH* (p=0.038), *MON* (p=0.024), *MON* % (p=0.047), *RBCs* (p=0.025), *Hct* (p=0.0060, *MCHC* (p=0.045) (Table 5a, Table 5b).

Table 5a. Dependent variables control from weight of food.
Note: df=degrees of freedom and p=significant set at <0.05.

	Dependent Variables	df	F	p
Weight of food	BMI	1	0.146	0.704
	REE	1	0.085	0.771
	VO_{2peak}	1	0.202	0.654
	Weight	1	0.146	0.703
	WHR	1	0.146	0.703
	DP	1	0.148	0.701
	SP	1	0.223	0.638
	KXT	1	1.249	0.267
	Overhydration	1	4.429	0.038

Table 5b. Dependent variables control from weight of food.

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Note: df=degrees of freedom and p=significant set at <0.05.

	Dependent Variables	df	F	p
Weight of food	nh_weight	1	0.180	0.672
	TBW	1	0.915	0.341
	ECW	1	0.148	0.701
	ICW	1	1.877	0.174
	E_I	1	1.811	0.182
	LTI	1	3.121	0.081
	FTI	1	0.043	0.836
	LTM	1	2.082	0.153
	LTM%	1	0.214	0.645
	ATM	1	0.042	0.838
	Fat (kg)	1	0.039	0.844
	Fat (%)	1	0.111	0.739
	BCM	1	2.506	0.117
	WBC	1	1.314	0.255
	LYM	1	0.083	0.773
	MON	1	5.303	0.024
	GRA	1	0.706	0.403
	LYM(%)	1	1.351	0.248
	MON(%)	1	4.069	0.047
	GRA(%)	1	0.060	0.807
	RBC	1	5.190	0.025
	HGB	1	0.550	0.460
	HCT	1	7.910	0.006
	MCV	1	0.251	0.671
	MCH	1	2.789	0.098
	MCHC	1	4.126	0.045
	RDW	1	0.287	0.593
	PLT	1	1.952	0.166
	MPV	1	0.727	0.396
	PCT	1	1.535	0.219
	PCW	1	1.854	0.177

Calories

The analysis indicated a statistically significant influence of calories on the following variables: *WBC* (p=0.026), *MON* (p=0.045), *MCH* (p=0.023), *MCHC* (p=0.028) and *MPV* (p=0.048) (Table 6).

Table 6. Dependent variables control from calories.
Note: df=degrees of freedom and p=significant set at <0.05.

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	Dependent Variables	df	F	p
Calories	BMI	1	0.009	0.924
	REE	1	0.003	0.957
	VO_{2peak}	1	0.350	0.555
	Weight	1	0.095	0.759
	WHR	1	0.946	0.333
	DP	1	0.022	0.881
	SP	1	1.076	0.302
	KXT	1	0.193	0.661
	OH	1	0.376	0.541
	nh_weight	1	0.082	0.776
	TBW	1	0.154	0.696
	ECW	1	0.147	0.702
	ICW	1	0.145	0.704
	E_I	1	0.041	0.840
	LTI	1	0.000	0.994
	FTI	1	0.005	0.941
	LTM	1	0.077	0.782
	LTM%	1	0.044	0.835
	ATM	1	0.033	0.857
	Fat (kg)	1	0.035	0.851
	Fat (%)	1	0.047	0.829
	BCM	1	0.058	0.810
	WBC	1	5.125	0.026
	LYM	1	2.390	0.126
	MON	1	4.125	0.045
	GRA	1	1.848	0.177
	LYM(%)	1	0.015	0.901
	MON(%)	1	1.321	0.254
	GRA(%)	1	0.482	0.489
	RBC	1	1.911	0.170
	HGB	1	0.829	0.365
	HCT	1	0.891	0.348
	MCV	1	1.215	0.273
	MCH	1	5.328	0.023
	MCHC	1	4.981	0.028
	RDW	1	2.867	0.094
	PLT	1	3.806	0.054
	MPV	1	4.030	0.048
	PCT	1	2.131	0.148
	PCW	1	0.387	0.535

Physical activity

The covariate analysis of PA showed a statistically significant influence on VO_{2peak} (r=0.276, p=0.003), *LTI* (r=0.207, p=0.023), *WBC* (r=-0.207, p=0.024) and *GRA* (r=-0.179, p=0.05) (Figure 8).

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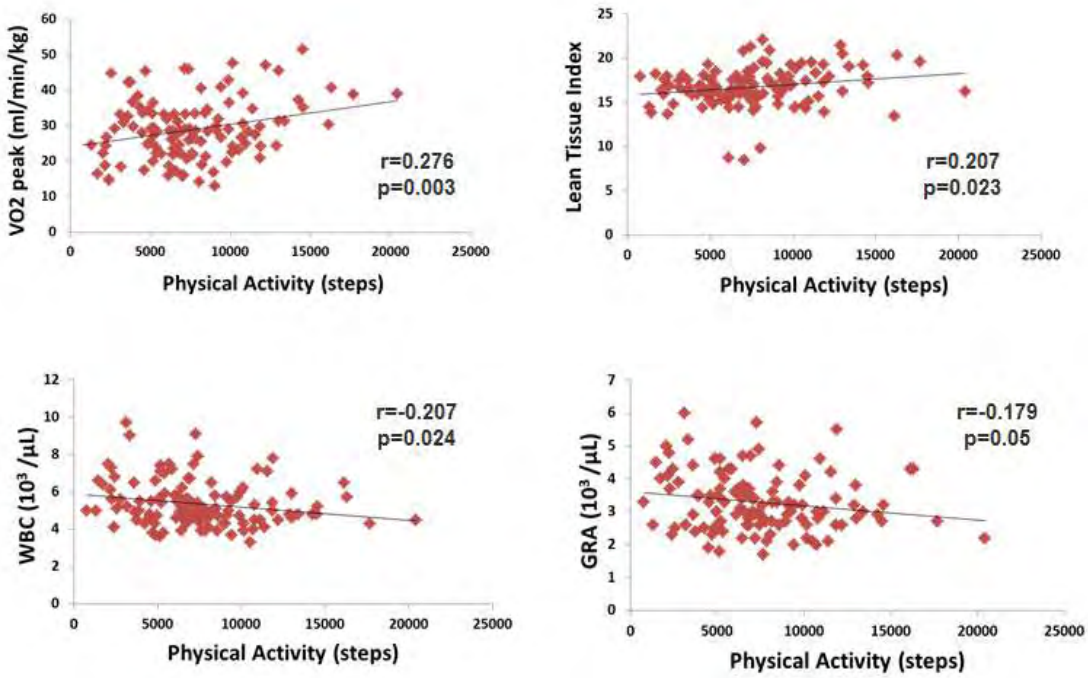
Figure 8. Correlations between physical activity and peak aerobic capacity, lean tissue index, white blood cells and granulocytes.



Protein

Statistically significant control from protein intake were observed on *MCH* (p=0.008), *MCHC* (p=0.022), *RDW* (p=0.020) and *MPV* (p=0.025) (Table 7).

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Table 7. Dependent variables control from protein.
Note: df=degrees of freedom and p=significant set at <0.05.

	Dependent Variables	df	F	p
Protein	BMI	1	0.440	0.509
	REE	1	0.006	0.940
	VO_{2peak}	1	0.619	0.433
	Weight	1	0.011	0.918
	WHR	1	0.254	0.615
	DP	1	0.399	0.529
	SP	1	0.001	0.976
	KXT	1	0.282	0.596
	OH	1	2.806	0.097
	nh_weight	1	0.001	0.981
	TBW	1	0.281	0.597
	ECW	1	0.310	0.579
	ICW	1	0.240	0.625
	E_I	1	0.006	0.939
	LTI	1	0.405	0.526
	FTI	1	0.161	0.689
	LTM	1	0.312	0.578
	LTM%	1	0.536	0.466
	ATM	1	0.034	0.854
	Fat (kg)	1	0.030	0.862
	Fat (%)	1	0.616	0.435
	BCM	1	0.097	0.757
	WBC	1	1.907	0.171
	LYM	1	0.043	0.836
	MON	1	0.109	0.742
	GRA	1	2.942	0.090
	LYM(%)	1	1.157	0.285
	MON(%)	1	1.801	0.183
	GRA(%)	1	3.228	0.076
	RBC	1	1.139	0.289
	HGB	1	3.001	0.087
	HCT	1	0.001	0.976
	MCV	1	2.663	0.106
	MCH	1	7.414	0.008
	MCHC	1	5.432	0.022
	RDW	1	5.651	0.020
	PLT	1	3.207	0.077
	MPV	1	5.209	0.025
	PCT	1	1.496	0.224
	PCW	1	0.302	0.584

Caffeine

The analysis showed a statistically significant control of caffeine intake on VO_{2peak} (p=0.016), *E_I* (p=0.042), *LTI* (p=0.044), *LTM* (p=0.026), *BCM* (p=0.025) and *MPV* (p=0.010) (Table 8).

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Table 8. Dependent variables control from caffeine.
Note: df=degrees of freedom and p=significant set at <0.05.

	Dependent Variables	df	F	p
Caffeine	BMI	1	0.134	0.715
	REE	1	1.139	0.289
	VO_{2peak}	1	5.994	0.016
	Weight	1	0.000	0.990
	WHR	1	0.134	0.715
	DP	1	2.594	0.111
	SP	1	1.707	0.195
	KXT	1	2.169	0.144
	OH	1	1.138	0.289
	nh_weight	1	0.053	0.819
	TBW	1	2.099	0.151
	ECW	1	0.464	0.498
	ICW	1	3.823	0.054
	E_I	1	4.239	0.042
	LTI	1	4.173	0.044
	FTI	1	0.602	0.440
	LTM	1	5.088	0.026
	LTM%	1	1.568	0.214
	ATM	1	0.457	0.501
	Fat (kg)	1	0.463	0.498
	Fat (%)	1	1.272	0.262
	BCM	1	5.199	0.025
	WBC	1	0.698	0.406
	LYM	1	0.833	0.364
	MON	1	0.607	0.438
	GRA	1	0.046	0.831
	LYM(%)	1	0.221	0.639
	MON(%)	1	0.304	0.583
	GRA(%)	1	0.591	0.444
	RBC	1	0.015	0.903
	HGB	1	0.279	0.599
	HCT	1	2.116	0.149
	MCV	1	2.053	0.155
	MCH	1	0.074	0.786
	MCHC	1	1.173	0.282
	RDW	1	0.033	0.857
	PLT	1	1.370	0.245
	MPV	1	6.966	0.010
	PCT	1	0.068	0.795
	PCW	1	0.291	0.591

Fat

Statistically significant control from fat intake was found on *WBC* (p=0.002), *LYM* (p=0.043), *MON* (p=0.027), *GRA* (p=0.029) and *PLT* (p=0.05) (Table 9a, Table 9b).

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Table 9a. Dependent variables control from fat.
Note: df=degrees of freedom and p=significant set at <0.05.

	Dependent Variables	df	F	p
Fat	BMI	1	0.078	0.780
	REE	1	0.051	0.823
	VO_{2peak}	1	0.632	0.429
	Weight	1	0.104	0.748
	WHR	1	0.564	0.455
	DP	1	0.010	0.922
	SP	1	1.587	0.211
	KXT	1	0.002	0.968
	OH	1	0.156	0.694
	nh_weight	1	0.102	0.750
	TBW	1	0.001	0.980
	ECW	1	0.011	0.917
	ICW	1	0.016	0.899
	E_I	1	0.325	0.570
	LTI	1	0.216	0.643
	FTI	1	0.181	0.671
	LTM	1	0.125	0.724
	LTM%	1	0.094	0.759
	ATM	1	0.227	0.635
	Fat (kg)	1	0.230	0.633
	Fat (%)	1	0.093	0.761
	BCM	1	0.146	0.703
	WBC	1	10.029	0.002
	LYM	1	4.232	0.043
	MON	1	5.061	0.027
	GRA	1	4.949	0.029
	LYM(%)	1	0.005	0.943
	MON(%)	1	0.829	0.365
	GRA(%)	1	0.273	0.603
	RBC	1	1.318	0.254
	HGB	1	0.347	0.557
	HCT	1	0.363	0.549

Table 9a. Dependent variables control from fat.
Note: df=degrees of freedom and p=significant set at <0.05.

	Dependent Variables	df	F	p
Fat	MCV	1	1.196	0.277
	MCH	1	3.138	0.080
	MCHC	1	2.082	0.153
	RDW	1	1.413	0.238
	PLT	1	3.935	0.050
	MPV	1	2.924	0.091
	PCT	1	2.583	0.111
	PCW	1	0.700	0.405

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CHAPTER 5: DISCUSSION

The main aim of this study was to investigate the effects of different types of exercise on REE, VO_{2peak}, body composition and complete blood cell count. The results did not show significant differences in any of the health indicators measured at any time-point of the study (at the end of the 8 week exercise period and at the end of the de training period). With respect to the effects of exercise on REE, the results of this study are in agreement with the study of Broeder et al (1992), which investigated the effect of 12 week high-intensity endurance or resistance exercise training on REE on 47 healthy, untrained males (18-35 years). Furthermore, in a previous study, during a training period of 22 weeks where 103 subjects were included in four groups: aerobic exercise training, resistance exercise training, combined aerobic and resistance exercise training and control group, no significant changes in REE were observed in any of the exercise groups (Jennings et al., 2009). A large body of studies using aerobic training groups has no significant changes in REE (Dolezal & Potteiger, 1998).

The results of the present study are in contrast with previously-published studies that have shown differences in REE after a chronic training period. Studies with resistance intervention protocols or some with combined aerobic and resistance exercise have found increases in REE, due to its strong correlation with free fat mass (Dolezal & Potteiger, 1998; Dolezal et al., 2000). In the present study no changes in FFM were observed and that could be a reason for no changes in REE. Also, the discrepancy between the current study and previous published studies can be due to the different exercise protocols. These differences relate to the total duration of exercise, the intensity, as well as the rest breaks between consecutive exercise bouts (Speakman & Selman, 2003). Finally, an important factor that can affect the results of REE pertains to the time of measuring REE from the last exercise session. It is known that REE can be elevated up to 24-48h after a single bout of resistance

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exercise (Jamurtas et al., 2004; Schuenke et al., 2002). In the present study, the time interval of REE was 72h after the last exercise session but many studies do not refer the time point of REE measurement in their methodology (Table 10). Our study is in contrast with the one which was demonstrated by Byrne and Wilmore (2001) who measured REE 72hours after the last exercise session. The latter study included three groups of participants (control, resistance and combined training group) and observed significant increase of REE in the resistance group, but a decrease in the combined exercise group (Byrne & Wilmore, 2001).

Table 10. Time point of REE measurement since the last exercise session.

Studies	Time point in hours
Poehlman et al (1988)	24
Pratley et al (1994)	24
Tremblay et al (1985)	36
Broeder et al (1992)	48
Sharp et al (1992)	36
Van Etten et al (1995)	30
Byrne and Wilmore (2001)	72

The present study did not detect significant differences in body composition in the exercise groups although we expected decrease in weight and total body FM in the aerobic and combined group and increase in free fat mass particularly in the resistance group. A possible reason for the unexpected lack changes in the body composition of the training groups is the method used here for estimating body composition. BIA is an accurate and safe method for estimating body composition and TBW and it can be strongly influenced by the hydration factor an lead to fake results (Deurenberg, 1996), although we controlled the hydration factor in our study.

We investigated the effect of the three exercise programs on VO_{2peak} and the results did not show significant changes after the training period. According to the majority of studies with resistance exercise protocols, the results of our study have similarly no

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significant changes in VO_{2peak} (Campos et al., 2002; Tanaka & Swensen, 1998). The observed lack of changes in VO_{2peak} in the aerobic and combined group were unexpected and are in contrast with many studies that have shown increases in VO_{2peak} (Cadore et al., 2011; Doncaster & Twist, 2012; Dudley & Djamil, 1985; Hunter, Demment, & Miller, 1987). The present results may be due to the exercise protocols used, the exercise intensity incorporated, and the number of participants included in each group.

As for the complete blood cell count, no changes were observed after the training period. This result is consisted with the results of Dorota Kostrzewa-Nowak et al (2014) where no significant changes in complete blood cell count were reported.

In summary, lack of changes in the tested health indicators can be probably be explained by:

- The type and intensity of the exercise groups
- The duration of the training period
- The sample size

A second aim of this study was to investigate changes in REE, VO_{2peak}, body composition and complete blood cell count after a de-training period (8 weeks in our study) and the present study was the first one to examine these changes on untrained males. The results did not reveal statistically significant changes in the tested values after the de-training period. There are not many studies that investigated changes in health parameters after a de-training period and most of them are using trained participants. These studies suggest that a de-training period in athletes or trained people leads to a decrease in VO_{2peak} and metabolic rate and an increase in body fat (Ormsbee & Arciero, 2012). The lack of changes during the de-training period in the present study is probably explained by the lack of changes observed during the training period.

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CHAPTER 6: CONCLUSIONS

The specific 8 weeks exercise protocols of the present study did not generate significant changes on resting energy expenditure, peak aerobic capacity, body composition and complete blood cell count. No changes on the health parameters were observed after the de-training period either.

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APPENDIX: INTERNAL ETHICS COMMITTEE

University of Thessaly
Department of Physical Education and Sport Science

Internal Ethics Committee

Trikala: 11/12/2013
Protocol number: 809

Application for approval of research entitled: The effects of prolonged exercise on brown-like
tissue formation in humans.
Scientist responsible – supervisor: Yiannis Koutedakis PhD, Professor in Applied Physiology
Departments: Department of Physical Education and Sport Sciences, School of Sport, Performing
Arts and Leisure
Institutions: Thessaly University, Wolverhampton University
Scientific advisor: Andreas D. Flouris PhD, Researcher in Environmental Physiology
Department: FAME Laboratoty
Institution: CERETETH
Main researcher – student: Ntinas Petros
Study program: PhD
Department: School of Sport, Performing Arts and Leisure
Institution: Wolverhampton University, UK (collaborating institution)
The proposed research relates to a:
Research grant Postgraduate thesis Undergraduate □ X thesis □ Independent research □
Contact phones: Yiannis Koutedakis, PhD: +302431047056; Andreas D. Flouris, PhD: +302431500601, Ntinas Petros: +306974010118
Contact emails: Yiannis Koutedakis, PhD: y.koutedakis@pe.uth.gr; Andreas D. Flouris, PhD:
andreasflouris@cereteth.gr; Ntinas Petros: petros.cd@gmail.com
The Internal Ethics Committee (IEC) of the Department of PE and Sport Science (DPESS), University of Thessaly, examined the proposal in its 3-2/11-12-2013 meeting and approves the implementation of the proposed research.

The Chair of the IEC – DPESS

ATHANASIOS TSIOKANOS, PHD

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Υπεύθυνη δήλωση

Η κάτωθι υπογεγραμμένη Παρασκευή Γκιάτα, ΑΕΜ: 0712 (μεταπτυχιακή φοιτήτρια του τμήματος Επιστήμης Φυσικής Αγωγής και Αθλητισμού του Πανεπιστημίου Θεσσαλίας του προγράμματος «Άσκηση και Υγεία»

δηλώνω υπεύθυνα ότι αποδέχομαι τους παρακάτω όρους που αφορούν

τα πνευματικά δικαιώματα και τη διαχείριση του επιστημονικού υλικού ή των ερευνητικών δεδομένων που θα έχω στη διάθεσή μου κατά την πορεία της συμμετοχής μου στο ερευνητικό έργο με τίτλο: " Η επίδραση διαφορετικών προγραμμάτων άσκησης σε διαφορετικούς δείκτες υγείας"

και του οποίου επιστημονικώς υπεύθυνος είναι ο καθηγητής κ.Κουτεντάκης Ιωάννης

1. Τα πνευματικά δικαιώματα του έργου αυτού μετά την αποπεράτωσή του ανήκουν στον/στην επιστημονικώς υπεύθυνο-η ή/και στον φορέα που υποστηρίζει ή χρηματοδοτεί το έργο.
2. Οποιαδήποτε επιστημονική δημοσίευση ή ανακοίνωση (αναρτημένη ή προφορική), ή αναφορά που προέρχεται από το υλικό/ δεδομένα της εργασίας αυτής και η πιθανή συμμετοχή του ονόματός μου σε αυτήν αποφασίζεται εκ των προτέρων από εμένα και τον/την επιστημονικώς υπεύθυνο-η του έργου και αυτό θα πιστοποιηθεί εγγράφως μεταξύ εμού και του/της υπεύθυνου-ης.
3. Η σχετική σύμβαση εργασίας και οι υποχρεώσεις μου από τη συμμετοχή μου στο έργο αυτό, ρυθμίζονται με βάση τους κανόνες και τις αρχές της Επιτροπής Ερευνών του ΠΘ.

Τέλος, δηλώνω ότι γνωρίζω τους κανόνες περί δεοντολογίας στην έρευνα της σχετικής με το συγκεκριμένο ερευνητικό έργο περιοχής, τους κανόνες περί λογοκλοπής και πνευματικής ιδιοκτησίας και ότι θα τους τηρώ απαρέγκλιτα καθ' όλη τη διάρκεια της συμμετοχής μου στο έργο αυτό και κατά την πιθανή μου συμμετοχή σε διαδικασίες δημοσίευσης που θα προκύψουν μετά το τέλος του έργου.

08/01/2016
Η δηλούσα
Παρασκευή Γκιάτα

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